able to enter the intima, whereas the larger ones are rejected.

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Mango Grafting in Eight Weeks

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Mango is commercially propagated by inarching. Age of the seedlings to be inarched varies from 1½ to 2½ years, and the grafts are separated from the parent tree in about 3 months. Thus, it takes 2–3 years before a mango graft is ready for transplanting in the field. During this period, the nurseryman must take very good care of the seedlings; besides, copious watering of grafts, essential for good union, makes the method cumbersome and expensive. Further, such grafts, being on 2-year-old seedlings, have a relatively poor root system. They also do not transport well.

Inarching of mangoes on 4-week-old seedlings was, therefore, tried by the author in order to overcome the serious disadvantages mentioned. Mango stones planted in the the first week of July started germinating by the end of the month. About 30 days after germination, the seedlings attained a height of approximately 1 ft and a girth of ⅛ in.–¼ in. One hundred such seedlings were lifted from the seedbed along with stones and sprouting roots, and the soil clinging to the stones was removed. The stones were then covered with wet sphagnum moss about ½ in. in thickness, held in position by a thin string. The seedlings were taken to the parent tree and inarched with new shoots of equal thickness in early September (Fig. 1).

FIG. 1.

Complete union took place in about a month, and the grafts were detached from the mother plant by the end of September and potted. Eighty per cent success was obtained. Watering was completely withheld since the entire operation was completed in the rainy season, when the rain water absorbed by the moss furnished the required moisture. This method also obviated the necessity of lifting of stock with a ball of soil for food material, as this was supplied by the stones.

Temperature-dependent Characteristics of an Adenylpyrophosphatase Preparation from Potatoes[1]

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A need for a means of selectively hydrolyzing the acid-labile phosphate groups in ATP arose in our studies (*1*) on the turnover of labeled phosphate in the ATP present in preparations from animal tissues. Although crystalline myosin (*2*) and purified myokinase (*3*) proved useful, the time and effort involved in the preparation of these enzymes, together with the lack of stability of myosin, prompted a study of other preparations (*4*) that might be both stable and easily available. We report here on a preparation from potatoes which, in suitable dilution, possesses the desirable property that at temperatures above 7° C it catalyzes the hydrolysis of the 2 acid-labile phosphates in ATP, and at 7° or below it catalyzes the hydrolysis of only the terminal group. The preparation is quite stable and may be prepared in a period of 24 hr. One sample, saturated with toluene, maintained its activity over a period of a year. Between periods when aliquots were withdrawn for use in the analysis of ATP, the solution was stored at 2°–5°. The usefulness of our preparation in the large-scale conversion of ATP to ADP is being studied.

Kalckar (*5*) and, later, Krishnan (*6*) reported on an enzyme preparation from potatoes catalyzing the hydrolysis of the acid-labile phosphates in ATP. Kalckar (*5*) suggested that a single enzyme was involved. Meyerhof (*7*) proposed that the name apyrase be reserved for the dephosphorylating enzymes that do not distinguish between ATP and ADP. Our preparation differs sufficiently from those reported by Kalckar and Krishnan to suppose that we are dealing with a different enzyme or a mixture of enzymes.

[1] This investigation was supported by a research grant from the National Advisory Heart Council, National Institutes of Health, USPHS.

Accordingly, it is not appropriate to attempt any classification at this time.

The enzyme is prepared in the following manner. Fresh potatoes[2] are ground in a Waring Blendor at 2° with one fifth their weight of water, and the mixture is passed through cheesecloth. The filtrate is permitted to stand at 2° to allow settling of the starch and the cell debris. The colored supernatant is decanted and the pH adjusted to 3.75 with 10% acetic acid at 0°. The supernatant from the acid precipitation is obtained by centrifugation and dialyzed against distilled water at 2° until free of chloride and phosphate. The slight amount of protein that precipitates during dialysis is removed by centrifugation.

The resulting supernatant is a clear, all but colorless solution showing an activity of 7,000–11,000 units/mg protein when tested under conditions of substrate excess according to the standard test of Krishnan (*6*). The preparation catalyzes the complete hydrolysis of the 2 acid-labile phosphates in ATP and shows negligible activity toward adenosine-5-phosphate and inorganic pyrophosphate. Like the preparations of Kalckar and Krishnan, it is activated by calcium ion. The activity of our preparation toward other phosphate esters can be judged, at this time, only by the result of several trials in which it was used to estimate the ATP content of preparations of rat brain homogenate (*8*). The values calculated for the ATP content agreed with those obtained by the use of crystalline myosin and differed markedly from those obtained by the use of 10-min acid hydrolysis. It would appear from these results that the preparation does not show marked activity toward the other phosphate esters present in an active rat brain preparation.

The results of preliminary studies of the kinetics of the hydrolysis of ATP as catalyzed by our enzyme preparation are given in Fig. 1. The time-dependent

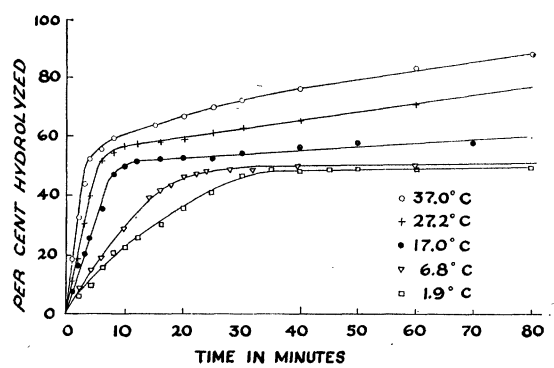

FIG. 1. The hydrolysis of the acid-labile phosphate in ATP.

course of the hydrolysis was established by determining the inorganic phosphate liberated at intervals throughout periods of 60 or 180 min, depending upon the temperature. As shown in Fig. 1, studies were conducted at 37.0°, 27.2°, 17.0°, 6.8°, and 1.9°. The procedure of Fiske and Subbarow (*9*) was used for the determination of phosphate. Each ml of the digestion mixture that was employed contained 0.5 mg calcium chloride, 0.5 ml $M/10$ succinate buffer pH 6.5, ATP equivalent to 48 μg acid-labile phosphorus, and 100 units of the enzyme. A solution of ATP and a solution containing the remaining components of the digestion mixture were equilibrated to the temperature of the bath and mixed at zero time. Aliquots were withdrawn at intervals and pipetted immediately into the acid molybdate reagent of Fiske and Subbarow. A control flask without enzyme served as a blank. A similar study, not reported in detail, was made wherein approximately equimolar amounts of purified ADP served as substrate.

The curves for the data obtained at 37.0°, 27.2°, and 17.0° show that inorganic phosphate is liberated in two steps, one much faster than the other, and indicate that the enzyme preparation is many times more active toward the terminal group (P_3) in ATP than toward the terminal group (P_2) in ADP. The data available are sufficient to afford an estimate of the respective rates. At the temperatures 37.0° and 27.2°, for example, the phosphate attributable to P_2 is liberated according to the first-order law, with little deviation except near the completion of the reaction. The constants for the hydrolysis of P_2 when ATP served as the substrate are: $K_{37} = 0.018$ min^{-1} and $K_{27} = 0.011$ min^{-1}; when ADP served as the substrate, $K_{37} = 0.017$ min^{-1} and $K_{27} = 0.012$ min^{-1}. An approximation of the constants for the hydrolysis of P_3 at the same temperatures yielded value thirty times greater than those calculated for the hydrolysis of P_2.

The curves for the data at 6.8° and 1.9° show that the hydrolysis stops sharply when 50% of the acid-labile phosphate has been made free and suggest that the liberated phosphate represents the exclusive and complete hydrolysis of P_3. Support for this suggestion is offered by the observation that, under the conditions of these experiments, several samples of the enzyme preparation showed only negligible activity toward ADP over periods of 90 min. In conformity with these observations, calculations according to the Arrhenius equation, using the constants for the hydrolysis of P_2 at 37.0°, 27.2°, and 17.0°, when either ATP (second stage of hydrolysis) or ADP served as substrate, suggest that the energy of activation increases as the temperature is lowered. Quite possibly, due to reversible inhibition (*10*) of the enzyme or other factors, very high values for the energy of activation are reached at temperatures near 7°. Studies in progress are directed toward a more complete understanding of the kinetics, the temperature characteristics, and other properties of this enzyme preparation.

The thirtyfold difference in the values for the hydrolysis constants for P_3 and P_2, in comparison to the approximately twofold differences reported by Kalckar (*5*) and Krishnan (*11*), suggest that we are not dealing with the same enzyme preparation reported by them. This difference raises the question

[2] White-skinned and red-skinned potatoes and yams, obtained from retail outlets, were used.

whether potato "apyrase" is a mixture of ATPase and ADPase, the ratio of which in any given preparation depends upon the procedure employed.

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Nudibranch Spicules Made of Amorphous Calcium Carbonate[1]

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The occurrence of amorphous calcium carbonate in nature has rarely been proved. The principal known case is that of the calcium carbonate in some arthropod exoskeletons. Mayer and Weineck (*1*) demonstrated by x-ray diffraction that the exoskeletons of *Astacus* and *Julus* contained amorphous calcium carbonate.

A second interesting case is that of the spicules in the tissues of the nudibranch mollusks. Fifty per cent of the dry tissue is made up of calcium carbonate spicules about .5 mm long. The mineralogical form of these spicules has been in dispute. Schmidt (*2*), using optical methods and specific gravity measurements, concluded that they were vaterite. But Rinné (*3*) found no x-ray diffraction pattern and concluded that the spicules were amorphous. Mayer and Weineck (*1*), on the other hand, found an x-ray diffraction pattern characteristic of vaterite. Their specimens had been preserved in 70% alcohol.

In the present study three careful attempts were made to obtain an x-ray diffraction pattern of the spicules in the dried tissue of *Archidoris*.[2] Only a faint halite pattern was obtained from the few halite crystals visibly scattered among the preponderance of calcium carbonate spicules in the dried tissues as teased under a microscope. After ashing, the x-ray diffraction powder pattern consisted of a strong calcite pattern and the same weak halite pattern. These studies are a confirmation of the presence of amorphous calcium carbonate in the spicules of the nudibranch *Archidoris*. It seems likely that the vaterite may occur as a transformation product resulting from conditions of preservation. The submicroscopic morphology of these amorphous but birefringent spicules is an unsolved colloid problem.

In the case of these spicules, as in the cases of other biological skeletons, a consideration of three levels of integration is required. Molecular patterns alone do not yield a complete description, for the arrangement of the molecular units at colloidal and microscopic levels is also a major aspect.

In their gross form the spicules of *Archidoris* resemble the calcite spicules of some octocorals and the opal spicules of some sponges. It was Schmidt (*2*) who generalized that organisms often build similar skeletons out of entirely different chemical substances.

[1] From a dissertation on The Biogeochemistry of Strontium, presented to the faculty of Yale University in partial fulfillment of requirements for the Ph.D. degree. Grateful appreciation is expressed to G. E. Hutchinson for his direction and to Horace Winchell, of the Brush Mineralogical Laboratory, Yale University, for use of x-ray facilities.

[2] Obtained by G. E. Hutchinson and H. W. Harvey from Plymouth, Eng.

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The Heparinoid Nature of a Serum Mucoprotein[1]

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A possible role of the serum mucoprotein fraction in blood coagulation mechanisms was suggested by its acidic properties and high polysaccharide content—characteristics common to heparin and to synthetic sulfonated polysaccharide esters (*1*) with anticoagulant activity. Increase in the polysaccharide/protein ratio and reduction of the protein component within

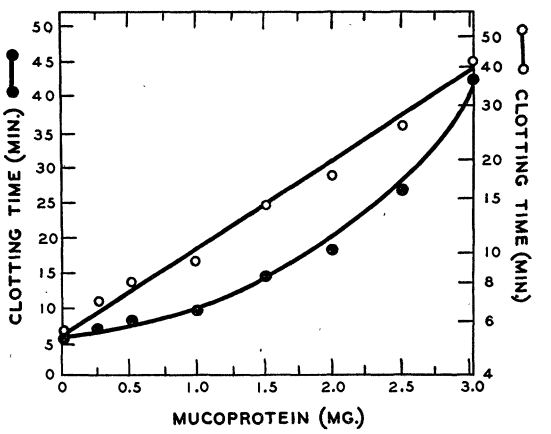

FIG. 1. Influence of mucoprotein concentration on the whole blood coagulation time (Lee-White) of 0.9 ml of fresh human blood. Fresh blood was added to 0.1-ml aliquots of an ox mucoprotein solution prepared in *M*/5 phosphate buffer (pH 7.4).

[1] A preliminary report. These studies were initially presented at the Conference on Folic Acid Antagonists in Neoplasia, March 11, 1951, The Children's Hospital, Boston, Mass.

[2] With the technical assistance of J. Dolores Johnson.