

collected on peach trees, weeds, and wild and cultivated shrubs were confined in cheesecloth bags on branches of phony trees. After 4 or 5 days the bags with their contained insects were transferred to test trees growing in the large cage. The bags were left on these test trees for 30 or more days, unless all the insects had died earlier. At the end of the test period the bags were carefully removed in such a manner that none of the insects escaped within the large screened cage. The test trees were Elberta June-buds in their second year from seed. In order to provide fully adequate controls, alternate trees were left as checks. Transmission tests were made from April to October. During the following winter the test trees and checks in the cage were dug and replanted in isolated plots in areas where there were no commercial orchards and where phony disease was rare or unknown.

Phony disease has a long incubation period in peach trees, apparently from about 18 months (or two growing

TABLE 1

Species	Total No.	Phony peach transmissions	
		Definite	Probable
<i>H. triquetra</i> .....	203	8	8
<i>C. costalis</i> .....	64	1	2
<i>O. undata</i> .....	26	3	0
<i>G. versuta</i> .....	20	2	0

seasons) to as much as 3 years. Although a few of the trees used in the 1945 tests showed suspicious symptoms in September 1947, it was not until the spring of 1948 that reliable diagnoses could be made. At that time 7 trees were positively phony, and 8 more were almost certainly so. Of the 7 positive cases, two had been inoculated by *G. versuta*, three by *H. triquetra*, and two by *Oncometopia undata* (F.). All of the 8 trees that were almost certainly diseased had been inoculated with *H. triquetra*. None of the check trees showed any symptoms of the disease.

In contrast with the 1945 series, a number of the trees used in the 1946 experiments showed positive symptoms in July 1948. At that time seven definite cases of phony peach were noted. One of these trees had been inoculated by *C. costalis*, one by *O. undata*, and five by *H. triquetra*. At least two other trees inoculated by *C. costalis* are almost certainly diseased. All check trees appeared to be normal.

The totals for definite and probable insect transmissions of phony peach obtained in the test series of 1945 and 1946 are presented in Table 1. One check tree was provided for each test tree, a total of 313 in all. All check trees are still normal.

The definitely positive results represent from 2 to 28% successful transmission by the different species. Although all these insects are general feeders, three species are known to be associated with peach trees at certain seasons. For the fourth one, *C. costalis*, a natural association is doubtful, but the insect survives for periods of

a month or more when confined on peach. This species has thus far been the least successful of the four in transmitting the disease. All four species belong to the subfamily Tettigellinae, and it appears quite possible that other members of this group may be able to transmit phony peach even though they may be of no real importance as vectors.

In 455 experiments involving the use of the remaining 49 species of insects that were tested, all results were definitely negative.

#### Reference

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## Inhibition of the Adenosinetriphosphatase Activity of Preparations of Myosin<sup>1</sup>

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During the past year we have been investigating the inhibition of the adenosinetriphosphatase activity of the myosin complex by extracts of the posterior pituitary

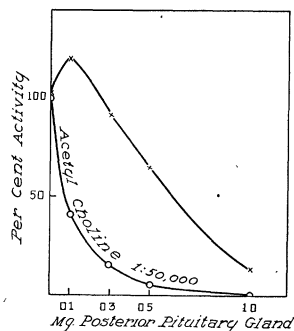


FIG. 1. ATPase activity in the presence of extract of posterior pituitary gland and acetylcholine. The percentages of the control values are plotted. Activity was determined by analysis for inorganic phosphate after 5-min incubation at 30° C in 1 M KCl buffered at pH 9.0 with 0.1 M NaHCO<sub>3</sub>-Na<sub>2</sub>CO<sub>3</sub>. One mg of CaCl<sub>2</sub>, 10 mg of ATP (sodium salt), and a solution of twice-precipitated myosin (0.2 mg of protein nitrogen) containing 0.001 M NaCN were present in a total volume of 5 ml. Acetylcholine and the soluble material of the desiccated posterior pituitary gland were added as indicated. The incubation was terminated by the addition of trichloroacetic acid to make a final concentration of 5%.

gland. Our interest in this casual observation was due to the finding that the inhibition was greatly reinforced by the addition of acetylcholine. Although a considerable mass of data has been accumulated, truly definitive experiments have not been devised, and it is considered

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desirable not to delay further the presentation of our results.

Results, typical of those we have obtained, are illustrated in Figs. 1 and 2. Twice-precipitated myosin from the rat, obtained by a procedure utilizing cyanide (1), was used in these experiments. Two methods were necessary in following the ATPase activity. For comparative studies, analysis for inorganic phosphate was sufficient, but, in order to follow rapid changes, manometric methods (2) were necessary. It is apparent from Fig. 1 that an extract of the posterior pituitary gland<sup>2</sup> is inhibitory at most concentrations but is capable of activation when

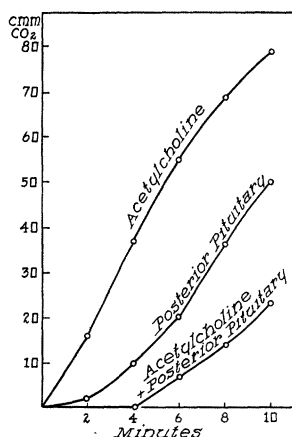


FIG. 2. Manometric measurement of inhibition of ATPase activity of myosin. The incubations were conducted in an atmosphere of nitrogen-carbon dioxide in 1 M KCl buffered with 0.1 M carbonate-bicarbonate. The initial pH (after saturation) was about 8.2; the total volume was 3.5 ml and only half the amount of myosin was used, but, otherwise, the substrates were the same as in Fig. 1. The soluble material from 1 mg of desiccated posterior pituitary gland and acetylcholine in a final concentration of 1:30,000 were used as indicated. After 30 min, the CO<sub>2</sub> production was equal in all cases, and the amounts of inorganic phosphate in the digests were identical within the range of error of the methods.

used in lower concentrations. If acetylcholine is supplied, the extract of posterior pituitary gland is inhibitory at all significant concentrations; acetylcholine was without effect when added without the extract of the posterior pituitary gland. It is possible that the inhibition with the crude extract of the posterior pituitary gland was due to a significant concentration of acetylcholine in the extracts of the gland. A factor not covered in Fig. 1 concerns the concentration of calcium; maximal inhibition was obtained with maximal activation of the enzyme by calcium ions. When the calcium level was low, little activity was observable, and the inhibition was difficult to demonstrate; when calcium was supplied in great

excess, the inhibition was greatly reduced. For example, with 1 mg of CaCl<sub>2</sub>, only 22% of the control activity (at 5 min) was observed, whereas the comparative value with 5 mg was 51%. In Fig. 2, typical data obtained by manometric methods are represented. It is seen that after a few minutes the inhibition disappeared; after this stage the ATPase activity proceeded at a normal velocity and to the same extent as the control. In studies of this release of inhibition it was found that the release is delayed by anaerobic conditions or by addition of eserine or DFP. In so far as could be determined, the release of inhibition is due to hydrolysis of acetylcholine and/or enzymatic or oxidative destruction of the factor in the extract of the posterior pituitary gland; i.e. inhibition was re-established, after release, by the addition of either extra acetylcholine or extra extract of the posterior pituitary gland in the presence of an excess of the other material. It was not possible to predict, within narrow limits, the amount of extract of posterior pituitary gland necessary to establish complete inhibition with different preparations of myosin; concentrations of acetylcholine varying from 1:10,000 to 1:100,000 were not found to differ with various preparations of myosin except in so far as the release of inhibition was concerned. Release of inhibition was delayed longer with higher concentrations of acetylcholine. In no case did we fail to obtain inhibition of the activity of myosin from rabbit or rat with the combination of posterior pituitary and acetylcholine.

It was found impossible to obtain adequate purified preparations of the known factors of the posterior pituitary gland for comparison, but certain observations have indicated a possible identity of our factor with one or more of the known principles of the posterior pituitary gland. The active material was found to dialyze through Cellophane membranes and to be inactivated by oxidation with bromine water, by treatment with 1 N NaOH for 3 hrs at room temperature, and by hydrolysis for 3 hrs at 94° with 1 N HCl. Activity was also destroyed by incubation of the extract of posterior pituitary gland with an extract of duodenum of the hog. Commercial preparations of "Pitressin" were more active than those of "Pitocin." Nervous tissue (brain of rats) was found to contain similar material, although in an apparently lower concentration than the posterior pituitary gland.

The significance of these results is difficult to assess at this time. If a relaxation mechanism for the transfer of energy to the myosin complex is assumed, the results would be grossly consistent with the known effects of acetylcholine and of the principles of the posterior pituitary gland. Studies of the physical aspects of this phenomenon have been seriously handicapped by the time factors involved, and our results do not allow for a unique interpretation as to whether or not the myosin complex is in the contracted state during the period of inhibition.

#### References

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2. SUMMERSON, W. H. Personal communication.

<sup>2</sup> Prepared from desiccated posterior pituitary glands supplied through the courtesy of Dr. Oliver Kamm, of Parke, Davis and Company. One hundred mg of the dried powder was extracted with 10 ml of saline, and the filtrate was used in the experiments reported here. The sodium salt of ATP was obtained through the courtesy of Dr. E. A. Osterberg, of Abbott Laboratories.