proteins by a series of purification steps. In the last step, serum transferrin and albumin were precipitated by their specific antisera after electrophoresis in agarose gel. As is shown in Figure 2, the autoradiographs of such immunoelectrophoretic plates showed a considerable darkening of the area corresponding to the serological precipitates of transferrin and of albumin. The darkening above rat serum albumin was stronger than that above transferrin. The specific activity of the antigen-antibody complex was determined quantitatively by extracting and counting the radioactivity of the immunological precipitates. The results obtained from slices and cell-free systems are summarized in the Table. In the case of experiments with slices the data represent the mean value of 4 to 5 immunoelectrophoretic runs per experiment. In the case of the cell-free preparations, 1 or 2 immunoelectrophoretic plates per experiment were extracted and counted. The incorporation into transferrin was one-third of that into albumin when calculated per mg antigen-antibody precipitate. In agreement with MILLER and BALE[2], the results indicate that liver is one of the sites of transferrin synthesis in rats. Furthermore, the data presented show that the incorporation of radioactive amino acid into the extensively purified protein is also manifest in slices and cell-free preparations[9].

Incorporation of ^{14}C-leucine into transferrin and albumin by slices and cell-free preparations of rat liver

Experiment	Counts/min/mg protein[a]		Ratio
	Transferrin	Albumin	Transferrin/Albumin
(a) Slices			
1	295 (110)	1430 (490)	0.21
2	460 (185)	1795 (590)	0.25
3	525 (170)	1440 (370)	0.36
4	605 (225)	2085 (620)	0.29
5	880 (345)	2490 (535)	0.35
(b) Cell-free systems			
1	27 (10)	105 (16)	0.25
2	60 (8)	200 (10)	0.31
3	115 (18)	300 (30)	0.39
4	120 (17)	455 (28)	0.26

The data given in parenthesis represent the total counts. [a] Antigen plus antibody. – Method of incubation: (a) Slice experiments: two 1.5 g portions of slices from rat liver were incubated in 10 ml of Krebs-Henseleit Ringer solution[5] containing 10 μc of DL-leucine (20 mc/mmole). Incubation was conducted for 3 h at 35°C under 6% CO_2–94% O_2. The purification of the proteins was then carried out as described previously[5,6]. (b) Cell-free preparations: These were incubated as described in Figure 2 and fractionated[5,6].

Zusammenfassung. Der Einbau von ^{14}C-Leucin in Serumtransferrin in vitro in Leberschnitten und in isolierte Lebermikrosomen der Ratte wurde gezeigt und mit demjenigen in Serumalbumin verglichen. Die gereinigten Proteine wurden immunoelektrophoretisch ausgefällt und ihre Radioaktivität bestimmt. Ebenso wurde die Reindarstellung von Transferrin aus Rattenserum beschrieben.

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Hydrated Density of Chondroitin Sulfate Preparations

Recent investigations reported particularly by OGSTON[1], LAURENT[2], SCHUBERT et al.[3] have indicated that polysaccharides of physiological importance exist, at high concentration, as rigid three-dimensional networks of extended linear chains, possess large effective volumes and act sterically to exclude other macromolecular materials from their respective domains. It has also been shown, more recently, that such molecules can act as effective plasticizers for native connective tissues, provided that a certain minimal degree of polymerization is exceeded and that the molecules are not oxidized to the corresponding dialdehyde starches[4,5]. The network model breaks down at low concentrations, where entanglement couplings decrease, and the macromolecules lose all or most of their aforementioned properties.

It might be predicted, therefore, that the hydrodynamic properties of polysaccharide sols would resemble those of viscoelastic materials at high concentrations and would behave like Newtonian fluids at lower concentrations. Data from one extremely simple type of experimental approach are presented in the following brief report, which substantiates the prediction from an essentially phenomenological point of view.

Samples of chondroitin sulfate containing approximately 26% protein and 4.2% nitrogen, were obtained (1961) from three different commercial suppliers (General Biochemicals Co., Chagrin Falls (Ohio), Nutritional Biochemical Corp., Cleveland (Ohio), and Mann Research Laboratories, New York and dispersed in triply glass-distilled water. The apparent hydrated densities of serial dilutions

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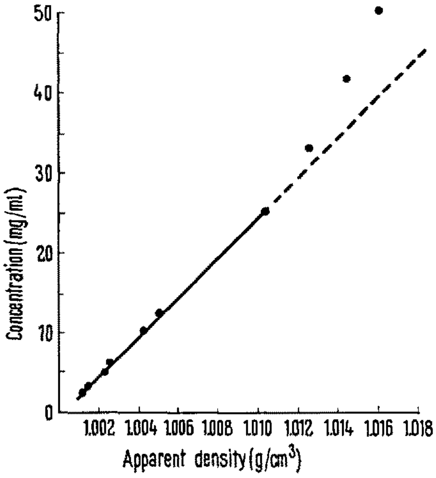
of freshly prepared stock solutions of each preparation were determined by the falling drop method[6] using calibrated xylene:bromobenzene (79:21, v/v) columns for the densiometer liquid phase (to provide a constant rate of shear) and a specially designed, micrometer-driven microliter syringe assembly. The time of descent of droplets of uniform size was determined to ± 0.1 sec by a stopwatch for both the solvent and solute phases. Apparent density was then calculated by the algebraic sum of the true density of water and the difference between the apparent densities of the water and chondroitin sulfate solutions, corrections being made for temperature effects. All studies were performed at 25 ± 1°C.

The mean values of 4–10 separate determinations of the apparent density of each of the three preparations (reproducibility, ± 0.00005) were then plotted for concentrations up to 50 mg/ml, which proved to be the upper limit of usefulness of the experimental method.

As demonstrated in the Figure, a remarkably linear relationship was found to exist between apparent density and concentration over the range of 2.5–25 mg/ml. Non-Newtonian behavior was clearly demonstrated beyond this range. At low concentrations, where randomly distributed chains presumably do not overlap and no entanglement couplings are formed, density increases monotonically with concentration. However, at higher concentrations, above 25 mg/ml, free rotation of chain segments appears to be constrained by the formation of entanglement couplings (and perhaps other electroviscous and charge interactions as well) so that, despite the increase in the total number of chains present in solution, each occupies a smaller relative volume than do freely orienting chains. The overall effect is thus a significant departure of the concentration-density relationship from linearity and a corresponding increase in the complexity of the kinematic viscosity formulation.



The resulting elastic as well as viscous properties[7,8] of such gel-like materials (which, in physiological articular cartilage, may exist in concentrations approximating 200 mg polysaccharide/ml 'tissue water'[9]) almost certainly accounts for the characteristic physical properties of specific native connective tissues. Selective loss of the carbohydrate moiety from connective tissues generally during the course of natural aging or disease processes, reported by a large number of investigations, would then also be predicted to result in profound alterations in the physical properties and hydration of these tissues, which accords with an expanding body of experimental evidence.

Résumé. La densité du sulfate de chondroitine-protéine en solution aqueuse a été déterminée par la méthode du «falling drop» utilisant des colonnes de xylène:bromobenzène. Un comportement Newtonien fut observé à des concentrations de 25 mg/ml. A des concentrations plus élevées (au niveau physiologique), les polysaccharides se comportent comme des liquides visco-élastiques, probablement à cause de l'enchevêtrement des différentes chaînes polymères.

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Effect of pH on Active Sodium Excretion

There are some data concerning the effects of changes in H^+-concentration on sodium transport in the frog[1-3]. This fact could be of functional importance, considering that the metabolic end-products of mammals are mostly of an acid nature. We tried to study this aspect following principally a well-known method[4].

We isolated the M. soleus and M. extensor digit. long., red and white muscles respectively, of rats. They were loaded in a modified K-free Krebs-Henseleit solution with a concentration of 160 m*M* Na at 3°C and pH 7.48 for $2^1/_4$ h whilst bubbling with a gas mixture of 93% O_2-7% CO_2. The changes in the Na and K content of the muscles (mEq/kg wet weight ± S.E. of mean) are shown in Table I. In these and the following experiments, we always compared companion muscles.

In the next series we recovered such Na-rich muscles in a fluid at 22°C containing 137 m*M* Na and 10 m*M* K at different pH values with adequate gas-bubbling for $2^3/_4$ h.

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