acid and that binding occurs at many sites on the lectin molecule. We refer to these as the nonspecific binding sites.

It is known that all cell membranes, regardless of whether they are derived from transformed cells or from normal cells, are extremely rich in sialic acid[11]. The binding of NANA may therefore explain the near equality in the binding of this lectin to both transformed and normal cells.

During previous studies on the binding of N-acetylglucosamine by WGA we reported that only 1 mol of this hapten was bound/mol of agglutinin[10] We assumed, from this, that binding of N-acetylglucosamine occurs at a specific location on the lectin molecule. Those studies wer
over the same hapten concentration range as the binding studies with NANA reported here. We conclude that over these concentration ranges WGA has a higher affinity for binding NANA than N-acetylglucosamine.

Table 1 Competitive Effect of N-Acetylglucosamine and N-Acetylneuraminic Acid for Binding to WGA

Concentration of N-acetylglucosamine *	Concentration of N-acetyl-[4-^{14}C]-neuraminic acid *	Mol NANA bound/mol agglutinin	% Inhibition
—	0.1 mM	1.9	—
0.01 mM	0.1 mM	2.1	—
0.05 mM	0.1 mM	1.6	15%
0.1 mM	0.1 mM	0.0	100%
Concentration of NANA *	**Concentration of N-[1-^{14}C]-acetylglucosamine ***	**Mol N-acetylglucosamine bound/mol agglutinin**	**% Inhibition**
—	0.1 mM	0.98	—
0.01 mM	0.1 mM	0.86	12%
0.05 mM	0.1 mM	0.62	35%
0.1 mM	0.1 mM	0.0	100%

Equilibrium dialysis was with 5×10^{-3} μmol WGA and was carried out as described in Fig. 1.

* Final concentration, at equilibrium, in the cell containing protein.

When the equilibrium dialysis of N-acetylglucosamine against WGA was repeated with a large excess of this hapten (approximately 500-fold molar excess over WGA) we observed that approximately 10 mol of N-acetylglucosamine was bound per mol of agglutinin. Thus at high N-acetylglucosamine concentrations it appears that nonspecific binding of this hapten by WGA can occur. To determine whether the binding of N-acetylglucosamine and NANA occurs at identical sites on the lectin molecule we studied the competitive effect of one hapten on the binding of the other (Table 1). The inhibitory effect of either hapten on the binding of the other increased as the concentration of the competing hapten increased, indicating that the nonspecific binding observed with NANA and with high concentrations of N-acetylglucosamine occurs at identical sites on the lectin. The question remains whether or not the binding of N-acetylglucosamine at low concentrations represents a specific binding property of WGA. If this were true it would be predicted that even high NANA concentrations would have no effect on the specific binding of N-acetylglucosamine, but this was not observed. However, as these haptens show some structural similarities the high concentrations of NANA may induce WGA to accept and bind this hapten at a site that is otherwise specific for N-acetylglucosamine.

On the basis of these results we propose that the initial step in the agglutination of cells by WGA is the nonspecific binding of the lectin to sialic acid residues on the cell surface. In transformed cells, this nonspecific binding possibly orients the lectin to bring the N-acetylglucosamine binding site into close proximity to molecules on the cell membrane that contain N-acetylglucosamine. Correct orientation of the lectin molecule is the prerequisite to agglutination. We therefore propose that either the membranes of normal cells do not contain N-acetylglucosamine residues at the required location for specific binding to WGA, or normal cells contain N-acetylglucosamine residues which are masked so that agglutination by WGA cannot occur.

This research was supported by a grant from the National Cancer Institute. One of us (P. J. G.) is a fellow of the Damon Runyon Memorial Fund for Cancer Research.

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Received July 4, 1972.

1 Burger, M. M., and Noonan, K. D., *Nature*, **228**, 512 (1970).
2 Eckhart, W., Dulbecco, R., and Burger, M. M., *Proc. US Nat. Acad. Sci.*, **68**, 283 (1971).
3 Ben-Bassat, H., Inbar, M., and Sachs, L., *J. Membrane Biol.*, **6**, 183 (1971).
4 Ozanne, B., and Sambrook, J., *Nature New Biology*, **232**, 156 (1971).
5 Liske, R., and Franks, D., *Nature*, **217**, 860 (1968).
6 Aub, J. C., Sanford, B. H., and Cote, M. N., *Proc. US Nat. Acad. Sci.*, **50**, 613 (1965).
7 Gannt, R. R., Martin, J. R., and Evans, V. J., *J. Nat. Cancer Inst.*, **42**, 369 (1969).
8 Burger, M. M., and Goldberg, A. R., *Proc. US Nat. Acad. Sci.*, **57**, 359 (1967).
9 Smith, D. F., and Walborg, E. F., *Fed. Proc. Fed. Amer. Soc. Exp. Biol.*, **31**, 411 (1972).
10 LeVine, D., Kaplan, M. J., and Greenaway, P. J., *Biochem. J.*, **129**, 847 (1972).
11 Abercrombie, M., and Ambrose, E. J., *Ann. Rev. Cancer Res.*, **22**, 525 (1962).

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Note added in proof. Pinck, M., Chan, S., Genevaux, M., Hirth, L., and Duranton, H., *Biochimie*, **54**, 1093 (1972), have shown that valine is bound to the viral genome of Okra Mosaic Virus and Eggplant Mosaic Virus, two viruses of the same family as TYMV.

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