stored in 0.3*M* NaCl (*3*). Before use they were treated with hydrogen peroxide, hexadecyltrimethyl ammonium bromide (Cetavlon), bis-(*p*-chlorophenyldiguanido)-hexane diacetate (Hibitane), and tetracycline according to the schedule of Bird. The first of the three culture media (*4*) permitted seed germination and also allowed early observation of any contaminating organisms. The second (*5*) aided penetration of roots by larvae and the development of galls. It, too, permitted detection of contaminants. The third, the nemin assay agar of Winkler, Kuyama, and Pramer (*6*), not only permitted further development of symptoms and counting of fungal traps but also maintained a *p*H range that kept spontaneous trap formation at a consistently low level.

To obtain three-membered cultures, 3-day-old sterile seedlings were transferred to the penetration agar where they remained for 4 days after inoculation with a nematode egg mass. Few larvae failed to penetrate the roots. Thus the number of larvae inside the roots was similar to the number in the control plates. During the 4-day period, galls began to form. The diseased plants containing active pathogens were transferred to the nemin assay agar. Any assay agar culture that had a contaminating external *Meloidogyne* larva carried over from the penetration medium was discarded. A day later young fungal hyphae were transferred to the assay plates on squares of cornmeal agar. Counts of traps were made 7 and 9 days afterward.

In three orientation trials which involved 18 to 20 plates of two-membered combinations there were always 800 to 1000 traps per 60-mm plate when *A. conoides* was combined with newly hatched *M. hapla* larvae (average, 900). In contrast, there were never more than 20 traps in plates containing sterile healthy tomato seedlings and the fungus (average, 8). Similar low numbers of traps formed spontaneously in pure cultures of *A. conoides* (average, 6). This is the first substantiation, based on intact plant tissue growing in axenic culture, of the earlier generalization of Deschiens and Lamy (*1*) that plants do not induce trap formation.

Of even more interest are the two experiments with three-membered cultures (Table 1). No further significant production of traps was observed on the 12th and 13th days. In cultures of both the diseased and healthy seedlings there was even a gradual breakdown of the few traps that had been produced. It is clear that the radical changes brought about by the pathogenic activities of the nematodes inside the host plant do not make the diseased root a source of available nemin. Unless an inductive compound is formed inside the parasitized roots and released later in the development of the syndrome, it is not to be expected that such diseased plants would contribute to the formation of traps by nemin-requiring fungi in the rhizosphere.

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8. Part of this investigation was supported by USPHS grant 2G-849. Samples of Hibitane diacetate and tetracycline were supplied by Ayerst and Lederle Laboratories, respectively. *Arthrobotrys conoides* was obtained from David Pramer of Rutgers University and diseased roots containing *Meloidogyne hapla*, from James Bloom of Pennsylvania State University.

18 August 1964

Human Blood Group A_1 Specific Agglutinin of the Butter Clam Saxidomus giganteus

Abstract. *Saline extracts of the butter clam* Saxidomus giganteus *specifically agglutinated human red blood cells of phenotypes* A_1 *and* A_1B. *The agglutinin was completely absorbed by* A_1 *and* A_1B *cells, partially absorbed by* A_2 *cells, and unaffected by B and O cells. Of 21 sugars tested, only* N-*acetyl*-d-*galactoseamine and* N-*acetyl*-d-*glucoseamine inhibited the agglutinin.*

Certain plants produce substances called lectins that agglutinate red blood cells of various species. Furthermore, some of these lectins react specifically with certain isoantigens, such as the A_1 antigen of human red blood cells. A recent review of lectins has been presented by Boyd (*1*), and references to blood group reactive substances in invertebrates will be found in Cushing *et al.* (*2*). I now report an agglutinin in clams that shows specificity toward human red blood cells of blood group A_1.

The agglutination of human red blood cells by a saline extract of the butter clam *Saxidomus giganteus* (Deshayes) (*3*) was initially observed while the clams were being studied for a different purpose (*4*). The clams were obtained from Oyster Bay, Southern Puget Sound, and were received shucked, washed, and frozen (*5*). Saline extracts were prepared by mixing equal amounts of ground clam and physiological saline (wt./vol.) and incubating the mixtures at room temperature for 30 minutes. The saline suspension of clams was centrifuged at 36,000*g* in a Servall RC-2 centrifuge for 30 minutes (15°C). The supernatant obtained was lytic for human red blood cells. The hemolytic effect was removed by diluting the extract 1:4 with physiological saline or by heating at 56°C for 30 minutes and recentrifugation at 36,000*g* for another 30 minutes. Both preparations produced the same agglutination reactions against red cells. Most of the data reported here were obtained with the preparation that had been inactivated by heat, since it could be used undiluted. Agglutinations were performed by mixing 0.1 ml of clam extract with an equal volume of 2 percent suspension of washed human red blood cells in 10 by 75 mm tubes. Tubes were incubated at room temperature for 30 minutes, centrifuged for 1 minute in a Serofuge (Clay-Adams), tapped gently to dislodge the cells from the bottom, and observed for clumping of red cells.

Data on the reactions of undiluted extract with human red cells obtained from 27 randomly selected donors are presented in Table 1, along with the ABO blood groups of the donors (*6*). The results are based on performance of the test on at least five different occasions. The same reactions were obtained each time, except that three samples of cells of blood group O gave equivocal positive reactions on one occasion. These three samples were considered negative against the clam extract. Cells of blood groups A_1 and A_1B gave unequivocal 4+ reactions with

Table 1. Reactivity of saline extract of *Saxidomus giganteus* with the red blood cells of 27 randomly selected donors.

Phenotype of donors	Samples tested with clam extract (No.)		
	Total	Positive	Negative
A_1	8	8	0
A_1B	2	2	0
A_2	3	0	3
B	4	0	4
O	10	0	10

Table 2. Absorption studies with saline extracts of the butter clam *Saxidomus giganteus* and red cells of various blood groups. Test cells were of blood group A_1.

Absorption at various dilutions of extract				
1:1	1:2	1:4	1:8	1:16
		Unabsorbed		
4	4	4	1	–
		Group A_1		
–	–	–	–	–
		Group A_1B		
–	–	–	–	–
		Group A_2		
4	–	–	–	–
		Group B		
4	4	4	1	–
		Group O		
4	4	4	–	–

the clam extract on all five occasions. The agglutinin appears specific, therefore, for a blood factor associated with the A_1 agglutinogen.

Absorption studies were carried out to elucidate the specificity of the agglutination. One volume of packed, washed red cells was mixed with three volumes of undiluted clam extract in 10 by 75 mm tubes and incubated at room temperature for 30 minutes. The supernatant obtained by centrifugation of the absorbed clam extract at 2500 rev/min for 10 minutes was tested against A_1 cells. Results from absorptions with cells of phenotypes A_1, A_1B, A_2, B, and O are presented in Table 2. Cells of groups A_1 and A_1B completely absorbed the agglutinin, while under the same conditions A_2 cells partially absorbed the agglutinin. Cells of blood groups B and O had no effect on the agglutinin. The absorptions support the data in Table 1. The partial absorption of the clam agglutinin by A_2 cells suggests, however, a structural similarity of the clam receptor on A_1 cells, with some chemical grouping on A_2 cells, even though A_2 cells were not clumped by the agglutinin.

In an effort to ascertain something of the chemical nature of the receptor for the clam agglutinin, inhibitions with various saccharides were carried out. The following saccharides were tested in 2000-μg amounts, contained in 0.1 ml, against 0.1 ml of undiluted clam extract; *d*-glucose, *d*-fructose, *d*-mannitol, dulcitol, maltose, *d*-melibiose, *d*-lactose, α-methyl-*d*-glucose, *l*-rhamnose, sucrose, *d*-xylose, raffinose, trehalose, salicin, *d*-galactose, *d*-mannose, *l*-arabinose, *d*-galactoseamine, *d*-glucoseamine, *N*-acetyl-*d*-galactoseamine, and *N*-acetyl-*d*-glucoseamine. Only *N*-acetyl-*d*-galactoseamine and *N*-acetyl-*d*-glucoseamine inhibited the agglutination of A_1 cells by the saline extract of the clam, which suggests that these sugars are associated with the receptor for the clam agglutinin.

The clam agglutinin was nondialyzable and is thus probably a large molecule. The agglutinin was inactivated when the clam extract was heated at 70°C in a water bath for 20 minutes and may, therefore, be a protein.

Preliminary studies on the anatomical location of the agglutinin have indicated that it is present in saline extracts of siphons of *Saxidomus giganteus* at about the same titer as in the whole clam extract. This observation suggests that the agglutinin is not partially digested residual food materials of the clam gut. In addition, since the clams were collected from unpolluted waters and were washed and frozen immediately after harvesting and kept frozen until examined, the possibility that the agglutinin results from microbial growth or microbial degradation of clam tissues appears unlikely. Certainly another potential source of agglutinins for red cell isoantigens has been demonstrated.

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4. The clams in this study were being used in the study of the haptenic properties of paralytic shellfish poison, a potent toxin produced by the dinoflagellate, *Gonyaulax catenella*, and concentrated in certain bivalves that use *Gonyaulax* as a food source. The clams used were not contaminated with the toxin.
5. The clams were sent by Dr. A. K. Sparks, University of Washington, Seattle, to Earl McFarren, of the Robert A. Taft Sanitary Engineering Center. I obtained them from McFarren.
6. Blood supplied by the Blood Bank, Cincinnati General Hospital.

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Complex Visual Concept in the Pigeon

Abstract. *Pigeons were trained to respond to the presence or absence of human beings in photographs. The precision of their performances and the ease with which the training was accomplished suggest greater powers of conceptualization than are ordinarily attributed to animals.*

It is well known that animals can use one or a few distinguishing features to discriminate stimuli such as simple visual arrays differing in size, shape, or color. In the experiment described here, however, pigeons were trained to detect human beings in photographs, a class of visual stimuli so diverse that it precludes simple characterization.

Five male racing (homing) pigeons between 1 and 2 years of age were obtained from a local breeder. Apart from the likelihood that they had been housed in outdoor coops, nothing was known about their past histories. All five were given approximately the same training and all performed similarly.

The pigeons were first fed on a minimal diet until their weights fell 20 percent. They were then fed enough food to maintain them at the reduced weights. Once a day each bird was placed in a box containing a hinged switch mounted on a wall next to a 5 cm by 5 cm translucent plate and a feeding device. During the first few sessions, the pigeons were trained to eat from the feeding device each time it was operated, when food was made available for approximately 3 seconds. Next, the pigeons were taught to peck at the hinged switch to trigger the feeder. At first, every peck at the switch operated the feeder, but, after two sessions, the procedure was changed so that pecks were effective only once a minute, on the average. An intermittent schedule of reward of this type produced relatively steady behavior, with little satiation of hunger. As a final stage in the preliminary training the pigeons were taught that only when the translucent plate next to the switch was illuminated with a uniform white light were pecks effective, but still only intermittently. When the plate was dark, pecks were entirely ineffective. The illumination changed randomly in time, averaging a change a minute, with the sole reservation that