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Purification of Mouse Lung-Conditioned Medium Colony Stimulating Factor (CSF)[1] (38073)

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(Introduced by N. F. Stanley)

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Colony stimulating factor (CSF) has been demonstrated in animal serum (1, 2), urine (3, 4), tissue extracts (5, 6), and medium conditioned by a variety of tissues and cell types (7–13). When stimulated by this specific factor certain hemopoietic progenitor cells can proliferate *in vitro* to form colonies of granulocytes and/or macrophages (7). CSF occurs in a variety of molecular forms that differ somewhat in several respects including resistance to various chemical (3, 9, 10, 13, 14) and enzymatic treatments (2, 3, 9, 10, 13–16), calcium phosphate chromatographic behavior (5, 6, 11, 13, 16), and electrophoretic mobility (2, 3, 5, 6, 10, 11, 14, 16, 17). With respect to size (zone sedimentation) CSF varies in apparent sedimentation coefficient (S_{20w}) from approximately 2.0–6.0S (2–4, 11–14, 17, 18). Size appears to be dependent not only on source (2–6, 9, 18) but according to whether a tissue injuring stimulus such as endotoxin has been used. In the hours following endotoxin injection, in addition to extractable tissue and serum CSF levels rising (17), CSF molecular size appears to fall (4, 11, 12, 14, 17). The approximately 2.0S CSF of mouse lung-conditioned medium probably represents an extreme example of this phenomenon (11, 14). This CSF has recently been investigated (11, 14), because of its interesting properties, its ease of preparation and its high sp act. For similar reasons purification was attempted.

Materials and Methods. S. typhimurium endotoxin (a gift from Dr. C. Jenkins, University of Adelaide) was dissolved in saline (25 μg/ml) then injected iv 0.2 ml per animal into 150 adult C57/B1 mice. Three hours after injection the mice were exsanguinated under ether anesthesia, the lungs excised, and each pair placed intact into an individual sterile capped 17 × 100 mm plastic tube (Falcon Plastics, Los Angeles) containing 5 ml of serum-free culture medium (11). After 48 hr incubation at 37°C in 10% CO_2 supplemented fully humidified air the medium was harvested.

The harvested medium was subjected sequentially to the following procedures:

1. Heat treatment and dialysis (14).
2. Block elution from calcium phosphate gel, dialysis, and lyophilization (14).
3. Ion exchange chromatography on a 90 × 1.8 cm DEAE cellulose column which was eluted at 4°C at a pressure head of 20 cm buffer giving a flow rate of 40 ml/hr. Elution was with 500 ml of 0.1 *M* Tris–HCl pH 7.4 containing 0.02% NaN_2 followed by a linear 1000 ml 0.00 *M* to 0.15 *M* NaCl gradient in the same buffer. The biologically active fractions were pooled, dialyzed (3 days, 3 changes of water, 4°C), concentrated by rotary evaporation at 40°C then lyophilized.
4. Gel filtration on an 80 × 1.7 cm Biogel P30 column eluted at 4°C at a pres-

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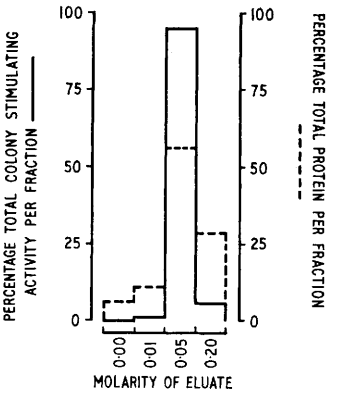

FIG. 1. Stage II of a purification of mouse lung-conditioned medium CSF (3-48E). Calcium phosphate elution profiles of both the CSF and total protein (Lowry) are indicated.

sure head of 12 cm buffer with 1000 ml of 0.03 *M* Tris–HCl pH 7.4 + 0.02% NaN_2. Fractions containing colony stimulating activity were pooled, dialyzed (3 days, 3 changes of water, 4°C), concentrated by rotary evaporation at 40°C then lyophilized.

5. With the aid of sample spacers up to 250 μg of the CSF preparation was applied per 5 mm width of the upper surface of each "Gradipore" 4–25% polyacrylamide continuous concave gradient gel slab of internal dimensions 83 × 73 × 3 mm (Townson & Mercer, Sydney). To the same gels but in different sample application spaces were applied 2-μliter samples of normal human serum (Hp 2-1 type) to aid in the identification and quantitation of the protein bands in the mouse CSF preparations. Electrophoresis was for 17 hr (75 V 4°C) in 0.6% Tris + 2.88% glycine (w/v) in water pH 8.3. At completion of electrophoresis the gel slabs were removed from their cells, sliced parallel to their faces, one side stained with Coomassie brilliant blue R-250 (Sigma), the other sliced across the slab in 2-mm fractions (parallel to the band fronts) that were individually crushed, extracted, and assayed for CSF.

Protein was estimated either by the method of Lowry *et al.* (19), or in the case of column chromatographic procedures, adsorption at 280 nm. CSF was assayed by methods previously described (20). CSF preparation potency was quantitated in units (units = colony count obtained from linear region of dose response curve multiplied by the dilution of the preparation in the culture).

Results. The results of each chromatographic procedure are sequentially shown; calcium phosphate chromatography (Fig. 1), ion exchange chromatography on DEAE cellulose (Fig. 2), and gel filtration on Biogel P30 (Fig. 3). Also shown is the result of the first of two final stage polyacrylamide gradient gel electrophoretic separations (Fig. 4). In this figure comparison can be made between the location of the stained protein bands and the eluted CSF of opposing sides of the sectioned gel slab. Also shown are the relative positions of the stained protein bands in two samples of human serum that had been coelectrophoresed through an adjacent area of the same gel. The band which corresponded in position to CSF, although just visible in the stained half of the sectioned gel, required intensification to be seen in Fig. 4. Estima-

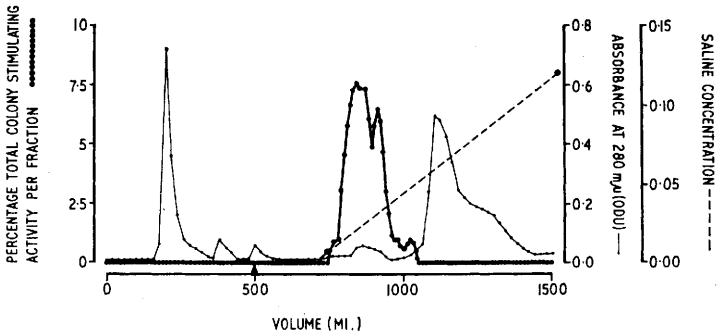

FIG. 2. Stage III of a purification of mouse lung-conditioned medium CSF (3-48E). DEAE cellulose chromatographic profile of both CSF and total protein are shown.

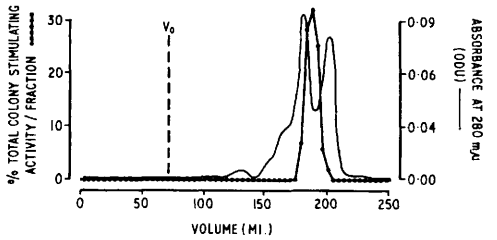

FIG. 3. Stage IV of a purification of mouse lung-conditioned medium CSF (3-48E). Biogel P30 elution profile of both CSF and total protein are shown. Vo = void volume, Ve/Vo CSF = 2.7.

tion of the protein content of the CSF-containing band was made by comparison with the staining intensity of the minor bands of known protein content in the co-electrophoresed human serum. In the subsequent gradient gel electrophoretic separation the CSF peak again exactly coincided with a band of similarly graded staining intensity to that seen in the first gel. The results are summarized in Table I.

Discussion. Due to size and/or charge heterogeneity stage V mouse lung-conditioned medium CSF did not form a sharp band on gradient gel electrophoresis. About 70% of the recovered biological activity was however resolved from both the preceding and trailing stainable bands. Because of the high sp act of the CSF in the peak region (estimated at 1.0×10^{10} units/mg protein), as compared with purified L cell-conditioned medium CSF (9) and purified E1 cell-conditioned medium CSF (18), it is considered likely that the former CSF was in a high state of purity.

Comparison of the specific activities of the purest lung-conditioned medium CSF [approx sp act 1×10^{10} units/mg protein, approx MW 20,000 (12)] and purified human urinary CSF [approx sp act 1.6×10^{8} units/mg protein (16), approx MW 45,000 (21)] as assayed on mouse bone marrow indicate that under these conditions the former CSF may be about 60 times as potent as human urinary CSF on a protein weight for weight basis or 25–30 times as potent on a mole for mole basis. This calculation assumes a direct relationship between molecular weight and Coomassie brilliant blue staining intensity. The higher apparent specific activity of mouse lung-conditioned medium CSF compared to human urinary CSF may indicate a higher binding or triggering effectiveness of the former CSF on CSF responsive mouse hemopoietic cells.

The final location of mouse lung-conditioned medium CSF on gradient gel electro-

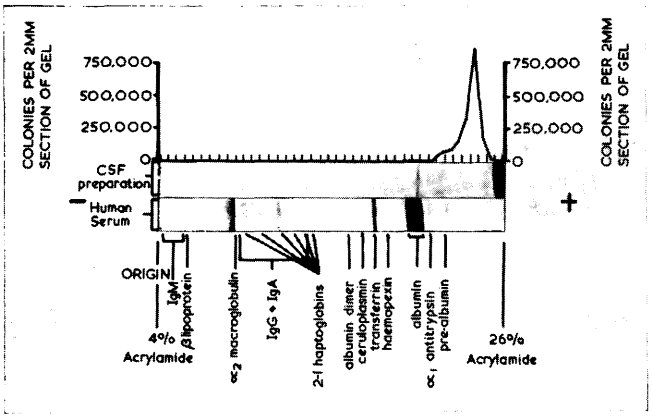

FIG. 4. Stage V of a purification of mouse lung-conditioned medium CSF (3-48E). The positions of both the eluted CSF and the corresponding stainable protein bands in a sliced 4–26% gradient polyacrylamide gel are shown. For comparison the positions of the stainable protein bands in a sample of human serum that was coelectrophoresed on an adjacent section of the same gel slab were included. N.B. The band corresponding in location to the CSF peak, although visible in the stained gel, required intensification to be reproduced in this figure.

TABLE I. Purification of Lung-Conditioned Medium Colony Stimulating Factor.

Stage	Purification procedure	Recoverable activity[a] (%)	Sp act[b] (units[c]/mg protein)
I	Heat and dialysis	100	6.0×10^4
II	Calcium phosphate chromatography	127	1.8×10^5
III	DEAE cellulose chromatography	39	2.2×10^6
IV	Biogel P30 chromatography	38	5.0×10^6
V	Gradient gel electrophoresis	27	1.0×10^{10}[d]

[a] % Recovery calculated with reference to stage I material. Compensation made for sequential reduction in total processed material due to taking of reference samples, at each stage, and staining of half of sliced gradient gel.

[b] Protein estimates stages I–IV according to method of Lowry *et al.* (19). Stage V protein estimate based on width and color intensity of band in stained half of sectioned gradient gel.

[c] CSF preparation potency (units) = colony count obtained from linear region of dose response curve multiplied by the dilution of preparation in the culture.

[d] Applies only to CSF in central region of peak which was clearly resolved from adjacent protein bands.

phoresis was confirmatory of its relatively low molecular weight.

Summary. A five-stage procedure was used to purify the low mol wt (approx 2.0 S) colony stimulating factor (CSF) in mouse lung-conditioned medium. The final stage, polyacrylamide gradient gel electrophoresis, separated a band with an estimated peak specific activity of 10^{10} units CSF/mg protein.

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1. Chan, S. H., Metcalf, D., and Stanley, E. R., Brit. J. Haematol. 20, 329 (1971).

2. Stanley, E. R., Robinson, W. A., and Ada, G. L., Aust. J. Exp. Biol. Med. Sci. 46, 715 (1968).

3. Stanley, E. R., and Metcalf, D., Aust. J. Exp. Biol. Med. Sci. 47, 467 (1969).

4. Sheridan, J. W., and Metcalf, D., Proc. Soc. Exp. Biol. Med. 144, 786 (1973).

5. Bradley, T. R., Stanley, E. R., and Sumner, M. A., Aust. J. Exp. Biol. Med. Sci. 49, 595 (1971).

6. Sheridan, J. W., and Stanley, E. R., J. Cell. Physiol. 78, 451 (1971).

7. Bradley, T. R., and Metcalf, D., Aust. J. Exp. Biol. Med. Sci. 44, 287 (1966).

8. Bradley, T. R., and Sumner, M. A., Aust. J. Exp. Biol. Med. Sci. 46, 607 (1968).

9. Austin, P. E., McCulloch, E. A., and Till, J. E., J. Cell. Physiol. 77, 121 (1971).

10. Watson, J., and Pritchard, J., J. Immunol. 108, 1209 (1972).

11. Sheridan, J. W., and Metcalf, D., J. Cell. Physiol. 81, 11 (1973).

12. Sheridan, J. W., and Metcalf, D., *in* "Proceedings Second International Workshop on Hemopoiesis in Culture" (W. A. Robinson, ed.), in press.

13. Stanley, E. R., Bradley, T. R., and Sumner, M. A., J. Cell. Physiol. 78, 301 (1971).

14. Sheridan, J. W., Metcalf, D., and Stanley, E. R., J. Cell. Physiol., in press.

15. Stanley, E. R., and Metcalf, D., Aust. J. Exp. Biol. Med. Sci. 49, 281 (1971).

16. Stanley, E. R., and Metcalf, D., *in* "Cell Differentiation" (R. Harris, P. Allin and D. Viza, eds.), p. 272. Scandinavian Univ. Books, Munksgaard (1972).

17. Sheridan, J. W., and Metcalf, D., J. Cell. Physiol. 80, 129 (1972).

18. Landau, T., and Sachs, L., Proc. Nat. Acad. Sci. USA 68, 2540 (1971).

19. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. 193, 265 (1951).

20. Metcalf, D., J. Cell. Physiol. 76, 89 (1970).

21. Stanley, E. R., and Metcalf, D., Proc. Soc. Exp. Biol. Med. 137, 1029 (1971).

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