CHROM. 4328

Chromatography of fish pituitary extracts on Sephadex G-100

A significant advance in endocrinological research with chromatography has been made possible by the introduction of cross-linked dextran polymers known as Sephadex (Pharmacia, Uppsala, Sweden). The development of the technique of molecular sieving on Sephadex by PORATH AND FLODIN[1] and PORATH[2,3] offered a simple and rapid method for the fractionation of water-soluble substances. Since then Sephadex has been extensively used for the separation and purification of bovine growth hormone (DELLACHA AND SONENBERG[4]), human growth hormone (REISFELD *et al.*[5], ROOS *et al.*[6]) and porcine growth hormone (PAPKOFF *et al.*[7]). Purification of a gonadotrophic factor of the hypophysis of the carp (*Cyprinus carpio* L.) has been achieved by FONTAINE AND GERARD[8]. Recently in 1968 YAMAZAKI AND DONALDSON[9,10] purified salmon pituitary gonadotrophin by gel filtration on Sephadex G-100. In the present investigation, separation of the water-soluble protein of the pituitary extracts of some fish into different components has been attempted by gel filtration on Sephadex G-100. Fish at their different stages of gonadal maturation have been chosen for this. They also differed in their spawning habits, *i.e.*, puntius (*Puntius gonionotus* Bleeker) and tilapia (*Tilapia mossambica* Peters) spawn very easily in captivity whereas grass carp (*Ctenopharyngodon idellus* Cuv. and Val.), bighead carp (*Aristichthys nobilis* Richardson) and silver carp (*Hypophthalmichthys molitrix* Cuv. and Val.) do not spawn at all in captivity.

TABLE I

PROPERTIES OF THREE GRADES OF SEPHADEX

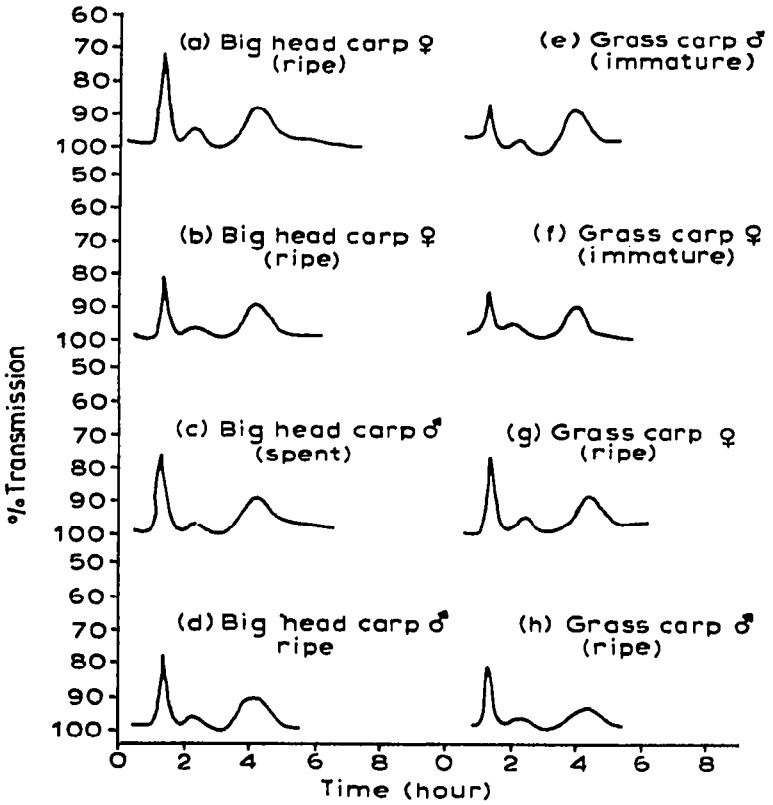
Type	*Water regain (g/g)*	*Size of molecule completely excluded (mol. wt.)*	*Suitable loading for fractionation (g/g dry gel)*	*Swelling factor (ml/g)*
G-50	5.0	8 000–10 000	0.18	10
G-100	10.0	100 000	—	20
G-200	20.2	200 000	0.18	40

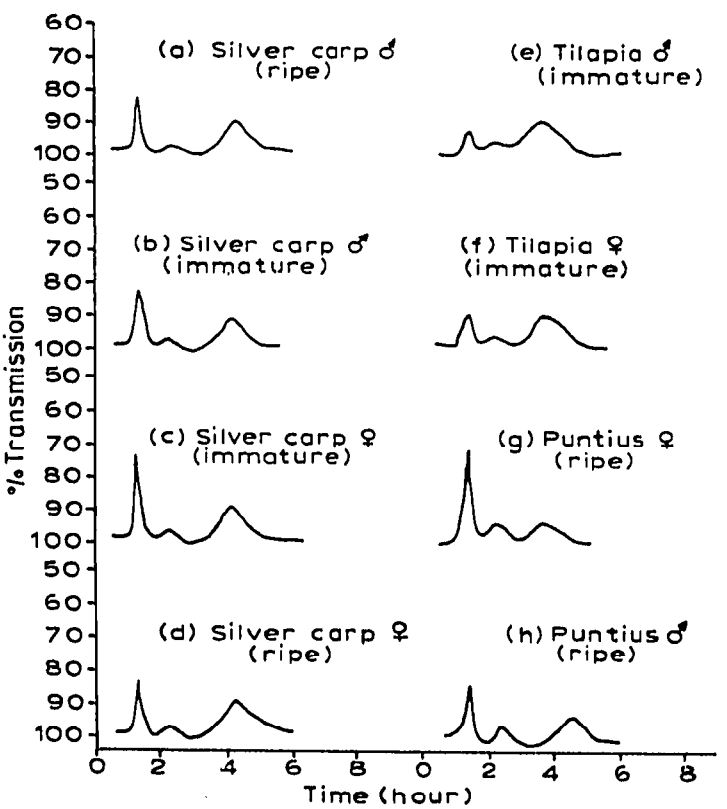
Pituitary glands of sexually immature and mature males and females of the above-mentioned fish and also of tilapia hybrid male (*Tilapia mossambica* Peters × *Tilapia hornorum* Trewavas) were homogenized with a Tri-R Tissue Homogenizer in the following eluants: 0.1 *M* sodium chloride; 0.02 *M* acetic acid (SANFELIPPO AND SURAK[11]); 0.2 *M* ammonium acetate (AURBACH AND POTT[12]); acetic acid–pyridine–water, 60:15:25 (PORATH AND LINDER[13]); phosphate–Tris–EDTA buffer (pH 4.0); lithium borate buffer, pH 8.6 (2.2 g lithium hydroxide and 23.75 g boric acid made 2 l with water). Then these were centrifuged in a Super Supragyro centrifuge at 4000 r.p.m. for about 15 min. The supernatant was directly applied to different Sephadex columns, 42 × 0.9 cm diameter, equilibrated with the particular eluants. Three different grades of Sephadex have been used, the properties of which are listed in Table I (after GORDON AND EASTOE[14]).

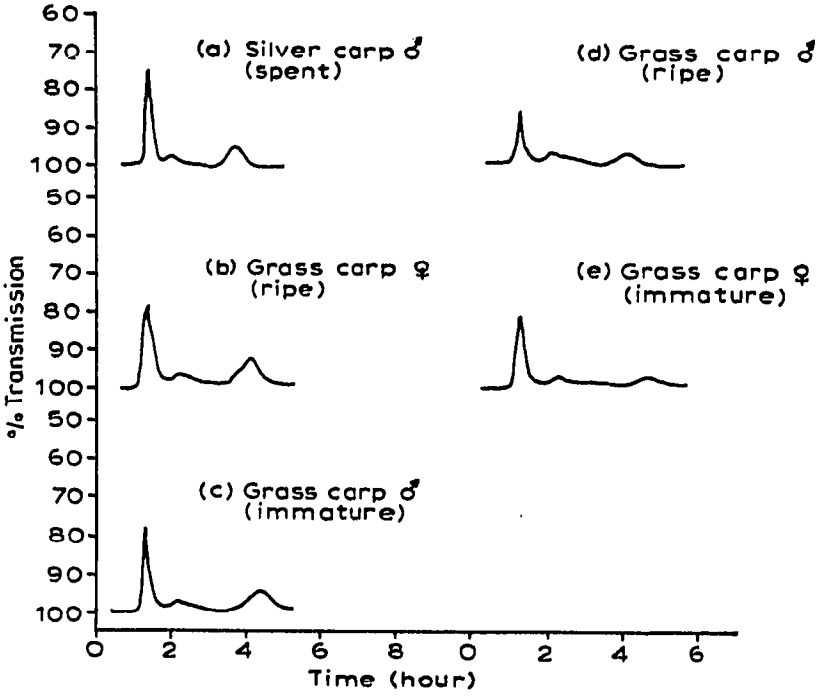
TABLE II

DETAILS OF THE FISH AND THEIR PITUITARY USED FOR CHROMATOGRAPHY

Fig. No.	*Eluent*	*Total dry weight of pituitaries (mg)*	*Fish*	*No. of fish*	*Range in length (cm)*			*Range in weight (g)*			*Sex*	*Condition of gonad*
					Min.	*Max.*	*Mean*	*Min.*	*Max.*	*Mean*		
1a	0.2 *M* ammonium acetate	6.7	Big head carp	1	—	—	75.5	—	—	4550	♀	Ripe
1b		4.8	Big head carp	1	—	—	64.5	—	—	4070	♀	Ripe
1c		5.0	Big head carp	1	—	—	64.0	—	—	3630	♂	Spent
1d		4.6	Big head carp	1	—	—	63.3	—	—	3290	♂	Ripe
1e		5.0	Grass carp	12	29.1	32.5	37.1	282	567	390.4	♂	Immature
1f		4.8	Grass carp	9				—	—		♀	Immature
1g		5.5	Grass carp	1	—	—	82.7	—	—	7390	♀	Ripe
1h		3.5	Grass carp	1	—	—	60.0	—	—	3010	♂	Ripe
2a	0.2 *M* ammonium acetate	4.5	Silver carp	1	—	—	60.0	—	—	2900	♂	Ripe
2b		3.5	Silver carp	1	—	—	53.0	—	—	2150	♂	Immature
2c		3.8	Silver carp	1	—	—	58.0	—	—	2600	♀	Immature
2d		4.4	Silver carp	1	—	—	58.3	—	—	2690	♀	Ripe
2e		8.2	Tilapia hybrid (*T. mossambica* × *T. hornorum*)	12	23.6	29.5	27.0	216	526	404.8	♂	Immature
2f		8.3	*Tilapia mossambica*	28	14.3	16.9	15.3	60	115	80	♀	Immature
2g		7.5	Puntius	6							♀	Ripe
2h		5.5	Puntius	6	24.1	30.5	27.2	183	409	281	♂	Ripe
3a	0.1 *M* sodium chloride	4.7	Silver carp	1	—	—	56.5	—	—	2540	♂	Spent
3b		5.5	Grass carp	1	—	—	77.0	—	—	5150	♀	Ripe
3c		4.0	Grass carp	1	—	—	68.0	—	—	3750	♂	Immature
3d		3.7	Grass carp	1	—	—	65.0	—	—	2930	♂	Ripe
3e		3.4	Grass carp	1	—	—	56.0	—	—	2970	♀	Immature


(a) Big head carp ♀ (ripe)
(e) Grass carp ♂ (immature)
(b) Big head carp ♀ (ripe)
(f) Grass carp ♀ (immature)
(c) Big head carp ♂ (spent)
(g) Grass carp ♀ (ripe)
(d) Big head carp ♂ ripe
(h) Grass carp ♂ (ripe)
% Transmission
Time (hour)


(a) Silver carp ♂ (ripe)
(e) Tilapia ♂ (immature)
(b) Silver carp ♂ (immature)
(f) Tilapia ♀ (immature)
(c) Silver carp ♀ (immature)
(g) Puntius ♀ (ripe)
(d) Silver carp ♀ (ripe)
(h) Puntius ♂ (ripe)
% Transmission
Time (hour)



Figs. 1–3. Sephadex G-100 chromatograms of fish pituitary extracts.

Mixtures of these in different proportions were also tried, with no success, to get better separation. The best separation was obtained with Sephadex G-100 alone.

Elution was carried out at room temperature at about 10 ml/h and was recorded with a LKB 4701 A Uvicord recorder at 253.7 nm at a recording speed of 10 mm/h. Of all the eluants tried, the best separations were obtained with 0.2 *M* ammonium acetate and 0.1 *M* sodium chloride. 0.02 *M* acetic acid did not give sharp peaks nor did the phosphate–Tris–EDTA buffer. With pyridine the recorder did not work. The fractions of the pituitary extracts eluted with lithium borate buffer were again examined by horizontal electrophoresis to see whether they further resolve into more components. But unfortunately no bands were obtained.

For most of the mature and big fish, an individual pituitary was usually sufficient for one run, but when it was not sufficient, pituitaries from a few fish of the same sexual developmental stage were mixed together for the run. Details on length, weight and gonadal condition are given in Table II.

Pituitary extracts showed positive ninhydrin reaction and so did the different eluted fractions. Figs. 1–3 show the chromatograms of the pituitary extracts. These clearly indicate that the water-soluble protein of the pituitary extracts has been fractionated into three distinct components. The biological activity of these fractions is being tested on fish and other animals. This study will be described in detail elsewhere (SINHA[15]).

Thus there are three eluted peaks all of which show very close resemblance in all the recordings, despite the fact that they are of different fish, of different spawning habits and of different sex, at their different stages of gonadal maturation. Because of the differences in the weight of the sample of the pituitary, no direct comparison can be made of the amount of the different eluted peak component at different stages. Yet it seems obvious that the difference may be purely quantitative, *i.e.*, different

amount of a single component, or partly qualitative, *i.e.*, difference in the relative amount of components. It is perhaps because of this similarity that very high percentages of positive results have been obtained when pituitary materials were injected in experimental fishes even though the donor and recipient were of different species (HASLER *et al.*[16]; CLEMENS AND SNEED[17]; YAMAZAKI AND DONALDSON[9,10]; YASHOUV *et al.*[18]).

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