INHIBITION BY CERTAIN POLYSACCHARIDES OF HEMAGGLUTINATION AND OF MULTIPLICATION OF INFLUENZA VIRUS

By R. H. GREEN, M.D.,* and D. W. WOOLLEY, Ph.D.

(From the Laboratories of The Rockefeller Institute for Medical Research)

(Received for publication, April 9, 1947)

Antagonism between structurally similar compounds provides a useful tool with which to investigate biological processes. An example of how it has been used as a guide to study virus-host relationships will be described in the present paper.

Examination of many cases of competition between structurally similar substances has led to the conclusion that the inhibitory analogs compete with their related metabolites when the latter function as substrates for a metabolic reaction (1). Now, if one assumes, as Hirst has done (2), that influenza virus, in causing hemagglutination, may be likened to an enzyme attacking a specific substrate in the red cell, then it might be possible to inhibit this activity of the virus by adding a suitably constituted analog of the substrate. Such an inhibition would be analogous to the blocking of the action of succinic dehydrogenase by malonate, a close relative of succinate (3), as well as to numerous other more recent examples (1). Although the postulated substrate which the virus attacks in the red cell is unknown, the observations of Hirst and Hotchkiss (4) that periodate destroys that part of the erythrocyte which reacts with the virus suggested that it is carbohydrate in nature. Therefore, in the work now to be described, a number of simple and complex carbohydrates have been tested for their ability to inhibit agglutination of chicken red blood cells by influenza A virus. The virus-erythrocyte system recommended itself as the simplest case of virus-cell relationship, and it was hoped that any agent found effective in relation to it might find application in an animal host in which multiplication of virus occurs (5).

A number of polysaccharides were found to be capable of inhibiting hemagglutination. Several of the effective agents examined contained large amounts of galacturonic acid, although some were related to other sugars. None of the simple carbohydrates tested showed any activity. Apple pectin, one of the most effective substances, was studied in some detail.

Evidence was obtained that apple pectin exerted an effect on the virus as well as on the erythrocytes. If the working hypothesis were correct, combination between the virus and the pectin would be expected, but reaction of the

* Fellow in the Medical Sciences of the National Research Council; aided by a grant from the National Foundation for Infantile Paralysis.

pectin with the erythrocytes would not be anticipated. The experimental results suggested that interaction occurred between every possible pair of the three participating substances (virus, polysaccharide, and cell). Therefore, it was of interest to find that from the erythrocyte itself a substance could be extracted which, like the carbohydrates, actively inhibited hemagglutination by the virus.

Because apple pectin was quite able to inhibit virus hemagglutination it was tested for ability to inhibit virus multiplication in embryonated eggs. It was found that pectin was able to do this too. This was true not only when pectin was given before inoculation with the virus but also when it was injected 1 to 2 hours afterwards.

Although the working hypothesis just outlined has led directly to positive experimental results it does not necessarily mean that this hypothesis is the correct one. The actual findings may have been coincidental and quite unrelated to the postulate that influenza virus reacts in an enzymic fashion with a carbohydrate substance in erythrocytes.

The fact that apple pectin inhibits multiplication of virus in embryonated eggs as well as hemagglutination is suggestive but does not prove that the two phenomena are related. However, it is of more than passing interest that alginic acid, a substance closely similar to apple pectin in both physical and chemical properties, showed relatively little activity as an inhibitor of virus hemagglutination and likewise showed little or no potency as an inhibitor of virus multiplication in the egg. On the other hand the substituted nitroacridin 3582, which has been demonstrated to have an inhibitory effect on the growth of influenza B virus in embryonated eggs, exerted no apparent effect on hemagglutination (6). Furthermore, some hemagglutination-inhibiting carbohydrates, *e.g.* citrus pectin, did not reduce multiplication of virus.

Materials and Methods

Virus Preparations.—Influenza A virus, PR8 strain, was used exclusively. This strain was obtained as an allantoic fluid preparation from Dr. Frank L. Horsfall, Jr., of the Rockefeller Institute. Allantoic fluids from the third and fourth egg passages made in this laboratory were the source of virus used in the present study. With slight modifications, passages were made and fluids collected and stored according to the directions of Hirst (2).

Sources and Preparation of Carbohydrates.—Apple and citrus pectins, gum acacia, gum myrrh, alginic acid, agar, corn starch, galactose, ribose, glucose, and mannose were commercial samples. Preparations of the specific polysaccharide of gum acacia (7), of the blood group A substance, and of cellobiuronic acid were supplied by Dr. W. F. Goebel of the Rockefeller Institute. Galacturonic acid was obtained from Dr. M. A. Stahmann of the University of Wisconsin.

The polyaldehyde of starch was prepared by the method of Jackson and Hudson (8), and the corresponding acid was made by treating an aqueous solution of the aldehyde with an excess of cold sodium hypobromite and dialyzing the reaction mixture to remove excess reagents. The sodium salt of the polysaccharide was then precipitated from the aqueous solution by

adding alcohol. Inositol galactoside tartrate was obtained from soybean lipositol as described by Woolley (9). Neville's method (10) was employed for the preparation of flaxseed mucilage, and the aldobionic acid of rhamnose and galacturonic acid was made from it according to the procedure of Anderson and Crowder (11). An extract from laked chicken RBC was obtained as follows: Stromata were prepared from 150 cc. of washed chicken erythrocytes after hemolysis with distilled water. These ghosts were washed 4 times with saline and then 3 times with water and finally suspended in 200 cc. of water and heated to 100°C. for 5 minutes. The suspension was cooled and a clear solution obtained by high speed centrifugation.

In testing for inhibition of hemagglutination the various materials were dissolved in water, usually in a concentration of 2 per cent, and adjusted to pH 7. Those which did not dissolve in water, *e.g.* alginic acid and gum myrrh, went into solution on neutralization. Infrequently, it was necessary to remove a small amount of insoluble residue by filtration.

Several methods of preparing solutions of apple pectin for injection into eggs have been tried but that described below was the only one that yielded uniformly good results. Powdered pectin, in small amounts at a time, was gradually sifted onto the surface of water heated to about 100°C. and stirred constantly. The solution was then neutralized with 1 N NaOH, autoclaved (11 pounds for 20 minutes), and when necessary, the pH readjusted to 7 by the addition of a sterile solution of NaOH. This method was time-consuming since about 3 hours' effort was required to obtain 100 cc. of a 5 per cent solution but it produced consistently clear solutions free of masses and insoluble residue. Departure from it often resulted in solutions which contained particulate matter and showed less successful results when tested in eggs.

Titration of Virus by Agglutination of Chicken RBC.—Titrations of virus were made by Hirst's procedure (2) except that readings were made by the pattern test reported by Salk (12). Serial twofold dilutions of allantoic fluid were made in saline,[1] using 0.5 cc. amounts. To each tube was then added 0.25 cc. of a 1 per cent suspension of washed RBC from individual, adult chickens. Results were read after the tubes had stood in a vertical position at room temperature (23–26°C.) for 2 hours. The highest dilution at which complete agglutination occurred was taken as the end-point and this amount of virus was arbitrarily considered one agglutinating unit.

The following criteria, identical in most respects with those described by Salk (12), were used in reading the tests: (1) A pattern considered characteristic of complete agglutination was designated as c (complete). This pattern consisted of a uniformly thin layer of cells completely covering the concave bottom of the tube. It was easily seen as a salmon-pink layer of cells when the tube was viewed either from the side or from the bottom. (2) A pattern considered characteristic of the absence of agglutination was designated as 0. Here, the cells did not stick to the margins of the bottom but slid down to the center forming a small, compact, dark red mass with sharply defined, smooth edges. When tubes containing such patterns were tilted horizontally the cells ran smoothly down the bottom and onto the side of the tube, much in the manner of a drop of ink running down an inclined plane surface. (3) A pattern considered intermediate between c and 0 was designated as p (partial). This type apparently consisted of large clumps of cells which had settled to the center of the bottom of the tube but invariably occupied a larger area than the patterns designated 0; had ragged edges, and did not run smoothly on horizontal tilting. Furthermore, such patterns frequently showed small areas of a thin layer of cells around their margins and a thin rim of cells still attached to the upper periphery of the concave bottom of the tube. (4) A pattern considered as closely approaching that described as 0 was designated t (trace). On casual inspection, this pattern could not be differentiated easily from 0, especially when viewed from

[1] Here, as elsewhere, saline means 0.85 per cent NaCl.

the side of the tube. It too consisted of a small, compact, dark red mass, occupying the center of the bottom of the tube. However, on closer inspection it was found that such patterns were slightly larger than the 0 type. In addition, the edges were somewhat granular and on horizontal tilting, when movement occurred, the cells tended to move as a single piece, rather than run smoothly.

Titration of Virus by Infectivity for Embryonated Eggs.—Serial tenfold dilutions of allantoic fluids to be tested were made in phosphate buffer[2] and 0.05 cc. amounts of appropriate dilutions injected into the allantoic sacs of each of 2 to 5 embryonated eggs on the 10th day of incubation. Incubation at 37.5°C. was continued for 48 hours, followed by chilling at 4°C. for 18 to 20 hours. Allantoic fluid was then removed from each egg, and tested for its ability to produce hemagglutination.

Inhibition of Virus Hemagglutination by Various Carbohydrates

Serial twofold dilutions of 2 per cent solutions of the substances tested were made in saline in 0.25 cc. amounts. To each tube was then added 0.25 cc. of a 1 per cent suspension of chicken RBC. This was followed by the addition of 0.25 cc. of virus-containing allantoic fluid so diluted in saline that each 0.25 cc. contained approximately 4 hemagglutinating units of virus (a 1–160 dilution of allantoic fluid). Positive controls consisting of only virus and cells and negative controls of only saline and cells were included and the results were read after 2 hours at room temperature.

The data presented in Table I show that several of the materials tested prevented the formation by influenza A virus of typical patterns of hemagglutination. Those showing a considerable effect were flaxseed mucilage, citrus pectin, apple pectin, blood group A substance, gum acacia, gum myrrh, and the extract of chicken RBC. Many of the substances showing inhibitory activity were polysaccharides rich in galacturonic acid. However, polygalacturonides were not unique in this respect because gum acacia and concentrates of the blood group A substance, both of which do not contain galacturonic acid, were effective. Alginic acid, a polysaccharide largely composed of mannuronic acid units, was practically inactive. Furthermore, complex carbohydrates such as starch and the polyaldehyde or polyacid prepared from it by oxidation caused no inhibition of hemagglutination. None of the simple carbohydrates, such as galactose, galacturonic acid, the aldobionic acid from flaxseed mucilage, etc., exhibited any activity. The chemical composition and homogeneity of such materials as the pectins, plant mucilages, and gums are not sufficiently established to justify exact conclusions as to the nature of the configuration required for inhibitory action.

Effect of Various Carbohydrates on Chicken RBC in the Absence of Virus

Because a wide variety of substances are known to cause hemagglutination (13, 14) experiments were performed to determine whether the materials studied had any effect on RBC in the absence of virus. Tests identical with those

[2] Except where otherwise indicated, phosphate buffer means 0.1 M sodium phosphate buffer at pH 7.

described in the preceding section, except that virus was replaced by saline, were set up. For facility in comparison these data also are presented in Table I. Several of the preparations tested in this manner caused some hemagglutination. Most of those capable of inhibiting virus hemagglutination did

TABLE I

Effect of Various Substances on Hemagglutination of Chicken RBC in the Presence of Influenza A Virus and Without Virus

Test substance	With influenza virus										Without influenza virus									
	Final concentration of test substance, γ/cc.										Final concentration of test substance, γ/cc.									
	6666	3333	1666	833	416	208	104	52	26	13	6666	3333	1666	833	416	208	104	52	26	13
Flaxseed mucilage		c	c	p	p	t	p	p	p	p		c	p	p	t	t	t	0	0	0
Galacturonic acid	c	c	c	c	c	c	c	c	c	c	0	0	0	0	0	0	0	0	0	0
Cellobiuronic acid	c	c	c	c	c	c	c	c	c	c	0	0	0	0	0	0	0	0	0	0
Inositol galactoside tartrate	c	c	c	c	c	c	c	c	c	c	t	t	t	0	0	0	0	0	0	0
Citrus pectin	p	t	t	t	t	t	p	c	p	p	p	t	0	0	0	0	0	0	0	0
Apple pectin	p	p	p	t	t	t	p	p	p	c	p	0	0	0	0	0	0	0	0	0
Blood group A substance	p	p	t	p	p	t	t	p	p	p	0	0	0	0	0	0	0	0	0	0
Galactose	c	c	c	c	c	c	c	c	c	c	0	0	0	0	0	0	0	0	0	0
Flaxseed mucilage aldobionic acid	c	c	c	c	c	c	c	c	c	c	c	p	0	0	0	0	0	0	0	0
Glucose	c	c	c	c	c	c	c	c	c	c	0	0	0	0	0	0	0	0	0	0
Mannose	c	c	c	c	c	c	c	c	c	c	0	0	0	0	0	0	0	0	0	0
Alginic acid	p	p	p	p	c	c	c	c	c	c	t	t	0	0	0	0	0	0	0	0
Soluble starch*	c	c	c	c	c	c	c	c	c	c	0	0	0	0	0	0	0	0	0	0
"Starch polyacid"	c	c	c	c	c	c	c	c	c	c	0	0	0	0	0	0	0	0	0	0
Gum acacia	c	c	c	p	p	p	t	p	p	p	c	c	p	0	0	0	0	0	0	0
RBC extract				p	p	t	p	p	p	p				0	0	0	0	0	0	0
"Starch polyaldehyde"	c	c	c	c	c	c	c	c	c	c	0	0	0	0	0	0	0	0	0	0
Gum myrrh*	p	p	p	p	p	p	p	p	p	p	0	0	0	0	0	0	0	0	0	0
Gum acacia specific polysaccharide	c	c	c	c	c	c	c	c	c	c	0	0	0	0	0	0	0	0	0	0
Ribose	c	c	c	c	c	c	c	c	c	c	c	c	p	0	0	0	0	0	0	0
Agar agar								c	c	c								p	0	0
Normal rabbit serum	0	t	t	t	t	t	p	p			0	0	0	0	0	0	0	0		

* Weight includes insoluble residue which was discarded.

so. However, a significant effect occurred only at concentrations higher than those at which there was inhibitory activity. The meaning of this was not clear, especially when it was found that some substances by themselves caused hemagglutination but showed no inhibitory action.

Additional evidence that the polysaccharides affected the RBC was obtained by showing that cells treated with a suitable concentration of apple pectin and then washed with saline did not behave in the same fashion, when mixed with influenza virus, as did untreated cells. Thus, 0.25 cc. of a 1 per cent suspension of cells mixed with 0.25 cc. of apple pectin (3 mg. per cc.) plus 0.25 cc. of saline showed no agglutination after 2 hours at 25°C. The tubes were then centrifuged lightly and the cells washed 5 times with saline and finally resuspended in

0.75 cc. saline. After the cells had sedimented 0.25 cc. of the supernatant was removed and replaced by 0.25 cc. of saline containing 4 agglutinating units of virus; the cells were resuspended and allowed to settle in the usual way. Readings then showed that only partial hemagglutination (readings of t or p) had occurred. However, when these cells were again washed several times and another 4 units of virus were added complete agglutination occurred. Furthermore, cells suspended in a hemagglutination-inhibiting concentration of apple pectin and treated with virus before washing behaved in a similar manner.

Effect of Apple Pectin on Virus

In order to determine whether apple pectin had any effect on the virus itself mixtures of the two were made and tested for infectivity in embryonated eggs.

1.8 cc. of a solution of apple pectin (10 mg. per cc.) in 0.08 M sodium phosphate buffer at pH 7 were mixed with 0.2 cc. of virus-containing allantoic fluid. After the mixtures had stood at 23–26°C. for 45 minutes they were titrated for infectivity in embryonated eggs as previously described. Control mixtures in which phosphate buffer was substituted for apple pectin were examined simultaneously. In one experiment alginic acid, in the same concentration a pectin, was used as a further control. The ID_{50} titers of virus treated with pectin in this manner were approximately 10^{-7} whereas those treated with alginic acid and phosphate buffers alone were approximately 10^{-8}.

The results of four such experiments showed that the titer of virus mixed with pectin was reduced by about 1 log.

Effect of Varying the Order of Mixing Apple Pectin, Cells, and Virus

The inhibition of virus hemagglutination effected by apple pectin was demonstrable when the reactants were mixed in the order previously described; *i.e.*, apple pectin, plus cells, plus virus. Moreover, when pectin and virus were mixed together and cells added to the mixture a similar inhibitory effect was observed. However, when virus and cells were first mixed and pectin was then added no inhibition was obtained. This finding indicated that the inhibition of hemagglutination was not due to a change in viscosity or some other physical property of the system, brought about by the pectin.

Difference between Inhibition of Hemagglutination Caused by Apple Pectin and That Caused by Normal Rabbit Serum

The fact that normal animal and human sera inhibit hemagglutination by influenza virus has been demonstrated repeatedly (2, 15, 16). The data in Table I illustrate this again. However, in contrast to the importance of the order of mixing for the inhibitory action of apple pectin, normal rabbit serum (heated at 56°C. for 30 minutes) caused inhibition of hemagglutination regardless of the order. Thus normal rabbit serum, when added after virus and cells were mixed, prevented hemagglutination, whereas apple pectin did not.

Inhibition by Apple Pectin of Virus Multiplication in Embryonated Eggs

1. When Pectin Was Injected ½ Hour before Virus.—One cc. of a 5 per cent solution of apple pectin was injected into the allantoic sac of each of a number of embryonated eggs on the 10th day of incubation. One-half hour later approximately 100 ID_{50} of influenza A virus, in a volume of 0.05 cc., were introduced into the allantoic sac. After incubation at 37.5°C. for 48 hours followed by chilling at 4°C. for 18 to 20 hours, the allantoic fluids were collected and titrated individually for the presence of virus by hemagglutination, and in some instances by infectivity for embryonated eggs. Eggs which had received virus alone or virus plus 1 cc. amounts of saline or a 5 per cent solution of alginic acid served as suitable controls.

Results illustrative of the best that were obtained are shown in Table II. As judged by the hemagglutination tests there had been considerable inhibition of virus multiplication in all of the pectin-treated eggs. In order to be sure that there was, actually, a reduction in the amount of virus, and that the presence of pectin in these allantoic fluids was not interfering with hemagglutination several of them were tested for infectivity. The results confirmed the hemagglutination findings and furthermore, showed that in the fluids tested no virus was detectable by this method. By subsequently adding known amounts of virus to these fluids it was shown that the presence of residual pectin in them did not significantly interfere with the detection of virus by means of hemagglutination.

Four experiments like the one depicted in Table II revealed that of a total of 37 eggs thus treated with pectin only 5 were positive for hemagglutination. All controls were positive. Alginic acid was chosen as a control because of its similarity in physical and chemical properties to apple pectin and because it showed only a slight ability to inhibit virus hemagglutination.

Furthermore, when judged by the results of hemagglutination tests, 25 mg. of apple pectin per egg afforded almost as much protection against 100 ID_{50} of virus as did 50 mg. A decrease in virus multiplication was observed when 10,000 and even when 1,000,000 ID_{50} of virus were inoculated after the injection of 50 mg. of pectin. For example, of 5 eggs given 10,000 ID_{50} of virus all 5 were negative and of 5 eggs given 1,000,000 ID_{50} 2 were negative.

Pectin injected into the yolk sac in 50 mg. amounts either ½ hour or 24 hours before inoculation of virus into the allantoic sac was not effective in inhibiting the multiplication of virus.

2. When Pectin Was Injected 1 Hour after Virus.—Embryonated eggs on the 10th day of incubation were inoculated with approximately 100 ID_{50} of virus and 1 hour later 1.0 cc. of a 5 per cent solution of apple pectin was injected into the allantoic sac of each. Appropriate controls were inoculated with virus alone and with virus followed by 1.0 cc. amounts of saline or 5 per cent solutions of alginic acid. The eggs were incubated and fluids harvested as previously described. Results were determined by hemagglutination and, in some instances, by infectivity also. Of a total of 25 eggs so treated the allantoic fluids of 15 or 60 per cent have not contained sufficient virus to produce hemagglutination.

TABLE II

Effect of Various Substances, When Injected 1/2 Hour before Virus, on the Multiplication of Influenza A Virus in Embryonated Eggs

Experiment No.	Substance injected	Amount		Egg No.	Dilutions of allantoic fluid										
					Hemagglutinin titers							Infectivity titers			
					10	20	40	80	160	320	640	0	10^3	10^5	10^6
		cc.	*mg.*												
1	Apple pectin	1.0	50	1	0	0	0	0	0	0	0	0		0	
				2	0	0	0	0	0	0	0	0		0	
				3	0	0	0	0	0	0	0	0		0	
				4	0	0	0	0	0	0	0	0		0	
				5	0	0	0	0				0	0	0	
				6	0	0	0	0	0	0	0	0		0	
				7	0	0						0	0	0	
				8	0	0	0					0		0	
				9	0	0	0	0	0	0	0	0		0	
				10	0	0	0	0	0	0	0				
				11	0	0	0	0	0	0		0	0	0	
				12	0	0	0	0	0	0	0	0		0	
				13	0	0	0	0	0	0	0	0	0	0	
				14	0	0	0	0	0	0	0				
				15	0	0	0	0	0	0	0				
				16	0	0	0	0	0	0	0				
				17	0	0	0	0	0	0	0				
				18	p	0	0	0	0	0		0	0	0	
				19	0	0	0	0	0	0	0				
				20	0	0	0	0	0	0	0				
1	Sodium chloride	1.0	8.5	21	c	c	c	c	c	c	c				
				22	c	c	c	c	c	c	c				0
				23	p	p	p	c	c	c	c				
				24	c	c	c	c	c	c	c				+
				25	c	c	c	c	c	c	c				+
				26	c	c	c	c	c	c	c				+
				27	c	c	c	c	c	c	c				
				28	c	c	c	c	c	c	c				
1	None			29	c	c	c	c	c	c	c				+
				30	c	c	c	c	c	c	c				+
				31	c	c	c	c	c	c	c				+
				32	c	c	c	c	c	c	c				
				33	c	c	c	c	c	c	c				
				34	c	c	c	c	c	c	c				
5	Alginic acid	1.0	50	35	c	c	c	c	c	c	c				
				36	c	c	c	c	c	c	c				
				37	c	c	c	c	c	c	c				
				38	c	c	c	c	c	c	c				
				39	p	c	c	c	c	c	c				

Results of a typical experiment are shown in Table III. It is evident that pectin given 1 hour after inoculation of virus exerted a significant inhibition of virus multiplication whereas alginic acid did not. The infectivity titers, however, showed that, in most instances, some multiplication had occurred. A less marked, but still appreciable, effect was obtained when the interval between injection of virus and pectin was increased to 2 hours.

TABLE III

Effect of Apple Pectin and Alginic Acid, When Injected 1 Hour After Virus, on the Multiplication of Influenza A Virus in Embryonated Eggs

Substance injected	Egg No.	Dilutions of allantoic fluid														
		Hemagglutinin titers									Infectivity titers					
		10	20	40	80	160	320	640	1280	2560	0	10^8	10^5	10^6	10^7	10^8
Apple pectin	1	0	0	0	0	0	0	0	0	0	+	+	±*			
	2	0	0	0	0	0	0	0	0	0	+	+	0			
	3	0	0	0	0	0	0	0	0	0	+	0	0			
	4	p	p	p	0	0	0	0	0	0						
	5	0	0	0	0	0	0	0	0	0	0	0	0			
	6	c	p	0	0	0	0	0	0	0		+	+	+	±	
	7	0	0	0	0	0	0	0	0	0	+	0	0			
	8	0	0	0	0	0	0	0	0	0						
Alginic acid	9	c	c	c	c	c	c	c	c	p			+	+	+	0
	10	c	c	c	c	c	c	c	p	p			+	+	+	+
	11	c	c	c	c	c	c	c	c	p			+	+	0	0
None	12	c	c	c	c	c	c	c	c	c				+	+	+
	13	c	c	c	c	c	c	c	c	c				+	+	+

* Indicates that, at this dilution, approximately half the eggs used for testing were positive.

Effect of Other Hemagglutination-Inhibiting Substances on Multiplication of Virus in Embryonated Eggs

A number of substances showing hemagglutination-inhibiting effect were tested, in the manner described above, for the ability to inhibit multiplication of influenza virus in embryonated eggs.

Citrus pectin, in 50 mg. amounts, flaxseed mucilage, in 10 mg. amounts, and undiluted normal rabbit serum in 1 cc. amounts, did not inhibit the multiplication of virus. Gum acacia, in 50 mg. amounts, did show a significant inhibitory effect when injected one-half hour before virus but showed no effect when injected one hour after the virus.

Toxicity of Apple Pectin for Embryonated Eggs

Apple pectin in 50 mg. amounts (1 cc. of 5 per cent solution) was relatively non-toxic when injected into the allantoic sacs of embryonated eggs on the 10th day of incubation. Thus, in a preliminary experiment all 3 eggs of a group so treated survived to the time of hatching. Subsequent experiments in which virus and 50 mg. amounts of pectin were injected into the allantoic sac showed that of 196 eggs 28, or about 14 per cent, died within the 48 hour incubation period.

SUMMARY

The complex carbohydrates apple pectin, citrus pectin, flaxseed mucilage, blood group A substance, gum acacia, and gum myrrh as well as an extract of RBC, when examined in a pattern test, were shown to inhibit the agglutination of chicken RBC by influenza A virus. A number of other simple and complex carbohydrates showed no inhibitory effect. The hemagglutination-inhibiting action of apple pectin was examined in some detail and evidence was adduced to show that it affected both virus and red cell. Apple pectin was also found to inhibit the multiplication of influenza A virus in embryonated eggs.

BIBLIOGRAPHY

1. Woolley, D. W., *Physiol. Rev.*, 1947, **27,** 308.
2. Hirst, G. K., *J. Exp. Med.*, 1942, **75,** 49; **76,** 195.
3. Quastel, J. H., and Wooldridge, W. R., *Biochem. J.*, 1927, **21,** 1224.
4. Hirst, G. K., and Hotchkiss, R. D., personal communication.
5. Burnet, F. M., Beveridge, W. I. B., McEwin, J., and Boake, W. C., *Australian J. Exp. Biol. and Med. Sc.*, 1945, **23,** 177.
6. Green, R. H., Rasmussen, A. F., Jr., and Smadel, J. E., *Pub. Health Rep., U. S. P. H. S.*, 1946, **61,** 1401.
7. Heidelberger, M., Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1929, **49,** 847.
8. Jackson, E. L., and Hudson, C. S., *J. Am. Chem. Soc.*, 1937, **59,** 2049.
9. Woolley, D. W., *J. Biol. Chem.*, 1943, **147,** 581.
10. Neville, A., *J. Agric. Sc.*, 1913, **5,** 113.
11. Anderson, E., and Crowder, J. A., *J. Am. Chem. Soc.*, 1930, **52,** 3711.
12. Salk, J. E., *J. Immunol.* 1944, **49,** 87.
13. Turner, A. W., *Australian J. Sc.*, 1946, **9,** 6
14. Popper, H., Volk, B. W., Meyer, K. A., and Kozoll, D. D., *Arch. Surg.*, 1945, **50,** 34.
15. Knight, C. A., *J. Exp. Med.*, 1944, **80,** 83.
16. Francis, T., Jr., *J. Exp. Med.*, 1947, **85,** 1.