ity. This suggests that thyroid hormone is directly involved in human ovarian function but the precise mechanism behind its involvement still remains to be elucidated.

1 Ball, J. N., Symp. zool. soc. London. *1* (1960) 105.
2 Leatham, J., in: Reproductive Biology, p. 857. Eds H. Ballin and S. Glasser. Exerpta Medica, Amsterdam 1972.
3 Sage, M., Am. Zool. *13* (1973) 899.
4 Fontaine, Y. A., in: Biochemical and Biophysical Perspectives in Marine Biology, vol. 2, p. 139. Eds D. C. Malins and J. R. Sargents. Academic Press, Orlando/Fla 1975.
5 Sen, S., and Bhattacharya, S., Ind. J. exp. Biol. *19* (1982) 408.
6 Gorbman, A., Dickhoff, W. W., Vigna, S. R., Clark, N. B., and Ralph, C. L., in: Comparative Endocrinology, p. 255. Wiley Interscience Publication, New York/Chichester/Brisbane/Toronto/Singapore 1983.
7 Chakraborti, P., and Bhattacharya, S., Gen. comp. Endocr. *53* (1984) 179.
8 Southren, A. C., Olivo, J., Gordon, G. G., Vittek, J., Briner, J., and Rafii, F., J. clin. Endocr. Metab. *38* (1974) 207.
9 Akande, E. O., Am. J. Obstet, Gynec. *122* (1975) 880.
10 Howland, B. E., and Ibrahim, E. A., Experientia *29* (1973) 1398.
11 Kato, R., Onoda, K., and Omori, Y., Endocr. Jap. *17* (1970) 215.
12 Schneider, G., Kopach, K., Chanian, H., Bonnefond, V., Mittler, J. C., and Ertel, N. H., Endocrinology *105* (1979) 674.
13 Chadrasekhar, Y., D'Occhio, M. J., and Setchell, B. P., J. Endocr. *111* (1986) 245.
14 Ingbar, S. H., and Woebar, K. A., in: Textbook of Endocrinology, p. 117. Ed. R. H. Williams. W. B. Saunders Company, Philadelphia/London/Toronto 1981.
15 Gordon, G. G., and Southren, A. L., Bull. N. Y. Acad. Med. *53* (1977) 241.
16 Chakraborti, P., Maitra, G., and Bhattacharya, S., Gen. comp. Endocr. *62* (1986) 239.
17 Jackson, V., and Chalkey, R., Biochemistry *13* (1974) 3952.
18 Lawson, G. M., Tsai, M. J., Tsai, S. Y., Mingletti, P. P., McClure, M. E., and O'Malley, B. W., in: Laboratory Methods Manual for Hormone Action and Molecular Endocrinology, p. 5. Eds W. T. Schrader and B. W. O'Malley. Houston Biological Ass. Inc. USA 1982.
19 DeGroot, L. J., and Torresani, J.. Endocrinology *96* (1975) 357.
20 Darling, D. S., Dickhoff, W. W., and Gorbman, A., Endocrinology *111* (1982) 1936.
21 Howell, S. H., in: Molecular Techniques and Approaches in Developmental Biology, p. 117. Ed. M. J. Crispeels. John Wiley and Sons. New York 1973.
22 Bernal, J., and DeGroot, L. J., Endocrinology *100* (1977) 648.
23 Oppenheimer, J. H., Koerner, D., Schwartz, A. L., and Surks, M. I., J. clin. Endocr. Metab. *35* (1972) 330.
24 Oppenheimer, J. H., Schwartz, H. C., and Surkes, M. I., Endocrinology *95* (1974) 897.
25 Schuster, L. D., Schwartz, H. L., and Oppenheimer, J. H., J. clin. Endocr. Metab. *48* (1980) 627.
26 Mukku, V. R., Kirkland, J. L., Hardy, M., and Stancel, G. M., Metabolism *2* (1983) 142.
27 Torresani, J., and DeGroot, L. J., Endocrinology *96* (1975) 1201.
28 Samuels, H. H., and Tsai, T. S., Proceedings of the 56th Annual Meeting of the Endocrine Society, Abstr. 74. Atlanta/Ga 1974.

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Vitamins and other metabolites in various sera commonly used for cell culturing

H. Baker, B. DeAngelis and O. Frank

New Jersey Medical School, Departments of Preventive Medicine and Community Health, and Medicine, Newark (New Jersey 07107, USA)
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Summary. Many cell culture media use different sera to enhance growth. We assayed vitamins and some related metabolites in different sera and identified the concentration of: thiamin, biotin, folates, riboflavin, pantothenates, nicotinates, vitamins B_6, B_{12}, A, E, C, and carotenes and some related metabolites: biopterins, free inositol, free and total choline, total carnitines in chicken, horse, rabbit, goat, pig, calf, newborn calf, fetal calf and human sera. Results indicate that vitamin and metabolite content of different sera vary. Such variations could produce fluctuant effects on cell culturings if the metabolite content of the serum is not documented.
Key words. Vitamins; cell culture.

Nearly all cell culture media have additions of serum, ascitic fluid, tissue extracts or the like for enhanced growth[1-4]. Cultured cells in defined or semi-defined media generally contain choline, folic acid, nicotinamide, pantothenate, pyridoxal, thiamin, cyanocobalamin and inositol[3]. Their concentrations in such media are in many instances very low, hence a quantitation of actual vitamin requirements may be obscured by crude supplements commonly employed. Additional vitamins, for example, are usually contributed by serum additions which moreover contribute various growth stimulants for different cells[1-4]. Because of difficulties in identification of significant growth stimulators in serum, defined media are being devised so constituents are kept as low as practicable[2,3]. Although defined media containing salts, amino acids, purines, pyrimidine and some vitamins provide excellent growth for many cell lines, sustained growth for many lines however require that the medium be fortified with an animal or human serum or some equivalent[3,5,6]; many kinds of sera are available for the purpose. However concentrations of vitamins and other metabolites contained in commercially available sera have, to our knowledge, been scantily documented. Such information could guide the investigator's choice of a serum for studying specific cellular metabolic pathways and growth patterns and may avoid confounding results obtained in cell culturings because of metabolite imbalances. Consequently we assayed vitamins and some related metabolites in various animal and human sera.

Materials and methods. Each of four different pooled batches of pathogen, antibiotic and drug-free serum from: chickens, horses, rabbits, goats, pigs, calves, newborn calves, fetal calves and humans were assayed for vitamins and several metabolites (vide infra). Except for human serum, all other sera were from Sigma Chemical Co. (St. Louis, MO) and

Hyclone (Logan, Utah). Pooled human sera were from 30 healthy laboratory personnel not taking drugs or vitamins; samples were not used immediately, but stored frozen (−20 °C) until analyzed. Several heat-inactivated sera were also assayed. Heat-inactivated serum denotes serum which is heat-inactivated in a water bath at 56 °C for 30 min to inactivate complement [7, 8]. Sera were estimated for thiamin, biotin, nicotinates, pantothenates, vitamins B_6, B_{12} and riboflavin with protozoa [9]; total folates were assayed with *Lactobacillus casei* (ATCC 7469) [9]. Vitamins, A, E and C and carotenes were determined spectrophotometrically [9]. Total choline (free choline + phospholipid choline) and free choline (choline + acetylcholine) were each determined with *Torulopsis pintolopessi* (ATCC 36231) [10]; total carnitine with *Torulopsis bovina* (ATCC 26014) (in preparation); biopterins: defined as biological active unconjugated pteridines, by *Crithidia fasciculata* (ATCC 12837) growth response [11]; free inositol analyzed with *Saccharomyces uvarum* (ATCC 9080) (in preparation). The methods used analyze for only the metabolically active forms of metabolites for man and higher animals [9, 12]. Deviation of the mean (±SD) for each serum sample was calculated [13].

Results. Vitamins and other metabolite levels in sera are tabulated in the table.

B_{12} (pg/ml): Rabbit serum had the highest titer: 94,444 ± 962 whereas chicken serum had the lowest 14 ± 2.1. Vitamin B_{12} levels in descending order were: rabbit, horse, human, goat, fetal and newborn calf, calf, pig, and chicken. Heat-inactivation of horse and calf serum did not lower B_{12}; it did lower newborn calf serum by more than 50%.

Folate (ng/ml): Fetal calf serum had the highest titer: 43.8 ± 7.7; goat the lowest: 3.0 ± 0.2. Folate levels in descending order were: fetal calf, calf, newborn calf, horse, chicken, pig, human, rabbit, and goat. Heat-inactivation lowered the folate level in newborn calf serum by approximately 82%.

B_6 (ng/ml): Calf serum had the highest level: 231.3 ± 50.5; newborn calf the lowest: 20.9 ± 1.3. Concentrations of sera in descending order were: calf, chicken, fetal calf, goat, human, rabbit, horse, pig, and newborn calf. Heat-inactivation lowered B_6 in calf serum by approximately 20%; it did not markedly change horse or newborn calf serum levels.

Thiamin (ng/ml): Rabbit serum had the highest thiamin levels: 124 ± 8.3; horse the lowest; 10.9 ± 0.8. Concentrations in descending order: rabbit, fetal calf, pig, chicken, calf, goat, human, newborn calf, horse. Heat-inactivation of horse, calf or newborn calf sera did not markedly affect thiamin levels.

Biotin (pg/mg): Fetal calf was highest: 35,140 ± 4,130; goat serum the lowest: 71.9 ± 34. Sera, in descending order of concentration were: fetal calf, chicken, calf, pig, rabbit, newborn calf, human, horse, goat. Heat-inactivation causes a 20% decline in calf serum biotin content, a 51% decline in newborn calf serum, and 66% decline in horse serum.

Pantothenate (ng/ml): Fetal calf serum had the highest level: 20,420 ± 2700; human serum the lowest: 201 ± 143; in descending order of concentration: fetal calf, newborn calf, chicken, pig, calf, horse, rabbit, goat, and human. Heat-inactivation decreased pantothenate in horse serum by about 48%, newborn calf serum by about 46%.

Riboflavin (ng/ml): Pig serum had the highest level: 326.7 ± 30.6; chicken the lowest: 77.5 ± 13.4; in descending order of concentration: pig, fetal calf, calf, newborn calf, rabbit, human, horse, goat and chicken. Heat-inactivation decreased horse serum by about 14%, newborn calf serum by about 20%, and calf serum by about 24%.

Nicotinate (ng/ml): Newborn calf serum had the highest level: 1260 ± 100, human the lowest: 30 ± 15; concentration in descending order: newborn calf, fetal calf, horse, goat, rabbit, calf, chicken, pig, and human. Heat-inactivation lowered nicotinate in newborn calf serum by 13% but horse or calf serum remained unchanged.

Vitamin A (ng/ml): Chicken serum was highest: 1360 ± 340; newborn calf the lowest: 160 ± 40; in descending concentration order: chicken, pig, human, rabbit, calf and goat, horse, fetal calf, and newborn calf. Heat-inactivation lowered vitamin A in newborn calf serum by 19%, but did not affect the vitamin A content of horse or calf sera.

Carotenes (ng/ml): Chicken serum had the highest: 9830 ± 2850; rabbit, goat, pig, newborn calf and fetal calf had levels below 100 (undetectible); in descending order: chicken, calf, human, and horse, and undetectible levels (below 100) in rabbit, goat, pig, newborn calf, and fetal calf. Heat-inactivation lowered carotene in horse serum from 260 ± 19 to below 100, not so for calf serum.

Vitamin E (μg/ml): Human sera was highest in vitamin E: 11.2 ± 1.2; chicken had 5 ± 1.4 and fetal calf 1.3 ± 0.9 whereas 1.0 or less than 1.0 (undetectable) of vitamin E is contained in the other sera tested.

Vitamin C (μg/ml): There is less than 0.5 μg/ml of ascorbate in all sera tested. The sera were not freshly drawn thus ascorbate is degraded in the processing procedure (see discussion).

Biopterins (ng/ml): Fetal calf serum had the highest: 28.3 ± 3.0; human serum had the lowest, 0.9 ± 0.2, in descending order: fetal calf, rabbit, chicken, pig, newborn calf, goat, horse, calf, human. Heat-inactivation reduced biopterins in newborn calf serum by approximately 87%, horse or calf sera were unaffected.

Inositol (μg/ml): Fetal calf serum had the highest: 152 ± 15.9; human serum had the lowest: 5.7 ± 2.4 in descending order: fetal calf, chicken, newborn calf, pig, goat, calf, horse, rabbit, human. Heat-inactivation did not affect horse or calf serum but lowered inositol in newborn calf serum by approximately 38%.

Carnitine (μg/ml): Goat serum had the highest: 28.3 ± 1.5; whereas fetal calf serum had the lowest, 4.0 ± 0.8, in descending order: goat, rabbit, calf, pig, human, horse, newborn, calf, chicken, fetal calf. Heat-inactivation did not affect carnitine concentration.

Free choline (μg/ml): Chicken serum had the highest: 52.8 ± 8.4; human serum the lowest, 4.6 ± 1.3; in descending order: chicken, pig, newborn calf, rabbit, fetal calf, calf, goat, horse, human. Heat-inactivation did not affect free choline concentration.

Total choline (μg/ml): Chicken sera had the highest total choline: 311 ± 35; fetal calf serum had the lowest, 112 ± 38. In descending order of concentration: chicken, calf, human, pig, rabbit, horse, goat, newborn calf, fetal calf. Heat-inactivation reduced the choline concentration in horse serum by approximately 30%, in calf serum by 12%, and in newborn calf serum by 8%.

Discussion. As repeatedly shown, sera as supplements of cell-culture media may decisively provide growth [3, 6]. In not-fully defined media, mammalian cells in culture satisfy their lipid requirements mainly from serum used in medium [14]; yeast and peptone digests can also serve as alternatives for serum in some instances [15]. Eagle's defined medium, with various modifications over the years, supplemented with 10% heat-inactivated human or fetal serum has been virtually standard for supplying undefined nutrients in large-scale screening for anti-cancer agents [16]. A drawback in growing many, if not most, cells in defined media is lack of essentials which sera provide. Because vitamins by definition can not be synthesized by cells a wide variety of forms and concentration are added to media. Presumably one of the functions of sera is to make up for inadvertent deficiencies in vitamin supply. One danger here is that excess of some vitamins may be detrimental. For example, excess vitamin A, as amply demonstrated, may cause damage to cell membranes [17]. Ex-

Vitamins, biopterins, inositol, carnitine, free and total choline in sera commonly used for cell culturing

	Chicken	Horse	Horse – heat inactivated	Rabbit	Goat	Pig	Calf	Calf – heat inactivated	Newborn calf	Newborn calf – heat inactivated	Fetal calf	Human
Vitamin B_{12} (pg/ml)	14.5 ± 2.1	4608 ± 462	4644 ± 92	94444 ± 962	490 ± 66	48 ± 13	183 ± 6	153 ± 3	282 ± 8	102 ± 27	469 ± 161	552 ± 258
Folate (ng/ml)	12.8 ± 1.1	16.3 ± 0.3	17.0 ± 1.0	8.2 ± 0.5	3.0 ± 0.2	12.1 ± 1.2	21.9 ± 0.7	21.4 ± 3.1	17.4 ± 1.0	2.9 ± 0.4	43.8 ± 7.7	10.9 ± 4.9
Vitamin B_6 (ng/ml)	90.6 ± 12.9	21.0 ± 2.5	21.3 ± 2.1	29.7 ± 3.1	41.3 ± 12.1	21.4 ± 3.1	231.3 ± 50.5	186 ± 23.3	20.9 ± 1.3	24.4 ± 4.0	43.5 ± 13.4	40.5 ± 12.
Thiamin (ng/ml)	48 ± 8.7	10.9 ± 0.8	11.5 ± 0.6	124 ± 8.3	29 ± 1.7	86 ± 7.9	43 ± 4.6	39 ± 1.2	17 ± 2.0	19.2 ± 1.6	87 ± 12.9	21 ± 6.0
Biotin (pg/ml)	3722 ± 255	427 ± 20	171 ± 26	1279 ± 76	71.9 ± 34	1345 ± 30	2033 ± 323	1610 ± 185	657 ± 33	307 ± 49	35140 ± 4130	639 ± 182
Pantothenate (ng/ml)	708 ± 113	506 ± 98	318 ± 20	429 ± 61	291 ± 34	619 ± 34	538 ± 13	582 ± 19	1917 ± 57	1042 ± 38	20420 ± 2700	201 ± 143
Riboflavin (ng/ml)	77.5 ± 13.4	125 ± 7.1	106.7 ± 5.7	169.3 ± 30.4	96.7 ± 15.3	326.7 ± 30.6	250 ± 42.4	191.8 ± 69.3	220 ± 52.9	174 ± 11.5	262 ± 47	126 ± 38
Nicotinate (ng/ml)	340 ± 100	510 ± 60	570 ± 80	480 ± 40	490 ± 50	150 ± 30	340 ± 50	370 ± 30	1260 ± 100	970 ± 40	1180 ± 410	30 ± 15
Vitamin A (ng/ml)	1360 ± 340	190 ± 50	180 ± 50	600 ± 150	420 ± 110	740 ± 200	420 ± 100	440 ± 120	160 ± 40	130 ± 40	164 ± 70	670 ± 140
Carotenes (ng/ml)	9830 ± 2850	260 ± 19	<100	<100	<100	<100	1750 ± 440	1640 ± 410	<100	<100	<100	1480 ± 460
Vitamin E (μg/ml)	5.0 ± 1.4	1.0 ± 0.3	<1	<1	<1	<1	1.0 ± 0.3	1.0 ± 0.3	1.0 ± 0.2	<1	1.3 ± 0.9	11.2 ± 1.2
Vitamin C (μg/ml)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Biopterins (ng/ml)	12.3 ± 1.8	3.6 ± 0.9	3.2 ± 0.3	14.7 ± 2.3	3.7 ± 0.7	7.3 ± 1.4	3.2 ± 0.5	2.8 ± 0.6	5.6 ± 0.9	2.3 ± 0.4	28.3 ± 3.0	0.9 ± 0.2
Inositol (μg/ml)	85 ± 7.1	8.2 ± 1.4	7.0 ± 0.9	7.1 ± 1.0	27 ± 4.4	29 ± 4.0	20 ± 5.0	20 ± 4.1	42 ± 5.7	26 ± 5.1	152 ± 15.9	5.7 ± 2.4
Carnitine (μg/ml)	4.4 ± 0.9	5.8 ± 0.8	5.5 ± 0.6	17.3 ± 2.1	28.3 ± 1.5	10.3 ± 0.4	10.5 ± 1.8	10.3 ± 0.4	5.6 ± 7.6	5.8 ± 0.3	4.0 ± 0.8	6.6 ± 2.4
Free choline (μg/ml)	52.8 ± 8.4	6.1 ± 0.8	5.3 ± 0.8	17.4 ± 2.9	6.3 ± 0.5	32.3 ± 6.1	8.8 ± 0.4	10.9 ± 0.9	18.0 ± 2.6	17.0 ± 2.6	16.8 ± 0.4	4.6 ± 1.3
Total choline (μg/ml)	311 ± 35	196 ± 12	137 ± 31	197 ± 39	137 ± 30	237 ± 9	301 ± 31	264 ± 9	140 ± 38	130 ± 28	112 ± 38	292 ± 37

cesses may be brought in by using sera that have high content of specific vitamins. As shown in the table 1, chicken serum is extremely high in vitamin A (1360 ± 340 ng/ml) compared with newborn calf serum (160 ± 40 ng/ml). Presumably then chicken serum should be avoided when one is concerned about cellular disruption. Different sera may contain varying levels of a particular vitamin (table), e.g. horse and rabbit sera contain high titers of B_{12} whereas calf serum contains approximately 500 times less than rabbit serum. Thus the need or absence of a particular vitamin depends on the medium as a whole, the serum used, and the cell types being cultured. The information in the table may help unravel some of the mishaps in cell culture. Possession of vitamin profiles for a particular serum sample used may provide useful guides to the choice of sera for cell culturings so as to avoid cell growth derangements. Instability of thiamin, riboflavin and vitamin C suggests that those vitamins might also become limiting, especially in serum-free culture media. Sera assayed here (table) contain virtually no vitamin C because of prolonged storage. Fresh animal and human sera (less than 12 h old) contains appreciable vitamin C[12, 18]. During a longer storage period, vitamin C activity disappears not only from sera (table) but from media as well[19] by oxidative degradation. We found that when fresh ascorbic acid (dissolved in phosphate buffered saline pH 7.2 or water) is added to RPMI series (1640) medium fortified with 2% human serum for leucocyte culture, the medium still retains ascorbate activity. If the ascorbate medium is incubated longer than 48 h, ascorbate activity completely dissipates. Heat-inactivation at 56 °C in many instances decreases some vitamins and metabolites in some sera (table). Thus, lowering of vitamin content in heat-inactivated sera should be taken into account when such sera are added to media. Also the sometimes needed anti-oxidant activity of vitamins such as vitamins C and E are lacking in stored sera (table). This may pose a problem when such vitamins are needed for cell culturings to insure anti-oxidant activity.

The titers of metabolites other than vitamins in different sera (table) must also be taken into account. Accordingly, carnitine, inositol, biopterins and choline reflects recognition that they too affect cell metabolism. The role of carnitine in cellular fatty acid oxidation, as an energy source, and other functions is being actively investigated[20]; the phosphorylated forms of inositol are important in mobilization of calcium especially in membrane function[21]; likewise the myriad functions of choline in lipid metabolism and methylations[22]. Biopterins, notably as tetrahydrobiopterin, cofactors the formation of indoleamines and catecholamines[23].

Because degradation of metabolites in sera occurs (even if stored frozen), as well as fluctuations in sera metabolite concentration due to differing dietary pattern of the donors, it becomes important to document the actual metabolite content of a serum sample before cell culture use to obtain proper cell-culturing reference points. Once reference points are obtained, metabolite concentrations can be adjusted by additions or dilutions to fit these points. This procedure then insures standardization of metabolite concentrations when depleted supplies of a specific serum for cell culture are replaced with a supply from new donors.

1 Ham, R. G., Uses and Standardization of Vertebrate Cell Cultures, p. 71. Tissue Culture Assoc., Gaithersburg 1984.
2 Ham, R. G., Methods for Preparation of Media, Supplements, and Substrates for Serum-free Animal Cell Cultures, p. 1. A. R. Liss, New York 1984.
3 Levenbow, L., and Eagle, H., A. Rev. Biochem. *30* (1961) 605.
4 Puk, T. T., Molecular Cell Genetics, p. 37. John Wiley, New York 1985.
5 Healy, G., and Parker, R. J., Cell. Biol. *30* (1966) 539.
6 DeLuca, C., Carruthers, C., and Tretsch, G., Exp. Cell Res. *43* (1966) 451.
7 Treglia, R. P., and Linscott, W. D., Molec. Immun. *17* (1980) 741.
8 Weiler, J. M., Ballas, Z. K., Feldbush, T. L., and Needleman, B. W., Immunology *45* (1982) 247.
9 Baker, H., and Frank, O., Clinical Vitaminology: Methods and Interpretation. John Wiley & Sons, New York 1968.
10 Baker, H., Frank, O., Tuma, D. J., Barak, A., Sorrell, M. F., and Hutner, S. H., Am. J. clin. Nutr. *31* (1978) 532.
11 Baker, H., Frank, O., Bacchi, C. J., and Hutner, S. H., Am. J. clin. Nutr. *27* (1974) 1247.
12 Baker, H., Frank, O., and Hutner, S. H., Modern nutrition in health and disease, 6th ed., p. 611. Lea & Febiger, Philadelphia 1980
13 Bancroft, H., Introduction to Biostatistics, p. 78. Hoeber-Harper, New York 1962.
14 Bailey, J. M., Proc. Soc. exp. Biol. Med. *115* (1964) 747.
15 Holmes, R., J. Biophys. biochem. Cytol. *6* (1959) 535.
16 Toplin, I., Cancer Res. *8* (1961) 1042.
17 Daniel, M., Dingle, J., Glauerb, A., and Lucy, J., J. Cell Biol. *30* (1966) 465.
18 Baker, H., Schor, S. M., Murphy, B. D., DeAngelis, B., Feingold, S., and Frank, O., Am. J. vet. Res. *47* (1986) 1468.
19 Mohberg, J., and Johnson, M. J., J. natl Cancer Inst. *31* (1963) 603.
20 Rebouche, C. J., and Paulson, D. J., A. Rev. Nutr. *6* (1986) 41.
21 Putney, J. W. Jr, TIPS *8* (1987) 481.
22 Shinozuka, H., and Katyal, S. L., Nutritional Pathology, p. 279. Marcel Dekker, Inc., New York 1985.
23 Gál, E. M., in: Handbook of Neurochemistry, vol 1, 2nd edn, p. 281. Plenum Press, New York 1982.

0014-4754/88/11-12/1007-04$1.50 + 0.20/0