squirrel atria the potassium concentration actually increased during hibernation. In only the hamster ventricle was this increase accompanied by a reciprocal decrease in sodium concentration. In hamster atria and ground squirrel ventricles, however, sodium content was lower in samples from hibernating individuals while the potassium content was unchanged.

The significance of the changes in sodium and potassium is unclear and may be different for each tissue. The changes do not reflect changes in plasma concentration, for neither plasma sodium (157 m*M* in hamsters and 149 m*M* in ground squirrels) nor plasma potassium (4.1 m*M* in hamsters and 3.9 m*M* in ground squirrels) was measurably altered during hibernation. Change in total tissue content of a cation could reflect either a change in its concentration in the tissue water or an increase or decrease in size of the tissue compartment containing that species of cation. For example, hydration (grams of water/gram dry wt) of hamster atria and ground squirrel ventricles decreases by 10 to 12 percent during hibernation (*5*). If about half of this loss of water represented a decrease in extracellular space, it would be sufficient to account for the observed decrease in tissue sodium. No attempt was made, however, to estimate extracellular space in kidney or the muscular tissues, and the contribution of extracellular sodium to the tissue sodium concentration cannot be stated. Consequently, the tissue potassium concentration is a more useful indication of changes in ionic steady state. (For example, with a plasma potassium concentration of about 4 m*M* and an extracellular space in a tissue of 25 percent the contribution of extracellular potassium to a tissue concentration of 300 microequivalents per gram dry weight would only be 1 percent.)

The possibility that the enormous increase in potassium content of ground squirrel kidney cortex is due to extracellular changes (for example, a great increase in hematocrit) seems to have been ruled out by subsequent experiments in which, as a routine procedure, the slices of kidney cortex were soaked for several minutes at room temperature in a phosphate-buffered Krebs medium containing calcium. In general, this treatment not only washes blood from the tissue but also brings about a decrease in concentration of tissue potassium (*7*, *8*). The slices of kidney cortex from the hibernating ground squirrel, when treated in this way, retained potassium at a concentration about 60 microequivalents per gram dry weight higher than that of slices from nonhibernating ground squirrels. It seems probable, therefore, that the high potassium content of kidney cortex during hibernation reflects a change either in the intracellular concentration of that ion or in the intracellular space.

The results thus demonstrate again the dramatic cold resistance and, therefore, uniqueness of tissues and mammalian hibernators.

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9. I acknowledge the able assistance of Christine Bullock. This work was supported by NIH grant GM 11494.

21 August 1964

Nematode-Trapping Fungi: Evaluation of Axenic Healthy and Galled Roots as Trap Inducers

Abstract. *Larvae of* Meloidogyne hapla *Chit. induced abundant trap formation in the predaceous fungus* Arthrobotrys conoides *Drechs. Under similar aseptic conditions, neither healthy roots of* Lycopersicon esculentum *Mill, nor roots parasitized by the root-knot nematode induced traps.*

Past investigations of nematode-trapping fungi have supplied information about the types of traps, the wide variety of animal tissue extracts that induce traps, the noteworthy absence of such induction by extracts of healthy plant parts, the chemical nature of the inducer (nemin), and the use of the fungi as biological controls of nematode-incited diseases (*1*). Bird (*2*) added greatly to the knowledge of the physiological changes in the host plant tissues brought about by the root-knot nematode, *Meloidogyne javanica* Treub., during pathogenesis. Using sterile conditions to study the formation of giant cells in diseased roots, he concluded that the initiation and growth of the galls depend upon the continuous activity of the endoparasite. Nothing was known, however, about the interaction between such a profoundly altered root and the nematode-trapping fungi. Indeed, it was not known whether intact healthy roots could induce trap formation in those fungi dependent on nemin-like compounds.

The purpose of this investigation was to bring together under aseptic conditions *Lycopersicon esculentum* Mill., *Meloidogyne hapla* Chit., and the nematode-trapping fungus *Arthrobotrys conoides* Drechs. in such a way that the influence of the nematode-parasitized roots on the formation of traps by the fungus could be studied in the absence of free larvae. To do this, a succession of treatments and culture media was required. The surfaces of tomato seeds were sterilized with $Ca(OCl)_2$. Nematode egg masses were collected from tomato plants and

Table 1. Trap formation by *Arthrobotrys conoides* Drechs. when combined in aseptic culture with (i) free larvae of *Meloidogyne hapla* Chit., (ii) healthy *Lycopersicon esculentum* Mill., and (iii) galled *L. esculentum* containing active endoparasitic larvae. Figures represent the average number of traps per 60-mm plate.

Trial 1		Trial 2	
7 days	9 days	7 days	9 days
Lycopersicon, galled*			
2	5	2	9
Lycopersicon, healthy†			
2	5	1	7
Meloidogyne larvae†			
800	850	850	900

* Averages of 41 to 57 cultures. † Averages of 5 cultures.

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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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