ANTIGENS OF VEGETABLE ORIGIN ACTIVE IN PNEUMOCOCCUS INFECTIONS

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In the course of the study of the antigenicity of polysaccharides of *Diplococcus pneumoniae* (pneumococcus), it seemed desirable to investigate the possible antigenicity of polysaccharides from common higher plants. In reviewing methods of isolation of plant polysaccharides, it was found that they have been extensively studied by biochemists with the view of isolation and identification of specific components, such as cellulose and starch in the structural fibers, and the glycosides, gums, hemicelluloses, which serve as reserve polysaccharides in the cell wall. The difficulty of separating the components has led to considerable confusion in their classification. In particular, hemicelluloses have been identified by Norman (1937) as "... those cell-wall polysaccharides which may be extracted from plant tissues by treatment with dilute alkalis, either cold or hot, but not with water, and which may be hydrolyzed to constituent sugar and sugar-acid units by boiling with hot dilute mineral acids". This method of separating the hemicelluloses from plants is similar to the method employed in this laboratory for isolating antigenic polysaccharide substances from animal tissues (Felton *et al.*, 1955). Accordingly, by using such an alkali method, as well as technique employed for isolating bacterial polysaccharides, similar polysaccharide substances have been separated from many plants. The isolation procedures and the tests of these hemicelluloses for immunizing ability against virulent pneumococci and the immunologic significance of the results comprise the subject matter of the present investigation. A report on the early part of this study was published in abstract (Felton *et al.*, 1947*a*).

Polysaccharides, isolated from several plant gums, have been found to give cross precipitation with antipneumococcus sera of several types (types I, II, and III) by Heidelberger, Avery, and Goebel (1929) and by Marrack and Carpenter (1938); also a polysaccharide component from oxidized cotton gave cross precipitation with type III serum (Heidelberger and Hobby, 1942). However, to our knowledge no study has been made of the antigenicity in mice of polysaccharides (hemicelluloses) from plants.

In the present study 70 different plant species, including several edible vegetables, were fractionated to obtain hemicelluloses and related substances, and the activity of the products as immunizing agents against pneumococcus infections was tested in mice. In certain instances, roots, stems, leaves, fruit, and seeds of plants were treated separately. In all, 437 fractions (not all polysaccharides) were isolated and tested in the usual *in vitro* precipitin tests with specific immune horse sera types I, II, and III, and in active immunization tests in mice against virulent pneumococci of the same three types. The report includes: methods of isolation, the distribution of immunizing activity among hemicelluloses from "edible" and "inedible" plants, the antigenicity of such products from different parts of the tomato plant, the degree of immunity following single and repeated injections, and a comparison of certain chemical analyses of some hemicelluloses of medium and some of high antigenicity.

METHODS OF ISOLATION OF HEMICELLULOSES

The methods used for extracting possibly antigenic constituents from the various plants have been adaptations of procedures employed in isolating antigenic polysaccharide components from bacteria or from animal tissues. Activity at the various steps was followed by the use of a precipitin reaction with antipneumococcus serum. After macerating the plant in a grinder, initial fractionation was made by expressing the

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soluble material in a hydraulic press under 4,000 pounds pressure. The hemicelluloses contained in the soluble extracts were then separated by one of the following procedures: (1) precipitation of the polysaccharide with calcium phosphate formed *in situ* at pH 9.0 as used for preparing pneumococcus polysaccharide from broth cultures (Felton *et al.*, 1935). The precipitate was then freed of calcium by dissolving in either 33 per cent acetic acid or hydrochloric acid at pH 3 in the cold (4 C) and reprecipitating the polysaccharide with three volumes of cold 95 per cent ethyl alcohol. Resolution in hydrochloric acid and precipitation with alcohol were sometimes necessary to remove residual calcium. After dissolving the precipitate in one per cent sodium acetate at pH 7, the hemicellulose was precipitated with one and one-half volumes of alcohol, and this precipitate was washed successively with methyl alcohol, acetone, and ether, and dried in a vacuum desiccator over calcium chloride. (2) In a few instances, the extract from the press was chilled and acidified directly to pH 3, and then precipitated with alcohol and the rest of the procedure carried out as above. (3) With dry substances, or those from which there was little or no liquid, the ground materials were extracted with organic solvents (ethyl or methyl alcohol, acetone, or other ketones), and then since the soluble fractions were usually found to be inert, the insoluble residue was taken up in water or if necessary for solution dilute sodium hydroxide at pH 9. With the soluble fraction one of the above procedures was followed. It should be pointed out that although these general methods were followed, it was necessary to adapt the procedure to the particular plant under study. No one method was found uniformly suitable for isolation of antigenic substance as followed by precipitin tests.

With most plants, the press cake from the original ground plant was found to be insoluble in water or even in dilute acid or alkali. Consequently to study this fraction, more rigorous solvents were employed, for example, 4-normal sulfuric acid or normal sodium hydroxide. However, as will be seen below, either there was little active material present, or some destruction occurred, for such fractions were in general of lower immunological titer than those from the original extract from the press.

For tests, saline solutions of the dried products were made containing 10 mg per ml, and serial dilutions were prepared for use in both the *in vitro* and the *in vivo* immunological procedures. Precipitin tests were run with 1 ml volumes of the serial dilutions of sample plus 1 ml of monovalent antipneumococcus horse serum (types I, II, and III) in that dilution which had been found to give maximum precipitation with specific pneumococcus polysaccharide. In the tests for active immunity in mice, the pneumococci used were strains of types I, II, and III of such virulence that one or two organisms caused the death of mice in 24 to 36 hours. The mice tested were of the C_3H strain selected because of their uniform susceptibility to pneumococcus infections (Felton and Stahl, 1937). The test for active immunity was carried out in two steps: (a) intraperitoneal inoculation into mice of serial dilutions of the isolated sample, with nine mice per dilution; and (b) seven days later intraperitoneal inoculation of 1,000 lethal doses of types I, II, and III pneumococci into above mice, with three mice per type per dilution of sample. The immunity end point was considered the least amount of sample which permitted survival of at least two of the three mice. Survivors were recorded for a minimum of 96 hours after infection.

RESULTS

(1) *Results from different isolation procedures.* Results obtained with products from six plants as examples of the above methods of isolation are shown in table 1. With collard greens and melon rind, the press extract was divided into two portions: the one (A) for isolation of polysaccharide by the calcium phosphate procedure, the other (B) for direct acidification to pH 3 with hydrochloric acid. With sunflower seed an aqueous extract was precipitated similarly with the calcium phosphate technique. With the other three plants, initial infusion in 0.5 normal sodium hydroxide preceded the calcium phosphate treatment. In all cases the usual repeated alcoholic precipitations from acid solution and finally from neutral solution were carried out. The biological tests were run on saline solutions of the final dried products. It would appear, from the table, that although each method resulted in some antigenic material, the calcium phosphate precipitation method yielded products of somewhat higher immunizing titer than did the others. However, with such a variety of source materials, a quanti-

TABLE 1

Antigenicity of vegetable hemicelluloses prepared by different methods

Vegetable	Method of Preparation	Immunological Tests					
		Precipitin titer Antipneumococcus sera			Immunity end point*		
		Type I	Type II	Type III	Pn I	Pn II	Pn III
					mg	*mg*	*mg*
Collard greens	Extract from press						
	(A) Calcium phosphate precipitation *in situ*	1:200	1:12,800	1:1,600	0.005	0.0005	0.5
	(B) Acidification	–	1:1,600	1:100	0	5.0	0
Melon rind (honeydew)	Extract from press						
	(A) Calcium phosphate precipitation *in situ*	–	1:128,000	1:16,000	0.05	0.05	0.005
	(B) Acidification	0	1:16,000	1:500	0.05	1.0	0
	Press residue + sulfuric acid	0	1:3,200	1:100	5.0	0.5	0
Wheat germ	(A) NaOH soluble + calcium phosphate *in situ*	1:800	1:800	1:400	0.05	0	0
	(A2) NaOH insoluble + sulfuric acid	0	0	1:100	0	0.0005	0
Grapefruit	(A) NaOH soluble	1:100	1:200	1:100	0	0.005	0
	(A2) NaOH insoluble + sulfuric acid	0	0	0	0	5.0	0
Sunflower seed	Extract from water infusion						
	(A) Calcium phosphate precipitation *in situ*	0	1:64,000	0	1.0	0.0005	0
	Water insoluble + sulfuric acid	0	1:6,400	0	0	5.0	0
	Sulfuric acid insoluble + NaOH	0	1:51,200	0	5.0	0.5	5.0
Apple (fruit) (winesap)	(A) NaOH soluble + calcium phosphate *in situ*	1:200	1:200	1:200	0	0.005	0
	Second fraction of (A)	1:6,400	1:3,200	1:1,600	0	0.05	0
	(A2) NaOH insoluble + sulfuric acid	0	0	0	0	5.0	0

* Immunity end point = least amount which immunized mice against 1,000 lethal doses of virulent pneumococci.

– Indicates not tested.

tative comparison of results from the different methods is hardly justifiable. It has furthermore been found that another lot of a given vegetable may require some modification of the technique applied here. These results are representative, however, in that in most instances the immunologic activity was found in the first extract. In other words, the antigenic components were generally soluble in their native state or on addition of dilute alkali. Some samples which failed to precipitate immune serum of one type stimulated immunity of high titer against that type, and conversely a few which did not immunize gave positive precipitin titers in low dilutions.

(2) *Distribution of immunizing activity among hemicelluloses from "edible" and "inedible" plants.* As an introductory survey of the results of active immunity tests on the isolated hemicelluloses and other fractions, the entire series was arbitrarily divided into products from commonly "edible" and those from commonly "inedible" plants, and these were listed in table 2 in order of the relative immunizing activity against virulent pneumococcus infections of types I, II, and III. Plus signs are used to indicate the degree of immunity, ranging from a high of three plus to indicate 0.0005 or 0.005 to one plus for 1.0 or 5.0 mg. In the instances in which several fractions

TABLE 2

Distribution of immunizing antigen among hemicelluloses from commonly "edible" and "inedible" plants

"Edible" Plants				"Inedible" Plants			
Source of hemicellulose	Immunity against 10^8 LD			Source of hemicellulose	Immunity against 10^8 LD		
	Pn I	Pn II	Pn III		Pn I	Pn II	Pn III
Melon honeydew (rind)	+++	+++	+++	Tomato greens (roots)	+++	++	++
Collard greens	+++	+++	++	Pumpkin (skin)	+++	++	+
Sunflower seed	+++	+++	+	Mosses	+++	++	0
Pumpkin pulp	+++	++	+	Nightshade	+++	0	0
Squash (fruit)	+++	+++	0	Grass, lawn	++	++	++
Peanut meal	+++	++	0	Cotton, nonabsorbent	+	+++	0
Tomato (fruit)	++	++	+++	Wood, maple	+	++	0
Watermelon rind	++	++	++	Wood, pine	++	++	++
Green bean (fruit)	++	++	++	Squash seed	+	+++	0
Parsnip greens	++	++	+	Ageratum plant	+	++	0
Pomegranate (fruit)	++	+	++	Cottonseed meal	+	+	0
Wheat germ	++	+++	0	Lupine seed	+	+	0
Cucumber	++	++	0	Mullen plant	+	+	0
Kale greens	++	++	0	Iris plant	0	++	0
Soybean meal	++	++	0	Spider lily	0	++	0
Rape greens	++	+	0	Gladioli (leaves and bulbs)	0	++	0
Lettuce	++	0	0	Corn husks	0	++	0
Rutabaga (root)	++	0	0	Nasturtium plant	0	0	++
Nutmeg powder	+	+++	++				
Onion bulb	+	+++	0	Chickweed plant	0	0	0
Lima bean	+	++	0	Geranium plant	0	0	0
Potato, white	+	+	+	Hydrangea plant (leaves and small stems)	0	0	0
Barley	+	0	0				
Sugar cane	+	0	0	Ivy, English	0	0	0
Yeast (baker's)	+	0	0	Japonica plant	0	0	0
Apple, Winesap	0	+++	0	Marigold	0	0	0
Grapefruit rind	0	+++	0	Morning glory plant	0	0	0
Irish moss (carrageen)	0	+++	0	Nicotiana plant	0	0	0
Oatmeal	0	+++	0	Rose	0	0	0
Rhubarb, leaves	0	+++	0	Spiderwort	0	0	0
Green pepper (fruit)	0	++	++	Sedum plant	0	0	0
Cabbage	0	++	0	Vetch seed	0	0	0
Coconut	0	+	++				
Cinnamon powder	0	0	++				
Carrot	0	0	+				
Broccoli greens	0	0	0				
Swiss chard greens	0	0	0				
Eggplant (fruit)	0	0	0				
Orange (rind)	0	0	0				
Pear (fruit)	0	0	0				
Pectin	0	0	0				
Radish root	0	0	0				
Rice (polished)	0	0	0				

+++ indicates immunity with 0.0005 mg or 0.005 mg of hemicellulose; ++ indicates immunity with 0.05 or 0.5 mg; + indicates immunity with 1.0 or 5.0 mg.

TABLE 3

Vegetable hemicelluloses with high immunizing activity against pneumococcus

Vegetable	Method†	Precipitin Titer Antipneumococcus Sera			Immunity End Point (against 1,000 LD)		
		Type I	Type II	Type III	Pn I	Pn II	Pn III
					mg	*mg*	*mg*
Tomato (tops and roots)	A	1:100	1:12,800	1:800	0.0005	0.05	5.0
Collard leaves (1)	A	1:200	1:12,800	1:1,600	0.005	0.0005	0.5
Tomato tops	B	*	*	*	0.005	0.05	5.0
Melon rind (1)	A	*	1:128,000	1:16,000	0.05	0.05	0.005
Tomato (green fruit)	A	1:400	1:12,800	1:1,600	5.0	5.0	0.0005
Nutmeg	C	1:400	1:400	1:400	5.0	0.0005	0.05
Squash, pattypan	C	1:400	1:400	1:400	0.0005	0.5	0
Tomato (tops, extract)	A	0	0	trace	0.0005	0.0005	0
Melon rind (2)	D	1:128,000	1:1,000	1:500	0.005	0.005	0
Pumpkin rind	A	1:2,000	1:2,000	1:2,000	0.005	0.05	0
Tomato tops	M1	*	*	*	0.005	0	0.5
Squash, winter	C	1:800	1:100	1:200	0.05	0.0005	0
Squash seed	C	1:100	1:400	1:400	0.5	0.0005	0
Onion	C	1:800	1:400	1:800	5.0	0.0005	0
Sunflower seed (1)	B	0	1:64,000	0	1.0	0.0005	0
Moss, Florida tree	C	1:1,000	0	0	0.0005	0	0
Peanut meal	C	1:100	1:400	1:400	0.0005	0	0
Pumpkin seed	D	1:3,200	1:200	1:200	0.0005	0	0
Pumpkin pulp	C	1:1,600	1:400	1:400	0.005	0	0
Sunflower seed (2)	A	0	0	0	0.005	0	0
Weed leaves	C	1:200	1:100	0	0.005	0	0
Tomato tops	M2	*	*	*	0	0.005	0.05
Collard leaves (2)	C	0	0	0	0	0.0005	0
Collard leaves (2b)	C	0	0	0	0	0.0005	0
"Moss, Irish"	A	0	1:500	1:1,000	0	0.0005	0
Rhubarb	D	1:800	1:400	1:800	0	0.0005	0
Wheat germ	B1	0	0	1:100	0	0.0005	0
Apple	B	1:200	1:200	1:200	0	0.005	0
Cotton	B2	0	1:1,000	0	0	0.005	0
Grapefruit rind	B	1:100	1:200	1:100	0	0.005	0
Oatmeal	B1	1:800	1:800	1:800	0	0.005	0
Squash, gooseneck	A	0	1:8,000	0	0	0.005	0

* Indicates not tested.

† Letters indicate initial step of isolation: A = calcium phosphate method; B = N NaOH; B1 = 0.5 N NaOH; B2 = 0.1 N NaOH; C = water; D = HCl; M1 = morpholine; M2 = diisopropylketone.

(1) and (2) indicate preparations made in different years.

Note: The C method frequently was preceded by removal of alcohol and/or acetone soluble constituents.

from a given plant, or products from different crops of a plant, have been studied, the one of highest titer is listed (see experiment 4 below). Several facts stand out: In the first place, the table shows more antigenic components from the "edible" group than from the "inedible"; second, in each group more than half immunized mice against two types and many against three types of pneumococci; third, in the active ones of either series, about 80 per cent immunized against

type II infection, 70 per cent against type I, but only some 40 per cent had measurable activity against type III. It should be mentioned that, except for broccoli greens, the plants listed as failing to demonstrate an antigenic component were not repeatedly studied. Hence it may be concluded only that the particular fractions isolated were devoid of antigenicity. Tests of products by another procedure or from another part of the plant, or another season's crop, might answer the question as to the possible presence of an active antigen in such plants. How widespread this characteristic may be is problematic, but indeed it is significant that such diverse plant species contain substances which, in minute amounts, immunize mice against virulent pneumococcus infections.

(3) *Hemicelluloses of high immunizing activity.* The high titer fractions are of greatest interest. Table 3 records a list of 32 hemicellulose components of high titer from 18 plants (only 3—nightshade, cotton, and tree moss—were inedible) together with the amount of isolated product which immunized mice against 1,000 lethal doses of pneumococci types I, II, and III, and the corresponding precipitin titers. The initial step of extraction is indicated in the second column, (A), (B), etc.; and the different parts of a given plant, or preparations from crops in different seasons, by the explanatory notes (1), (2), etc. It should be noted that approximately half of these preparations stimulated immunity against more than one type of pneumococcus. All but six preparations immunized against type II pneumococci; only one produced a high degree of immunity against type III. The immunity produced by a given product was seldom of the same titer against different types of pneumococci. The relationship between immunity and precipitin titer was not proportional. It is evident, however, that, whereas three of these samples failed to precipitate a specific serum of any of the three types, more than half of them precipitated all three types. To determine whether these results indicate a single antigen with polyvalent immunological characteristics or a mixture of monovalent antigens remains for subsequent investigation.

Several of these highly active samples were retested against larger numbers of pneumococci and with five mice per culture dilution. The significant results were as follows: collard greens' hemicellulose in concentration of 0.05 mg protected against 10,000 lethal doses of type I and 1,000,000 lethal doses of type II; tomato tops' (leaves and stalks) polysaccharide (0.5 mg) protected mice against 1,000,000 lethal doses of type II; from six other plants the isolated fraction (0.5 mg) protected against 10,000 lethal doses of type I; and from another group of six, 0.5 mg protected against 100,000 lethal doses of type II.

(4) *Antigenicity of hemicelluloses from different parts of the tomato plant.* In the preceding table, it could be seen that the hemicelluloses from various parts of a given plant differed appreciably in antigenicity. This might be expected from the fact that the function and chemical structure of the various parts are so diverse. In several experiments, different parts of the mature tomato plant were investigated separately for the presence of antigenic components. As seen in table 4, by the method of isolation employed in one

TABLE 4

Antigenicity of hemicelluloses from different parts of the tomato plant

Experiment No.	Part of Tomato Plant	Precipitin Titer Antipneumococcus Sera			Immunity End Point (against 1,000 LD)		
		Type I	Type II	Type III	Pn I	Pn II	Pn III
					mg	*mg*	*mg*
1	Green fruit	1:400	1:12,800	1:1,600	5.0	5.0	0.0005
	Root	trace	1:2,000	trace	0	1.0	0
	Stalks	1:400	1:25,600	1:400	5.0	0.5	5.0
	Tops	1:200	1:25,600	1:3,200	5.0	0.5	0
	Pool of roots, tops and stalks	1:100	1:12,800	1:800	0.0005	0.05	5.0
2	Ripe fruit	1:400	1:1,600	1:400	0.05	0.05	5.0
	Pool of roots and tops	1:200	1:51,200	1:800	0	0.05	0

experiment, the hemicellulose recovered from the root alone was of low immunizing titer for type II and negative for types I and III. The stems and the leaves were of low titer although a sample derived from a mixture of roots, stalks, and tops was of high titer (0.0005 mg) against type I infection. The hemicellulose from the green fruit was of high titer against type III, while ripe fruit yielded a fraction of moderate activity, 0.05 mg. Eleven batches of tomato leaves and vines have been investigated over a period of several years, and the products from six prepared in the same manner (calcium phosphate procedure) were antigenic for all three types in doses ranging from 5.0 to 0.0005 mg. The roots studied from four batches were, with the exception of the one above, devoid of antigenic substance. Hence it is possible that the more active antigens may be components of the developing rather than the storage areas of the plant.

(5) *Comparison of immunity following single and triple injections of vegetable hemicelluloses.* For comparative purposes, two samples of hemicellulose of high activity for at least one type of pneumococci were injected into mice in a single dose and in a series of three doses on consecutive days, and the mice were tested a week later with four dilutions of culture. It is evident, in table 5, that the products selected for the type I test immunized against 50,000 lethal doses when three injections of sample were given; those for type II against 50,000 lethal doses with either single or triple injections; and those for type III, against 5,000 lethal doses with triple injections. In other words, at least with these products multiple doses stimulated a higher degree of immunity against type I or III than a single injection, while with type II immunity was equally high whether single or multiple injections were given. This is in contrast to the results with specific pneumococcus polysaccharides, for the bacterial polysaccharides stimulate maximum degree of immunity by one injection of an immunizing dose (Felton *et al.*, 1955).

(6) *Some chemical studies in relationship to biologic activity.* Analytical studies were initiated to determine whether there might be a significant relationship between the degree of antigenicity and a measurable chemical or physical characteristic. Several products of high titer and others of moderate activity were tested for content of reducing sugars after hydrolysis (calculated as glucose), for pentose after hydrolysis, and for nitrogen content. Optical rotation and viscosity measurements were made on most of these samples. Results of these tests, arranged in the order of decreasing content of reducing sugars (glucose), are listed in table 6 in two groups based on the immunizing titer. It is seen that no parallelism existed between the content of reducing sugars and the immunity titer, for immunity was stimulated with 0.005 mg of hemicellulose whether the sugar content was as high as 36 per cent or was too low to measure. Unless the high optical rotation in two instances, wheat germ and sunflower seed, was significant, the other

TABLE 5

Comparison of immunity following single and triple injections of vegetable hemicelluloses

Vegetable	Number of Injections	Dose of Hemicellulose	Type of Pneumococcus	Active Immunity* Lethal Doses of Culture			
				500,000	50,000	5,000	500
		mg					
Ripe tomato	1	0.5	I	1*	1	4	2
	3	0.125	I	1	5	4	3
		0.25					
		0.5					
Collard	1	0.05	I	3	1	4	2
	3	0.0125	I	2	4	4	5
		0.025					
		0.05					
Tomato pool	1	0.5	II	0	5	3	5
	3	0.25	II	2	2	2	2
		0.5					
		1.0					
Collard	1	0.05	II	3	4	4	5
	3	0.0125	II	2	4	4	5
		0.025					
		0.05					
Grass	1	2.0	III	0	0	0	2
	3	0.5	III	1	0	3	3
		1.0					
		2.0					
Collard	1	0.05	III	0	0	0	3
	3	0.0125	III	0	0	4	2
		0.025					
		0.05					

* Numbers indicate survivors of five mice per dilution.

TABLE 6

Chemical and immunological tests of some vegetable hemicelluloses

Hemicellulose	Chemical and Physical Tests					Immunological Tests					
	Reducing sugar† (glucose)	Pentose after hydrolysis	Nitrogen	Optical rotation $[\alpha]_D$	Viscosity (water = 1.00)	Precipitin titer Antipneumococcus sera			Immunity end point against 1,000 LD		
						Type I	Type II	Type III	Pn I	Pn II	Pn III
	%	%	%						*mg*	*mg*	*mg*
Wheat germ	38.8	13.6	0.6	+17.3	1.41	0	0	1:100	0	0.0005	0
Grapefruit rind	36.4	14.0	0.8	−17.3	0.87	1:100	1:200	1:100	0	0.005	0
Collard	28.2	*	0	+17.3	1.04	1:200	1:12,800	1:1,600	0.005	0.0005	0.5
Sunflower seed	21.7	14.7	0.5	−20.8	1.25	0	0	0	0.005	0	0
Apple	1.2	0.4	0	0	0.98	1:200	1:200	1:200	0	0.005	0
Oatmeal	0	*	0.9	−62.3	1.01	1:800	1:800	1:800	0	0.005	0
Parsnip green	73.8	*	0	+121.2	*	1:800	1:6,400	1:400	0.05	0.05	5.0
Grass	58.8	*	0	+69.2	*	1:25,600	1:25,600	1:6,400	0.5	0	0.05
Moss, Iceland	49.6	11.2	0	−79.6	1.10	1:100	0	1:400	0.05	0	0
Excelsior, pine	36.6	*	0	−55.4	*	1:200	0	1:1,600	0.5	0.05	0.5
Hay	36.0	*	0	+17.3	*	1:100	1:51,200	1:1,600	0.5	0.05	0.5
Squash, Hubbard	30.0	10.2	0	*	1.03	0	0	0	5.0	0.05	0
Pumpkin pulp	29.2	7.1	0	+72.7	1.03	*	1:12,800	1:200	0.05	0.05	5.0
Tomato, ripe	23.9	*	0	+20.8	*	1:400	1:1,600	1:400	0.05	0.05	5.0
Green bean	23.5	*	0	+34.6	*	*	1:8,000	1:500	0.5	0.05	1.0
Watermelon (a)	20.8	9.2	0	+24.2	1.07	0	0	1:100	0.5	0.05	0.5
Watermelon (b)	7.6	4.1	0	+34.6	1.02	0	1:8,000	0	0.05	0.05	0
Pomegranate	5.2	2.2	0	+27.7	0.98	0	1:3,200	1:200	5.0	5.0	0.05
Kale	2.8	1.4	0	+27.7	0.99	0	0	0	0	0.05	0
Melon, honeydew	2.2	2.2	0	+58.9	1.05	1:100	0	1:200	5.0	0.05	5.0

* Indicates not tested.

† After hydrolysis, calculated as glucose.

analyses were not conclusive as to any correlation with the biological activity, either immunity or precipitin titer. It is of interest that nitrogen was less than one per cent or absent. Also the hemicelluloses yielded enough pentose on hydrolysis to indicate the possible presence of a pentosan in some of the samples tested. In conclusion, it would appear that the hemicelluloses derived by the above methods were not distinct chemical entities, and that the chemical characteristics did not parallel the variations in titer of antigenicity against pneumococcus infections.

DISCUSSION

The plant substances which are termed hemicelluloses comprise a diversity of complex polymers which yield on hydrolysis hexoses, pentoses, and uronic acids. The amount present in the cell walls of a plant varies greatly from one species to another, depending upon the state of development, the degree of hydration of the part, and upon environmental factors, such as exposure to sunlight, moisture. Furthermore, excision of any part of a plant, especially the leaves, rapidly alters the distribution of carbohydrates (Loehwing, 1948; Bonner, 1950). Hence it is not surprising that analyses of the plant products isolated for this study were so diverse as reported in table 6. The unexpected observation was that about half of all the fractions tested in the present series possessed characteristics of an immunological nature, namely, the ability to precipitate antipneumococcus sera and to stimulate active immunity against virulent pneumococci in mice. At this stage of the study it is obviously impossible to say whether a plant hemicellulose of given analysis might be expected to be antigenic, or even to predict that a given plant might have a component with potential immunological activity. However, it is pertinent to the latter that all the fractions obtained by one procedure (calcium phosphate

precipitation) from six batches of extract of tomato tops gathered from three years' crops were consistently antigenic. Although the studies have been in several cases largely of the inedible portion of a species, it was found that when an active component was present in the leaves (tomato) or rind (pumpkin or squash), the fraction isolated from the edible fruit or pulp was also antigenic. In other words, the presence of an antigen in any one part indicates the likelihood of an active component in another growing part. Thus it may be justifiable to assume that, by proper methods of isolation, an active antigen would regularly be found in, for example, tomato, collard, pumpkin, or certain vegetables.

Since many of these products possessed immunizing capacity over a range of doses but failed to immunize mice with larger amounts, it seemed plausible that in these larger doses they might "paralyze" the immunological defense in the same manner as was found with relatively large amounts of specific pneumococcus polysaccharides. This possibility was confirmed in one experiment in which two such products isolated from pine wood and from watermelon rind, respectively, were found to inhibit with 5.0 mg doses the immunizing activity of type I and type II pneumococcus polysaccharides against specific pneumococcus infections in mice. It was also observed with a small group of vegetable polysaccharides that those which failed to immunize had no apparent effect on the usual immunizing activity of the pneumococcus polysaccharides as tested either simultaneously or seven days after injection.

Our main interest in this problem stems from its possible relationship to the incidence of lobar pneumonia in man. The immunological fate of these vegetable antigens in edible plants, when the plants are taken orally into the animal body, is at present unknown. For the fact, that an isolated hemicellulose component was found to stimulate immunity in the animal host, does not necessarily indicate that the original food substance would be similarly broken down and an antigenic component liberated. Yet such activity seems plausible inasmuch as the human being, accustomed to a varied diet which may include many of these vegetables, has been found to retain in the tissues a polysaccharide component which is antigenic for pneumococci (Felton, 1949). Whether the presence of such vegetable antigen is advantageous or detrimental to the welfare of the host from the immunological viewpoint may depend upon the amount accumulated. For, if pneumococcus antigen from this source (food) is accumulated in the tissues, along with any pneumococcus antigen that might be present in a carrier, or following an attack of pneumonia, the quantity might reach the level corresponding to that found in tissues of mice which had become immunologically paralyzed following a large (0.5 mg) dose of pneumococcus polysaccharide (Felton *et al.*, 1947*b*), and thus a similar state might be developed, such that the host could no longer be immunized and would be hypersusceptible to pneumococcus infection. This possible connection with the incidence of lobar pneumonia has been discussed in more detail in an earlier publication (Felton, 1949). Suffice it to say here that the finding of a pneumococcus antigen in foodstuffs may well have a hitherto unrecognized connection with the immunological status of the host.

SUMMARY

Hemicelluloses have been isolated from 70 members of the plant kingdom and tested for antigenicity in mice against virulent strains of types I, II, and III, *Diplococcus pneumoniae*.

Whereas many of the 20 plants whose hemicelluloses were devoid of antigenic activity were flowering plants, the active ones were mostly edible vegetables.

The 202 active fractions from 50 plants were found to vary greatly in immunizing titer against 1,000 lethal doses of pneumococci. An immunizing dose ranged from 5 mg to 0.0005 mg.

Several of those of highest titer (0.0005 mg) were found to immunize against as much as 50,000 lethal doses of pneumococci type I or II, and 5,000 lethal doses of type III. One, collard greens' hemicellulose, immunized thus against all three types.

The immunity in many cases was polyvalent for two or even three types. In most instances, however, type II predominated.

Several samples of high or medium titer antigenicity have been analyzed for nitrogen, reducing sugar after hydrolysis (glucose), pentose, optical rotation, and viscosity and compared as to antigenicity and precipitin titer. Correlation between chemical and biological activity was low in this group of hemicelluloses.

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