Vol. 126, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 16, 1985 Pages 15-24

THE EXPRESSION OF CERULOPLASMIN, AN ANGIOGENIC GLYCOPROTEIN, BY MOUSE EMBRYONIC FIBROBLASTS

Fong-Fong Chu[1] and Kenneth Olden[1,2]

[1]Howard University Cancer Center, Department of Oncology,
2041 Georgia Avenue, N.W.,
Washington, D.C. 20060

[2]Membrane Biochemistry Section
Laboratory of Molecular Biology
National Cancer Institute
Bethesda, Maryland 20205

Received November 8, 1984

Balb/c 3T3, Swiss 3T3 and Rous sarcoma virus transformed Balb/c 3T3 mouse embryonic fibroblasts produced ceruloplasmin in vitro, whereas primary cultures prepared from the Balb/c mouse embryos did not produce ceruloplasmin. The amount of ceruloplasmin synthesis by the Balb/c 3T3 cell line is enhanced by Rous sarcoma virus-transformation (1.5-3 fold) and by treatment with dexamethasone (about 2.4 fold). The protein was identified as ceruloplasmin by (i) immunoprecipitation with ceruloplasmin-specific polyclonal antibody, and by similarity of (ii) peptide maps, and (iii) subunit molecular weight (135,000 dalton) to that of authentic ceruloplasmin from primary cultures of mouse hepatocytes.

Ceruloplasmin is a 135,000 m.w. α_2-glycoprotein that is responsible for binding of 90-95% of blood plasma copper (1). Among the various functions ascribed to this glycoprotein include: induction of angiogenesis or neovascularization due to its copper binding property, (2, 3), copper transport and homeostasis, participation as an acute phase reactant in response to a number of physiologic and disease states, mediation of ferroxidase, amine oxidase and superoxide dismutase activities (1, 4), and growth promotion of testis cells (5). Patients with a variety of cancers show substantial elevations in serum copper concentration that are due mainly to increased serum ceruloplasmin (6, 7). In normal animals, ceruloplasmin is mainly

The abbreviations used are: DMEM, Dulbecco's minimal essential medium; EDTA, ethylenediaminetetraacetate; HEPES, N-2-hydroxyethyl-piperazine-N'-2-ethane sulfonic acid; PMSF, phenyl methyl sulfonyl fluoride; RSV-3T3, Rous sarcoma virus transformed Balb/c 3T3; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

0006-291X/85 $1.50

synthesized by the liver (8); however, it is not known whether this is the case in tumor bearing animals, or whether the tumor cells also contribute to serum ceruloplasmin.

Previous studies by Klagsbrun et al. (9) have shown that angiogenesis was stimulated by the medium of cultured contact-inhibited (nontransformed) Balb/c 3T3 and SV40 transformed Balb/c 3T3 cells, however the angiogenic factor(s) was not identified. They interpreted their finding to mean that the production of tumor angiogenesis factor is an early event in the cell transformation process that precedes the loss of contact inhibition of growth in vitro. Recently, Gullino and associates reported that ceruloplasmin is angiogenic in the corneal assay (2), and that mouse skin fibroblasts acquired angiogenic capacity by the seventh passage in culture, but were only able to produce tumors after the 18-20th passage (10). These results raised the interesting possibilities that (i) the production of ceruloplasmin by contact-inhibited, preneoplastic Balb/c 3T3 cells might be involved in the anomalous expression of tumorgenicity in these cells (11, 12) and (ii) that the tumor cells might actually be the source of ceruloplasmin as the ability to elicit vascularization is critical for their growth and survival. We presently report that three 3T3 mouse embryonic fibroblast cell lines produce ceruloplasmin. This finding is discussed in terms of the possible involvement of ceruloplasmin in tumorigenicity.

MATERIALS AND METHODS

Reagents: High molecular weight protein standards with [^{14}C] label were purchased from Bethesda Research Laboratories, [^{35}S]-methionine and Enlightning reagent were obtained from New England Nuclear, rabbit antibodies against different human serum proteins from Accurate Scientific, Protein A-bacterial adsorbent and S. aureus V8 Protease from Miles Scientific, dexamethasone and type IV collagenase from Sigma Chemical Co. Tissue culture media, sera, L-glutamine, penicillin and streptomycin were purchased from GIBCO, and twelve-well tissue culture dishes from Costar.

Cell Culture: Balb/c 3T3, Swiss 3T3, NIH 3T3, SV-T2, M-MSV-Balb/c 3T3 mouse embryonic fibroblast cell lines, and Madin-Darby canine kidney (MDCK) epithelial cell lines were obtained from American Type Culture Collection, Rockville, MD. Balb/c 3T3, RSV-3T3 and NIH/MoLV-KiSV-3T3 were also obtained from Miss Elizabeth Lovelace of the Laboratory of Molecular Biology, National Cancer Institute, Rat 1 and RSV Rat 1 fibroblasts from Dr. Richard Hynes of the Massachusetts Institute of Technology, Chinese hamster (CH) fibroblasts from Dr. Martin Humphries of

Howard University and the National Cancer Institute, and the hepatitis B virus antigen-free (13) Hep G2 was obtained from Dr. Barbara Knowles of the University of Pennsylvania. Primary culture of mouse hepatocytes was prepared following the procedure described by Seglen (14) with minor modifications: the calcium-free perfusion buffer was composed of 0.14 M NaCl, 6.7 mM KCl, 44 mM $NaHCO_3$ and 25 mM HEPES adjusted to pH 7.4, and collagenase buffer contained 0.05% of type IV collagenase freshly dissolved in Dulbecco's minimal essential medium with high glucose and 25 mM HEPES at pH 7.4. Primary cultures of mouse embryonic fibroblasts were prepared from 14-18 day old Balb/c embryos by trypsin digestion of the whole embryos or eviscerated embryos as described by Aaronson and Todaro (15).

All the cells were grown in Dulbecco's minimal essential medium (DMEM) with high glucose except Hep G2 which was grown in regular MEM. Medium for the mouse fibroblasts contained 10% heat-inactivated calf serum, that for rat fibroblasts contained 5% heat-inactivated calf serum, and that for CH fibroblast, MDCK cells, Hep G2 cells and primary hepatocytes contained 10% heat-inactivated fetal calf serum. Media were also supplemented with 2 mM of L-glutamine, 50 IU/ml of penicillin and 50 ug/ml of streptomycin.

Immunoprecipitation of metabolic labeled proteins: Cells were plated on 12 well dishes before labeling. Nearly confluent cultures were labeled with 0.1 mCi [^{35}S]-methionine in 0.5 ml of culture medium for 20-24 h. Media were collected by spinning out detached cells or residual vesicles, centrifuged at 12,000 x g for 15 min, PMSF was added to a final concentration of 1 mM and stored at -70°C. Ceruloplasmin was immunoprecipitated at room temperature with 10 ul of rabbit anti-human ceruloplasmin antibody for 60 min followed by incubation with 50 ul of 10% v/v protein A-bacterial adsorbent for 30 min. The immune complexes were sedimented by centrifugation, washed five times with 1 ml of solution containing 50 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.1% SDS, and 1 mM PMSF, pH 7.4. Ceruloplasmin was eluted by boiling in 50 ul of 50 mM Tris-HCl, 4% SDS, pH 6.7. Supernatant was collected after centrifugation, and 10 ul of mixture containing 50% glycerol, 2% β-mercaptoethanol, and 0.25 mg/ml bromophenol blue was added and analyzed on SDS-polyacrylamide gels (16). Fluorograms were obtained with a minor modification of Bonner and Laskey's method (17). Polyacrylamide gels were impregnated with Enlightning prior to exposure to Kodak XAR5 X-ray films.

In many cases, sequential immunoprecipitation was performed with different rabbit antibodies against various human serum proteins. Among the antibodies used besides anti-ceruloplasmin were: anti-α_1-acid glycoprotein, anti-transferrin, anti-albumin, anti-α-haptoglobin, anti-α_1-antitrypsin, and anti-α-fetoprotein. Rabbit anti-bovine fibronectin antibody was a gift from Dr. Wen-Tien Chen of the Department of Anatomy, Georgetown University.

Peptide Mapping: Partial digestion of ceruloplasmin was performed with different concentrations of S. aureus, V8 protease by electrophoresing the protein band from a SDS-gel into a second SDS-polyacrylamide gel as described by Cleveland (18).

Other procedures: Two dimensional gel electrophoresis was performed as described by O'Farrell (19) and protein determination according to the Lowry procedure (20).

RESULTS

Several normal and transformed fibroblastic cell lines were monitored for the production of ceruloplasmin by immunoprecipitation from media or cell lysates of [^{35}S]-methionine labeled cultures. We found, to our surprise, that

Table 1: Expression of Ceruloplasmin by Different Types of Cells

Cells	Origin	Virus	Cp
Balb/c 3T3	Balb/c mouse embryos	-	+
Swiss 3T3	Swiss mouse embryos	-	+
NIH 3T3	NIH mouse embryos	-	-
RSV-3T3	Balb/c 3T3	Rous sarcoma virus (Schmidt-Ruppin)	+
SV-T2	Balb/c 3T3	SV40	-
M-MSV-Balb/3T3	Balb/c 3T3	Murine sarcoma virus (Moloney)	-
NIH/MoLV-KiSV-3T3	NIH 3T3	Moloney leukemia virus with Kirsten sarcoma helper virus wt 3	-
Rat 1 (or F2408)	Fisher rat embryos	-	-
RSV Rat 1 (or FRD-4)	Rat 1	Rous sarcoma virus	-
CH Fibroblast	Chinese hamster lung	-	-
MDCK	Dog kidney	-	-
Hep G2	Human liver tumor	-	+
Hepatocyte	1° culture of Balb/c mouse liver	-	+

The expression of ceruloplasmin was determined by immunoprecipitation of [^{35}S]-methionine labeled secretory proteins. The precipitates were resolved by SDS-PAGE. Fluorograms were obtained to determine the presence of ceruloplasmin in different cell types.

normal Swiss- and Balb/c-3T3 cells synthesized and secreted a glycoprotein precipitated with ceruloplasmin specific antibody (Table 1). The level of production of this protein is enhanced (1.5-3 fold) in Rous sarcoma virus (Schmidt-Ruppin) transformed Balb/c 3T3 (RSV-3T3) cells, but is not detectable in established cell lines transformed by SV40 and murine sarcoma virus (Moloney). Quantitatively, this protein represents about 0.2-0.6% of the total protein secreted by RSV-3T3. In fact, except for hepatocytes, no other normal nor transformed cell linestested produced detectable amounts of this specific protein (Table 1).

To determine if the protein was actually ceruloplasmin or an antigenically related protein, authentic ceruloplasmin was immunoprecipitated from primary cultures of adult Balb/c mouse hepatocytes and compared to the protein produced by the Balb/c fibroblasts with respect to SDS-PAGE. It is obvious from inspection of the fluorogram shown in figure 1 (left panel) that the

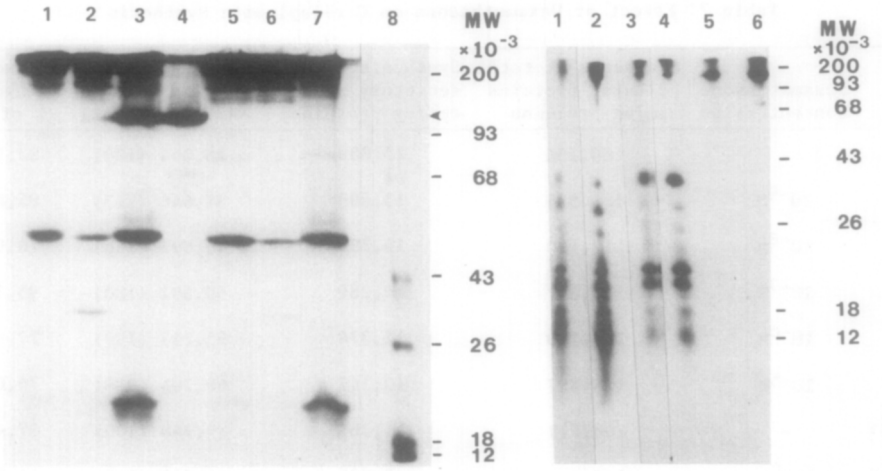

Fig. 1. Left panel. Fluorogram of [^{35}S]-methionine labeled proteins resolved on a 7.5% SDS-polyacrylamide gel. From left to right: immunoprecipitates of secretory proteins from M-MSV-Balb/3T3 (lane 1), NIH/MoLV-KiSV-3T3 (lane 2), RSV-3T3 (lane 3), and primary culture of hepatocytes prepared from Balb/c strain mouse (lane 4) with rabbit anti-human-ceruloplasmin antibodies. The arrow indicates the location of ceruloplasmin. Rabbit anti-human α_1-acid glycoprotein was used as control for background of immunoprecipitation. Immunoprecipitation of secretory proteins from medium of M-MSV-Balb/3T3 (lane 5), NIH/MoLV-KiSV-3T3 (lane 6), and RSV-3T3 (lane 7) with anti-α_1-acid glycoprotein showed nonspecific immunoprecipitable bands. [^{14}C]-labeled high molecular weight standards are indicated in lane 8. Right panel. Peptide mapping of ceruloplasmin from RSV-3T3 cells and Balb/c hepatocytes. From left to right, ceruloplasmin from RSV-3T3 cells (lane 1) and hepatocytes (lane 2) were digested with S. aureus, V8 protease at 1 ug/ml. Shown in lanes 3 and 4 are the banding patterns of ceruloplasmin from RSV-3T3 and hepatocytes digested with 0.2 ug/ml of V8 protease. Lanes 5 and 6 are reruns of ceruloplasmin from a 7.5% gel into a 10% polyacrylamide gel without addition of protease, and lane 5 is ceruloplasmin of RSV-3T3 origin while lane 6 is of hepatocyte origin.

two proteins comigrate with an apparent molecular weight of 135,000 dalton. More definitive identification was obtained by comparative analysis of peptide fragments. Mouse hepatocyte ceruloplasmin and the 135,000 molecular weight protein from mouse fibroblasts were subjected to limited proteolysis by treatment with V8 protease of S. aureus, and the proteolytic fragments generated were separated by SDS-PAGE. It is apparent from inspection of figure 1 (right panel) that identical peptide fragments were obtained with the immunoprecipitated protein from these two sources. Therefore, we conclude that the protein produced by the mouse fibroblasts is ceruloplasmin.

Since ceruloplasmin, like most other serum proteins, is normally produced by hepatocytes, its unexpected production by the mouse fibroblastic cells led us to assay the culture medium for the presence of other plasma proteins.

Table 2: Effect of Dexamethasone on Ceruloplasmin Synthesis

Cells	Dexamethasone concentration	Synthesis of total cellular proteins cpm/ug proteins	Synthesis of total secretory proteins cpm/ug proteins	Ceruloplasmin cpm/mg proteins (% of control)	Fibronectin cpm/mg proteins (% of control)
RSV-3T3	-	108,154	12,089	28,054 (100)	82,378 (100)
RSV-3T3	10^{-9}M	126,585	13,698	31,646 (113)	86,636 (105)
RSV-3T3	10^{-8}M	119,120	15,794	46,693 (166)	76,071 (92)
RSV-3T3	10^{-7}M	120,857	14,289	50,591 (180)	95,336 (116)
RSV-3T3	10^{-6}M	109,129	12,374	55,253 (197)	77,938 (95)
RSV-3T3	10^{-5}M	114,897	13,167	66,704 (238)	74,014 (90)
Balb/c 3T3	-	108,511	11,761	19,748 (100)	87,427 (100)
Balb/c 3T3	10^{-8}M	118,544	9,826	24,852 (126)	41,185 (47)

Cells were pretreated with various concentrations of dexamethasone for 24 h. Media were immunoprecipitated with rabbit anti-fibronectin antibody first, then with anti-ceruloplasmin antibody. No further induction of ceruloplasmin synthesis on RSV-3T3 cells occurred at concentrations higher than 10^{-5}M. Apparent cytotoxicity occurred at 10^{-3}M for RSV-3T3 cells, whereas Balb/c 3T3 cells were sensitive to 10^{-7}M dexamethasone. The experiment was performed twice.

However, we were unable to detect albumin, transferrin, α_1-acid glycoprotein, α-haptoglobin, α_1-antitrypsin and α-fetoprotein using specific antibodies, suggesting that other liver specific proteins were not produced by the fibroblasts.

Ceruloplasmin synthesis in hepatocytes is under hormonal regulation, and treatment of primary hepatocytes with dexamethasone doubles the secretion of this glycoprotein (21), therefore its effect on ceruloplasmin production by the mouse fibroblasts was determined. Dexamethasone stimulated the production of ceruloplasmin by about 2.4 fold in RSV-3T3 cells at 10^{-5}M shown in Table 2. A similar concentration of dexamethasone was toxic to nontransformed Balb/c 3T3 cells, however a slight stimulation of ceruloplasmin synthesis was obtained with a lower concentration (10^{-8}M). The specificity of the dexamethasone induction was determined by monitoring the amounts of 24 major secretory proteins separated by two dimensional gel electrophoresis, and induction of synthesis was found for only 2 of the 24 proteins analyzed, otherwise the amounts of specific secretory proteins were not significantly affected by dexamethasone treatment (data not shown). For example, fibronectin,

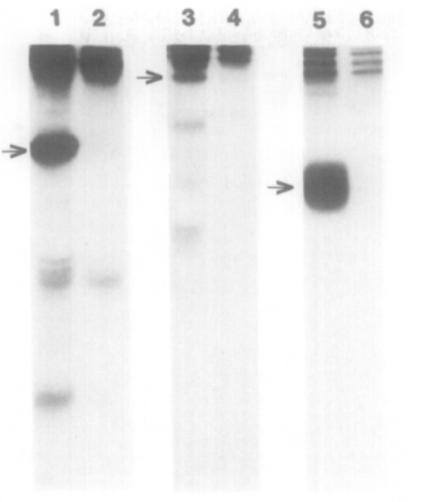

Fig. 2. Fluorograms of immunoprecipitates from culture media of primary and tertiary cultures of mouse fibroblasts from whole embryos. Sequential immunoprecipitation was performed with rabbit anti-human transferrin antibody, rabbit anti-human ceruloplasmin antibody and rabbit anti-human albumin antibody. Protein in immunoprecipitates was revolved by 7.5% SDS-PAGE. Lanes 1, 3 and 5 are immunoprecipitates from primary cultures of mouse embryonic fibroblasts, and lanes 2, 4 and 6 are immunoprecipitates from tertiary cultures. Lanes 1 and 2 are precipitates of anti-transferrin antibody. The arrow indicates the location of transferrin. Lanes 3 and 4 were precipitated with anti-ceruloplasmin antibody, and the arrow shows the location of ceruloplasmin. Lanes 5 and 6 are precipitates of albumin, and the arrow indicates the location of albumin.

a known secretory protein of these cells, was quantitated by immunoprecipitation and no effect of dexamethasone treatment was evident.

To determine if primary cultures of Balb/c mouse embryonic fibroblasts also produce ceruloplasmin, or whether its synthesis is an acquired characteristic of Balb/c 3T3 cells during passage in vitro, cultures were prepared from Balb/c mouse embryos and successive passages were monitored for ceruloplasmin production. While ceruloplasmin was detected in the medium of primary cultures of whole embryos (figure 2, lane 3), it was possibly due to contamination by hepatocytes since other plasma proteins were also produced in large quantities (figure 2, lane 1 and 5). However, ceruloplasmin and other plasma proteins were not present in the medium of tertiary cultures depleted of hepatocytes (figure 2, and lanes 2, 4 and 6), since hepatocytes cultured in serum containing medium do not divide (22). When primary cultures of fibroblasts were made from eviscerated embryos, none of the hepatic proteins including ceruloplasmin were detected (figure 3).

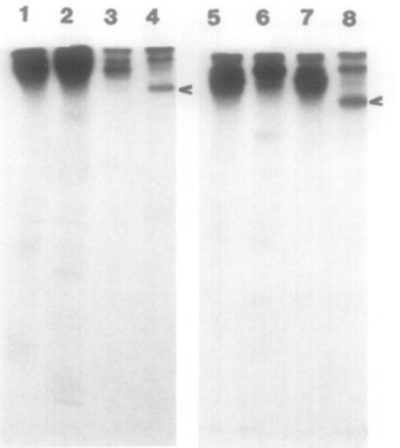

Fig. 3. Fluorograms of immunoprecipitates from culture media of primary and tertiary cultures of fibroblasts from eviscerated embryos and RSV-3T3 cells. For the primary culture, sequential immunoprecipitation was performed with anti-albumin antibody first followed with anti-ceruloplasmin antibody. For the tertiary culture, media was precipitated first with anti-transferrin then with anti-ceruloplasmin. RSV-3T3 cells were similarly treated as controls for ceruloplasmin localization on a 7.5% SDS-polyacrylamide gel. Lane 1 is immunoprecipitate of media from primary culture of eviscerated embryo fibroblasts with anti-albumin antibody. Lane 2 is immunoprecipitate of media from RSV-3T3 cells with anti-albumin antibody. Lane 3 is immunoprecipitate of media from primary culture of fibroblasts with anti-ceruloplasmin antibody. Lanes 4 and 8 are immunoprecipitates of media from RSV-3T3 with anti-ceruloplasmin antibody. Lane 5 is immunoprecipitate of media from tertiary culture of fibroblasts with anti-transferrin antibody. Lane 7 is immunoprecipitate of media from tertiary culture of fibroblast with anti-ceruloplasmin antibody. Arrows indicate the location of ceruloplasmin.

DISCUSSION

The results presented show, for the first time, that Balb/c 3T3, Swiss-3T3 and RSV-Balb/c 3T3 cell lines produce the angiogenic factor ceruloplasmin, and its production is stimulated by dexamethasone treatment. However, it is not produced by primary cultures of Balb/c mouse embryonic fibroblasts. The production of ceruloplasmin by the three mouse embryonic fibroblast cell lines is not an artifact peculiar to cells obtained from a single source, as initial stock cultures originated from two independent sources.

Expression of hepatic proteins by non-hepatocytes is a rare but not exclusive event. For example, during early gestation several organs of embryos can synthesize hepatic proteins (23). The yolk sac for instance can synthesize relatively large amounts of albumin, prealbumin, α_1-antitrypsin,

α-fetoprotein and transferrin; lung can occasionally synthesize trace amounts of transferrin; kidney or placenta can synthesize trace amounts of α-fetoprotein, and embryonic peripheral blood cells can synthesize α_1-antitrypsin, β-lipoprotein and α_2-macroglobulin. Some adult non-liver tissues can also synthesize liver glycoproteins. For example, primary cultures of Sertoli cells can synthesize and secrete at least 12 serum specific proteins (24); normal human lymphocytes synthesize and release α_1-acid glycoprotein (25) and express transferrin mRNA (26). In the wild-derived mouse Mus caroli, α_1-antitrypsin mRNA is uniquely expressed in kidneys at levels approaching that in liver, while other strains of inbred or wild mice do not exhibit this property (27).

The mouse 3T3 cell lines are widely regarded as normal cells in culture, and have been extensively used in studies of cell growth and viral transformation. However, it has been shown that highly vascularized malignant hemangioendotheliomas are produced from a subcutaneous inoculum of glass or plastic beads coated with Balb/c 3T3 cells (11, 12). Therefore, they are not truly normal even though a number of transformed characteristics are not yet evident, and apparently have already acquired the neoplastic characteristic essential for neovascularization and tumorigenicity. In this respect, the synthesis and secretion of ceruloplasmin, an angiogenesis factor, may be part of a cascade of events essential for tumor growth. Studies are currently under way to determine (i) whether the ceruloplasmin produced by the embryonic fibroblastic cell lines induces angiogenesis in the rabbit cornea, and (ii) at which cell passage ceruloplasmin production can first be detected.

ACKNOWLEDGEMENTS

We thank Dr. R. Steven Esworthy for his helpful suggestions, and Mrs. Lucy Kane for typing the manuscript. This work was supported by NIH grants GM 29804 and CA 34918. The abstract of this work has been published in the J. Cell Biol. 99, 339a (1984).

REFERENCES

1. Takahashi, N., Ortel, T.L., and Putnam, F.W. (1984) Proc. Natl. Acad. Sci. USA 81, 390-394.
2. Raju, K.S., Alessandri, G., Ziche, M., and Gullino, P.M. (1982) JNCI 69, 1183-1188.

3. McAuslan, B.R., Reilly, W.G., Hannan, G.N., and Gole, G.A. (1983) Microvas. Res. 26, 323-338.
4. Koj, A. (1974) Structure and Function of Plasma Proteins, Vol. 1, pp. 73-131, Plenum Press, London and New York.
5. Mather, J.P. (1982) In Vitro 18, 990-996.
6. Linder, M.C., Bryant, R.R., Lim, S., Scott, L.E. and Moor, J.E. (1979) Enzyme 24, 85-95.
7. Linder, M.C., Moor, J.R., and Wright, K. (1981) JNCI 67, 263-275.
8. Cederblad, G. (1979) Plasma Proteins, pp. 96-99, John Wiley and Sons, Chichester, New York, Brisbane, Toronto.
9. Klagsbrun, M., Knighton, D., and Folkman, J. (1976) Cancer Res. 36, 110-114.
10. Ziche, M. and Gullino, P.M. (1982) JNCI 69, 483-487.
11. Boone, C.W. (1975) Science, 188, 68-70.
12. Boone, C.W., Takeichi, N., Paranjpe, M., and Gilden, R. (1976) Cancer Res. 36, 1626-1633.
13. Knowles, B.B., Howe, C.C., and Aden, D.P. (1980) Science 209, 497-499.
14. Seglen, P.O. (1976) Methods in Cell Biol. 13, 29-83.
15. Aaronson, S.A., and Todaro, G.J. (1968) J. Cell Physiol. 72, 141-148.
16. Laemmli, U.K. (1970) Nature 227, 680-685.
17. Booner, W.M. and Laskey, R.A. (1974). Eur.J. Biochem. 46, 83-88.
18. Cleveland, D.W., Fischer, S.G., Kirschner, M.W. and Laemmli, U.K. (1977) J. Biol. Chem. 252, 1102-1106.
19. O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007-4021.
20. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
21. Weiner, A.L. and Cousins, R.J. (1983) Biochem. J. 212, 297-304.
22. Enat, R., Jefferson, D.M., Ruiz-Opazo, N., Gatmaitan, Z., Leinwand, L.A., and Reid, L.M. (1984) Proc. Natl. Acad. Sci. USA 81, 1411-1415.
23. Gitlin, D., and Gitlin, J.D. (1975) The Plasma Proteins. Structure, Function, and Genetic Control, Vol. 1, pp. 264-319, Academic Press, New York, San Francisco, London.
24. Wright, W.W., Musto, N.A., Mather, J.P., and Bardin, C.W. (1981) Proc. Natl. Acad. Sci. USA 78, 7565-7569.
25. Gahmberg, C.G., and Andersson, L.C. (1978) J. Exp. Med. 148, 507-521.
26. Lum, J.B., Infante, A.J., Yang, F., and Bowman, B.H. (1984) J. Cell Biol. 99, 334a.
27. Berger, F., and Baumann, H. (1984), J. Cell Biol. 99, 143a.