ther the glycosylated or deglycosylated form of the HA molecule. Of the ten antibodies to influenza HA peptide sequences which do not contain sites of glycosylation, three improved in reactivity after deglycosylation of the HA molecule, four were unchanged in reactivity (Fig. 3D), and three showed no reactivity. Thus, most site-specific antibodies to a peptide sequence of the influenza HA molecule containing a site of glycosylation improved in reactivity against the HA molecule after removal of carbohydrate.

These results suggest that the carbohydrate moieties of glycoproteins play a role in the host's immune recognition and response of these molecules. The experiments with monoclonal antibodies to the R-MuLV gp70 molecule and the antibodies to specific peptide regions of the influenza HA molecule (containing sites of carbohydrate attachment) show that the carbohydrate portion of these glycoproteins can effectively block the interaction of antibodies with the underlying polypeptide regions (*8*). In the influenza system this was true even with regions of the HA molecule that did not have attached carbohydrate, presumably by some distal effect. In contrast, heteroantisera prepared to either R-MuLV or influenza virus lost virtually all reactivity toward the gp70 and HA molecules, respectively, after deglycosylation. This result implies that not only can the carbohydrate moieties block the interaction of antibodies to protein antigenic determinants but that the carbohydrate either becomes the major immunogenic target of the glycosylated protein or directs the immune response to areas under influences of carbohydrate attachment. Thus, the carbohydrate moieties perform a dual role by masking certain polypeptide sites and at the same time directing the immune response, acting as immune decoys.

STEPHEN ALEXANDER
JOHN H. ELDER
*Department of Molecular Biology,
Research Institute of Scripps
Clinic, La Jolla, California 92037*

References and Notes

1. S. C. Hubbard and R. J. Ivatt, *Annu. Rev. Biochem.* **50**, 555 (1981).
2. For a review, see N. Sharon, H. Lis, in *The Proteins*, H. Neurath and R. L. Hill, Eds. (Academic Press, New York, ed. 3, 1982), vol. 5, pp. 1–144.
3. J. H. Elder and S. Alexander, *Proc. Nat. Acad. Sci. U.S.A.* **79**, 4540 (1982).
4. H. L. Niman and J. H. Elder, *Virology* **123**, 189 (1982).
5. W. Min Jou *et al.*, *Cell* **19**, 683 (1980).
6. N. Green *et al.*, *ibid.* **28**, 477 (1982).
7. The antisera were directed to peptides that encompass all seven sites of glycosylation in the X47 influenza HA molecule.
8. Along these lines, Skehil *et al.* [*Proc. Natl. Acad. Sci. U.S.A.* **81**, 1779 (1984)] reported that the addition of a glycosylation site at positions 63 to 65 in a variant of the 1968 Hong Kong influenza virus hemagglutinin inhibited the ability of a monoclonal antibody to the parent virus to recognize the variant.
9. U. K. Laemmli, *Nature* **227**, 680 (1970).
10. H. Towbin, T. Staehelin, J. Gordon, *Proc. Nat. Acad. Sci. U.S.A.* **76**, 4350 (1979); D. A. Johnson, J. W. Gautsch, J. R. Sportsman, J. H. Elder, *Gene Anal. Technol.* **1**, 3 (1984).
11. J. H. Elder, F. C. Jensen, M. L. Bryant, R. A. Lerner, *Nature (London)* **267**, 23 (1977).
12. The sera were obtained from the National Resources Branch of the National Cancer Institute.
13. Supported by Public Health Service grants CA25533 and CA25803 (J.H.E.). We thank R. A. Lerner for providing the antisera to the peptide regions and H. Alexander for help and advice during the course of the work; J. Gautsch, D. Pallotta, and T. Shinnick for comments on the manuscript; and A. Cibulsky and V. Maleckar for technical assistance. Publication 3547 MB from the Research Institute of Scripps Clinic.

14 June 1984; accepted 10 September 1984

Pollen Feeding in an Orb-Weaving Spider

Abstract. *Juvenile orb-weaving spiders appear in spring, when insect prey are scarce but when aerial plankton, such as pollen and fungus spores, is abundant. Microscopic organic matter may be the main food of orb-weaving spiderlings, with insects providing only a dietary supplement. Pollen, which is caught on the sticky spirals of* Araneus diadematus *orb webs, doubles the life expectancy of spiderlings and alters their web-spinning behavior, so that they spin more frequently than do fasting controls. Fungus spores do not have the same nutritional value as pollen and may be deleterious to the spiderlings.*

Orb-weaving spiders take down and eat their old webs at fairly regular intervals—a well-documented behavior (*1*) that is usually explained as a mechanism for recovering some of the costs of producing silk (*2*). Speculations about other possible benefits of web eating were raised after we observed young orb-weavers (Araneidae) spin and dismantle several successive webs without apparently capturing any insect prey.

Because the energy costs of web build-

Table 1. Means and confidence intervals for four variables tested on second and third instars of *Araneus diadematus*. Abbreviations: FF, fungus-fed (*9*); PF, pollen-fed (*10*); S, starved controls; *N*, number of spiderlings; and *n*, number of observations. Means followed by the same letter are not statistically different from each other [Student-Newman-Keul's a posteriori test (*28*)]. Probabilities determined by analysis of variance (ANOVA) (*29*).

Instar	Treatment	Mean	Confidence interval (95 percent)	*N*	*n*	*P*
		*Life-span (12-hour intervals)**				
2†	FF	10.96a	8.10 to 14.82	22		0.005
	PF	20.17	13.86 to 29.34	22		
	S	9.90a	7.25 to 13.51	22		
3	FF	17.22b	13.07 to 21.38	9		0.05
	PF	27.11	18.88 to 35.34	9		
	S	17.33b	9.11 to 25.06	9		
		*Frequency of spinning (webs spun per total number of 12-hour intervals alive)**				
2†	FF	0.25c,d	0.19 to 0.30	21		0.018
	PF	0.30c	0.25 to 0.36	21		
	S	0.19d	0.13 to 0.25	21		
3	FF	0.15	0.10 to 0.19	9		0.001
	PF	0.26e	0.22 to 0.30	9		
	S	0.21e	0.17 to 0.25	9		
		Number of 12-hour intervals with each web remaining intact‡				
2	FF	2.94	2.63 to 3.25		66	0.55
	PF	3.10	2.89 to 3.30		204	
	S	3.22	2.58 to 3.85		46	
3	FF	3.57	2.46 to 4.67		23	0.31
	PF	3.13	2.72 to 3.55		67	
	S	3.32	2.86 to 3.78		28	
		Number of 12-hour intervals between webs‡				
2	FF	3.29	2.38 to 4.20		66	0.001
	PF	1.98	1.71 to 2.24		204	
	S	4.11	2.78 to 5.44		46	
3	FF	5.39	3.47 to 7.32		23	0.003
	PF	2.72	2.04 to 3.39		67	
	S	4.57	3.27 to 5.87		28	

*Probabilities based on one-way ANOVA. †These data did not meet the assumption of equal variance of ANOVA and were log- or arcsin-transformed. Means and confidence intervals represent back-transformed values. (*28*). ‡Probabilities based on Kruskal-Wallis one-way ANOVA (*29*).

ing exceed returns from recycling old webs (*3*), it seemed possible that spiderlings were also ingesting microorganisms captured on their viscid spirals. Benefits from eating microorganisms would be particularly important for second-instar spiderlings (that is, those in the first web-building stage) because individuals at this stage have already survived one molt and a period of about 1 or 2 weeks (*4*) with no food other than yolk reserves. Even taking into account that spiders can sacrifice 50 percent of their body weight to produce silk (*5*), these young spiders would soon exhaust their potential to produce silk and to build webs if they had no other source of nutrients.

Microorganisms can be seen when webs of second-instar spiderlings are examined under a light microscope. While some of this microscopic filtrate is comprised of insects and nematodes, the bulk is made up of plant pollen and fungus spores. Other investigators, notably Lubin (*6*), have remarked that webs can sometimes be so densely coated with pollen and dust that their effectiveness in the capture of insect prey is reduced. If juvenile spiders do feed on the plant materials and fungi on their webs, then our understanding of the feeding habits of spiders requires radical revision. Thus, spiders would best be described as omnivores rather than the carnivores that arachnologists have always assumed them to be (*7*). This study was undertaken to test the hypothesis that juvenile orb-weavers consume and obtain substantial nutrition from the pollen and fungi on their webs.

Second instars (I_2) of *Araneus diadematus*, the garden cross-spider, were taken from a communal group and put into individual cages (*8*). Once the spiderlings had spun their first web, 66 of them were randomly assigned to one of three treatment groups: their webs were sprinkled with scrapings from fungus (*9*), grains of birch pollen (*10*), or nothing (starved) (*11*). A fourth group of 60 I_2 spiderlings was fed one aphid (*12*) per day and observed until the spiderlings molted or died. This was done to compare survival rates between the experimental groups and spiderlings on an insect diet (*13*). The experiment was repeated with 27 third-instar (I_3) spiderlings taken from this aphid-fed group after they had molted.

Young spiders not only fed on the pollen but also substantially increased their chances of survival by doing so (Table 1). Compared with their starved (S) and fungus-fed (FF) siblings, I_2 spiderlings that were fed pollen (PF) doubled their mean life-span. They also spun significantly more webs per 12-hour interval than their starved siblings. The group receiving fungus did not differ significantly from the starving group, nor was there a significant difference in the number of 12-hour intervals that individuals of each group maintained their webs, suggesting that the PF and FF groups were not simply taking down their webs because the webs were full of debris. However, time spent between taking down an old web and the spinning of a new one was significantly shorter for the PF group, perhaps indicating that it was more difficult for the FF and S groups to manufacture the silk required for spinning a new web (Table 1).

Third-instar spiderlings in the PF group also outlived their fungus-fed and starved siblings (Table 1). Among I_3's, the frequency of web-spinning did not differ between PF and S groups, but the FF group spun significantly less often. Like the I_2's, the I_3's did not show a significant difference in the number of 12-hour intervals during which webs were kept up, but again the PF group took less time than the other groups to spin new webs after old ones had been taken down.

The period during which an orb-weaving spider leaves its web intact is crucial for survival. Prolonged exposure of a functional web, which increases the chances of catching an insect, is particularly important in springtime, when prey of appropriate sizes for these small (0.5 to 1.0 mg) spiderlings are scarce. Second-instar spiderlings receiving pollen pursued a different strategy than their starved or fungus-fed siblings; they spun more often. However, among the I_3 spiderlings it was the fungus-fed group that

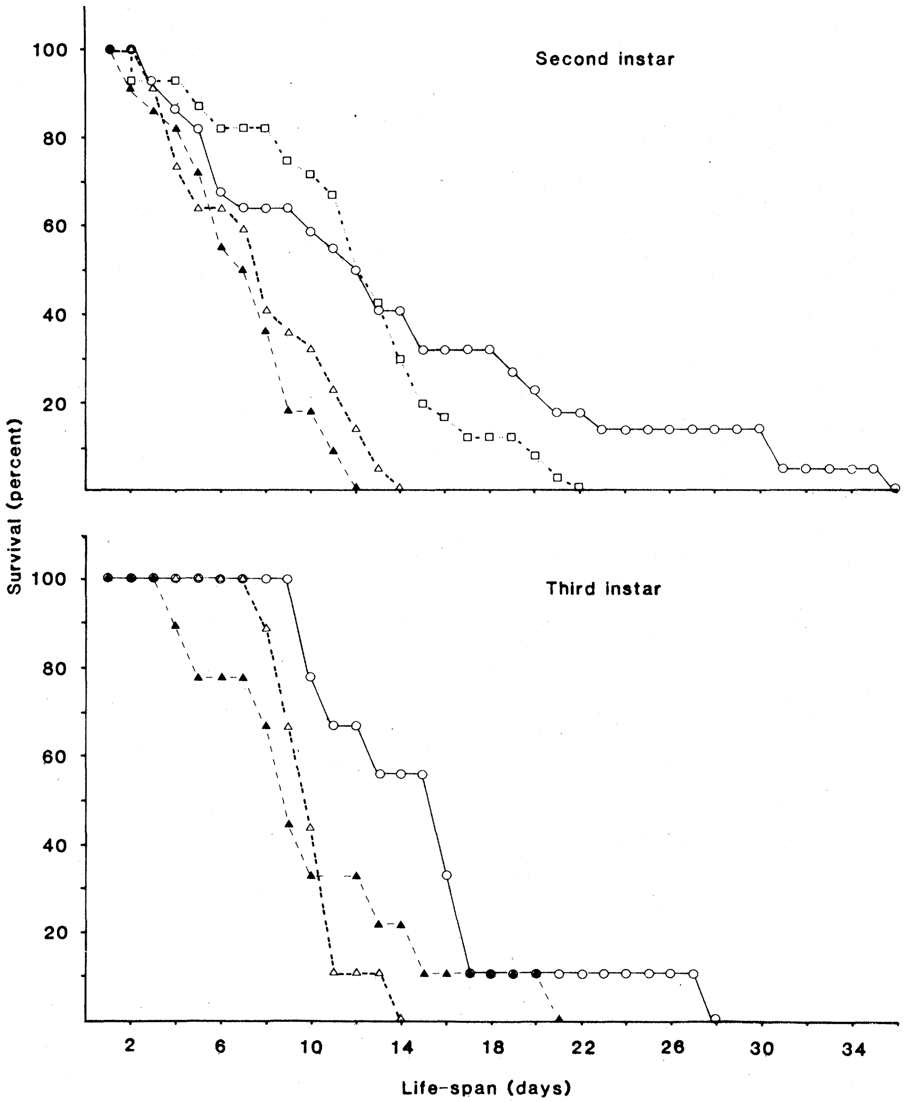

Fig. 1. Percent survival of second- (top) and third- (bottom) instars of *Araneus diadematus* subjected to different treatments. Numbers of second- and third-instar spiderlings, respectively, are given in brackets: (▲) starved [22, 9]; (△) fungus-fed [22, 9]; (○) pollen-fed [22, 9]; and (□) aphid-fed [60]. For the aphid-fed group, the curve represents animals remaining alive in the second-instar stage. Those that died and those that molted were summed.

differed by spinning less frequently. Some individuals in the starved group outlived the fungus-fed group in these older spiderlings (Fig. 1, bottom). This, coupled with the decrease in web-spinning frequency, suggests that the fungous material was ingested and had a deleterious effect on the spiderlings.

Interestingly, the only spiderlings to molt were those in the fourth group, fed on aphids. Of the 60 aphid-fed individuals, 19 died before molting, revealing that mortality may be relatively high in the early instars of *A. diadematus*. Members of this group molted between days 8 and 19 and were either in their next instar or dead by day 21 (Fig. 1, top). Some of the PF animals lived as long as 36 days. Although the cause of death in the PF group is unknown, the observation that some individuals of this group outlived those of the aphid-fed group suggests that the pollen-fed spiderlings might have died because they could not molt rather than from actual starvation.

There are distinct differences in nutritional value among species of pollen (*14*). For example, under experimental conditions pollen from wind-pollinated (anemophilous) plants is nutritionally poorer for bees than pollen from insect-pollinated (entomophilous) plants (*15*). Some wind-transported pollens contain only trace amounts of tyrosine (*16*), an amino acid essential for the formation of new cuticle for both insects (*17*) and spiders (*18*). It would not be surprising if a tyrosine deficiency in the anemophilous birch pollen used in this experiment were responsible for the inability of spiderlings to molt. Spiderlings fed a single aphid at the beginning of an experimental period were able to molt, even when subsequently maintained on an insect-free diet after their first meal; those receiving only pollen or fungus throughout the trial never molted.

Fungus spores (3 to 30 μm in diameter) and pollen grains (20 to 50 μm in diameter) (*19*) are too large to pass through the cuticular platelets of a spider's pharynx. Particles larger than 1 μm are passively filtered out (*20*). Because chitinase is present in the digestive fluid of spiders (*21*), they should have no difficulty in digesting the chitinous outer coat of fungus spores (*22*) when consuming an old web by extraintestinal digestion. Enzymes capable of degrading pollen exine have not been actively sought among web-building spiders because such enzymes reputedly occur only rarely in the animal kingdom (*23*). Our results suggest that such a search might be fruitful.

We examined only two species of microorganisms in isolation. One of them, birch pollen, proved to be a valuable source of nutrition for I_2 and I_3 spiderlings of *A. diadematus*. The other, fungus spores, provided no apparent nutritional value and exerted some deleterious effects on spiderlings. Because pollen is an important nutrient for many terrestrial arthropods (*24*), it is not surprising that it can be a source of food for spiderlings. In the field many kinds of small plants and animals are trapped by orb webs. A complement of several microorganisms might provide a sufficient diet to enable spiderlings to reach the next instar during times of low availability of insect prey. In addition, spider silk constitutes a rich proteinaceous substrate, potentially enabling the growth of some organisms. Waste and by-products of some microorganisms may thus increase the nutritional benefits of web eating.

The existence of several adaptations in spiders for surviving starvation has led to the general conclusion that spider populations frequently exist under conditions of food deprivation (*25*). Field studies support this hypothesis (*26*). The small first webs [2.5 to 4.0 cm in diameter (*27*)] of I_2 cross-spiders are probably better at capturing aerial plankton than they are at detaining insects. Microorganisms may be the main food of these spiderlings, with insects providing only a dietary supplement.

RISA B. SMITH
Department of Plant Science and
Institute of Animal Resource Ecology,
University of British Columbia,
Vancouver V6T 1W5 Canada
THOMAS P. MOMMSEN*
Biology Department, Dalhousie
University, Halifax, Nova Scotia
B3H 4J1 Canada

References and Notes

1. A. L. Breed, V. L. Levine, D. B. Peakall, P. N. Witt, *Behaviour* **23**, 43 (1964); W. J. Gertsch, *American Spiders* (Reinhold, New York, 1978).
2. D. B. Peakall, *J. Exp. Zool.* **176**, 257 (1971).
3. ——— and P. N. Witt, *Comp. Biochem. Physiol. A* **54**, 187 (1976); K. N. Prestwich, *ibid.* **57**, 321 (1977).
4. T. Burch, *J. Arachnol.* **7**, 1 (1979); P. N. Witt, J. O. Rawlings, C. F. Reed, *Am. Zool.* **12**, 445 (1972).
5. P. N. Witt, *Arch. Environ. Health* **7**, 10 (1963).
6. Y. D. Lubin, *J. Arachnol.* **6**, 31 (1978).
7. R. Foelix, *Biology of Spiders* (Harvard Univ. Press, Cambridge, Mass., 1982), p. 1.
8. Communal webs were collected on 28 and 29 May 1983 from Westham Island, British Columbia, Canada. Cages were plywood frames (20 cm^2 by 9 cm deep) with a removable glass window on one side and clear acetate on the opposite side. A 10-cm square was cut in the acetate and a piece of cotton mesh inserted to provide ventilation. Experiments were conducted in a greenhouse under natural photoperiod between 31 May and 15 July 1983. Temperatures fluctuated between highs from 30° to 33°C and lows from 15° to 18°C.
9. *Cladosporium herbarum* was collected by pressing *A. diadematus* webs against plates of potato dextrose agar and incubating at room temperature. Several species of fungous colonies grew, but *C. herbarum* was by far the most abundant. This is a common mold of decaying vegetation and constitutes about 33 percent of all spores caught in inertial traps in the air of western North America [J. Lacey, in *Biology of Conidial Fungi*, T. Cole and B. W. Kendrick, Eds. (Academic Press, Toronto, 1981), p. 382]. The *C. herbarum* used was from subcultures of the original colonies. Spores were applied by brushing the fungous colonies lightly with a sable brush and then tapping the brush over webs. The black spores were highly visible, so newly made and therefore spore-free webs were easily identified for subsequent treatment.
10. Birch (*Betula papyrifera*) catkins were collected locally between late March and early April and air-dried on fine copper screens and the pollen was sieved. Spiderlings were already in webs when the birch was in flower. The bright yellow pollen grains were applied with a sable brush.
11. The webs of starved spiderlings were marked with a fine wisp of cotton. All cages were sprayed with distilled water twice a day to prevent dehydration.
12. Alate *Macrosiphum euphorbiae*.
13. Smaller cages (glass dishes, 10 cm in diameter by 8 cm high, with glass tops) were used. Cardboard frames (8 cm^2 by 1 cm wide) were placed inside as supports for webs. Web size did not differ from those spun by spiderlings kept in the larger wooden cages (*8*).
14. *Pinus sylvestris* and *P. nigra* [R. G. Stanley and H. F. Linskens, *Pollen: Biology, Biochemistry, Management* (Springer-Verlag, New York, 1974), pp. 154–161].
15. ———, *ibid.*, p. 104.
16. ———, *ibid.*, p. 158.
17. R. F. Chapman, *The Insects, Structure and Function* (Elsevier, New York, 1969), p. 443.
18. K. G. Collatz and T. Mommsen, *Comp. Biochem. Physiol. A* **52**, 465 (1975).
19. M. J. M. Hirst and G. W. Hirst, in *Airborne Microbes* (17th symposium of the Society for General Microbiology), P. H. Gregory and J. L. Monteith, Eds. (Cambridge Univ. Press, New York, 1967), pp. 307–344.
20. R. Foelix, *Biology of Spiders* (Harvard Univ. Press, Cambridge, Mass., 1982), pp. 47–48.
21. T. P. Mommsen, *Comp. Biochem. Physiol. A* **60**, 371 (1978); *Biochim. Biophys. Acta* **612**, 361 (1980).
22. J. M. Aronson, in *Biology of Conidial Fungi*, T. Cole and B. W. Kendrick, Eds. (Academic Press, Toronto, 1981), p. 459.
23. R. G. Stanley and H. F. Linskens, *Pollen: Biology, Biochemistry, Management* (Springer-Verlag, New York, 1974), pp. 110–111.
24. ———, *ibid.*, pp. 109–111.
25. For review, see S. E. Riechert and J. Luczak, in *Spider Communication*, P. N. Witt and J. S. Rovner, Eds. (Princeton Univ. Press, Princeton, N.J., 1982), p. 353.
26. A. C. Janetos, *Behav. Ecol. Sociobiol.* **10**, 19 (1982); A. Kajak, *Ekol. Pol. Ser. A* **32**, 718 (1965); R. B. Smith also conducted a field study in which more than 1400 webs of second-, third-, and fourth-instar *Araneus diadematus* were examined, and 87 percent of them captured no prey.
27. H. Pfletschinger, *Einheimische Spinnen* (Kosmos Gesellschaft der Naturfreunde, Stuttgart, 1979).
28. R. R. Sokal and R. J. Rohlf, *Biometry* (Freeman, San Francisco, ed. 2, 1981), pp. 400–428. An outlier greater than 3 standard deviations from the mean was removed from the starving group. To maintain the orthogonal design, we randomly removed one datum from the other two groups, leaving 21 individuals in each group. Student–Newman-Keul's a posteriori test at the 0.05 level of significance was used to determine differences between pairs of treatments [R. R. Sokal and R. J. Rohlf, *Biometry* (Freeman, San Francisco, ed. 1, 1969), pp. 239–241].
29. S. Siegel, *Non-Parametric Statistics* (McGraw-Hill, Toronto, 1956), p. 190. The parametric analysis of variance test was not used because these data did not meet the assumption of equal variances even after a transformation. The Student–Newman-Keul's test was not used because it is only appropriate for data in which the means have equal variances.

30. We thank W. G. Wellington, B. D. Frazer, D. Berner, H. Levi, and C. Villars for reading the manuscript and offering valuable suggestions and F. Vollrath for input into the original concept. This work was supported by grants from the Natural Sciences and Engineering Council of Canada (W.G.W. and T.P.M.).

* Present address: Department of Zoology, University of British Columbia, Vancouver V6T 2A9 Canada.

5 December 1983; accepted 1 June 1984