

Dilute gels (0.3—2.0 %) of certain poly-electrolytes (desoxyribonucleic acid, carboxymethylcellulose) and of a low molecular weight substance (dibenzoylcystin) give similar diffraction patterns with small but characteristic differences from that of pure water at the same temperature. The major peak at $(\sin\theta) / \lambda = 0.16 \text{ \AA}^{-1}$ shifts toward smaller angles and the height of the minor peak at 0.23 \AA^{-1} increases relative to the properties of pure water. There also seems to be a tendency for the minor peak to shift toward larger angles.

From diffraction data it is known that the structure of water changes with temperature¹. With decreasing temperature the tendency of a water molecule to bond itself tetrahedrally to four other neighboring molecules increases. This is observed as a shift in the major peak towards smaller angles and of the minor peak towards larger angles and in an increase in the height of the minor peak.

Thus the changes on gelation are qualitatively similar to those obtained in pure water when the temperature is decreased; that is, the gel-forming substances produce a decrease in the "structural temperature" of the water. The effect is opposite to that found with electrolytes which are known to increase the "structural temperature"².

The experimental results confirm the Forslind theory of gelation³ according to which the thermal vibrations in the water lattice are reduced through a specific coupling to the non-aqueous component of the gel. The stabilization of the water is the result of a structural similarity between the ideal, four-coordinated water lattice and the positions of the hydrogen bond forming atoms on the surface of the non-aqueous phase.

An identical theory was developed in order to explain certain physico-chemical properties of desoxyribonucleic acid solutions⁴. Dielectric properties, viscosity and proton magnetic resonance data were explained through assuming the existence of large hydration shells with a higher degree of water lattice order than that prevailing in pure water. The present X-ray diffraction data are in agreement with the theory that certain asymmetric macromolecules can stabilize the water lattice over distances of some hundred Å.

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Carboxymethyl Chitin, a New Substance Suitable for the Determination of Chitinase Activity

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The chitinases have not hitherto been investigated as extensively as could be expected for enzymes that break down such a common substance as chitin. This substance occurs in large quantities both in several animals (*e. g.*, crustaceans, insects and molluscs) and in various plants (*e. g.*, fungi). Chitinases are also known from various sources, *e. g.*, the "liver" of snails, insect larvae, sperm, a variety of bacteria, molds, and almonds.

The following substrates have been used for the determination of chitinase activity: native chitin, regenerated chitin (precipitated by diluting a solution of chitin in cold concentrated hydrochloric acid), chitosan (deacetylated chitin), chitotriose and chitobiose. As chitin is insoluble in water, it is not suitable as a substrate for the determination of enzymic activity. Chitosan is better for it is soluble in buffer solutions with pH less than about 6.5. In more alkaline solutions it is insoluble and hence not suitable. It is surely not the same enzyme^{1,2} that attacks chitin and its oligosaccharides (*cf.* amylase and maltase).

A new chitin derivate, not described previously, carboxymethyl chitin, is suggested now as a substrate for the determination of chitinase activity. It has not the disadvantages of the substances used previously.

The preparation of carboxymethyl chitin. Raw chitin is prepared in the well-known way, *e. g.*, by treating shells of crustaceans with a dilute acid. The chitin is dissolved in supersaturated cold hydrochloric acid and precipitated by adding water or alcohol. The regenerated chitin is heated on a water bath with chloroacetic acid and a strong solution of sodium hydroxide in the way well-known for the preparation of carboxymethyl cellulose. Native chitin does not react with sodium hydroxide and chloroacetic acid under these conditions. The reaction product is dissolved in water and after neutralization the sodium salt of carboxymethyl chitin is precipitated by adding alcohol.

The determination of chitinase activity. A solution of carboxymethyl chitin is rather viscous, and so it is possible to run the determination of chitinase activity viscosimetrically in the well-known way³. This is advantageous

because viscosimetric methods usually are considerably more sensitive than other methods. As the substrate is a polyvalent acid, the ionic strength in the reaction mixture must be kept constant (*e. g.*, 0.1), and the concentration of multivalent cations should be as low as possible. Addition of cysteine or other compounds containing sulphhydryl groups causes a steady decrease in the viscosity of carboxymethyl chitin solutions. However, this difficulty can be overcome — in the same way as in the case of carboxymethyl cellulose⁴ — by adding a small amount of potassium ferri-cyanide.

Senju and Okimasu⁵ have described the preparation of hydroxyethylchitin, which is probably a suitable substrate for chitinase determination but this has not yet been proved.

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The Zimmermann Method

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This method¹ is the most frequently used in steroid analysis. The sample is dissolved in ethanol, made alkaline with KOH, and stained with *m*-dinitrobenzene. If the sample contains steroids with a ketogroup in position 17, the extinction curve will show a flat maximum at 5 300 Å. In their absence the curve will only slope towards longer wave-lengths. The reactions causing the staining are not known. None of the many variants of the method is accurate. One source of error is the oxidation of ethanol by the nitro compounds. The aldehyde thus formed polymerises in alkaline solution.

m-Dinitrobenzene is soluble in most organic solvents. As no staining will occur unless $pOH < 2$, the only suitable solvents for the reaction are the simplest alcohols. The tertiary butanol is the only alcohol not oxidised by *m*-dinitrobenzene. It was used for an investigation of the Zimmermann reaction. The following results were obtained:

1. Alkaline solutions of pure *m*-dinitrobenzene in tert. butanol have the same extinc-

tion curves as those described for stained 17-ketosteroids.

2. The intensity of the colour depends mainly on the concentration of OH⁻. It explains why the colours disappears after a couple of days.

3. Some carbonyl compounds as acetaldehyde, acetone, cyclohexanone, and 17-ketosteroids increase the extinction, and shift the absorption maximum over to longer wave-lengths (5 600 Å). Easily enolised ketones and formaldehyde have no or only slight influence on the colour.

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The Incorporation *in vivo* of Amino Acids into Subfractions of Cytoplasmic Particles

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It has been shown previously^{1,2} that in chick liver the incorporation *in vivo* of labeled amino acids is particularly high in the sub-microscopic particulate components of the cytoplasm.

¹⁴C-glycine, ¹⁵N-glycine, or ¹⁵N-DL-alanine were injected intravenously into chicks or rats. The animals were killed 1—5 minutes after the administration of the isotope. Fractions of large and small submicroscopic particles were prepared from homogenates of the livers by differential centrifugation. (After removal of the mitochondrial fractions, the homogenates were centrifuged for 20 minutes at 20 000 g, and subsequently for 40 minutes at 105 000 g). After several washings, the particles were repeatedly treated at 0° C with 0.2 M NaHCO₃, pH 8.4, and with 0.6 % desoxycholate, pH 8.4. The cholate extracts were further fractionated by means of ethanol at low temperatures³.

The isotope contents of the proteins were consistently higher in the large microsomes than in the small ones. In both fractions, the carbonate extracts (which contained a major part of the ribonucleic acid of the particles) showed the highest isotope contents. In the desoxycholate extracts, the proteins of the higher ethanol fractions showed a higher isotope level than the proteins of the more readily precipitable fractions. The non-soluble residues obtained after the desoxycholate treatments, had considerably lower isotope contents.