

has set, the ungelled solution behind the slot former is aspirated and discarded. The apparatus is then carefully placed on the stand in the bottom buffer reservoir without disturbing the gel, and cold water is circulated through the cooling plates. The slot former is slowly removed and the slot is cleaned with a fine spatula to eliminate any threads of gel remaining in the slot. After insertion of the sample the electrodes are connected to a power supply and electrophoresis is begun. On completion of the experiment the gel may be sliced or stained as such with a suitable dye (amido black 10 B). For preparative work, marker strips are sectioned longitudinally from the center and the lateral margins, and are stained. The background of the marker strips is destained and the strips are approximated to the unstained portions of the gel. The latter are then sectioned into selected horizontal segments. The fractionated material can be isolated from the unstained gel sections by extraction with buffer in a tissue grinder, centrifugation, and dialysis.

Electrophoretic patterns of bovine serum albumin obtained with the apparatus described were similar to those reported with commercially available equipment<sup>5</sup>. 50–150 mg of protein mixtures can be fractionated on the apparatus and approx. 65–70 % of the material recovered.

The apparatus has also been used successfully to resolve and isolate a family of four glycoproteins obtained from bovine aorta from material presumably "homogeneous" after repeated zone electrophoresis on starch blocks<sup>6</sup>. Glycoproteins in the polyacrylamide gum have been stained with PAS, and distinguished from other types of proteins.

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### **Thermal transitions in different mammalian collagen-bearing structures**

Collagen always occurs naturally in association with small quantities of non-collagenous protein and varying percentages of mucopolysaccharide, especially hyaluronic acid and the chondroitin sulphates. In its purest form, tendon, there is less than 1 % by dry weight of polysaccharide. Mammalian skin contains about 1 % polysaccharide but approx. 20 % lipid, again on a dry weight basis. At the other extreme

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(apart from bone) hyaline cartilage contains up to 40 % of chondroitin sulphate and fibro-cartilage about 15 %.

The question sometimes arises as to the possible effect of the small amounts of polysaccharide upon the shrinkage temperature,  $T_s$ , of tendon and skin samples<sup>1,2</sup>. Such an effect should be most marked in collagen associated with large amounts of polysaccharide, and it has been reported<sup>3</sup> that human costal cartilage has a  $T_s$  of 83–87°, which is about 20° higher than that of mammalian tendon. However, in these measurements it is important to distinguish between the overall gross shrinkage of the structure under test, and the behaviour of the collagen contained in the structure. For these reasons it was decided to measure  $T_s$  and the transition temperature in HCl solution at pH 1,  $T_t$ , of a number of cartilages and to compare them with values obtained for tendon and skin.

The following cartilages from humans of various ages were used: costal (2 years) an example of hyaline cartilage, and semi-lunar from knee (44 years) and intervertebral disc (69 years), both fibro-cartilages. These were compared with human skin (25 years) and rat-tail tendon.  $T_s$  was measured in 0.9 % NaCl. The details of the experimental procedure have been given elsewhere<sup>4,5</sup>, but in essence  $T_s$  and  $T_t$  are obtained by observing the sudden increase in stress when a sample constrained at its natural length is heated. This method does not rely upon visual observation of shape changes in the sample, but is sensitive to any tendency of the sample to contract. Concerning the measurement of  $T_t$  it has been found<sup>5</sup> that when the collagen sample is immersed in HCl solution at pH 1 and at room temperature, it undergoes a first stage of contraction which is reversible, but that upon being heated it suddenly contracts at a characteristic temperature into a second stage from which it cannot be recovered. This temperature we call the acid transition temperature  $T_t$ .

TABLE I  
SHRINKAGE AND TRANSITION TEMPERATURES OF CARTILAGE

Sample	$T_s$ (°C)	$T_t$ (°C)
Rat-tail tendon	60	35
Human skin	60	37
Costal cartilage	60	36
Knee cartilage	62	37
Intervertebral disc	60	43

The results are given in Table I. It can be seen that the  $T_s$  values agree among themselves, and the value 60–61° is typical of mammalian collagen. The  $T_t$  values agree among themselves also, except for that of the intervertebral disc. The intervertebral disc was the only sample which did not exhibit two-stage contraction even after immersion for 12 h in acid. 10 min is usually sufficient time to complete first-stage contraction<sup>5</sup>. The reason for this behaviour in the disc is not known, but it could be connected with the age of the sample (69 years); it has been reported that the ability of human collagen to swell in acid decreases with age<sup>6</sup>. However, any change due to ageing would be expected to reflect itself in  $T_s$  also. An observation made some time ago by PARTRIDGE<sup>7</sup> is in harmony with our results. He found a marked correlation between the conditions necessary to obtain good yields of chondromucoid from cartilage and those giving rise to thermal shrinkage of free rat-tail

tendon. GUSTAVSON<sup>1</sup>, also, reports that for various collagens, chemical methods indicate that polysaccharides do not stabilize the structure.

Apart from the high value for  $T_t$  in the intervertebral disc sample, the results obtained by physical methods show that collagen retains its characteristic thermal behaviour even when associated with large amounts of polysaccharide.

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### A mass-spectroscopic study of the EMERSON enhancement effect

During 1955-1958, the late Professor R. EMERSON discovered a synergistic effect on the rate of photosynthesis when algae were simultaneously illuminated with two light beams of different wavelength<sup>1</sup>. The discovery of the EMERSON enhancement effect<sup>2-8</sup> has been interpreted to mean that photosynthesis involves two separate photo-reactions, sensitized by two pigment systems. In view of the effect of light on respiration<sup>9</sup>, and the limitations of the methods (manometry<sup>1,2</sup> and polarography<sup>3-7</sup>) so far employed, an isotopic study of the EMERSON effect permitting the separation of concurrent evolution and uptake of O<sub>2</sub> during illumination appeared desirable.

We have used the mass spectrometer inlet system of HOCH AND KOK<sup>10</sup> permitting continuous sampling of gases dissolved in liquids. The experimental methods were similar to those described earlier<sup>11</sup>. Light beams of appropriate wavelengths were obtained from a 750-W tungsten lamp, filtered by a 12-in water filter to remove the infrared radiation and isolated by the combination of a second-order Bausch and Lomb interference filters (half bandwidth, 10 m $\mu$ ) with suitable sharp cut off coloured glasses. In these experiments, the two light beams were brought to focus on the surface of the cuvette at an angle of 30°. Several neutral-density wire screens were used to vary the intensity of the incident light.

A suspension of *Chlorella vulgaris* (growth conditions: 25°; 1000 ft candles; modified Knop's medium; 3% CO<sub>2</sub> in air; 2-day-old culture) served as the experimental material. Heavy oxygen (<sup>18</sup>O<sub>2</sub>) was introduced by shaking it with an algal suspension in a syringe.

The rate of photosynthesis caused by a single beam of light, increases linearly with light intensity; then the light curve ( $P = f(I)$ , where  $P$  = rate of photosynthesis and  $I$  = intensity of light) bends, and finally saturation is reached. A part of this

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