

# Effects of Ionizing Radiation on Two Gelatin Fractions II

## Studies on Viscosity, Sedimentation Velocity, and Molecular Weight Effects

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The effects of subdenaturant levels of irradiation on two fractions, F-I and F-II, isolated from commercial pigskin gelatin, were investigated through viscosity and sedimentation studies in 2*M* potassium thiocyanate solutions. The source of irradiation was a 3 Mev Van de Graaff accelerator. When used as controls, F-I and F-II were found to have intrinsic viscosity values of 0.675 and 0.550, respectively, while their respective average molecular weights were determined to be 173,000 and 86,000. After subjection to variable irradiation dose, the log of intrinsic viscosity values for F-I were directly proportional to the log of absorbed dose while an inverse proportionality occurred with F-II. Determination of Svedberg constants showed a similar pattern prevailed in the respective fractions when observed under sedimentation velocity conditions. Data obtained indicate random coil configuration was substantially undisturbed as radiation was applied, but bond rupture and linkage processes did occur.

IN OUR PREVIOUS PAPER (1) we described the isolation of the two fractions F-I and F-II from commercial pigskin gelatin by alcohol precipitation. We also discussed their acid/base titration behavior and the technique applied in irradiating these fractions in the form of 1% solutions, 5% gels, and dry films under the electron beam of a 3 Mev Van de Graaff Accelerator. We now report the results obtained in viscosity studies on both fractions before and after irradiation, their behavior under sedimentation velocity conditions, and the molecular weight determinations based on these data.

Viscosity measurements constitute a critical parameter in the evaluation of higher molecular weight polymers because of relationships to molecular weight, sedimentation rate, diffusion constant, and osmotic pressure.

The viscosity of a polymer solution (of which gelatin is a species) is a concentration-dependent factor. Huggins (2) established that the variation of viscosity with concentration of high polymer solutions can be expressed by

$$\frac{\eta_{sp}}{c} = [\eta] + k'[\eta]^2c \quad (\text{Eq. 1})$$

where  $\eta_{sp}/c$  is the reduced viscosity,  $[\eta]$  is referred to as the intrinsic viscosity and is defined as

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$$\lim \left( \frac{\eta_{sp}}{c} \right) \text{ as } c \rightarrow 0$$

and  $k'$  is a polymer-solvent factor (also known as the Huggins constant or the hydrodynamic interaction constant).

The shape of the gelatin molecule in solution is generally accepted as being random coil. Boedtker and Doty (3) have so asserted on the basis of light scattering experiments. Williams, Saunders, and Cicirelli (4) established that the sedimentation rate constant for proteins in solution is proportional to the square root of the molecular weight; they concluded that gelatin molecules in solution exist in the form of a random coil. The equation of Staudinger in logarithm form appears to express best the relationship between intrinsic viscosity and molecular weight

$$\log [\eta] = \log K + \alpha \log M \quad (\text{Eq. 2})$$

where  $K$  is a solvent constant independent of molecular weight and  $\alpha$  has a value dependent upon the size and shape of the molecule in question. For random coil gelatin Williams found the  $\pm \alpha$  value to be 0.62.

Pouradier and Venet (5), working with homogeneous fractions of Eastman Kodak F-74 gelatin (isoelectric point 4.75 and an average molecular weight 65,000), calculated  $\alpha$  and  $K$  values through Eq. 2. Their values of 9.885 and  $1.66 \times 10^{-5}$  were for  $\alpha$  and  $K$ , respectively.

The variable response of proteins to irradiation can be shown by their behavior under sedimentation velocity conditions. When considered together with intrinsic viscosity data, this information is useful in determining changes in molecular

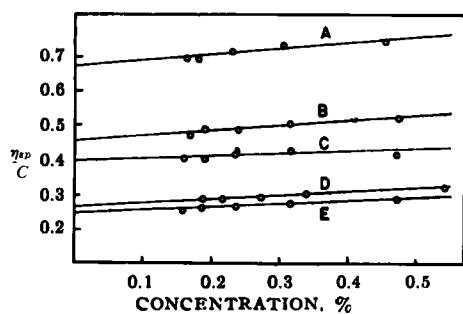


Fig. 1.—Plot of reduced viscosity vs. concentration to obtain intrinsic viscosity values for F-I before and after aqueous solutions were irradiated. One per cent solutions were irradiated ( $B = 0.17$  Mrads.;  $C = 0.44$  Mrads.;  $D = 1.27$  Mrads.,  $E = 2.80$  Mrads.) and used as control ( $A$ ). Viscosities were determined in  $2 M$  KCNS solvent.

weights. That such changes do occur has been shown by Alexander, *et al.* (6), who found that irradiation damage on solid crystalline bovine serum albumin (2 Mev electron beam) containing 4 to 6 per cent water (in the absence of oxygen) occurs in three stages. Primary ionization changes the shape of the molecule (as reflected in changes in sedimentation behavior) at doses insufficient to cause appreciable shifts in weight average molecular weights. This is the result of a breakdown of many secondary valency bonds, brought about by a single event which occurs on the average every 45 ev. A second ionization event opens the molecule still further so that half the disulfide bonds are accessible. Further irradiation causes extensive aggregation by intermolecular hydrogen bonds.

### EXPERIMENTAL

**Viscosity Studies.**—Although considerable work has been reported on viscosity measurements of gelatin solutions, diverse opinion exists as to optimal solvent systems, pH, and temperature conditions. For this reason, preliminary runs were made under variable conditions to determine those most suitable for characterizing properly the particular fractions described here.

Water at  $25^\circ$  and  $50^\circ$  and sodium acetate-acetic acid buffer pH 4.8 at  $25^\circ$  and  $50^\circ$  gave unsatisfactory curve responses with respect to linearity and reproducibility. Sodium chloride  $0.15 M$  at  $25^\circ$  and  $50^\circ$  gave positive straight-line plots with F-I and F-II but was not adopted as the experimental solvent because of its inability to inhibit aggregation (3) and to bring about reproducibility.

Straight-line plots of  $\eta_{sp}/c$  as a function of concentration were obtained with F-I and F-II in  $2 M$  KCNS adjusted to pH 10.4. Viscosity determinations on irradiated 5% gels and dry films of F-I and F-II, after subsequent dilution to flowable 1% solutions, were conducted by pipetting a 5-ml. volume of the gelatin solution slowly into an Ubbelohde viscometer immersed in a water bath maintained at  $25^\circ \pm 0.02^\circ$ . An equal volume of  $4 M$  KCNS was

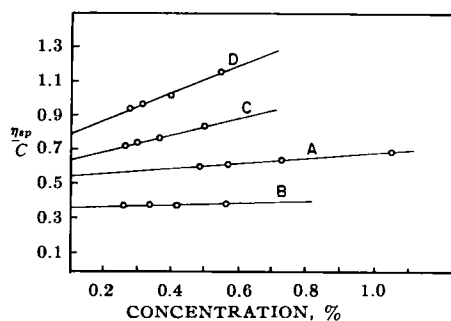


Fig. 2.—Plot of reduced viscosity vs. concentration to obtain intrinsic viscosity values for F-II before and after aqueous solutions were irradiated. One per cent solutions were irradiated ( $B = 0.16$  Mrads.;  $C = 0.47$  Mrads.;  $D = 1.19$  Mrads.) and used as control ( $A$ ). Viscosities were determined in  $2 M$  KCNS solvent.

TABLE I.—INTRINSIC VISCOSITY VALUES FOR CONTROL AND IRRADIATED FRACTIONS I AND II

F-I		F-II	
Dose, Mrads.	$[\eta]$	Dose, Mrads.	$[\eta]$
Control	0.675	Control	0.550
0.17	0.460	0.16	0.370
0.44	0.400	0.47	0.650
1.27	0.275	1.19	0.800
2.80	0.250	....	....

then added to the viscometer and (after gentle mixing) was allowed to come to temperature equilibrium. Subsequent dilutions were made with 5-ml. volumes of  $2 M$  KCNS to maintain ionic strength constancy. Solvent flow time was 89.3 seconds. A sufficient number of readings were taken at each dilution level to assure reproducibility within  $\pm 0.05$  second. A minimum of four dilutions followed the initial reading at 0.5% concentration. Solutions used in viscosity experiments were passed through a Seitz filter before irradiation.

Reduced viscosity values plotted as a function of concentration for F-I and F-II are shown in Figs. 1 and 2. Intrinsic viscosity data are given in Table I. Log dose vs. log  $[\eta]$  relationships were linear for both fractions; proportionality was inverse in the case of F-I and direct in the case of F-II.

The extrapolated  $[\eta]$  value 0.675 obtained for F-I control corresponds closely to the 0.690 reported by Gouinlock, *et al.* (7), for their F-3 fraction, despite a  $5^\circ$  difference in temperature ( $30^\circ$ ) and half the molar concentration (1  $M$ ) of aqueous KCNS solvent. Pouradier's (5) value of 0.520 for his fraction Z1 (characterized as heterogeneous) is in reasonable agreement with our F-II, 0.550. The 0.640 value for his 5111 fraction is proximate to our F-I, 0.675.

**Sedimentation Velocity Studies.**—Rates of sedimentation of F-I and F-II in the form of 1% solutions as controls and irradiated in an aqueous environment were observed in the Spinco model E ultracentrifuge. Single and double sector cells were used on controls. Double sector cells were used exclusively on all irradiated samples. Sedimentation temperature was maintained at  $25^\circ$ , then corrected to  $20^\circ$ . In the case of controls run singly, instrument speed was set at 59,780 r.p.m. Double sector runs in all cases (except F-II irradiated at approximately

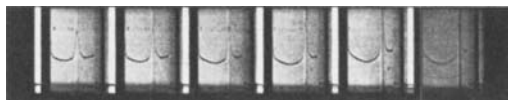


Fig. 3.—Sedimentation velocity run of 1% F-I in 2 *M* KCNS at 25°. Sedimentation boundary in a single sector cell was observed from right to left at 16-minute intervals using rotor speed of 59,780 r.p.m.

0.4 and 1.0 Megarad levels) were made at 39,460 r.p.m. Speeds in the excepted runs were reduced to 31,410 r.p.m. and 14,290–12,590 r.p.m., respectively, because of rapid boundary movement.

Figure 3 illustrates a sedimentation velocity run on F-I at 1% concentration in a single sector cell conducted at 59,780 r.p.m. at 25° in 2 *M* KCNS solvent. The sedimentation boundary was observed from right to left at 16-minute intervals. A similar peak was obtained at 0.7% concentration.

Single peaks were observed with F-I in double sector cells at 0.5% and 0.25% concentrations, at 39,460 r.p.m. observed at 32-minute intervals; single peaks with F-II at 0.9% and 0.45% concentrations, at 39,460 r.p.m. were observed at 16-minute intervals. Single peak characteristics prevailed in both fractions under all levels of irradiation employed.

Since irradiation was conducted on an aqueous system, sufficient quantities of potassium thiocyanate were subsequently added to each sample to bring the salt concentration to 2 *M* for sedimentation studies.

Sedimenting boundaries were analyzed with the aid of the Gaertner microcomparator. Plots of  $\log x$  (where  $x$  is in cm. from axis of rotation) vs.  $t$  (in minutes) were constructed and extrapolated to zero time from which the uncorrected sedimentation coefficient was obtained through

$$S = \frac{dx/dt}{\omega^2 x} \quad (\text{Eq. 3})$$

Corrections of  $S$  to 20° were made through the relationship

$$S_{20w} = S_{obs} \left( \frac{\eta_t}{\eta_{20}} \right) \left( \frac{\eta}{\eta_0} \right) \left( \frac{1 - \bar{V} \rho_{20w}}{1 - \bar{V} \rho_t} \right) \quad (\text{Eq. 4})$$

where  $\eta_t$  is the viscosity of H<sub>2</sub>O at 25° (8.949 × 10<sup>-3</sup> poise);  $\eta_{20}$  is the viscosity of H<sub>2</sub>O at 20° (10.087 × 10<sup>-3</sup> poise);  $\eta/\eta_0$  is the factor for relative viscosity of 2 *M* KCNS to that of H<sub>2</sub>O (0.915 × 10<sup>-2</sup>);  $\bar{V}$  is the partial specific volume of gelatin (0.695);  $\rho_{20w}$  is the density of the solvent (2 *M* KCNS) at 25° (1.090 Gm./ml.).

The  $\rho_t$  value was determined by experiment. Viscosity and density data for water are given by Svedberg (8). The specific volume factor and the  $\eta/\eta_0$  relationship are those of Boedtker and Doty (9).

Plots of  $1/S_{20w}$  vs. concentration were extrapolated to zero concentration and the reciprocal of the intercept taken as the coefficient value  $S^0$  (Svedberg units). Results are shown in Table II and Figs. 4 and 5.

**Molecular Weight Determinations.**—Since the energy levels of irradiation imparted to F-I and F-II were designedly of low order, the extent of denaturation occurring in the irradiated solutions was small, and it was assumed that the random coil configura-

TABLE II.—EXPERIMENTALLY DETERMINED SVEDBERG CONSTANTS ( $S^0$ ) FOR F-I AND F-II AFTER IRRADIATION

F-I		F-II	
Sample Dose, Mrads.	$S^0$	Sample Dose, Mrads.	$S^0$
Control	4.25	Control	2.85
0.17	9.52	0.16	4.49
0.44	8.33	0.47	14.28
1.27	5.88	1.19	22.22
2.80	5.50		

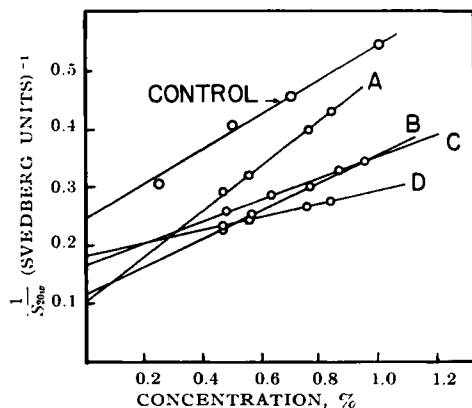


Fig. 4.—Determination of Svedberg constants by plotting the reciprocal of the corrected sedimentation coefficient of irradiated 1% aqueous solutions of F-I as a function of dilution in 2 *M* KCNS solvent. The doses imparted were: A = 0.17 Mrads., B = 0.44 Mrads., C = 1.27 Mrads., and D = 2.80 Mrads.

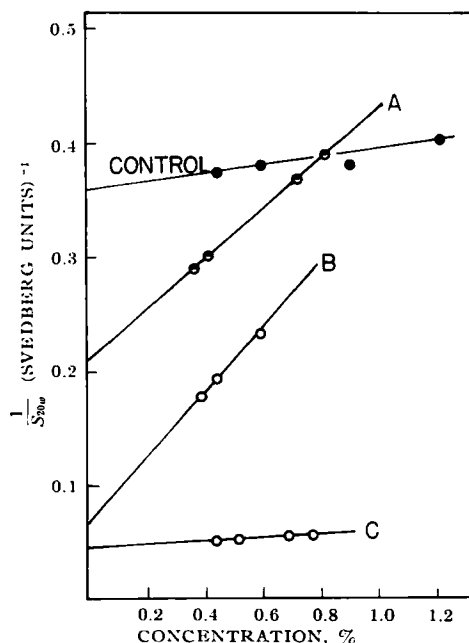


Fig. 5.—Determination of Svedberg constants by plotting the reciprocal of the corrected sedimentation coefficient of irradiated 1% aqueous solutions of F-II as a function of dilution in 2 *M* KCNS solvent. The doses imparted were: A = 0.16 Mrads., B = 0.47 Mrads., and C = 1.19 Mrads.

TABLE III.—MOLECULAR WEIGHT DATA AND  $\alpha$  VALUES AS FUNCTIONS OF RADIATION DOSE

F-I		
Sample, Mrads.	Molecular Weight	$\alpha^a$
Control	173,000	0.64
0.17	479,500	0.56
0.44	365,500	0.56
1.27	180,000	0.56
2.80	150,000	0.56
F-II		
Sample, Mrads.	Molecular Weight	$\alpha^a$
Control	86,000	0.66
0.16	140,000	0.60
0.47	1,065,000	0.55
1.19	2,254,000	0.54

<sup>a</sup> From Eq. 2.

tion remained essentially intact. Intrinsic average molecular weights were calculated through the Scheraga-Mandelkern equation derived from the hydrodynamic ellipsoidal model (10)

$$M = [\eta]^{1/2} \left( \frac{S^0 \eta_0 N}{\beta - (1 - \bar{v}_p)} \right)^{3/2} \quad (\text{Eq. 5})$$

A value of 0.915 for the viscosity of 2 M KCNS is in agreement with Boedtker and Doty (3). Taking  $2.16 \times 10^6$  as the average beta value (11), molecular weights for F-I and F-II as well as their irradiated counterparts were obtained. The values are given in Table III along with  $\alpha$  values determined from Eq. 2. A plot of the log of intrinsic viscosity vs. the log of molecular weight was linear for both fractions, as was the relationship of sedimentation constant to the square root of molecular weight, thus establishing maintenance of random coil configuration (4).

## DISCUSSION

**Viscosity Studies.**—Since low order irradiation of F-I at 1% concentration produces successively lower intrinsic viscosity values in logarithmic relationship to dose, the question is posed whether scission plays a dominant role in reducing the viscosity of dilute gelatin solutions irradiated at these energy levels. According to Pasynsky (12) the rupture of peptide linkages, even if it does occur under the influence of biological radiation doses (0.3–2.5 R.), could not sufficiently change the configuration of the protein (serum albumin; gelatin) molecule. This, allegedly because the coiled state of the protein with numerous intramolecular bonds between chain links, is insensitive to radiation of this magnitude. As the dose is increased, internal structural changes take place, *i.e.*, aggregation, as reflected in an increase of absorption in the ultraviolet spectrum.

It is conceivable that these phenomena may occur in F-II, which shows progressive increase in viscosity after an initial decrease (with respect to the control). If the principle of proportionality between intrinsic viscosity and molecular weight is applied, one may assign responsibility for weight increases to (a) covalent linkage, or (b) excitation or activation of the molecules followed by regrouping the structural changes, *i.e.*, aggregation.

Bello, *et al.* (13), contend it is not likely that high intrinsic viscosities in unsolubilized gelatin are due to aggregation, but to crosslinking. Their experi-

mental evidence, corroborated by the generally accepted capability of potassium thiocyanate to maintain gelatin solutions in a state of disaggregation, tends to negate the argument that aggregation is accountable for increased viscosity rather than crosslinking.

That large solvent:solute ratios are responsible for producing experimentally detectable intrinsic viscosity changes at low order irradiation levels is borne out by negligible and erratic viscosity changes in irradiated 5% solutions and films. Results obtained with films particularly give impetus to the proposition that secondary solvent-solute interactions during the ionization event are factors in producing shifts in intrinsic viscosity values. Impact of ionizing radiation on solid gelatin causes absorption of energy by the protein, but the absence of water during impingement deprives the molecules of freedom of movement and, consequently, interaction with solvent.

Wiederhorn, *et al.* (14), observed that thermally contracted (denatured) collagen obeys the kinetic theory of rubber elasticity. Assuming collagen to be a network polymer consisting of long chains tied together at intervals along their length, the average molecular weight of the chain between points of primary valence crosslinking was shown to be in the order of 55,000. This was based on the premise that the molecular weight between points of crosslinking can be determined from stress-strain behavior of a thermally contracted sample. From this quantity Wiederhorn deduced a method for calculating the number of crosslinks per unit volume of mass. The following mathematical relationship correlates the retractive force with elongation

$$f = \frac{v_2^{1/3}}{Mc} RT\rho \left( \alpha - \frac{1}{\alpha^2} \right) \quad (\text{Eq. 6})$$

where  $f$  is the retractive force per unit cross section;  $\alpha$  is the relative elongation of the sample, *i.e.*, the ratio of the stretch length to the initial length;  $\rho$  is the density of the unswollen sample;  $T$  is the absolute temperature;  $R$  is the gas constant  $8.478 \times 10^4$  if the force is given in Gm./sq. cm. and the density in Gm./ml.;  $Mc$  is the average molecular weight of the chains between points of crosslinks; and  $v_2$  is the volume fraction of the elastic constituent in the sample.

Employing a modified Wiederhorn device, stress-strain measurements were made on a series of commercial pigskin gelatin films irradiated as follows: control, 2.5, 5.0, 10.1, and 25.0 Megarads. It was found that the average molecular weight between crosslinks in the control varied from about 40,000–82,000; at the 2.5 Megarad level, it varied from 198,000–304,000, at the 5.0 Megarad level from 251,000–345,000, and at the 10 Megarad level from 499,000– $1.2 \times 10^6$ . Because of the brittleness of the sample and experimental difficulties, the stress-strain measurements on a 25.0 Megarad sample could not be obtained.

Determinations of molecular weight between crosslinks in irradiated F-I and F-II were unsuccessful. Films of both fractions irradiated at high (2.5 and greater Megarad) levels, succumbed to water at 60°, 65°, 75°, and 80°, indicating that thermostable crosslinking did not occur or, if it did, such crosslinking was of sufficiently low order to es-

cape Wiederhorn detection. Dry films (retained over calcium sulfate) and moist films (retained 12 hours over a water-saturated atmosphere), the latter theoretically providing the necessary electrons through an aqueous environment to enhance crosslinking, met with identical lack of success.

#### Sedimentation and Molecular Weight Studies.—

It has been shown (4) that the relationship establishes random coil configuration of gelatin molecules in solution and entrapment of solvent within the coil where the sedimentation constant is a linear function of the square root of the molecular weight. On this basis, the proposition that irradiation at subdenaturant levels may have at least a partially scissive effect on the protein structure seems plausible. The decrease in molecular weight values (F-I) may be explained as due to a combination of moderate deaggregation and scission. The reverse effect (increase in molecular weight) would indicate a tendency toward crosslinking and weak bond formation. Further support for this suggestion is provided through linearly related sedimentation velocity *vs.* intrinsic viscosity and Svedberg constants ( $S^0$ ) *vs.* the square root of molecular weight for both fractions.

One can now examine more closely the relationship between intrinsic viscosity and molecular weight as defined in the modified Staudinger equation (Eq. 2). The value of the semiempirical proportionality constant  $\alpha$  is two for rigid rods, one for free-draining random coils and 0 for impenetrable spheres. Although independent of molecular weight, it is dependent on the flexibility of the polymer chain and solvent:solute characteristics. Assuming that the  $K$  factor of  $2.9 \times 10^{-4}$  is valid (4) for randomly coiled gelatin molecules, and that the exponential value  $\alpha$  lies between 0.6–0.9 for a combination of the free-draining linear molecule and the swollen molecule with immobilized solvent, values of 0.64 for F-I and 0.66 for F-II are in reasonable agreement (Table III) with the established 0.62 value for randomly coiled gelatin. It appears justifiable to conclude that prior to irradiation both fractions had random coil convoluted configuration with solvent entrapment. In addition, it is evident from Table III that departure from the minimal 0.60  $\alpha$  value occurred on irradiation of both fractions.

It is interesting to note that Flory (15) sets an upper limit for  $\alpha$  of 0.80 on the basis of randomly coiled linear polymer theory and a lower limit of 0.50. Since the constant is a function of solvent:solute interaction, the latter value would indicate the limit of a solvent capable of dissolving the polymer. The  $\alpha$  values in all irradiated samples meet this requirement. It can be stated, therefore, that 2 *M* KCNS was capable of maintaining the gelatin fractions studied in a molecularly dispersed state and that random coil configuration was preserved.

A surprising result with respect to both fractions was the gross change in molecular weights imparted by small doses of energy on 1% solutions. This would seem contrary to prevailing opinion that low quanta of ionizing electrons produce relatively minor changes in protein (amino acid) structure. Although conceivably applicable in the case of solid states, it is not necessarily true of irradiated solutions. Considering that the higher molecular

weight fraction (F-I) of the two studied nearly tripled in value on exposure to extremely low level irradiation and that (as the energy levels were increased) the molecular weight values became progressively lower, it is conceivable that the random coil configuration enters into a linking reaction, the extent of which diminishes in proportion to the intensity of irradiation. The lower molecular weight fraction (F-II) behaves conversely, *i.e.*, small quanta of radiation force linking of the protein. As the intensity of energy increases, so does the molecular weight, indicating direct proportionality between radiation and molecular weight. This behavior suggests that when gelatin molecules are subjected to excitation at high dilution they are oriented toward each other by forces induced through the mechanism of low order irradiation, causing a partial unfolding of the random coil, lengthening of the chain (as indicated in the initially high intrinsic viscosity value), and crosslinking. Provided that the energy imparted to the protein remains quantitatively below that required to rupture critical linkages, the attraction will induce an increase in molecular weight.

It seems reasonable to assume that the two fractions examined had random coil configuration with solvent entrapment and that an assignment of 0.6+ for the  $\alpha$  value in the modified Staudinger equation is justified. Immobilization of the solvent within the swollen random coil can be inferred from the linear relationship between sedimentation constant and the square root of the molecular weight. Although F-I and F-II molecular weight values were related in 2:1 ratio, no experimental evidence was elicited which would establish substantial differences in the nature of the links holding the coiled entities together; the evidence suggested merely that a larger number prevailed. One can postulate as the most probable linkages van der Waals forces, weak hydrogen bonds (16), salt links, and covalent bonds joining the coils in a network structure. The lack of substantial coil bond strength is indicated by the lability toward denaturation by heat and, particularly in F-I and F-II, susceptibility to denaturation in aqueous solution on exposure to low order irradiation energy.

It is interesting to note that  $\alpha$  decreases on irradiation of both gelatin fractions but does not in any case fall below 0.54. Evidently, application of stress to the freedraining random coil configuration effects increased coiling with consequent displacement of entrapped solvent as well as immobilization of solvent not displaced. This tendency follows the  $[\eta]$  *vs.*  $\sqrt{M_w}$  relationship already established.

Although (as noted previously) 2 *M* KCNS is acceptable as a solvent for maintaining gelatin in a molecularly dispersed state, some significance must be attached to the values obtained for  $k'$  in the modified Huggins equation (Eq. 1). This factor has been found to be a parameter of solute:solvent interaction. According to Thomas, *et al.* (17), for linear polymers in good solvents  $k'$  has values between 0.3–0.4. For F-I and F-II controls, F-I irradiated up to about 1.0 Megarad, and F-II irradiated to about 0.2 Megarad, the  $k'$  values lie between 0.3–0.6, in good agreement with the requirements of Thomas. In excess of these

energy levels, however,  $k'$  for both F-I and F-II rises above 1.0. Since intrinsic viscosity is dependent on the shape of the molecule in solution and the shape in turn depends on solute:solvent interaction,  $k'$  values vary with solvent power and are inversely proportional to intrinsic viscosity. This would implicate 2 *M* KCNS as an inadequate solvent for measuring viscosity of gelatin irradiated at levels greater than 0.5 Megarad.

Nevertheless, on the basis of favorable experiments with 2 *M* KCNS by others (3) and data obtained by us, this solvent was elected and found to be quite suitable for viscosity as well as sedimentation studies. In 1% concentration, F-I and F-II gave reproducible straight-line plots of  $\eta_{sp}/c$  vs.  $c$  at 50° and at 25°. Constant values could not be obtained with either F-I or F-II when 5% solutions and films were diluted to 1% concentrations. This would appear to confirm the admonition that comparisons cannot be made on gelatins whose thermal, solution, and aging histories are not identical. Deviations invariably give misleading results because configurations of the protein molecule are not the same in sol and in gel states. For example, gelatin films prepared by hot evaporation are in a fold where hydrogen bonds are intramolecular, whereas cold-evaporated gelatin contains intermolecular hydrogen bonds and forms more stable films (18). Similar reasoning would apply to comparison of 1% and 5% gelatin solutions. In the former, solution can be effected more readily, and the molecular state is characterized by relative freedom of motion, whereas the latter requires a somewhat longer period for final dissolution of the protein, which is more compact and has less movement capability.

Therefore viscosity studies on dilute solutions, gels, and films are not susceptible to comparison even though the same protein is involved because the preparation of more concentrated forms followed by dilution, but interrupted by a short aging period, disorients the molecule. The configuration of the converted form is not the same as that in the 1% solution prepared initially. Hence, the behavior of the final solutions in viscosity studies differ appreciably. This reasoning is supported by differences in viscosity behavior of the dilute solutions, and gels and films converted to sols, following exposure to low order irradiation. The 1% solutions of F-I and F-II gave sharply definable plots of  $\eta_{sp}/c$  vs.  $c$ , readily extrapolatable to zero concentration to yield  $[\eta]$ . Irradiated gels and films converted to 1% solutions gave erratic results—overlapping straight-line plots and low  $[\eta]$  values which could not be reproduced. It appears that irradiation doses of the order 0.4–2.5 Megarads are capable of inducing discrete physical changes in the molecular make-up of gelatin in 1% solutions. The order of change in gels and solid states is comparatively small. Because of their compactness in 5% solution and their total immobility in the solid state, impingement of small quanta of ionizing radiation on gelatin molecules in these forms fails to disrupt their makeup to a degree sufficient for useful application of viscometric measurements.

The characteristically progressive reduction in  $[\eta]$  values for F-I and the shifting viscosity behavior of F-II may be attributed to changes in the size

and shape of the molecules and alterations in net charge with increased application of irradiation dosage. If scission or crosslinking (or both) occur, the increase or decrease in molecular weight is a function of scission and crosslinking rates which, in turn, are proportional to radiation intensity (19). The probability of crosslinking is proportional to the integrated dose  $R$  (in Megarads) of the radiation

$$q = \alpha R$$

where  $\alpha$  is a proportionality constant having units of mass/energy. With respect to scission

$$p = P_0 + \beta R$$

where  $\beta$ , the proportionality constant for scission, has the dimensions of  $\alpha$ , and  $p$  is the overall scission probability. Shultz (20) has evaluated the ratio  $\beta/\alpha$  in terms of the effect of radiation-induced branching on intrinsic viscosity in the pregelation region. By plotting  $[\eta]_{\text{branched}}/[\eta]_{\text{unbranched}}$  as a function of  $R/R^*$  where  $R^*$  is the critical radiation dose, it was shown that when  $\beta/\alpha = 0$  no scission occurs,  $[\eta]$  increases until  $R$  approaches  $R^*$ , then drops. The drop occurs sooner as the scission gains dominance. When  $\beta/\alpha > 1$ ,  $[\eta]$  decreases at the start, although molecular weight is increasing. The latter behavior is precisely that of F-II.

It is apparent that one cannot rely exclusively on intrinsic viscosity data to interpret changes in molecular weights of irradiated proteins. A more conservative approach would be to assume that several events occur during irradiation and that the final effects on the protein represent the total of the transformations on the entire molecule which would include scission, crosslinking, endlinking, charge transfer, polymerization, and deaggregation.

The drop in intrinsic viscosity of F-II with concurrent increase in molecular weight tends to support the proposition that if the  $\beta/\alpha$  ratio (20) is greater than unity,  $[\eta]$  decreases initially though the molecular weight is increasing, and that full reliance cannot be placed on  $[\eta]$  values to interpret changes in molecular weights of irradiated polymers. Here again one must look to the three principal reactions which may occur following free radical formation. In the lower molecular weight F-II, the lowest radiation level causes an immediate twofold increase in molecular weight. At the next level, the molecular weight becomes substantially greater and continues in the same direction. This would indicate recombination and coupling reactions between free radicals resulting in copolymerization by crosslinking and/or endlinking.

The interdependence of intrinsic viscosity, sedimentation constant, and molecular weight values for irradiated F-I and F-II has been established. Gouinlock's F-2 and F-3 fractions (7) showed the same relationship as did irradiated F-I—namely, a decrease in  $[\eta]$  reflected a decrease with respect to both  $S^0$  and  $M_w$ . The reverse pattern, nevertheless interdependent, was manifested in irradiated F-II. If it is accepted that 2 *M* KCNS is capable of maintaining gelatin in solution in a molecularly dispersed state, it will necessarily follow that changes in intrinsic viscosity can be relied upon to reflect sedimentation and molecular weight changes. Since intrinsic viscosity is related to the size of the molecule, an increase in this value in the presence

of KCNS would indicate the presence of forces other than those responsible for aggregation to account for increases in molecular weight.

In our next and final paper in this series we will show experimental data which indicate that molecular weight changes in the two fractions studied occur through a free radical mechanism.

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## Antacid Properties of Calcium, Magnesium, and Aluminum Salts of Water-Insoluble Aliphatic Acids

By STUART P. ERIKSEN†, GEORGE M. IRWIN, and JOSEPH V. SWINTOSKY

The antacid properties of several calcium, magnesium, and aluminum salts of aliphatic acids were studied. A number of the salts showed potential antacid properties *in vitro*. Of this group calcium laurate also showed promising results by *in vivo* tests in dogs and humans. Along with their acid-neutralizing capacity, higher molecular weight metal salts have several interesting physical properties, for example, their insolubility in aqueous fluids above approximately pH 5, and their neutralizing properties below this pH. They tend to float on the surface of solutions and cling to the walls of a container before they react with acidic solutions. Following reaction with hydrochloric acid, salts of higher molecular weight aliphatic acids form insoluble aliphatic acids which are reputed to retard emptying of stomach fluids. A possible drawback to the higher molecular weight salts is a lower weight-to-weight antacid capacity when compared to antacids such as calcium carbonate and aluminum hydroxide.

ALKALI AND ALKALINE earth metal salts such as carbonates, bicarbonates, and glycinates have been of considerable use over the years as antacids. They possess the ability to neutralize the acid of the stomach efficiently but in some cases replace it with a considerable concentration of hydroxide ion. Recently, the authors have investigated the antacid properties of a series of metal-organic acid salts. It appeared from consideration of their physical properties that these substances might be useful antacids with interesting "secondary" properties. The

*in vitro* and *in vivo* studies of these metal salts are reported in this paper.

The calcium, magnesium, and aluminum salts of long chain aliphatic acids possess several interesting properties which might counterbalance their high equivalent weight (low acid-combining power). Since they are essentially non-wetted, they tend to float on aqueous solutions, coat wet surfaces, and offer the possibility of good protective action. They react with acid only below a characteristic pH, depending on the compound. Because the acid product of the neutralization reaction is poorly dissociated and is often relatively water-insoluble, we may consider these metal salts as hydrogen ion-exchange compounds—hydrogen and metal ions being in equilibrium with the two solid forms according to the equation

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