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Mechanical Transmission of a Plant Tumor Virus to an Insect Vector

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ions on orientation of the water molecule by hydration with their influence on the amount of coagulation during freezing. As predicted, coagulation varies with salt concentration and goes through a minimum at low concentrations. Under favorable conditions coagulation can be entirely inhibited. These effects would appear to have various biological applications.

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Antithiamine Effect of Oxythiamine and Neopyrithiamine. A Comparative Study¹

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According to Wilson and Harris (3), pyrithiamine does not have the structure assigned to it. These workers have synthesized a new compound which they call "neopyrithiamine," and which shows chemical and physical properties that would be expected from the pyridine analogue of thiamine. When this compound became available,² an experiment was set up with a view of comparing its antithiamine effect with that of oxythiamine, which was shown in this laboratory to act as an antagonist for thiamine (2).

Mice of the Swiss and C-57 Black strains from our stock colony were used. The animals were placed at weaning on a thiamine-deficient diet which consisted of casein (Labeo) 25%, sucrose 53%, hydrogenated vegetable oil 10%, lard 5%, Osborne and Mendel salt mixture³ 5%, and Ruffex 2%, and contained the following supplements per kg of diet: riboflavin 10 mg, pyridoxin 10 mg, calcium pantothenate 100 mg, α -tocopherol 40 mg, β -carotene⁴ 20 mg, vitamin D (Drisdol) 5000 units, and choline chloride 1.5 g. When the animals had become steady in weight, they were maintained on 1 μ g of thiamine per day, injected in aqueous solution subcutaneously for one week. Daily injections of oxythiamine or neopyrithiamine were then started together with 1 μ g of thiamine in a ratio (thiamine:antivitamin) of 1:50. Control animals, three for each group, received thiamine alone. The results are shown in Table 1.

In the case of both compounds the effect on the food intake was apparent overnight. There was a drop in

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² We are indebted to Dr. K. Folkers of Merck & Co., Inc. for the neopyrithiamine.

³ The quantity of manganese was doubled.

⁴ A mixture of 90% beta and 10% alpha carotene (GBI) was used.

the food intake of the animals and a loss in weight. The data show that the antivitamin effect of neopyrithiamine is more pronounced than that of oxythiamine.

TABLE 1
COMPARATIVE ANTITHIAMINE EFFECT OF OXYTHIAMINE AND NEOPYRITHIAMINE

Substance tested	No. of mice	Wt of mice at start of injections of anti-vitamin g	Incidence of polyneuritis %	No. of days before polyneuritis developed	Survival time in days
Oxythiamine	9	10.6-13.6	0	...	13-21
Neopyrithiamine	9	10.2-13.8	100	5-7	7-8

This manifested itself in the development of polyneuritic symptoms in the animals treated with neopyrithiamine and in a shorter survival period. The polyneuritic syndrome was similar to that described by Morris (1). In addition, we observed that the mice were apt to hold their heads on one side, and that they developed complete paralysis of the hind legs. The controls maintained their weight, and they survived until they were sacrificed two weeks after the last animal in the corresponding experimental group had died.

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Mechanical Transmission of a Plant Tumor Virus to an Insect Vector¹

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Most plant viruses transmitted by leafhoppers have not been demonstrated to be infective in extracts. The virus dealt with in this paper causes tumors in certain susceptible plants, is carried by leafhoppers, and like others in that group has proven difficult to detect in extracts. It is our purpose to recount briefly the variety of methods that failed to transmit the virus and to report on the success of insect-to-insect transmission by injection.

The virus, known as wound-tumor virus, *Aureogenus magnivena* Black (2), is transmitted from plant to plant by the leafhoppers *Agalliopsis novella* (Say), *Agallia constricta* Van Duzee, and *Agallia quadripunctata* (Provancher). Attempts by the authors to transmit the virus

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mechanically from plant to plant have been made, using various methods of inoculation, both with untreated juice from infected plants and also after the addition of materials to the juice to alter the osmotic pressure, pH, and oxidation-reduction potential with the aim of stabilizing the virus. Juice has been rubbed onto the leaves and injected into the stems and crowns of plants by different techniques. Several species of susceptible plants have been used in these experiments, but an infection has never been obtained by any of these methods. The inoculation of plants with juices from infective insects also failed to produce infection, even though such juice was proved to have infective virus when tested by the successful technique described here. Leafhoppers fed on juices from diseased plants, from viruliferous insects, and on concentrates of these juices prepared by high speed centrifugation did not become infective. It is possible, of course, that more extensive trials with these procedures or variations of them might yield positive results.

Storey (5) was the first to transmit a plant virus from insect to insect by inoculating virus-free vector leafhoppers, *Cicadulina mbila* Naude, with juice from insects carrying maize-streak virus, *Fractilinea maidis* (Holmes) McKinney (3). Using fine glass capillaries, Black (1) transmitted the aster-yellows virus (*Chlorogenus callistephi* H. var. *vulgaris* H.) by injecting juice from viruliferous into nonviruliferous leafhoppers (*Macrostelus divinus* Uhler). In view of these earlier successes, it seemed logical to attempt transmission of the virus by injecting healthy vector leafhoppers with extracts from insects carrying wound-tumor virus.

Three hundred and fifty nonviruliferous leafhoppers (*Agallia constricta* Van Duzee) were caged on crimson clover plants (*Trifolium incarnatum* L.) showing pronounced symptoms of wound-tumor disease and were kept there for four weeks at about 25° C. Maramorosch had previously (4) obtained good transmission of the virus at 25° C with this vector, after a minimum incubation period in the insect of two weeks. The insects were then collected and ground at 0° C with an equal weight of 0.25 M NaCl. The suspension was centrifuged at 3500 rpm for 5 min, and the supernatant used without further dilution for the injection of 28 virus-free nymphs. The solution was drawn up into a fine glass capillary, and a small amount forced into the insect through a puncture made in the abdomen with a glass capillary. Twelve of the 16 insects which survived the injection proved to be infective when tested on crimson clover plants. Although no controls were included in this experiment, the results are considered significant because there were no accidental infections with this virus in the greenhouse during the course of this experiment or the two previous years.

A second experiment was carried out to confirm these results as well as to obtain a first approximation of the dilution end point. An extract of viruliferous insects was prepared as in the first experiment, and 0, 10⁻¹, 10⁻², 10⁻³, and 10⁻⁴ dilutions were made with 0.25 M NaCl. Groups of 20-30 insects were inoculated with each dilution, and an equal number of uninoculated insects were

kept as controls. All insects were tested individually on two sets of crimson clover plants over a period of two months, and the plants were then observed in the greenhouse for an additional period of two months. None of the control insects became infective, but some insects inoculated with each dilution of viruliferous insect extract up to and including 10⁻³ did. There was a long incubation period before inoculated insects were able to infect healthy plants with wound-tumor virus.

These experiments show that wound-tumor virus can be detected in extracts from insects, and the experiments also make possible the determination of some of the physical and chemical properties of the virus. The fact that three leafhopper-transmitted plant viruses have proved transmissible by this method of insect inoculation suggests that the method may succeed in certain instances where other techniques have failed.

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The Chromatographic Estimation of Lysine and Some Applications of the Method¹

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In exploring the various published chromatographic techniques, it occurred to us that paper chromatography of the copper salts of amino acids might provide a simple means of estimating some of the amino acids. The salts were prepared as described in our modification of the Pope and Stevens procedure for determination of the amino acid N (1) and 0.02-cc aliquots of the resulting solution partitioned on paper strips in an aqueous phenol atmosphere for 6 hr by the capillary ascent principle described by Williams and Kirby (2). After drying in air the strips were developed by painting with a freshly prepared 10% solution of aqueous iron ferrocyanide. The characteristic pink color of copper ferrocyanide, which appears distinctly on drying, indicates the position and relative amounts of various amino acids on the strip. Tests with 60 γ of purified specimens showed glutamic and aspartic acid to have overlapping R_f values in the area 0.08-0.10. Lysine occurred in the region 0.42-0.50. Arginine and histidine overlapped in the 0.55-0.62 R_f zone. The other amino acids, glycine, serine, threonine, valine, leucine, isoleucine, tyrosine, proline, hydroxypro-

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