of the dystrophic pigment epithelium was below that of the normal pigment epithelium at age 5 days, after which time it was steadily lower than normal (Figure B).

To conclude, our findings show that in affected rats aged 10 to 15 days (which coincides with the postanatal period when cell death occurs most rapidly) retinol content increased, whereas the amount of retinyl esters was apprecialby reduced. The decrease of retinyl esters content in epithelium cell layers could be the consequence of a defective or absent retinol esterifying activity. It is probable that, in the diseased eye, not only the protein pattern of the retina is defective[15] but the pigment epithelium may also lack some enzymatic proteins. Retinal hereditary degeneration related to a deficiency of several proteins would be then caused by multigenetic defect.

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Purification of Plasminogen Activator(s) from Human Seminal Plasma[1]

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Summary. Human seminal plasma contains an activator of plasminogen which was chromatographically purified. The molecular weight was determined to be 67,000.

The conversion of plasminogen into plasmin can be effected by various naturally occurring activators, such as tissue activator, blood activator, urine activator (urokinase) and trypsin. Also, in seminal plasma, an activator of plasminogen has been found[2–6]. Apart from its physiological significance, it is also of interest in the search for thrombolytic enzymes.

This paper concerns chromatographic purification and characterization of the plasminogen activator in human seminal plasma.

Material and methods. Semen samples were obtained from the Fertility Laboratory. They were centrifuged at 15,000 *g* for 1 h and the supernatant was decanted. 60 ml of the clear supernatant was dialysed in 3 × 2 l, 0.1 *M* acetate buffer, pH 5.0 for 3 × 12 h. A precipitate formed, which was removed by centrifugation at 15,000 *g* for 1 h. This procedure was repeated.

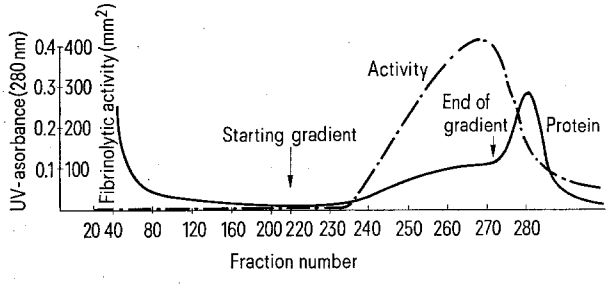

Fig. 1. Elution profile of seminal plasma on CM-Sephadex C-50 (see text).

The dialyzed seminal plasma with a volume of 100 ml was made 0.2 *M* in NaCl. Ionic exchange chromatography was then performed on a column of CM-Sephadex C-50, 4.4 × 10 cm (Pharmacia Fine Chemicals, Uppsala, Sweden), in 0.1 *M* acetate buffer, pH 5.5, 0.2 *M* NaCl. The column was first eluted with starting buffer and then a linear NaCl-gradient was applied (0.2–0.5 *M* NaCl). Fractions of 15 ml were collected at a flow rate of 60 ml/h. The absorbancy of each fraction was read at 280 nm. The fibrinolytic activity of the fractions was tested on unheated and plasminogen-free fibrin plates[7]. The fibrinolytically active fractions were concentrated by ultrafiltration (Diaflo ultrafiltration cell, membrane PM 10) prior to gel filtration.

Gel filtration was performed on a column of Sephadex G-200, 2.5 × 100 cm (Pharmacia Fine Chemicals, Uppsala, Sweden), in 0.02 *M* *Tris*-HCl, pH 7.4, 0.3 *M* HCl. 5 ml of the concentrate was applied. Fractions of 5 ml were collected at a flow rate of 18 ml/h. The absorbancy and fibrinolytic activity were determined as previously described.

The molecular weight was measured on a column of Sephadex G-100, 2.5 × 100 cm (Pharmacia Fine Chemicals, Uppsala, Sweden), in 0.05 *M* *Tris*-HCl, pH 7.4, 0.1 *M* HCl. The column was calibrated with Blue Dextran (V_o),

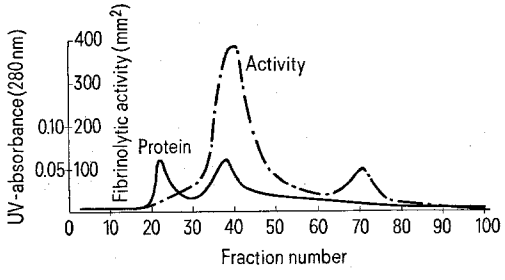

Fig. 2. Elution profile of seminal plasma on Sephadex G-200 (see text).

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and K_2CrO_4 (V_t). Cytochrome C, ovalbumin, bovine serum albumin and transferrin were used as reference substances.

Polyacrylamide gel electrophoresis was performed at pH 9.5 according to HJERTÉN[8]. The gels were cut longitudinally into 2 halves, one of which was then cut in 2 mm thick slices. The slices were examined for fibrinolytic activity by putting them directly into fibrin plates and incubating for 24 h. The other half was stained for protein with Coomassie Blue.

Results and comments. Cellular fragments and subcellular elements were thus removed from the seminal plasma by centrifugation. The precipitate formed during dialysis against acetate buffer contained no fibrinolytic activity.

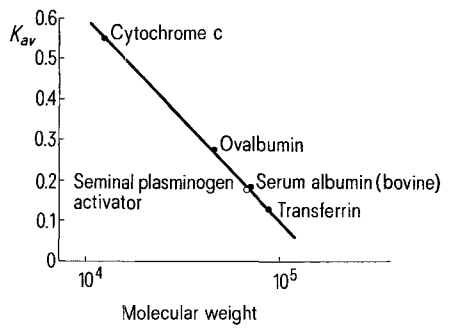

Fig. 3. Molecular weight determination of seminal plasminogen activator (see text).

When the centrifuged seminal plasma dialyzed to pH 5.5 was applied to a column of CM-Sephadex ,a large amount of the protein passed through the column without being bound (Figure 1). No fibrinolytic activity was found in this peak. The fibrinolytic activity was then eluted with a linear NaCl-gradient. In order to exclude unspecific proteolytic activity, it was checked that the active fractions had no activity on plasminogen-free or heated fibrin plates.

The active fractions were concentrated and chromatographed on Sephadex G-200. The elution pattern is shown in Figure 2. The activity was eluted in the second peak. Although the absorbancy was low in all peaks, the procedure resulted in no complete separation as the activity did not coincide completely with the second peak. This was further shown by electrophoresis in polyacrylamide gels, which revealed 5 different protein bands. By slicing the gels and examining the fibrinolytic activity in the slices, it was shown that the fibrinolytic activity did not coincide with any of these bands. Thus only a minor portion of the protein in peak 2 is contributed by the fibrinolytic activator.

The molecular weight of the seminal fibrinolytic activator was estimated by gel chromatography on Sephadex G-100. The molecular weight was determined to 67,000 (Figure 3). The K_{av} on Sephadex G-100 was 0.177.

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Binding of Mercury and Zinc to Cadmium-Binding Protein in Liver and Kidney of Goldfish (*Carassius auratus* L.)[1]

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Summary. Mercury and zinc, besides Cadmium, are incorporated into cadmium-binding protein found in liver and kidney of goldfish. In the liver, the Cd-BP incorporates more zinc (40%) than in the kidney (1.2%), while mercury show similar affinity for the Cd-BP of this two organs (17 and 12% respectively).

It has been demonstrated that in various animals the administration of cadmium increased the level of a cadmium-binding protein (Cd-BP), present in the soluble cytoplasmatic fraction of liver and kidney[2–4]. This protein, first isolated and characterized from the equine kidney cortex[5], has been found able to bind other metals such as mercury and zinc[4–6]. Recently the Cd-BP has been isolated from the liver and kidney of fishes, and it seems that the physico-chemical characteristics are similar to the Cd-BP isolated from mammalian tissues[7,8]. Although the biological function of Cd-BP seems to be a detoxication mechanism for cadmium, some suggestion of a possible role of Cd-BP in the metabolism of various metals has been reported in the literature[9,10].

Since heavy metals represent environmental pollutants frequently found in inland waters, it seemed interesting to investigate the contribution of Cd-BP to the distribution of cadmium, mercury and zinc in the liver and kidney of fish.

Eight goldfish were injected i.p. with a mixture of $CdCl_2$, $HgCl_2$ and $ZnCl_2$ and their radioactive tracers ^{109}Cd, ^{203}Hg, and ^{65}Zn, at a final dose of 30 nmoles of each salt per single fish. After 24 h, the fish were anesthetized by urethane dissolved in the water and sacrificed by decapitation. The dissected liver and kidney were minced and homogenized in 0.25 *M* sucrose and their soluble cytoplasmatic fraction separated by ultracentrifugation at 105,000 × *g* for 90 min. The Cd-BP was isolated from

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