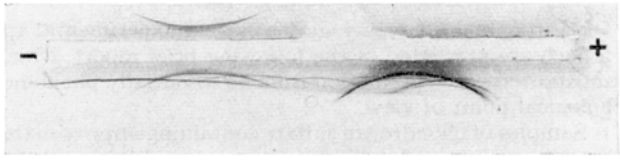
Incorporation of ^{14}C-Leucine into Serum Transferrin by Slices and Cell-Free Preparations of Rat Liver

Transferrin is an iron-binding protein of serum. Its electrophoretic mobility is that of a β_1-globulin[1]. It has been shown[2,3] that labelled amino acids are incorporated into this protein by perfused isolated rat liver. The experiments to be described here indicate that slices and cell-free preparations from rat liver are able to incorporate radioactive leucine into transferrin. After incubation the protein was isolated by means of salt fractionations and electrophoreses. Finally, transferrin was precipitated by its specific antiserum. The incorporation of ^{14}C-amino acid into transferrin was compared with the incorporation into rat serum albumin by the same preparations. Synthesis of rat serum albumin in vitro has been shown earlier by CAMPBELL et al.[4].

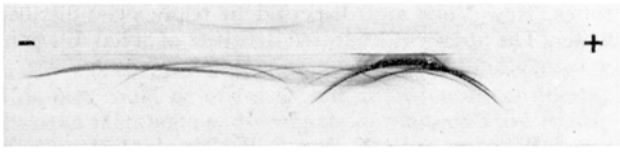
The present experiments, including those with slices and cell-free systems, were carried out in a way similar to that which has been described for the in vitro labelling of ferritin[5,6]. For experimental details concerning incubation of slices or cell-free systems and fractionation of the proteins after incubation, reference is made to the previous papers[5,6]. For the preparation of antiserum, transferrin was isolated from rat serum which was saturated with iron (Iron-Dextran, Benger Laboratories). The serum was precipitated with $(NH_4)_2SO_4$ at 40% saturation at 20°C[7]. The precipitate was discarded and to the supernatant $(NH_4)_2SO_4$ was added until a saturation of 65% was reached. The precipitate was redissolved in 0.01 *M tris*, pH 6.4, and dialysed for about 15 h against the *tris* which was changed several times. The dialysed solution was first filtered through DEAE-Sephadex and then through CM-Sephadex. Both resins had been equilibrated previously with 0.01 *M tris*, pH 6.4. The final eluate essentially consisting of transferrin, hemopexin and some γ-globulin, was concentrated by dialysis under reduced pressure against maleate buffer, ionic strength 0.05, pH 6.35. The solution concentrated to about 1 ml was subjected to starch block electrophoresis in the maleate buffer for 18 h (4 V/cm). Two coloured bands were seen, one mainly containing hemopexin, and the other one transferrin. Because of the somewhat incomplete separation, the two proteins after elution and concentration were subjected again to the starch block electrophoresis.

Figure 1 shows an immunoelectrophoretic plate of the two preparations showing the purity of the transferrin and of hemopexin when tested with rabbit antiserum against whole rat serum. Only one precipitation line can be seen for each protein. For the preparation of antiserum rabbits were injected with the preparation of transferrin mixed with Freund's adjuvant[8].

After the incubation of slices or cell-free systems with radioactive amino acids under appropriate conditions, transferrin and albumin were freed from the bulk of other



(a) Transferrin



(b) Haemopexin

Fig. 1. Immunoelectrophoretic pattern at pH 8.6 of the purified rat serum transferrin and hemopexin tested with antiserum against rat serum. Upper well, serum transferrin (Figure 1a), hemopexin (Figure 1b). Lower well, rat serum. Central trough, rabbit antiserum against rat serum.

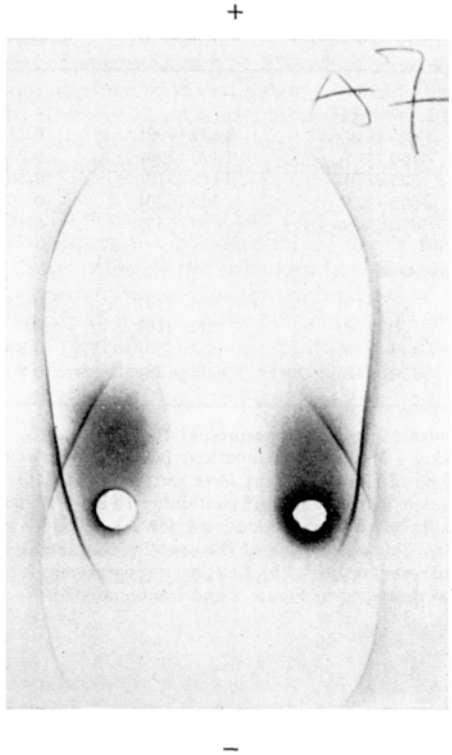


Fig. 2. Autoradiograph of immunoelectrophoretic plate. Time of exposure, 6 days. Outer troughs, antiserum against transferrin mixed with antiserum against albumin. The central wells contain purified proteins mainly consisting of transferrin and albumin. Two experiments are shown. The proteins were labelled by cell-free preparations as follows: Microsomes obtained from 10 g of liver and cell sap (100 mg of protein) were incubated in a final volume of 13 ml. In addition, the mixture contained 10 m*M* phosphoenolpyruvate, 1 m*M* ATP, 0.20 m*M* GTP, 250 μg pyruvate kinase, 5 m*M* reduced glutathione, 4 m*M* $MgCl_2$, 0.05 *M* KCl, 0.035 *M tris* buffer, pH 7.8, 0.25 *M* sucrose, and 20 μc ^{14}C-DL-leucine. After incubation for 1 h at 35°C the proteins were purified as described earlier[5,6].

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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] Transferrin	Albumin	Ratio 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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