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

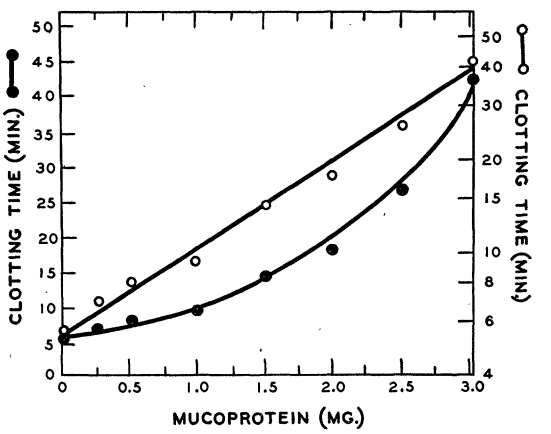

FIG. 1. Influence of mucoprotein concentration on the whole blood coagulation time (Lee-White) of 0.9 ml of fresh human blood. Fresh blood was added to 0.1-ml aliquots of an ox mucoprotein solution prepared in *M*/5 phosphate buffer (pH 7.4).

[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.

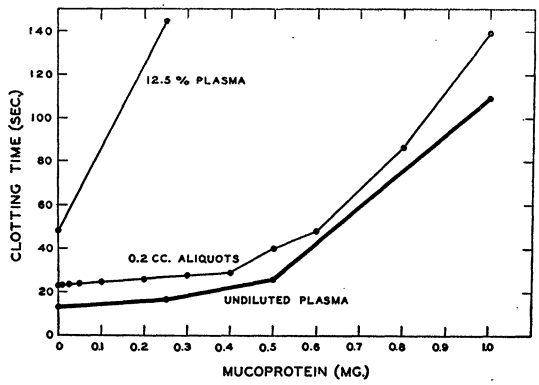

FIG. 2. Relationship of mucoprotein concentrations to the clotting time of recalcified normal human plasma. The wide lower line shows the results of addition of 0.1-ml aliquots of ox mucoprotein in *M*/5 phosphate buffer to 0.1 ml of human plasma clotted by the Quick method. The clotting times observed with 0.2-ml aliquots of mucoprotein, plasma, and thromboplastin, as well as the effect of 0.1-ml aliquots of mucoprotein on the clotting of 12.5% plasma (Shapiro-Link method), are indicated by the thin lines above.

the serum mucoprotein fraction were recently observed (*2*) in patients with either uncomplicated hepatic insufficiency (hepatitis, portal cirrhosis) or diseases characterized by abnormal globulin formation (multiple myeloma, kala-azar, etc.).

Mucoproteins were isolated from dog, ox, and human serum by two methods: (*a*) ammonium sulphate (*3*) precipitation followed by dialysis; (*b*) precipitation (*3*) with phosphotungstic acid. Both methods employ precipitation with perchloric acid for the initial exclusion of the serum albumins and globulins. Mucoprotein fractions isolated by method (*a*) showed no anticoagulant activity. A clot-decelerating action on whole blood (Fig. 1) and recalcified plasma (Fig. 2) was observed with buffered solutions of mucoproteins obtained from the three species by method (*b*). Precipitation of pepsin, protamine, or human serum albumin with phosphotungstic acid did not yield proteins with anticoagulant activity, although similar treatment of several polysaccharide-rich globulin fractions (IV-4, IV-6, and IV-7 of Cohn) resulted in complexes with a clot-retarding activity approaching that of the mucoprotein.

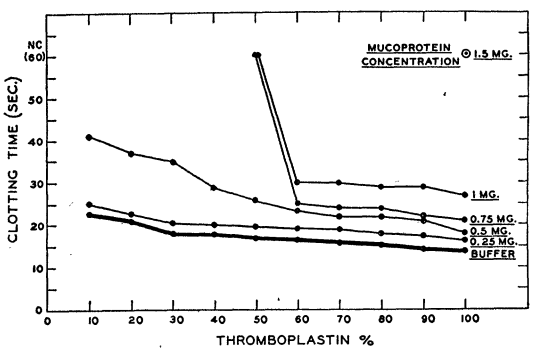

FIG. 3. Effect of mucoprotein on the activity of thromboplastin. Illustrated are the clotting times of plasmas in which 0.1-ml aliquots of various concentrations of thromboplastin (Difco) were added to 0.1-ml samples of an ox mucoprotein prior to recalcification. The results with the buffer control (*M*/10 barbital) are shown in the lower wide line; the increasing concentrations of mucoprotein from 0.25 to 1.5 ml are shown in ascending order. Incubation of mucoprotein with the thromboplastin for 5 min according to Tocantins' method showed no evidence of a direct antithromboplastic action. N. C. (60) = no clot at 60 sec.

Optimum clot-deceleration of mucoprotein solutions occurred at pH range 6.2 to 7.5 in phosphate, barbital, borate, or imidazole buffers. Solutions were stable on standing for 24 hr and were not inactivated by heat of 65° C for 20 min. The approximate anticoagulant activity of several mucoproteins on whole blood was 1/50 to 1/150 that obtainable with a commercially available heparin (Abbott). The activity of isolated thromboplastin (*4*), thrombin (*5*), or fibrinogen (*5*) was not inhibited by direct addition of mucoprotein. However, the clot-decelerating effect of mucoprotein on plasma could be easily prevented by the addition of an excess of either thromboplastin (Fig. 3) or, to a lesser extent, of thrombin. Mucoproteins produced a metachromatic reaction with toluidine blue. Complex-formation or binding of an ox mucoprotein by protamine solutions blocked the deceleration of whole blood clotting time (Fig. 4) and of pro-

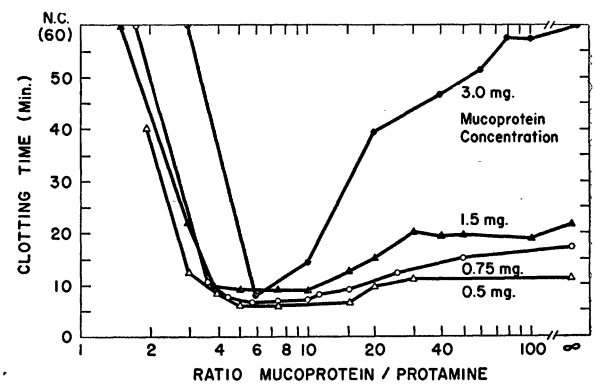

FIG. 4. Mucoprotein-protamine titrations on the clotting time of fresh whole blood; 0.1-ml aliquots of protamine (Lilly) and an ox mucoprotein (*M*/10 barbital buffer, pH 7.4) were placed in Lee-White clotting tube. After 5 min incubation, at 28° C, 1 ml of fresh human blood was added. The timing was started when blood was added to the first tube. N. C. (60) = no clot at 60 min.

thrombin time. The optimal ratio for binding was 4 to 10 parts of mucoprotein to 1 part of protamine (Lilly). The addition of 1.5 mg of mucoprotein increased the protamine titration (*6,7*), performed by the Allen (*8*) technique, in a representative experiment, from 80 to 120 μg. A definite relationship between protamine binding and the mucoprotein levels of sera from normal or abnormal subjects could not be established by means of either the protamine titration (Allen) on heparinized whole blood, or the binding capacity of fresh unheparinized whole blood (measured by the excess of protamine, which, in the uncombined state, inhibits clot formation). However, all sera in which increased protamine binding was observed also manifested an elevated mucoprotein level. Since the serum mucoprotein fraction obtained by method (*b*) combines with protamine or toluidine

blue, fails to influence certain isolated components of the clotting mechanism, and acts principally on the first phase of coagulation, its relatively weak anticoagulant action may be termed "heparinoid" in nature. The relationship of various methods of isolation to anticoagulant property will be reported elsewhere (*9*). No correlation of the serum mucoprotein level with certain hemorrhagic syndromes reported to be associated with the presence of heparin-like substances (*10, 11*) in the serum has thus far been observed.

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The Percutaneous Absorption of Water Vapor[1]

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A recent paper by Szczesniak, Sherman, and Harris (*1*) described an experiment which demonstrated the percutaneous absorption of water by rats immersed in 40% deuterium oxide solution. This prompts us to report some observations we have made on the percutaneous absorption of water vapor, using tritium oxide as a tracer. Although there have been many studies and some controversy over the percutaneous absorption of water from the liquid state (reviewed by Szczesniak *et al.* [*1*]), there has not, to our knowledge, been any attempt to demonstrate such absorption of water vapor.

Our experimental procedure involved the exposure of a shaved area of the abdominal skin of 300- to 400-g rats to an atmosphere of tritium-labeled water vapor. The animals were under Nembutal anesthesia, and exposures were in most cases of 1 hr duration.

The exposure chamber consisted of the female member of a glass ball-and-socket type ground joint with a cross-sectional area at the open end of 6.6 cm^2. A small cup to hold the aqueous solution of tritium oxide was suspended in the chamber, and the open end of the exposure chamber was held against the rat skin by slight pressure. In some of the initial exposures the chamber was sealed to the skin with collodion, but later this precaution was found to be unnecessary.

[1] Based on work performed under Contract No. W-31-109-Eng-52 for the Atomic Energy Commission.

[2] Thanks are due R. C. Thompson for his cooperation and suggestions, and R. C. Thorburn for radioanalysis of the many samples required.

TABLE 1

Percutaneous Absorption of Water Vapor by the Rat from a Saturated Atmosphere at 30° C

Method of sampling	Total No. exposures	μg water/cm^2 skin/min
Immediate blood sample	66	2.5 ± 1.3
24-hr blood sample	23	4.2 ± 1.2
Total body water sample	49	3.0 ± 1.5

The liquid in the cup does not come in contact with the skin but serves as a reservoir for the maintenance of an atmosphere saturated with water vapor.

The amount of water entering the animal was determined by radiometric analysis for tritium in either: (*a*) a blood sample withdrawn from the heart immediately following exposures, (*b*) a blood sample taken approximately 18–24 hr after the exposure, or (*c*) a sample of the body water obtained by azeotropic distillation of the ground animal with benzene. Analytical results obtained on the 24-hr blood samples were corrected for the biological half-life of tritium oxide in the rat, which time has been determined to be approximately 3 days. Details of the procedure used in the radioanalysis of samples and in determination of the biological half-life will be reported elsewhere.

About two thirds of the data were obtained from single exposures. The rest were obtained from experiments in which animals were exposed repeatedly at 24-hr intervals, the amount absorbed in a single exposure being determined by the difference in activity between pre-exposure and post-exposure blood samples. The variation between different exposures of a given animal was as large as that between exposures of different animals. The statistically summarized results are given in Table 1. It is obvious that complete equilibration of the absorbed water vapor with the body water did not occur until after the immediate post-exposure blood sample was taken. It has been shown previously that subcutaneously injected water in the human does not become equilibrated with the body fluids for a matter of several hours (*2*).

Considering the absorption/cm^2/min to be 4 μg, and taking 500 cm^2 as the total surface area of a 350-g rat (*3*), then 2,000 μg of water is absorbed by the rat per minute from an atmosphere saturated with water vapor. This is an interesting quantity compared with the water intake via the lungs which, assuming an air intake of 60 ml/min, would absorb 2,000 μg/min from the same atmosphere. The calculations for all data involving micrograms of water penetrating the skin were made with the assumption that tritium acts like hydrogen, even though this is probably not strictly true.

The effect of varying water vapor pressure and the extent of absorption in other animals are being investigated and will be reported in detail later.

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