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.

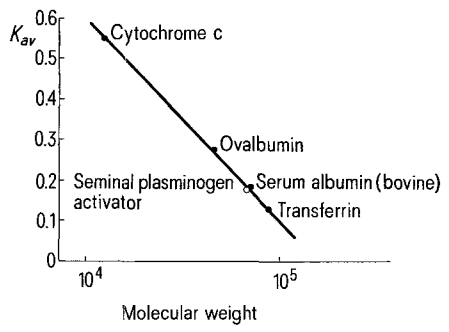

Fig. 3. Molecular weight determination of seminal plasminogen activator (see text).

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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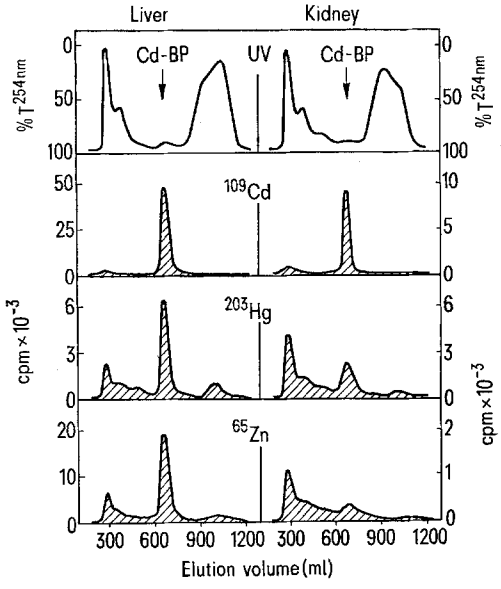
Distribution of ^{109}Cd, ^{203}Hg and ^{65}Zn, in liver and kidney of *Carassius auratus*, 24 h after i.p. injection of 30 nmoles of each labelled metal (% of the dose)

	109 Cadmium	203 Mercury	65 Zinc
Liver			
Total	27.5	6.9	8.1
Cytosol	26.3	2.3	4.9
Cd-BP	24.5	1.2	3.2
Kidney			
Total	7.4	4.0	1.6
Cytosol	6.1	1.3	0.3
Cd-BP	5.2	0.5	0.03



Gel filtration on Sephadex G-75 of the cytosol of liver and kidney of goldfish injected with 30 nmoles of ^{109}Cd, ^{203}Hg and ^{65}Zn, 24 h before the sacrifice.

the cytosol by the chromatographic procedure of SHAIK and LUCIIS[11] on a Sephadex G-75 columns, 100 × 3 cm, equilibrated with *Tris*-Cl buffer 10 m*M* at pH 8.2. The UV-transmission of the eluate was continuously monitored at 254 nm, and the radioactivities measured in the collected fractions (5 ml).

The Figure shows the elution profiles obtained from the gel-filtration of the liver and the kidney cytosol. Besides the cadmium, there was also mercury and zinc incorporated into Cd-BP, both in the liver and in the kidney. In these 2 organs, all the cadmium radioacvity was recovered in the Cd-BP fractions, while for mercury and zinc the radioactivity was found both in the Cd-BP and the higher molecular weight proteins.

The results reported in the Table show the distribution of ^{109}Cd, ^{203}Hg and ^{65}Zn in the liver and in the kidney. It was found that most cadmium was present in the cytosol of the liver, and that it was incorporated into the Cd-BP. In the kidney, the fraction bound to the Cd-BP represents only 70% of that found in the total organ. The results for Hg indicate, in comparison to Cd, lower proportions found in the cytosol (around $^1/_3$) and in the Cd-BP (12 to 17%). These proportions appear similar for liver and kidney.

As far as the distribution of ^{65}Zn is concerned, a strong difference was observed between liver and kidney. In kidney, the Cd-BP incorporates much less zinc (1.9%) than in liver (39.5%). This suggests the possibility of the presence in fish of an hepatic zinc-binding protein, with characteristics similar to the Cd-BP, as reported for the human liver by BÜHLER and KÄGI[12]. Although some differences were observed between the 3 metals in their affinity for the Cd-BP of liver and kidney, the results reported indicate that in fish, as in mammals, this metal-binding protein represents an important binding-site for the II B elements of the periodic table. Since cadmium stimulates the synthesis of the Cd-BP, the incorporation of different metals in the same component would explain some interactions observed in the metabolism of such metals in fish exposed to polluted water[13].

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The Response of Ca-mediated Action Potentials and Contractile Activity in Mammalian Ventricular Myocardium Towards Alkalosis

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Summary. Alkalosis (pH 7.8) produced by reduction of CO_2 concentration augmented both upstroke velocity of Ca action potentials and isometric contractile force of mammalian heart muscle. If the increase of pH to 7.8 was achieved by a raise of HCO_3 concentration (with simultaneous reduction of CO_2 concentration), the positive inotropic response was not accompanied by an augmented Ca current. Obviously, the well-known positive inotropic effect of alkalosis does not only depend upon the enhancement of transmembrane Ca influx during excitation, but can be mediated alone by affecting intracellular Ca movements as well.

It is a well-known fact that changes of pH in the extracellular fluid induce alterations of contractile activity of the myocardial cell. Thus, a decrease of the H concentration exerts a positive inotropic effect[1]. This reflects a larger amount of activator Ca available at the myofibrils which could be caused by a promoting action of alkalosis upon Ca release from stores (NAKAMARU and SCHWARTZ[2]) if changes of extracellular pH lead to concomitant alterations of the H concentration in the cell. On the other hand, it would be conceivable that alkalosis enhances transmembrane Ca inward current. In order to obtain a more precise insight in the mechanism under-