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Effect of Polyunsaturated Fatty Acids on Fetal Mouse Brain Cells in Culture in a Chemically Defined Medium

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Abstract: The biochemical and morphological effects of polyunsaturated fatty acids on fetal brain cells grown in a chemically defined medium were studied. Fetal brain cells were dissociated from mouse cerebral hemispheres taken on the 16th day of gestation. After cells had grown in chemically defined medium for 8 days, the proportion of polyunsaturated fatty acids of cultured cells was only one-half of that observed at day 0 and about 1.5 times less than that of cells grown in serum-supplemented medium. Fatty acid 20:3(n-9) was present in cultured cells grown in either chemically defined or serum-supplemented medium, demonstrating the deficiency of essential fatty acids. The reduced amount of polyunsaturated fatty acids in cells grown in the chemically defined medium was balanced by an increase in monounsaturated fatty acids. The saturated fatty acids were not affected. When added at the seeding time, linoleic, linolenic, arachidonic, or docosahexaenoic acid stimulated the proliferation of small dense cells. Besides, we demonstrate that each of the four fatty acids studied was incorporated into phospholipids. Adding fatty acids of the n-6 series increased the content of n-6 fatty acids in the cells, but also provoked an increase in the n-3 fatty acids. Among several combinations of fatty acids, only 20:4 and 22:6, when added to the culture in a ratio of 2:1, restored a fatty acid profile similar to controls (i.e. *in vivo* tissue taken at postnatal day 5). **Key Words:** Fetal brain cells—Chemically defined medium—Polyunsaturated fatty acids. **Bourre J. M. et al.** Effect of polyunsaturated fatty acids on fetal mouse brain cells in culture in a chemically defined medium. *J. Neurochem.* **41,** 1234–1242 (1983).

Brain tissue contains substantially higher amounts of polyunsaturated fatty acids in phospholipids than do other tissues, particularly arachidonic 20:4(n-6) and docosahexaenoic 22:6(n-3) acids. Various kinds of studies have shown that these polyunsaturated fatty acids cannot be synthesized by mammalian tissue, and are derived from external source. For example, the dietary importance of polyunsaturated fatty acids on developing brain has been extensively investigated (Dobbing, 1972; Mead and Dhopeshwarkar, 1972; Paoletti and Galli, 1972; Svennerholm et al., 1972; Crawford and Sinclair, 1972). It is also known that before myelination, the early period of cell proliferation is characterized by a high content of polyunsaturated fatty acids in brain (Sinclair and Crawford, 1972), as well as in peripheral nerve (Yao, 1982) lipids. Furthermore, an increased amount of polyunsaturated fatty acids, in particular 20:4(n-6), was found in regenerated nerve following Wallerian degeneration (Yao, 1982). How neural tissue accumulates the polyunsaturated fatty acids and whether these fatty acids have any widespread action in the nervous tissue are important and unsolved questions.

However, the influence of other organs cannot be totally ruled out in the above *in vivo* studies. Therefore, *in vitro* systems have been used, such as neuroblastoma cells and dissociated cells taken from fetal rat brain and grown in serum-supplemented media. Yavin et al. (1975) have shown that polyunsaturated fatty acids may be important at the time of neurite elongation and synaptogenesis; however, the cultured brain cells are unable to synthesize appreciable amounts of 22:6(n-3) from 18:3(n-3) (lin-

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Abbreviations used: 18:2(n-6), Linoleic acid; 18:3(n-3), Linolenic acid; 20:4(n-6), Arachidonic acid; 22:6(n-3), Docosahexaenoic acid.

olenic acid) (Yavin et al., 1975; Robert el al., 1977; 1978). Thus the question that 22:6(n-3) is an essential fatty acid for nervous tissue can be raised, although it is not even known if n-3 precursor is an essential nutriment for mammals (Tinoco et al., 1979).

Most of the studies on brain cells in culture were carried out in serum-supplemented medium, which is rich in polyunsaturated fatty acids. Therefore, their effect might be difficult to analyze. Serum-free, chemically defined medium (Bottenstein and Sato, 1979) provides better conditions to study the polyunsaturated fatty acid requirements of nerve cells *in vitro,* since it contains very minute amounts of 18:2(n-6) (linoleic acid) and essentially no 18:3(n-3) (Ham, 1965).

The present work was undertaken (1) to determine the fatty acid composition of brain cells grown in this chemically defined medium and to compare their composition with those of the starting cells, of the cells grown in serum-supplemented medium, and of the *"in vivo"* tissue at the same total age; (2) to investigate whether the presence of 20:3(n-9) acid in brain cells results from essential fatty acid deficiency in the chemically defined medium; (3) to determine if adding linoleic, linolenic, arachidonic, or docosahexaenoic acid provokes changes in the fatty acid composition, morphology, and multiplication of the cells; (4) to study the essentiality of both precursors (18:2 plus 18:3) or the products (20:4 plus 22:6) of polyunsaturated fatty acid series in brain cells grown in the chemically defined medium. These results have been presented in abstract form (Bourre et al., 1982).

MATERIALS AND METHODS

Cell culture

Dissociated fetal brain cells were obtained from cerebral hemispheres of 16-day embryos of Swiss mice. Cells were mechanically dissociated in the presence of 10% fetal calf serum as described previously (Faivre-Bauman et al., 1981) and seeded on dishes precoated with gelatine-polylysine and fetal calf serum (Uriel et al., 1981). The dissociated cells were then grown in either medium supplemented with 10% fetal calf serum or a chemically defined medium (Bottenstein and Sato, 1979) containing 1 p*M* 17-β-estradiol (Faivre-Bauman et al., 1981).

Various fatty acids were added to the cultures at the seeding time and renewed at the change of medium on day 5. They were introduced as fatty acids bound to fatty acid-free bovine serum albumin (100 mg/ml). When added alone the amount of polyunsaturated fatty acid in 1 ml of culture medium was 1 μg per 25 μg fatty acid-free bovine serum albumin. When added in combination the concentrations were as follows: 1 μg 18:2 or 20:4 per 25 μg fatty acid-free bovine serum albumin and 0.5 μg 18:3 or 22:6 per 12.5 μg fatty acid-free bovine serum albumin.

Fatty acid analysis

Analyses were performed on (a) freshly dissociated cells at day 0; (b) cells grown in serum-supplemented medium, or (c) cells grown in chemically defined medium for 8 days; (d) the postnatal tissue taken at day 5 (which correspond to the age of the analyzed culture). It was also performed on cells grown in chemically defined medium supplemented with linoleic or linolenic or arachidonic or docosahexaenoic acids at different concentrations. Several combinations of these fatty acids in different concentrations were also tried.

Each analysis was carried out on cells grown in parallel in serum-supplemented medium or in chemically defined medium with or without polyunsaturated fatty acids, from the same dissociated cells.

Cultured cells were first washed three times with normal saline solution and harvested with a rubber spatula. The washed cells were pelleted at 500 *g* and lyophilized. Postnatal brain tissue was dissected and kept dry at −40°C until extraction was performed.

The procedure for lipid extraction has been described elsewhere (Folch et al., 1957; Bourre et al., 1977; Pollet et al., 1979). Free fatty acids were removed from the lipid extract by thin-layer chromatography (Eto and Suzuki, 1971). The lipids remaining at the origin were methylated according to the method of Morrison and Smith (1964). The fatty acid methyl esters were analyzed by gas chromatography on an SE 52 and Carbowax column (Bourre et al., 1978) and on an open tubular capillary column (0.0025 mm diameter, 80 m long) coated with Carbowax 20 M as previously described (Bourre et al., 1981). Measurements were performed on at least four different cultures. On each culture two lipid analyses were made.

[^{3}H]Thymidine incorporation and autoradiography

After different times *in vitro,* cell cultures were incubated at 37°C with 0.5 μCi/ml of [^{3}H]thymidine (26 Ci/mmol) in the chemically defined medium for 8 h. They were extensively rinsed with the culture medium at 4°C, allowed to stand overnight in 10% trichloroacetic acid, and scraped. After centrifugation, the trichloroacetic acid precipitate was dissolved in 0.1 *M* NaOH, and an aliquot was counted for radioactivity.

For autoradiography, following [^{3}H]thymidine incorporation the cultured cells were fixed in 4% paraformaldehyde for 2 h at room temperature. After rinsing with the same medium, the fixed cells were coated with Kodak NTB2 emulsion solution diluted twice and developed after 3 days at 4°C. Cells containing silver grains in their nuclei were counted along two perpendicular axes in six fields of the culture dish.

Neuron identification

Cell cultures were incubated at 4°C for 30 min with 5 μg tetanus toxin (a gift of Dr. Bizzini, Institut Pasteur, Garches). The cultures were then successively exposed to a rabbit anti-tetanus toxin serum (1/50) and fluorescein-conjugated goat anti-rabbit immunoglobulin at 4°C for 30 min each. The cultured cells were then fixed in ethanol-acetic acid (95:5, vol/vol) at 4°C for 10 min. The tetanus toxin-positive cells were examined under an epifluorescence Leitz microscope, and counted along two perpendicular axes of the culture dish. This gave an index of the number of neurons in the dishes.

Electron microscopy

The cells were fixed with glutaraldehyde and embedded within the culture dish as previously described (Benda et

al., 1975). Embedding was performed in Epon, with hyroxy-propyl-methacrylate as dehydrating agent according to Brinkley et al. (1967). Interesting areas were selected with an ocular marker and mounted on araldite blocks according to Picart and Tixier-Vidal (1974). Ultrathin sections were made in a plane either parallel to the surface of the culture or in a perpendicular direction and then subjected to electron microscopy (Siemens Elmiskop I apparatus).

RESULTS

Fatty acid composition of cultured cells

After 8 days *in vitro*, cerebral hemisphere cells obtained from 16-day mouse embryos contain two cell populations, (a) a basal layer of glial cells displaying different stages of differentiation, and (b) on top of it, clusters of small dense cells and refringent neuronal cells with elongating processes. In chemically defined medium, the basal cell layer is far less developed than it is in serum-supplemented medium (Faivre-Bauman et al., 1981).

The fatty acid composition of fetal brain cells grown in either serum-supplemented or chemically defined medium showed a lower proportion of polyunsaturated fatty acids than that of freshly dissociated brain cells or postnatal brain tissue (Table 1). This reduction of polyunsaturated fatty acids, particularly 20:4(n-6) and 22:6(n-3), was more pronounced in cultured cells grown in chemically defined medium than in those grown in serum-supplemented medium. In addition, 20:3(n-9) acid, which is the product resulting from essential fatty acid deficiency, was also found in the cultured cells. Concomitantly, there was an increase of monounsaturated fatty acids. The saturated fatty acids were not, however, substantially affected (Table 2).

Generally speaking, the fatty acid profile of freshly dissociated cells was similar to that of postnatal brain tissue (Tables 1 and 2), except for some differences in minor fatty acids (20:0, 20:1, 20:3, 22:0, 22:1, and 22:5).

Chemically defined medium supplementation of individual polyunsaturated fatty acids

Morphological effects. Two essential fatty acids, 18:2(n-6) and 18:3(n-3), or their metabolic products, 20:4(n-6) and 22:6(n-3), were added to the culture medium in the concentration range 0.1–10 μg/ml. As judged by phase contrast microscopy, the appropriate concentration of polyunsaturated fatty acids for cell growth was 1 μg/ml. Higher doses were toxic, as shown by an increase in the number of floating and dying cells.

Fatty acid-free bovine serum albumin alone caused a spreading of the cultured cells and a slight mitogenic effect, as compared with nonsupplemented chemically defined medium (Fig. 1, a and b). Each of the four polyunsaturated fatty acids (1μg/ml) exerted a greater mitogenic effect than did bovine serum albumin. Particularly, clusters of small, dense, proliferating cells were observed (Fig. 1c). Autoradiographic experiments were performed on days 1, 5, and 7. The highest number of mitoses was found on day 5 in control as well as in treated cultures. Furthermore, on day 5, these experiments confirmed the mitogenic effect of polyunsaturated fatty acids. Two types of labeled cells were observed: flat, big cells and small, dense cells (Fig. 2). The number of radiolabeled nuclei counted by autoradiography was more than twice as high in brain cells grown in the chemically defined medium containing polyunsaturated fatty acids as in control cells (Table 3; Fig. 2). A similar result was also obtained by measuring radiolabeled DNA after precipitation with 10% trichloroacetic acid (Table 4).

Preliminary experiments were performed with the aim of identifying the actively dividing cells. Neurons were visualized by their tetanus toxin binding capacity. When brain cells were grown in the medium containing polyunsaturated fatty acids, the number of neurons was not modified (e.g., 1079 ± 78 neurons in the 20:4(n-6)-treated culture medium versus 1201 ± 84 neurons in the control medium).

TABLE 1. *Percentage distribution of various fatty acid series in cultured brain cells grown with or without polyunsaturated fatty acids*

				CDM					
	D_0	PN5	SSM	—	+18:2	+18:3	+20:4	+22:6	+20:4 + 22:6
Polyunsaturated	34.8	32.7	28.0	19.9	37.7	22.2	34.0	32.1	33.5
n-6	20.9	17.1	15.8	10.7	29.5	7.9	24.9	13.7	18.1
n-3	13.9	15.6	9.0	2.8	8.2	14.3	9.1	18.4	15.4
n-3/n-6	0.66	0.91	0.57	0.26	0.27	1.8	0.36	1.3	0.85
Saturated	38.3	43.3	37.1	35.8	43.0	48.5	40.1	44.9	41.5
Monounsaturated	25.9	24.0	34.9	44.3	19.3	29.3	25.9	22.9	25.0
Saturated/ monounsaturated	1.5	1.8	1.1	0.80	2.2	1.6	1.5	1.9	1.7

Cells were grown for 8 days in different culture conditions. SSM = 10% fetal calf serum-supplemented medium; CDM = chemically defined medium; D_0 = dissociated cell pellet at the seeding time (day zero); PN5: "*in vivo*" tissue taken at postnatal day 5 (5-day-old pups). Results are expressed in percentage of total fatty acids; n-6, linoleic series; n-3, linolenic series.

TABLE 2. *Percentage distribution of fatty acids in cultured brain cells grown with or without polyunsaturated fatty acids*

				CDM					
	D_0	PN5	SSM	—	+18:2	+18:3	+20:4	+22:6	+20:4 +22:6
16:0	16.2	12.6	16.2	14.7	4.3	6.9	6.0	8.1	9.2
16:1	2.9	1.6	3.4	6.0	1.2	1.7	1.9	0.4	1.8
18:0	21.6	28.7	20.6	21.1	36.8	38.3	30.3	31.2	28.4
18:1	21.7	18.7	30.6	37.1	16.2	24.7	19.8	17.8	19.5
18:2(n-6)	0.9	0.9	0.2	1.0	12.8	—	1.6	2.5	1.1
18:3(n-3)	tr	—	—	—	—	0.7	0.8	2.4	—
20:0	0.2	1.2	0.3	tr	0.8	1.8	1.7	2.3	1.8
20:1	0.9	2.5	0.9	1.2	0.7	2.0	2.4	4.1	2.8
20:3(n-9)	—	—	3.2	6.4	—	—	—	—	—
20:3(n-6)	0.3	0.7	0.6	5.1	0.6	—	3.2	1.6	1.2
20:4(n-6)	13.9	11.4	11.5	4.0	6.9	4.6	11.1	4.4	11.8
22:0	0.3	0.8	—	—	1.1	1.5	2.1	3.4	2.1
22:1	0.4	1.2	—	—	1.2	0.9	1.8	0.6	0.9
22:4(n-6)	4.1	3.7	2.1	0.4	6.2	3.0	7.5	4.6	3.6
22:5(n-6)	1.7	0.4	1.4	0.2	3.0	0.3	1.5	0.6	0.4
22:5(n-3)	tr	0.4	1.4	—	1.5	2.0	0.8	0.5	0.4
22:6(n-3)	13.9	15.2	7.6	2.8	6.7	11.6	7.5	15.5	15.0

Abbreviations as in Table 1.

Electron microscopic observations of cultures grown in media supplemented with polyunsaturated fatty acids confirmed the presence of numerous small neuroepithelial cells associated to form clusters of four to six cells (Fig. 3). Some of these cells were larger and possessed several features in common with brain oligodendrocytes in culture as previously described by McCarthy and De Vellis (1980). The cytoplasm displays a granular matrix and contains an irregular network of smooth tubules, as well as numerous microtubules. Conspicuous free polysomes are scattered in the cytoplasm or sometimes concentrated in a small area beneath the plasma membrane. The well-defined Golgi zone is limited by stacks of four or five saccules and displays in its core numerous small vesicles, coated or uncoated (Fig. 4). Such cells often send out cytoplasmic extensions, either irregular or unipolar (Fig. 4). Many intermediary stages between these cells and primitive neuroepithelial cells could be seen.

Changes in fatty acid composition. When linoleic acid 18:2(n-6) bound to fatty acid-free bovine serum albumin was added to the chemically defined medium at the seeding time, it was largely incorporated as such into the cell lipids. It was found to be about 1% of total fatty acids in normal postnatal brain tissue, and 0.2% in cells grown in the culture medium without supplementation of polyunsaturated fatty acids. Addition of 18:2(n-6) to the chemically defined medium resulted in an increase of not only the n-6 series (18:2, 20:4, and 22:4) but also the n-3 series (22:5 and 22:6) of polyunsaturated fatty acids in the cultured brain cells as compared with controls (cells grown in the defined medium without polyunsaturated fatty acids).

A minute amount of 18:3(n-3) was found in the cultured brain cells when 18:3(n-3) was added to the culture medium. However, the elongated and desaturated products of 18:3(n-3), 22:5 and 22:6, were substantially higher in treated cells than in control cells (Table 2). The proportions of total polyunsaturated fatty acids in cultured brain cells were still far below than those found in freshly dissociated cells or postnatal brain tissue (Table 1).

The proportion of 20:4(n-6) was approximately three times higher in brain cells grown in the chemically defined medium supplemented with 20:4(n-6) than in control cells. In addition, the proportions of 22:4(n-6), 22:5(n-6), and 22:6(n-3) were also markedly increased (Table 2).

In contrast to 18:3(n-3), 22:6(n-3) when added to the chemically defined medium was largely incorporated into the cultured brain cells (Table 2). The proportions of total polyunsaturated, monounsaturated, and saturated fatty acids were then essentially the same as in the fetal brain tissue and the freshly dissociated cells (Table 1).

Generally, addition of any individual polyunsaturated fatty acid tested to the chemically defined medium led to an increase of 18:0, 20:0, 22:0, and 22:1 and decrease of 16:0, 18:1, and 20:3(n-6) in the treated brain cells as compared with the control cells (Table 2). The 20:3(n-9) acid was not detected. Addition of fatty acid-free bovine serum albumin to the cultured medium did not cause any significant change in the fatty acid profile of brain cells.

Chemically defined medium supplementation with combined polyunsaturated fatty acids

To test whether brain cell growth requires the presence of either precursors or products of polyunsaturated fatty acids in the chemically defined

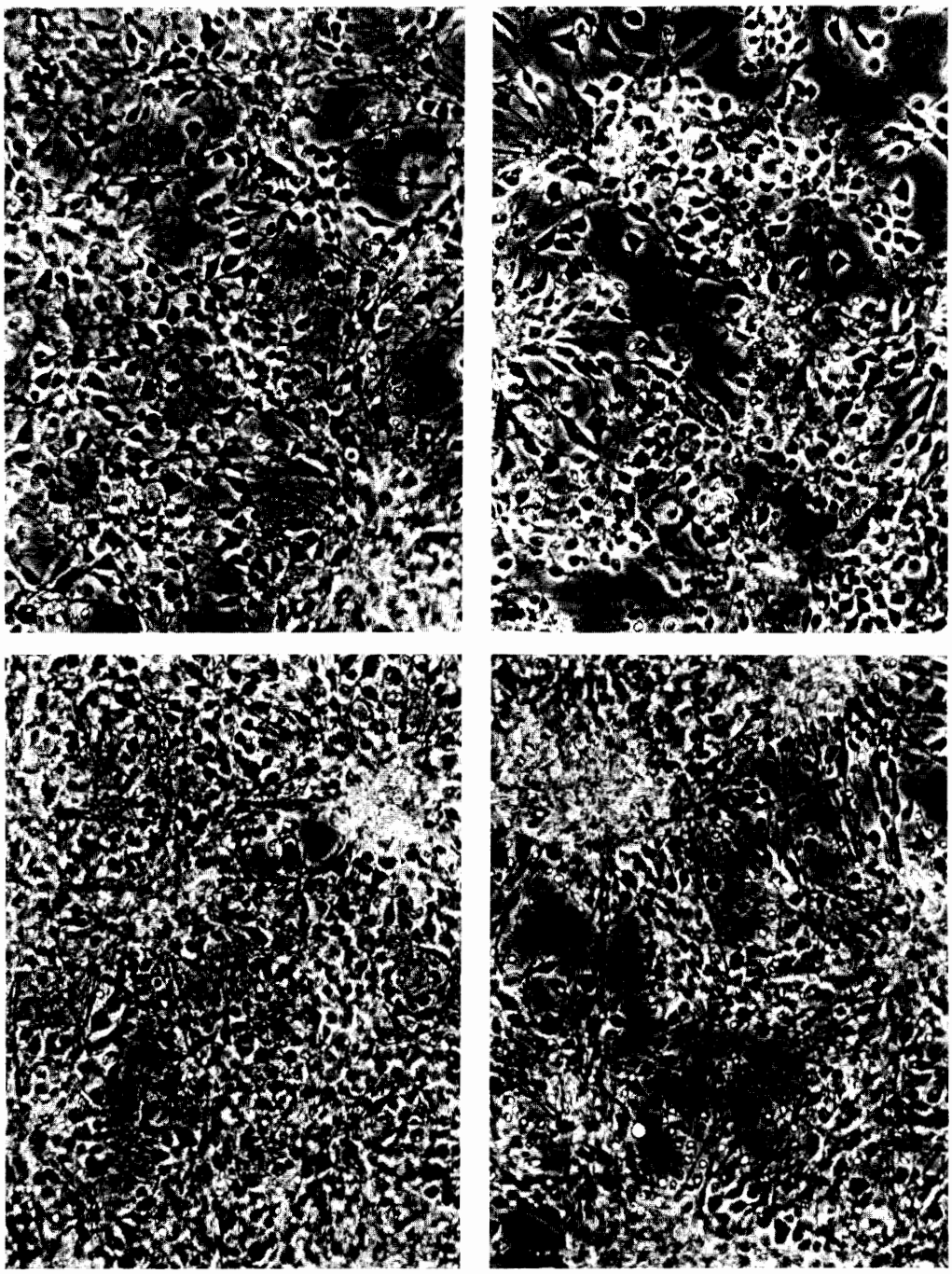

FIG. 1. Morphological effect of polyunsaturated fatty acid supplements. Living cells, observed by phase contrast microscopy, after 6 days *in vitro* (×212). **(a)** Control culture grown in chemically defined medium (CDM). **(b)** CDM + fatty acid-free bovine serum albumin alone (25 μg/ml). **(c)** CDM + 18:2 (1 μg/ml carried by 25 μg/ml fatty acid-free bovine serum albumin). **(d)** CDM + 20:4 (1 μg/ml + 22:6, 0.5 μg/ml, carried by 37.5 μg/ml fatty acid-free bovine serum albumin).

medium, different combinations of polyunsaturated fatty acids from n-3 and n-6 series were tested. When both 18:2(n-6) and 18:3(n-3) (1 μg/ml and 0.5 μg/ml, respectively) were added to the chemically defined medium, the proportion of 20:4(n-6) decreased as compared with cells grown with 18:2(n-6) alone, but increased as compared with cells grown with 18:3(n-3) alone (data not shown). On the other hand, the fatty acid profile of cells grown in the presence of both 20:4(n-6) and 22:6(n-3) (1 μg/ml and 0.5 μg/ml, respectively) was not substantially different from that of postnatal tissue (Table 2).

Addition of combined polyunsaturated fatty acids from the n-3 and n-6 series also resulted in mito-

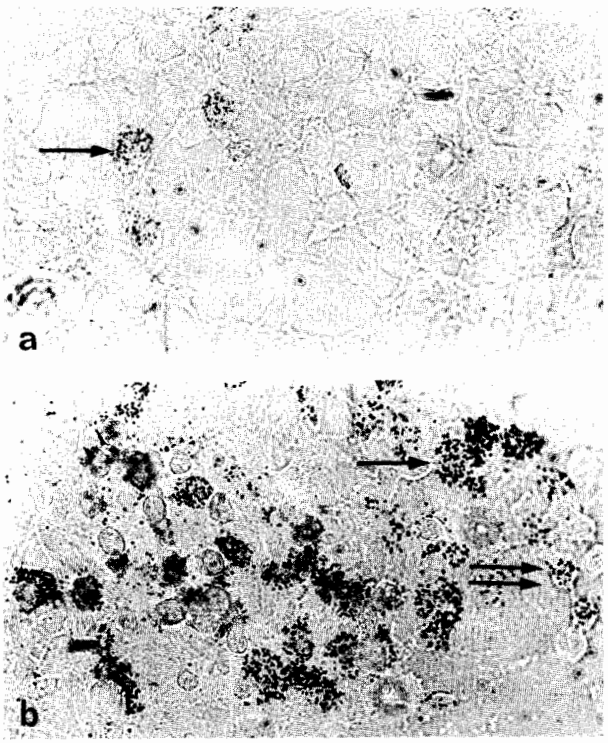

FIG. 2. Autoradiography of [^{3}H]thymidine-labeled cells. On day 5 *in vitro*, cells were incubated for 8 h with [^{3}H]thymidine and processed for autoradiography. **(a)** Fatty acid-free bovine serum albumin-treated culture, exhibiting mainly flat, large labeled cells. **(b)** 22:6(n-3) (1 μg/ml)-treated culture. Two types of labeled cells are observed, flat cells (single arrows) as in the controls, and small rounded cells (double arrows) (×424).

genic effects on non-neuronal cultured cells as judged by either phase contrast microscopy (Fig. 1d) or by [^{3}H]thymidine incorporation (Table 4).

DISCUSSION

Brain cells grown in chemically defined medium, and to a minor extent in serum-supplemented medium, presented a dramatic defect in polyunsaturated fatty acids and biochemical symptoms of essential fatty acid deficiency as shown by the presence of 20:3(n-9). The polyunsaturated fatty acids present in the starting cells were probably largely preserved and reutilized (approximately 70% of the 22:6 as measured with external standard), but they were not sufficient to maintain a normal unsaturation index. Thus it can be speculated that the fluidity of the membrane and physicochemical and, therefore, biochemical properties of the cells were altered. This might account, for example, for th defective morphological maturation of the synapses (Puymirat et al., 1982).

TABLE 3. *Number of radiolabeled cell nuclei in cultures incubated with [^{3}H]thymidine*

Supplements to chemically defined medium	% Control	
	Experiment 1	Experiment 2
None	100	100
18:2(n-6)	198	275
18:3(n-3)	207	256
20:4(n-6)	220	250
22:6(n-3)	272	288

Cultures were grown for 5 days and incubated for 8 h with 0.5 μCi/ml [^{3}H]thymidine. The dishes were processed for autoradiography. In each experiment, the number of radiolabeled nuclei was counted on two dishes, on six fields.

Any individual fatty acid—18:2(n-6), 18:3(n-3), 20:4(n-6), or 22:6(n-3)—added to the chemically defined medium was incorporated into the cell phospholipids. This addition prevented the accumulation of 20:3(n-9) in the cultured brain cells. In the case of linoleic acid, 18:2(n-6), our results obtained with cells grown in serum supplemented-medium confirmed the observation of Yavin et al. (1975) that it does not accumulate in the brain cells. However, addition of 18:2(n-6) to the chemically defined medium resulted in a high proportion of 18:2(n-6) in the cultured cells. This is probably due to substrate saturation and limited desaturase (or elongating) activity within the culture.

As n-6 and n-3 series are not interconvertible, addition of combined polyunsaturated fatty acids was tested. Only when both 20:4(n-6) and 22:6(n-3) were added to the culture medium, at concentrations of 1 μg/ml and 0.5 μg/ml, respectively, could a fatty acid composition of brain cells similar to that of postnatal tissue be obtained.

In this study, polyunsaturated fatty acids were incorporated into the cells when bound to fatty acid-free serum albumin. However the *in vivo* carrier is not known. α-Fetoprotein, which can bind 20:4(n-6)

TABLE 4. *[^{3}H]Thymidine incorporation into DNA (percentage of control)*

		CDM Supplemented with:				
CDM	BSA (100 μg)	18:2(n-6)	18:3(n-3)	20:4(n-6)	22:6(n-3)	20:4(n-6) + 22:6(n-3)
100	125	179	226	271	209	271

Cultures were grown for 5 days and incubated for 8 h with 0.5 μg/ml [^{3}H]thymidine. DNA was precipitated and radioactivity was counted. Each point represents the mean of two dishes. Concentrations of BSA and fatty acids are described in Materials and Methods.

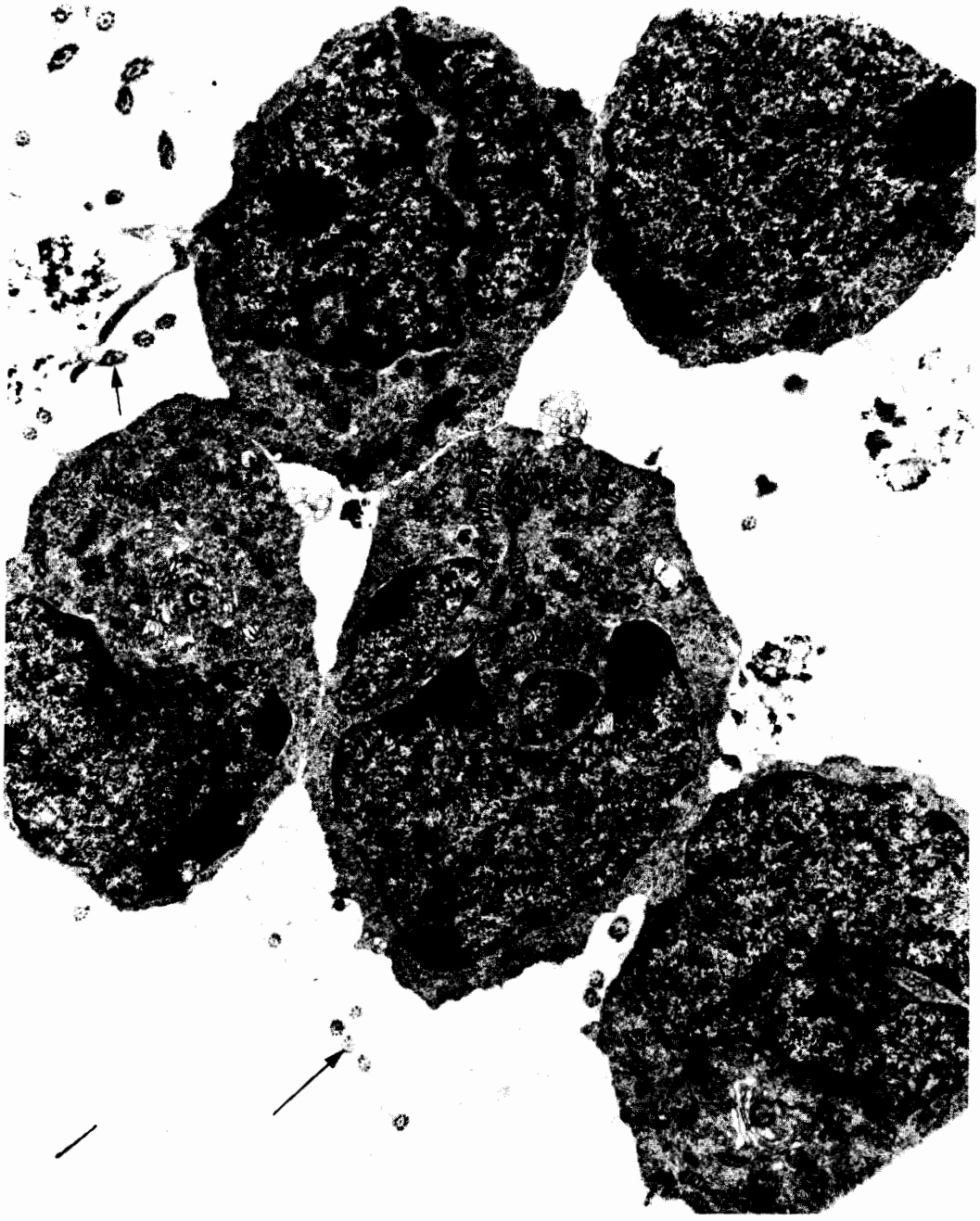

FIG. 3. Electron micrograph of 22:6(n-3)-treated cells. Brain hemisphere cells taken from 16-day-old mouse fetuses were grown for 10 days in serum-free medium supplemented with 1 μg/ml 22:6(n-3). Section through a cluster of five neuroepithelial cells. Note the electron-dense cytoplasm with scattered conspicuous polysomes. Two cells display a conspicuous Golgi zone (G). One can see several transverse sections of ciliae belonging to ependymal cells which are not rare in such cultures (arrows) (×7200)

and 22:6(n-3) (Parmelee et al., 1978; Piniero et al., 1979; Benassayag et al., 1980) is a good candidate.

Addition of polyunsaturated fatty acids to the chemically defined medium not only modified the fatty acid composition but also altered the morphology of the cultured brain cells. They appeared to be mitogenic for glial cells, since the number of neurons remained constant. A similar mitogenic effect was found in C6 glioma cells grown in culture medium containing 18:2(n-6) (Wolfe et al., 1980). In our cultures, several facts suggest indirectly that most of the dividing cells are precursors of oligodendrocytes: first, the morphological observations at the ultrastructural level; second, the biochemical determinations showing an increased amount of saturated and monounsaturated very-long-chain fatty

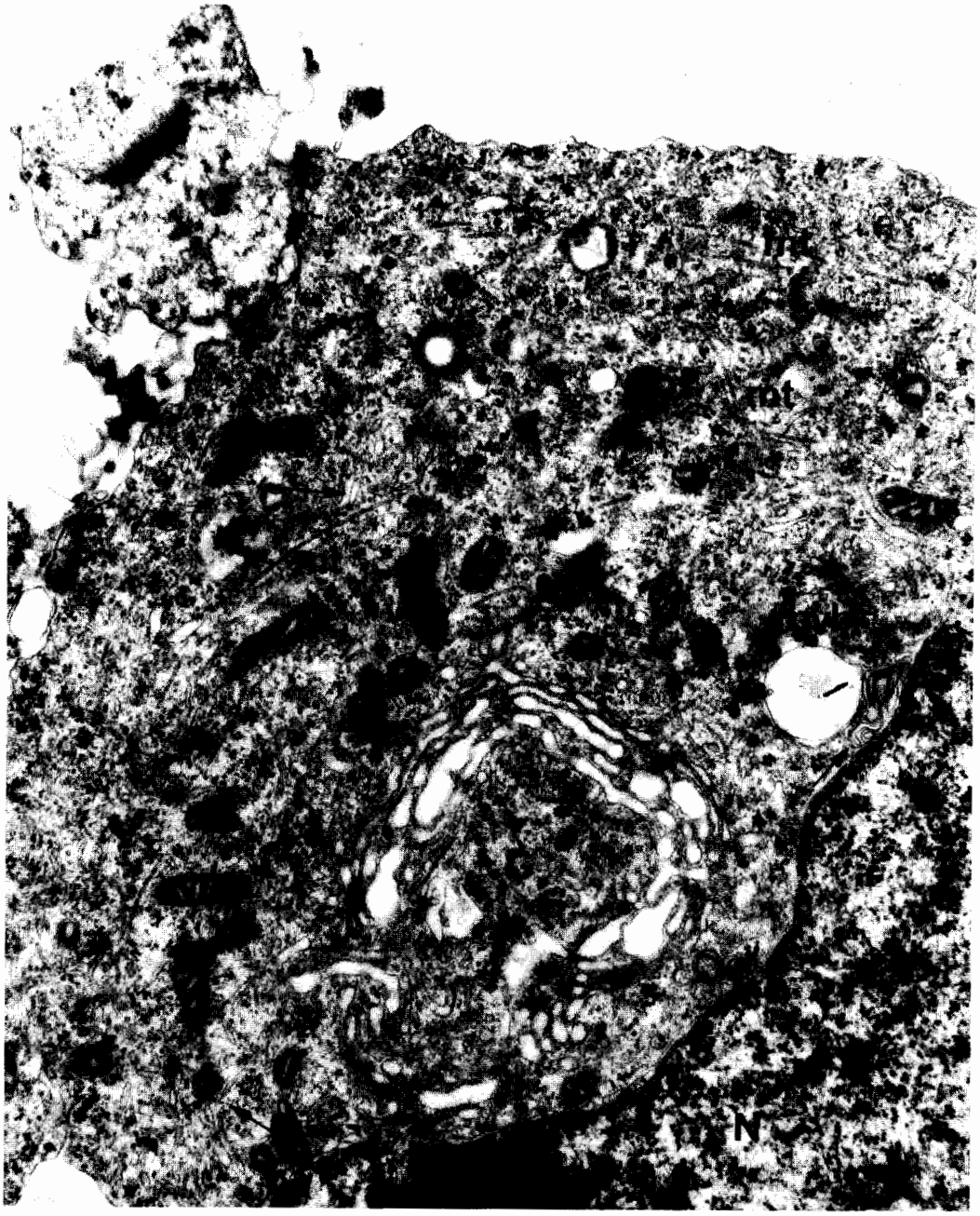

FIG. 4. Electron micrograph of 20:4(n-6)-treated cells. Same culture conditions as in Fig. 3. Higher magnification showing the organization of the cytoplasm and of the Golgi zone in a more mature cell. N:Nucleus; G: Golgi zone; mt: microtubule; mvb: multivesicular body; →, smooth network (×24,000).

acids, which are known to be contained in oligodendrocytes (Morand et al., 1981); third, in preliminary experiments, the lack of mitogenic effect of polyunsaturated fatty acids added to cultured cells from the hypothalamus, a poorly myelinated brain region (manuscript in preparation). In any case, some additional experiments using specific oligodendrocyte markers to confirm this hypothesis are needed.

The hypothesis that the dividing cells are precursors of oligodendrocytes would be in agreement with the results of Honegger and Matthieu (1980) showing that addition of 18:2(n-6) to cultured aggregating cells grown in chemically defined medium can result in increased activity of myelin-related enzymes. In the absence of 18:2(n-6), however, these enzyme activities (Matthieu et al., 1978) and myelin lipid synthesis (Bourre et al., 1979) were relatively reduced.

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