parent therefore that a transmissible, filterable leukemogenic agent could be activated in at least 11 different C3H mice by total body x-ray irradiation. Selecting a potent extract, the agent could be passed serially through 4 consecutive cell-free passages of newborn C3H hosts.

Whether the filterable leukemic agent, designated "passage X" is distinct from leukemic virus designated passage A which originated from spontaneous Ak leukemia and which has been also passed serially in C3H mice, remains to be determined. Since the concentration of the virus in extracts used is not known, it is not possible to differentiate virus A from virus X on the basis of serum neutralization tests here reported. Thus, the assumption that the leukemic viruses A and X are distinct, although possibly related, remains a working hypothesis, and still requires experimental confirmation.

Summary. 1. A filterable leukemic agent designated "virus X" recovered from a C3H female in which leukemia was induced by x-ray irradiation, was passed serially through 4 consecutive cell-free inoculations of suckling C3H mice. 2. Of 40 mice inoculated, 26 thus far developed leukemia (65%) at ages varying from 3 to 11 months. 3. Inactivated (56°C ½ hr) rabbit and guinea pig serum, prepared with agent X filtrates, only partially neutralized passage A leukemic agent; passage A immune rabbit serum had a distinct, though not complete neutralization effect on the passage A agent.

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Site of Reaction of Wax Bean Hemagglutinin with Rabbit Erythrocytes.* (24539)

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Many plant seeds contain substances which agglutinate animal erythrocytes(1). Experiments reported here reveal several features of rabbit erythrocyte agglutination common to wax bean hemagglutinin (WBH) and to influenza and Newcastle Disease viruses. This suggests that both may act on the same site of the red cell membrane.

Materials and methods. Preparation of WBH. A partially purified preparation was obtained as follows. To 50 g of finely pulverized stringless wax beans‡ (*Phaseolus vulgaris*) was added 500 ml distilled water, and pH of suspension was adqusted to pH 7. Insoluble material was removed from suspension by centrifugation, and pH of supernatant was acidified to pH 4.6. Material precipitating out at this pH was discarded, and the supernatant fully saturated with $(NH_4)_2SO_4$. The precipitated protein was dialyzed against distilled water and finally lyophilized. The *receptor destroying enzyme* (RDE) was prepared from *Clostridium perfringens* as described by Popenoe and Drew(2). *Virus-treated blood cells* were prepared by adding 10 ml of chorioallantoic fluid [obtained from 10-day-old embryonated eggs inoculated with influenza (PR 8) or Newcastle Disease (NDV) virus§ and harvested after incubation at 37° for 48 hours] to 30 ml of 1.5% suspension of trypsinated rabbit erythrocytes(3). 0.9% NaCl replaced

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‡ Purchased from Farmer Seed and Nursery Co., Faribault, Minn.

§ Generously provided by Dr. J. T. Syverton, Dept. of Bacteriology, Univ. of Minnesota, Minneapolis.

TABLE I. Substances Showing Inhibition of Agglutination by WBH.

Substance tested	Inhibitory activity, I.U./mg	Sialic acid, %	Source or method of preparation
Ovomucin	520	5.8	(5)
Ovomucoid	10	2.0	(6)‡
Orosomucoid	350	16.1	(7)§
Serum viral inhibitor	475	12.2	§
Rabbit serum	16*	16.0*	
Bovine "	100*	26.0*	
Meconium	300	4.5	(8)
Sialyl-lactose	170	47.5	(9)
Sialic acid (meconium)	0	100 †	(10)
(ovomucin)	2	85	(11)

* Inhibitor activity and sialic acid expressed as I.U./ml and mg % respectively.

† Assumed to be 100%; used as standard in determination of sialic acid(4).

‡ Purchased from Worthington Biochemical Corp., Freehold, N. J.

§ Gift from Dr. R. J. Winzler, Univ. of Illinois, Chicago.

‖ Gift from Dr. R. K. Silver, Univ. of Pennsylvania, Philadelphia.

the virus for control. After 2 hours at 4°, the cells were washed twice with cold 0.9% NaCl and resuspended in 50 ml of saline. The cells were held overnight at 37°, washed 3 times, and finally suspended in sufficient 0.9% NaCl to give a red cell concentration of 1.5%. *RDE-treated serum* was prepared by mixing 1 ml rabbit serum, 1 ml RDE solution (replaced by 0.015M $CaCl_2$ in control), and 3 ml 0.015 M $CaCl_2$. Following incubation for 2 hrs at 37°, enzymic action was stopped by heating at 56° for 90 min. Filtrates, obtained by adding 5 ml of 5% trichloroacetic acid to 2 ml aliquots of each solution, were analyzed for sialic acid, the difference between RDE-treated and control sera representing net enzymic release of sialic acid from the serum. The remainder of each solution, after dialyzing against 0.9% NaCl, was tested for inhibitor activity. *Hemagglutination inhibition.* The substance to be tested was dissolved in 0.9% NaCl, and 2-fold serial dilutions of this solution were made up in final volume of 0.5 ml using 0.9% NaCl as the diluent. To each tube was added 0.5 ml of WBH solution containing 2 γ protein equivalent to 25 hemagglutinating units as defined by Liener(3). After 30 min. at room temperature, 1 ml of 1.25% suspension of trypsinated rabbit erythrocytes was added to each tube and mixed. The tubes were read photometrically at the end of 2-2½ hrs., and dilution of inhibitor corresponding to 50% sedimentation of cells was calculated(3). One inhibitor unit (I.U.) was arbitrarily defined as that amount of test substance which caused 50% inhibition of complete agglutination under the conditions specified above. The source or method of preparing the various substances tested for inhibitory activity against WBH is shown in Table I. *Sialic acid* content of these materials was determined according to the method of Svennerholm(4).

Results. Inhibition by mucoproteins. In Table I is presented a partial list of substances tested for inhibitory effect on hemagglutinating activity of WBH.‖ In general the most potent inhibitors of WBH belonged to that class of soluble mucoproteins which contain sialic acid and act as inhibitors of viral agglutination(12,13). Inhibition by sialyl-lactose may be compared to inhibition of viral agglutination by a similar, if not identical, compound isolated by Kuhn and Brossmer (14) from cow colostrum. Sialic acid itself, which did not inhibit WBH, likewise has no inhibitory effect on viral agglutination(15). These features of similarity between WBH and viral agglutination suggest a mechanism of inhibition analogous to that postulated for the virus, namely that mucoproteins, by virtue of their chemical constitution (involving sialic acid), combine with virus (or WBH) and prevent their attachment to similar sites on the surface of the red cell.

Agglutination of virus-treated cells. Erythrocytes agglutinated by influenza virus, and from which the latter has been spontaneously eluted, can no longer be agglutinated by the same virus(16). This has led to the concept that virus enzymically "destroys" the receptor site on the cell membrane involved in agglutination. If this site is identical with that involved in the combination of WBH with red cells, pretreatment of the latter with virus should reduce their susceptibility to agglutina-

‖ Not included in Table I are about 20 simple sugars and oligosaccharides which displayed little or no inhibition. Of these only α-lactose and D-galactose were found to exhibit measurable inhibition (0.2 and 0.3 I.U./mg respectively).

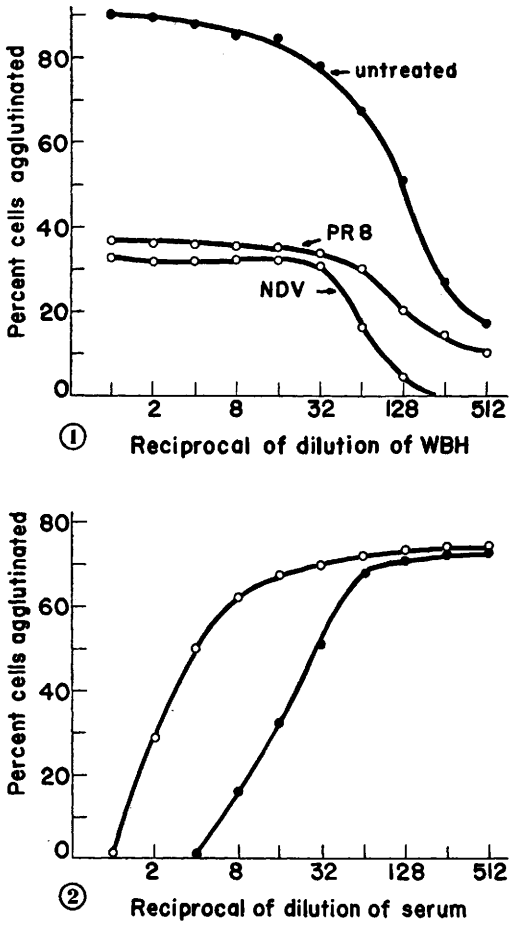

FIG. 1. WBH agglutination of rabbit erythrocytes pretreated with influenza (PR8) or Newcastle disease (NDV) compared with untreated cells. Two-fold serial dilutions of WBH were made from stock solution containing 20 γ protein/ml.

FIG. 2. Inhibition of WBH agglutination by RDE-treated rabbit serum (○—○) compared to untreated serum (●—●). Two-fold serial dilutions of sera were made from final solutions equivalent to 0.2 ml serum/ml. Level of WBH in each tube = 2 γ protein.

tion by WBH. The results of an experiment designed to test this hypothesis (Fig. 1) showed that untreated cells were more readily agglutinated by WBH than cells which had been pretreated with PR 8 or NDV virus. Concentrations of WBH which caused almost complete agglutination of untreated cells caused only partial agglutination of virus-treated cells. Attempts to demonstrate a similar effect by replacing virus with RDE were not successful. It had been previously noted that sensitivity of red cells to agglutination by WBH is considerably enhanced by treatment with proteolytic enzymes. Since no claim can be made for the purity of the RDE preparation used here(2), the possibility exists that blood cells may have been sensitized by peptidases contaminating this preparation of RDE(17), thus obscuring any effect RDE might have had in destroying the receptor site.

Inhibition by RDE-treated serum. Mucoprotein viral inhibitors, subjected to the action of RDE, no longer inhibit viral agglutination, an effect accompanied by release of sialic acid(12,13). Fig. 2 presents the results of an experiment in which rabbit serum, an effective inhibitor of WBH (Table I), was treated with RDE and tested for residual inhibition. A distinct difference in inhibition was obtained between the RDE-treated serum and the untreated control. Arbitrarily selecting 50% agglutination as a reference point, the dilutions corresponding thereto for the treated and untreated sera were 1:4 and 1:32 respectively. From sialic acid determinations, it was calculated that 73% of the protein-bound sialic acid residues originally present in the serum had been cleaved by RDE.

Discussion. Gottschalk(12) summarized the salient features of virus-cell-mucoprotein interaction as follows: (a) certain mucoproteins inhibit viral agglutination of red cells, (b) when pretreated with virus or purified enzyme (RDE), mucoproteins no longer inhibit and cells are no longer agglutinated, and (c) concomitant with these effects, a small molecular weight compound (sialic acid) is released from mucoprotein or cellular stroma.

Evidence has been presented here to sustain the view that the phenomenon of phytoagglutination (at least in the case of WBH) conforms rather strikingly to these characteristics. Thus, (a) all mucoproteins which inhibited WBH agglutination have been reported to be potent inhibitors of viral agglutination, (b) blood cells treated with 2 different types of virus became more refractory to agglutination, and finally (c) inhibitory activity of serum against WBH was considerably reduced (8-fold) when pretreated with RDE, an effect which was associated with release of about

75% of the bound sialic acid of serum. By analogy, therefore, it may be presumed that the combination of WBH with red blood cells involves the same or similar site on the surface of the cell which serves as a substrate for the viral enzyme or RDE.

There are two further observations of a negative nature. First, WBH could not be eluted from the erythrocytes once agglutination had occurred, and secondly, WBH was unable to cleave sialic acid from the various mucoproteins shown in Table I or from sialyllactose. Of the 3 successive steps involved in viral agglutination-adsorption leading to agglutination, enzymic destruction of receptor site, and elution of virus—the enzymic and elution stages can therefore be clearly excluded in the case of WBH. Thus the mode of action of WBH most closely resembles that of the heat-inactivated or "indicator" virus which retains the capacity to agglutinate red cells, an effect which can be inhibited by mucoproteins, but which can no longer be eluted due to the loss of its enzymic function.

Summary. Mucoproteins known to be inhibitors of viral agglutination inhibited agglutination of rabbit erythrocytes by the wax bean hemagglutinin (WBH). Pretreatment of red blood cells with influenza (PR 8) or Newcastle Disease virus reduced susceptibility of such cells to agglutination. The enzymatic release of sialic acid from rabbit serum by a receptor-destroying enzyme prepared from *Cl. perfringens* was accompanied by a loss in inhibitory activity of serum against WBH agglutination. These and related observations suggest that the site of reaction of WBH with erythrocytes is similar, if not identical, to that of the viral receptor site on the surface of the cell.

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A Mechanism of Action for Antithyroid Activity of Reserpine.* (24540)

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Since the introduction of reserpine as a therapeutic agent, reports have appeared ascribing an antithyroid activity to this substance. Chronic reserpine therapy reportedly resulted in "normalization" of the basal metabolic rate in 15 hyperthyroid patients(1), and in peripheral inhibition of thyroxin by reserpine(2,3), as well as a more direct action by reserpine upon the thyroid(4,5). Determination of basal metabolic rate or degree of iodine

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