Large Scale Preparation of Liver Growth Factor for Cultivation of Nematodes[1] (35080)

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Cultivation of metazoa under bacteria-free conditions is valuable for biochemical and nutritional studies and avoids the complexity introduced by associated organisms. For such studies the nematode has particular advantages.

Investigation of the nutritional requirements of the free-living nematode *Caenorhabditis briggsae* (1) led to development of a chemically defined medium (2) which required addition of a low level of an organic supplement to obtain continued reproduction. One very effective supplement is a partially purified protein designated growth factor (GF) (3). This has been used in the culture of free-living nematodes (4) and parasitic helminths (2, 5).

GF was originally prepared by a slow, laborious, stepwise elution from hydroxylapatite columns (3). In the present paper a rapid, efficient "batch" technique is described. Using this new method, batches yielding more than 2 g of GF have been prepared easily in a day.

Materials and Methods. The starting material, liver protein fraction (LPF), was prepared from lamb liver homogenate by fractional precipitation with ammonium sulfate, taking a cut between 30 and 50% or between 40 and 60% saturation (1, 3). Potassium phosphate buffer was prepared as a 1.0 *M* stock solution, pH 7.0, and diluted as required. The LPF was dialyzed against 0.15 *M* buffer until free of ammonium sulfate and then centrifuged at 18,000*g* for 30 min at 4°. The protein concentrations in different preparations ranged from 25 to 50 mg/ml. Dry hydroxylapatite (HA) (Bio-Rad Laboratories, Richmond, California) was mixed with 0.15 *M* buffer and, after overnight soaking, filtered through Whatman No. 1 paper on a Buchner funnel. The volume of the retained buffer, *i.e.*, hold up volume, was determined by wet cake weight. Suction was controlled, and discontinued immediately upon the disappearance of liquid at the surface of the cake. Wet HA cake was stirred in a beaker with LPF in 0.15 *M* buffer for 2 hr, LPF being added in a ratio of 60 mg/g of dry HA.

The 0.15 *M* eluate was removed by filtration through a Buchner funnel. The HA cake was overlaid with a volume of 0.15 *M* buffer equivalent to the holdup volume, and suction applied. This rinse procedure was repeated 3 or 4 times until the protein concentration of the filtrate was less than 1 mg/ml. This eluate and the washings were discarded.

Growth factor was eluted from the HA cake with 0.5 *M* buffer. The HA cake was placed in a beaker and 0.85 *M* buffer added in a volume equivalent to the hold up volume, so that the final molarity was 0.5, making allowance for the volume of 0.15 *M* buffer retained by the HA. The mixture was stirred for 30 min, then filtered through a Buchner funnel. The filtrate containing GF had a protein concentration of 6 to 8 mg/ml. The HA cake was overlaid with a volume of 0.5 *M* buffer equivalent to the hold up volume, and the filtrate was combined with the first 0.5 *M* eluate.

All procedures were carried out in either a cold room or an ice bath. Protein solutions were stored at —20°. The HA was prepared

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TABLE I. Recovery of Growth Factor in 0.5 *M* Buffer from Hydroxylapatite Suspended in Potassium Phosphate Buffer, pH 7.0.

	Distribution of protein as % of total		
Filtrate	0.15 *M*	0.5 *M*	1.0 *M*
First eluate	37.6	11.1	0.3
Rinse 1	17.3	6.7	0.1
2	11.3	1.7	—
3	7.4	0.4	—
4	3.6	0.2	—
5	1.5	0.1	—
Total for buffer	78.7	20.2	0.4

for re-use by eluting with 1.0 *M* buffer, followed by several washes with 0.15 *M* buffer.

Samples were sterilized by passage through Millipore filters, first of pore size 0.45 μ, and then 0.3 μ. Biological activity was ascertained as previously described (6) using *C. briggsae* as the assay organism. GF was added to *C. briggsae* Maintenance Medium (CbMM) (Grand Island Biological Company, Grand Island, N. Y.) at 10 to 100 μg/ml. Activation treatments (7) included freezing in the defined medium, addition of 10% Ficoll, or heating for 6 min at 53°, or for 24 to 48 hr at 37°.

Protein concentrations were determined by absorbancies at 280 and 260 mμ (8) or by the method of Lowry *et al* (9). Electrophoreses were done on the Millipore Phoro-Slides, using a Veronal buffer, pH 8.6, ionic strength 0.06, and 100 V for 17 min.

Results. The distribution of protein (9) in the successive filtrates is shown in Table I; GF was recovered in the 0.5 *M* filtrate and rinsings. The fractionation was followed electrophoretically and is shown in Fig. 1. The main band in the electrophorogram of GF is located between α_2 and β serum globulins. This band is present in the starting material, LPF, but is greatly decreased in the 0.15 *M* filtrate.

Biological activity was assayed after activation by freezing in defined medium. The Relative Potencies (6) were: GF, 100; LPF, 33; 0.15 *M* filtrate, 3; and 1.0 *M* filtrate, 0. When activated by freezing, heating, or addition of Ficoll, activity was comparable with that of GF previously prepared by the column method (3).

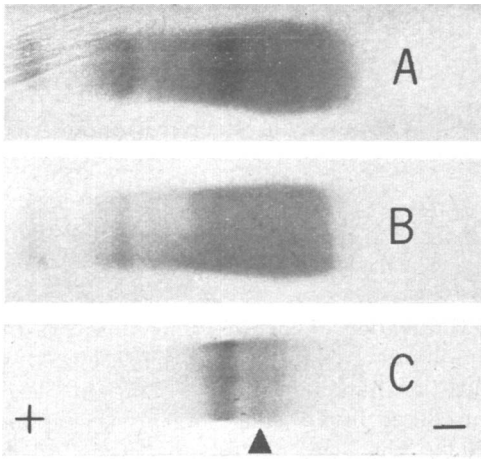

FIG. 1. Electrophorograms of fractionation: (A) LPF; (B) 0.15 *M* filtrate; (C) 0.5 *M* filtrate (GF). The arrow indicates the application point.

The two GF preparations were similar also in other respects, *viz,* both had a straw-yellow opalescent appearance, with a tendency to form a stringy precipitate in dilute solution, less than 1 mg/ml; the yields were 20 to 25% of the LPF; the electrophorograms were similar (Fig. 2); the ratios of OD at 280 mμ to OD at 260 mμ were 1.1–1.2; the ratios of protein as determined by UV absorbance to protein as determined by Lowry method were 1.5–1.6; both were stable to lyophilization. In immune electrophoresis against serum of a rabbit immunized with

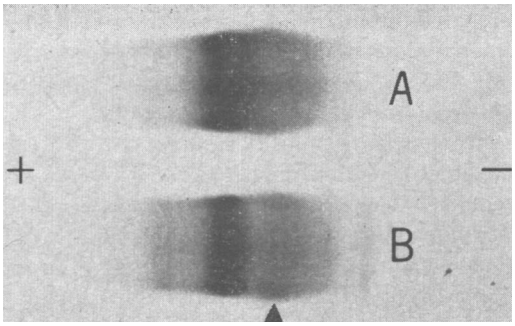

FIG. 2. Electrophorograms of growth factor (GF) prepared by batch (A) or column (B) method. The samples were concentrated to 15 mg/ml with Sephadex G-200.

column GF, similar precipitin bands were shown by GF prepared by the column and batch methods.

In culture media GF is used at 50 to 100 μg/ml in CbMM for free-living nematodes, and at 250 μg/ml for continuous column cultures (4) of *Neoaplectana carpocapsae*. A single batch preparation therefore provides material for several months of work. Stored frozen, there is no detectable loss of activity.

Summary. A rapid, simple and efficient preparation of growth factor from lamb liver for cultivation of nematodes is described.

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