ions. These ions include Sr, Pb, Ba, Ca, Cs, K, Na, NH_4, Ag, and Ce. Among them are several elements of interest with respect to radioactive wastes and fallout. The alunite type of structure is suited to the housing of relatively large cations, and it is primarily this factor that accounts for the observed preferential substitution of Sr for Ca in crandallite over coexisting Ca minerals, such as calcite and apatite, in which the cation coordination number is smaller.

Crandallite readily exchanges Ca for dissolved Sr. Since the 12-coordinated cation sites are not interconnected, as are the sites in cation-exchangers of the channeled zeolitic and layered phyllosilicate types, the exchange is limited to surface positions. For example, a freshly prepared gel of synthetic crandallite, a gel coarsened to microscopic crystals by hydrothermal treatment at 250°C, and crude Senegal crandallite rock crushed to pass 200 mesh were placed in 0.02M solutions of strontium nitrate. The amounts of solution and of solid phase were adjusted so that each contained the same number of Ca and Sr ions. In neutral and weakly alkaline solutions the percentages of exchange between Ca and Sr in the three preparations were roughly 31, 18, and 4 percent, respectively. Below *p*H 6.5 the amount of exchange decreases markedly. Natural crude Senegal crandallite rock was found to have an appreciable acidity-reducing capacity, presumably due to admixed basic aluminum phosphates and silicates. The reverse exchange, that of Ca for Sr in crystalline $SrAl_3(PO_4)_2(OH)_5H_2O$, was found to be quite small, in line with the relative solubilities and ionic adsorptive powers of Ca and Sr in general.

The mineral apatite, $Ca_5(PO_4)_3(OH)$, also takes up Sr in solid solution and has an appreciable adsorptive capacity for Sr in neutral solutions. The known reserves of natural phosphate rock (apatite) have been estimated at upwards of 40 billion tons suitable for conventional commercial applications. Apatite, however, is soluble in alkaline and carbonated ground waters and, unlike crandallite, is removed from the soil profile. Admixture of this mineral with crandallite as a top dressing might be useful both in controlling *p*H and in transiently affording ordinary Sr to the ground water. Natural phosphate rock contains roughly 0.01 to 0.20 per cent SrO by weight. The Sr and the Ca afforded by dissolution may act as a diluent for Sr^{90} available to plant life either directly from the ground water or by biochemical decomposition of the scavenger mineral.

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Rate of Swelling of Collagen

Abstract. The osmotic pressure gradient across the collagen solid matrix-solution interface forces the solid to imbibe fluid and thereby increases its volume. Concurrently, the decrease in entropy of the solid matrix produces a force which tends to resist any further increase in volume. The instantaneous velocity at which the swelling takes place is related to the difference between the osmotic and entropic forces. Finally, an explicit relationship is obtained between volume and time.

Swelling is an important property of polymers and is an outstanding characteristic of collagen. The two types which can be distinguished are (i) osmotic and (ii) lyotropic (bond scission). In type i the matrix imbibes fluid due to an osmotic pressure gradient across its solid-solvent interface; the restraining pressure of the matrix finally equals the osmotic pressure so that an equilibrium is attained. Certain fluids react with the matrix so as to loosen bonds (type ii); the swelling then proceeds to a greater extent because the restraining pressure of the matrix is reduced.

In a polyelectrolyte such as collagen, the equilibrium swelling volume depends upon the *p*H of the solvent (*1*). A minimum of swelling occurs near the isoelectric point, whereas pronounced swelling develops in the acid and alkaline *p*H regions. Acetic acid, particularly, is effective in both the solubilization and swelling of collagen.

Studies of types i and ii have been done particularly from measurements based on equilibrium swelling. Little attention has been given to the rate of the swelling process. The purpose of this report is to describe a rate mechanism of the swelling process which embodies both osmotic and lyotropic attributes of the system, collagen–acetic acid–water.

The isoelectric point of limed hide powder collagen is about *p*H 5.5. When this is placed in water, hydrogen ions leave the matrix to a certain extent and dissolve in the interstitial water phase. The matrix becomes an anion and its counter-ion is trapped inside. When it is placed in dilute acetic acid at *p*H 2 to 3, hydrogen ions are forced on to the matrix which thereby becomes a cation; its counter-ion again is trapped inside. This description of type i may be formulated to give the pressure inside the matrix which produces swelling—that is,

$$\pi = nRT/V = A/V,$$

where π is the osmotic pressure of the interstitial fluid phase of volume V for n moles of ions at a temperature T. Since the gas constant (R), n, and T are independent of the volume they may be combined to form A. The increased volume of the matrix forces random chains of collagen to become more uniformly aligned, with a subsequent decrease in their entropy. This reduction in entropy causes an increase in the matrix pressure which resists further volume increases. The retarding pressure of the matrix may be considered to be linearly related to its volume—that is, $P = \alpha V$, where α is a constant characteristic of the matrix restraining ability. The rate of change of volume with time can now be stated as being proportional to the difference in osmotic pressure π and the restraining pressure P in Eq. 1.

$$dV/dt = k(\pi - P) = k(A/V - \alpha V) \quad (1)$$

At swelling equilibrium Eq. 1 equals zero. Thus, Eq. 2 shows the dependence

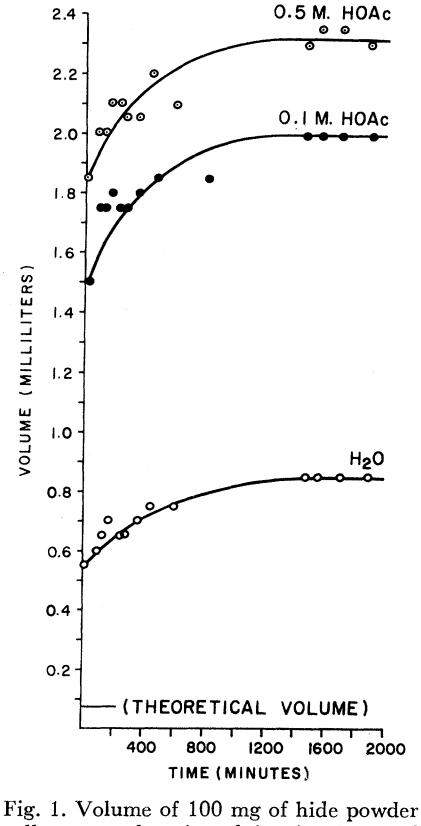

Fig. 1. Volume of 100 mg of hide powder collagen as a function of time in water and in 0.1 and 0.5*M* acetic acid at temperature 23° to 25°C.

Table 1. Values of constants.

Constant	Water	Acetic acid	
		0.1M	0.5M
V_0^2	0.29	2.17	3.30
V_∞^2	0.72	4.00	5.42
$2\alpha k$	0.0018	0.0025	0.0025

of the equilibrium volume V_∞ on the parameters A and α.

$$V_\infty = (A/\alpha)^{1/2} \quad (2)$$

When Eq. 1 is integrated and solved explicitly for V^2, Eq. 3 is obtained.

$$V^2 = V_\infty^2 - (V_\infty^2 - V_0^2)e^{-2\alpha kt} \quad (3)$$

If measurements of V^2 are made at sufficiently short times so that $e^{-2\alpha kt}$ can be approximated by $(1 - 2\alpha kt)$, then Eq. 4 is formed.

$$V^2 = V_0^2 + (V_\infty^2 - V_0^2)2\alpha kt \quad (4)$$

The following is an application of this theory to the swelling data of collagen in water and dilute acetic acid (0.1 to 0.5M).

One hundred milligram samples of hide powder collagen (*2*) were placed in 15-ml graduated centrifuge tubes. Then, 10 ml of water or dilute acetic acid was added. The tube was shaken for 2 to 3 minutes and spun at 3000 rev/min for 10 minutes to pack the swollen fibers. The volume of the sediment was then read to the nearest 0.1 ml, and the mixture was shaken again for 2 to 3 minutes. It was allowed to settle until the next volume was read. This process was repeated at 30-minute intervals during the initial stages of swelling but later at longer intervals. Water and aqueous solutions of acetic acid (0.1 to 0.5M) were used to swell collagen at temperatures of 23° to 25°C. The force and time at which the samples were centrifuged determines the volume of the sediment. However, both of these factors were constant throughout the study.

In Fig. 1 the volume of collagen is shown as a function of time for swelling in water and in 0.1 and 0.5M acetic acid at 23° to 25°C. The experimental data were plotted according to Eqs. 4 and 5 and were in conformity with the theoretical requirements except at zero time. The latter points always deviated from theoretical. The interpretation is given that collagen swells in two steps. Initially, the fibers imbibe solvent at a rapid rate until they reach the volume V_0. Thereafter, they swell from V_0 to V_∞ by the proposed osmotic mechanism. Table 1 shows the values of the constants determined for the swelling of collagen in water and dilute acetic acid. The influence of solvent on V_∞ is probably due to a reduction in α by the more active acetic acid. Since $2\alpha k$ showed only slight alteration with solvent, then, the specific rate constant k must have been elevated in acetic acid in comparison to water. V_0 presumably is also a measure of collagen solvation and its swelling by a particular solvent. In going from water to 0.5M acetic acid, the greater V_0 could be due to a discharge of carboxylate-basic salt-links. The lower pH of the acetic acid solutions would free the ionic attraction of chains previously bound by such bridges.

An analysis of the sorption of water by rubber (*3*) similarly was based on the osmotic pressure gradient of a solvent in the matrix as presented by Testor. If V_∞ were considerably greater than V_0, the rubber swelling equation would be identical with that for collagen. It appears then that processes other than Fick diffusion mechanisms determine the swelling and sorption in some types of polymers.

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Measurement of Precipitin Reactions in the Millimicrogram Protein-Nitrogen Range

Abstract. Exploitation of newer instrumentation and a dye-binding method for protein measurement made possible reduction in volume down to 1/1000 that commonly used, with no greater error. The procedure was tested at two levels, 1- and 10-μl volumes, with human gamma globulin and rabbit antiserum. Of the dyes tested, bromsulfalein proved best for the protein estimation.

The value of a method which would require a few drops to less than one drop of blood for replicate measurements of the precipitin reaction, particularly in pediatric practice and for studies with small laboratory animals, is obvious. Such a method should also be applicable to the microsamples involved in certain quantitative histo- and cytochemical studies. Exploitation of newer instrumentation (*1*) and a dye-binding method for determination of protein in millimicrogram amounts (*2*) made it feasible to modify and scale down the commonly used procedure, involving the so-called "micro" Kjeldahl analysis of the antigen-antibody precipitate (*3*), to the level required. With the method herein described, interaction of 10 μl of antigen solution with 10 μl of antiserum provides over 1 ml of colored solution for final measurement. Since this measurement can be carried out on less than 10 μl [for example, with the Beckman DU spectrophotometer adapted to microcuvettes (*1*)], it should be possible to use volumes of antigen and antiserum down to 0.1 μl, although in this study volumes down to only 1 μl were used. A comparison of four acid dyes previously employed for protein measurement or dye-protein binding studies (*2, 4*) was included in this investigation (*5*).

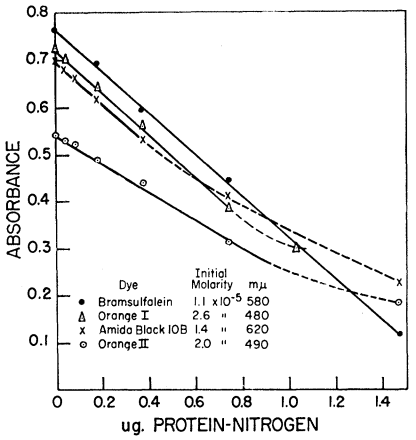

Fig. 1. Dye-binding of bovine serum albumin in 1 mg per 100 ml of bromsulfalein (Hynson, Westcott, and Dunning) and other dyes (Hartman-Leddon), at pH 2.

For the dye preparations used, maximal absorbance was given at the wavelengths shown in Fig. 1, and maximal binding to bovine serum albumin (Armour, fraction V) was obtained at pH 2 in 0.1M citric acid-sec phosphate buffer. Readily available albumin was used, since the dye-binding is comparatively nonspecific. The data in Fig. 1 were obtained by mixing 50 μl of buffered dye solution (12 mg/100 ml) with 20 μl of albumin solution and letting the mixture stand for 15 minutes, centrifuging it at 3000g for 5 minutes, adding 60 μl of the clear supernatant to 0.5 ml of distilled water, and measuring the absorbance. A blank was prepared by replacing the albumin solution with water. The dye concentrations listed in Fig. 1 are those of the blank solutions. Commercial dye preparations were used without further purification, since this sufficed for the practical purposes at hand. Dye-binding was linear at low albumin concentrations in all cases, but only bromsulfalein maintained linearity over the whole range tested; in fact, under the conditions of tissue analysis, its linearity extends to 2.25 μg of protein-nitrogen, with a reduced slope (*2*). Although both Amido