The Molecular Weight of Colony-Stimulating Factor (CSF) (35721)

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Specific progenitor cells in the bone marrow are able to proliferate in semisolid agar and form colonies of granulocytes and/or macrophages if stimulated by a factor termed the colony stimulating factor (CSF). CSF is demonstrable in the serum of urine of mice and humans (1–3), and appears to be a humoral regulator of granulopoiesis and macrophage formation with properties similar (4) to those of erythropoietin. Since the number of colonies forming in culture is determined by the CSF concentration, the culture method serves as a quantitative assay system for CSF (2, 5). Preliminary studies (4) on CSF in human urine indicated a large discrepancy between the apparent molecular weights determined by gel filtration on Sephadex gels (approx 190,000) and by zone sedimentation in sucrose gradients ($S_{20,w}$ approximately 3.3 S; mol wt approx 45,000). Similar large discrepencies in the apparent molecular weights determined by these methods have been found with other sources of CSF [mouse L-cell conditioned medium (6), mouse embryo cell conditioned medium (7), and mouse serum (8)]. This report describes experiments with human urine CSF which suggest that the zone sedimentation data form a more reliable means of estimating the apparent molecular weight of CSF than gel filtration on Sephadex gels.

Materials and Methods. The culture method using mouse bone marrow cells for the assay of CSF has been described in detail elsewhere (2, 5). Sephadex G-150 and blue dextran 2000 were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Columns (dimensions 150 × 2.3 cm) were equilibrated in Tris–HCl buffers, pH 7.5, with ionic strengths of 0.03 or 0.10. Whenever buffer or temperature conditions were altered, the void volume (V_0) of the column was redetermined using blue dextran 2000. Determination of the apparent molecular weight of CSF was based on the behavior of ^{125}I-bovine serum albumin (^{125}I-BSA), ^{131}I-ovalbumin, and ^{125}I-ribonuclease and was calculated after the method of Andrews (9). Zone sedimentation on 10–25% (unpurified urine concentrates) and 5–20% (purified CSF) sucrose gradients was carried out after the method of Martin and Ames (10), using labeled marker proteins, as described earlier (4). Apparent $S_{20,w}$ values for CSF were obtained by comparison with those of the ^{125}I-BSA marker protein (4.41S) (11) and agreed closely with those calculated by comparison with the ^{125}I-ribonuclease marker protein (1.88 S) (12). Apparent molecular weights were calculated from the apparent $S_{20,w}$ values by the approximation given by Martin and Ames (10).

CSF was partially purified as described previously (4) (100-fold purified with respect to protein) and by a modification of this procedure (13) (500-fold purified with respect to protein). In both cases, gel filtration on Sephadex columns represented the final stage in the purification. The 5000-fold purified preparation was obtained by gradient elution chromatography of the 500-fold purified preparation on hydroxylapatite at pH 6.6 (13).

Results and Discussion. The differences in the gel-filtration behavior on Sephadex G-150 of the colony-stimulating activity of human urine preparations of varying purity are shown in Table I. As shown, there was a gradual decrease in the apparent mol wt from approximately 190,000, in unpurified urine concentrates to approximately 60,000 in 500-fold purified CSF preparations. No such

TABLE I. Gel-Filtration Behavior of Various Preparations of Human Urine CSF on Sephadex G-150.

Source of CSF	V_e/V_0 (CSF)	Apparent mol wt of CSF
Unpurified urine	1.33	~190,000
Purified, 100-fold	1.44	~105,000
500-fold	1.76	~ 60,000

difference in behavior was observed for any one preparation between columns run at 4° and those run at room temperature or with changes in ionic strength in the range 0.03–0.10.

In contrast to the relationship between purity of the preparation and elution behavior on gel filtration, sedimentation of both unpurified and purified preparations gave rise to a constant apparent $S_{20,w}$ value of approximately 3.4 S (Table II). Data shown in Table II also demonstrate that, as far as could be measured, this value for the sedimentation coefficient was independent of temperature and to a certain extent, pH (sedimentation at pH 3.0 resulted in loss of activity), and ionic strength. Thus all zone sedimentation experiments have suggested that human urine CSF has an apparent molecular weight of approximately 45,000.

Further evidence suggesting a molecular weight of approximately 45,000 was derived from application of the gradient polyacrylamide gel technique of Margolis and Kenrick (14) to give a "pore limit" separation. Purified human urine CSF (500-fold purified with respect to protein) was found in approximately the same region as α_1-antitrypsin (mol wt 45,000) (15), although the behavior of the CSF of this same preparation on Sephadex G-150 suggested an apparent molecular weight of approximately 60,000. In addition, disc electrophoresis using the method of Davis (16) has supported the sedimentation and gradient gel data suggesting that CSF has a molecular weight of around 45,000, irrespective of contaminating protein. For preparations ranging in purity from 80-fold to 5000-fold with respect to protein, CSF was always found to migrate immediately behind the ^{125}I-BSA marker protein on 7.5% gels.

TABLE II. Sedimentation Behavior of Two Preparations of Human Urine CSF.

Source of CSF	Temp (°)	pH	Ionic strength	Apparent $S_{20,w}$
Unpurified urine	4.5	7.0	0.02	3.36
	4.5	7.5	0.10	3.30
Purified, 500-fold	4.5	7.5	0.10	3.43
	4.5	10.5	0.10	3.32
	12.5	7.5	0.10	3.30
	12.5	10.5	0.10	3.42
	~22	7.5	0.10	3.45
	~22	10.5	0.10	3.41

The basis of the anomalous behavior of CSF on Sephadex gel filtration would appear to be related to an association of CSF with other macromolecules under the gel-filtration conditions employed. For this reason, caution should be exercised in ascribing any changes in the elution characteristics on Sephadex of CSF of impure preparations treated with various enzymes to a direct effect of the enzyme involved on CSF [*e.g.*, Ref. (6)]. Polymerization of CSF cannot be eliminated, although this would probably necessitate the existence of a monomer unit of less than 45,000 mol wt for which there is no evidence at present.

Summary. The apparent molecular weight of human urine CSF determined by gel filtration on Sephadex gels varied inversely with the purity of the preparation (190,000–60,000 mol wt). The apparent molecular weights calculated from zone sedimentation data on sucrose gradients and gradient polyacrylamide gel electrophoresis, as well as indirect evidence from polyacrylamide disc electrophoresis, were all consistent with an estimate of 45,000, irrespective of the purity of the preparations used.

1. Robinson, W. A., Metcalf, D., and Bradley, T. R., J. Cell. Physiol. **69**, 83 (1967).

2. Metcalf, D., and Stanley, E. R., Aust. J. Exp. Biol. Med. Sci. **47**, 453 (1969).

3. Foster, R., Metcalf, D., Robinson, W. A., and Bradley, T. R., Brit. J. Haematol. **15**, 147 (1968).

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

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

6. Austin, P. E., and Till, J. E., Proc. Can. Fed. Biol. Sci. 13, 100 (1970).

7. Stanley, E. R., Bradley, T. R., and Sumner, M. A., J. Cell. Physiol., in press.

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

9. Andrews, P., Biochem. J. 91, 222 (1964).

10. Martin, R. G., and Ames, B. N., J. Biol. Chem. 236, 1372 (1961).

11. Phelps, R. A., and Putnam, F. W., Plasma Proteins 1, 158 (1960).

12. Schachman, H. K., Methods Enzymol. 4, 32 (1957).

13. Stanley, E. R., and Metcalf, D., Proc. Aust. Biochem. Soc. 3, 67 (1970).

14. Margolis, J., and Kenrick, K. G., Anal. Biochem. 25, 347 (1968).

15. Schultze, H. E., and Heremans, J. F., "Molecular Biology of Human Proteins," Vol. 1, p. 190. Elsevier, Amsterdam (1966).

16. Davis, B. J., Ann. N.Y. Acad. Sci. 121, 404 (1964).

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