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Effect of starvation on glucose transport and membrane fluidity in rat intestinal epithelial cells

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Studies on the surface area of microvilli (MV), fluidity of brush border membranes (BBM) and D-glucose uptake were carried out in rat intestinal epithelial cells (IEC) during progressive starvation and under re-feed conditions. The surface area of MV, fluidity of BBM and D-glucose transport through IEC membranes showed an increase during starvation when compared to well-fed controls. Re-feeding experiments restored the control values of all the three parameters within a short time. The results showed that the increase in D-glucose transport through IEC membranes during starvation is due to increased surface area of MV and increased fluidity of BBM.

D-Glucose transport; Membrane fluidity; Surface area of microvilli; Starvation; Re-feeding; Rat intestine

1. INTRODUCTION

During progressive starvation, events such as depletion of stored glycogen, increase in lipolysis, ketogenesis and irreversible glucose disposal take place. In other words, the changes in metabolic processes during starvation are such that they ensure the maintainance of an adequate supply of glucose to tissues which have an obligatory requirement for it for the performance of vital functions of the body during the stress condition [1–4]. The microvillus (MV) membrane of enterocytes is highly specialized for digestive functions: the surface is metabolically very active and plays an important role in the state of digestion and the active transport of nutrients [5]. In morphological studies of fasting, it has been reported [6,7] that there was a marked reduction in villus height. However, no information is available on the structural and physiological aspects of the MV membrane during starvation and re-feed conditions.

We, therefore, examined the effects of progressive starvation on functional aspects of brush border membranes (BBM) of enterocytes of the small intestine. Parameters such as surface area of MV, membrane fluidity of BBM and D-glucose transport through intestinal epithelial cell (IEC) membranes of rat were undertaken in the present study. The data indicate that during starvation this membrane system becomes more active for transport of D-glucose by changing its surface area and fluidity. However, following re-feeding these membranes initially become more active, and revert to control conditions later.

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2. MATERIALS AND METHODS

2.1. *Preparation of animals*

Adult male (90 ± 5 days with an average weight of 280–320 g) Wistar rats were used for the present study. These animals were housed in a room with constant temperature (24 ± 2°C) until they were killed. To avoid cannibalism during starvation each rat was housed separately. Groups of 5 rats, for each time point, were fasted for periods ranging from 1 to 8 days; the animals were provided with water ad libitum. The rats in control group were given food and water ad libitum. After 7 days of starvation rats were re-fed for 1–12 h time periods. The control, the starved, and the re-fed animals were killed by cervical dislocation at the appropriate time and anterior portions of the small intestines were dissected out.

2.2. *Electron microscopy*

Small pieces of intestine from control and experimental rats were collected and fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 3 h. After washing, post-fixation in 1% osmium tetroxide in phosphate buffer was carried out for 3 h. The fixed samples were washed, dehydrated and embedded in Araldite. Ultrathin sections were stained with 2% uranyl acetate, post-stained with 0.2% lead citrate and examined in a Jeol 100 CX electron microscope. The number of MV per unit area was counted and their surface area was calculated.

2.3. *Preparation of BBM fraction*

The proximal region of the intestines from well-fed, starved (day 1 to day 7) and re-fed rats was taken and rinsed thoroughly with ice-cold 0.15 N NaCl. The intestinal cells were collected by scraping the inverted intestine with a glass side in buffer I (50 mM mannitol, 2 mM Tris/HCl, pH 7.1). BBM was prepared according to the method of Kessler et al. [8]. The protein content of the fraction was estimated by Lowry's method [9]. Membrane purity was checked by alkaline phosphatase assay [10], and observation of negatively stained preparations using an electron microscope.

2.4. *Measurement of fluidity*

Fluorescence measurements for membrane fluidity using pyrine as fluorescent probe were recorded on a Hitachi F-4000 fluorescence

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spectrophotometer operated in the ratio mode with 5 nM excitation and emission band pass. Pyrene, to a final concentration of 4 μM, was incorporated into BBM preparations in stabilizing buffer containing 60 mM KCl, 1 mM EDTA, 10 mM imidazole and 1 mM $MgCl_2$ (pH 7.2). The excitation wave length used was 333 nm, the monomer emission was observed at 372 nm and 392 nm and the eximer emission was at 470 nm. The emission spectra of free pyrene were recorded from 350 to 500 nm. The eximer/monomer intensity ratio was calculated as a ratio of the intensities at 470 nm and 372 nm, respectively. Spectra were recorded with increasing protein concentration until the saturation point was reached.

2.5. D-glucose transport

About 2 cm of the proximal region of the intestine was inverted and incubated in 4 ml of Hank's buffer (pH 7.4) containing cold D-glucose in various concentrations, (100 μM to 1 mM) and 5 μCi of ^{14}C-labelled D-glucose (specific activity 210 mCi/mM, obtained from BARC, Bombay, India). Incubation was done for 5–30 min at room temperature with constant gentle shaking. The concentration of cold D-glucose and the period of incubation was standardized. Intestinal pieces from control and experimental rats were incubated for the fixed (20 min) time and washed in 40 ml of washing solution (0.154 M NaCl, 10 mM HEPES/Tris, pH 7.4) as suggested by Treves et al. [11]. IEC were scraped from the washed intestine, homogenized and radioactivity was counted in Packard liquid scintillation counter 2200 CA TRICARD. To find the effect of Na^+, ethanol, temperature, etc., on D-glucose transport, experiments were carried out in the presence and absence of Na^+ or ethanol in Hank's buffer. Temperature-dependent D-glucose transport was studied by incubating the intestines at 4, 25 and 37°C.

3. RESULTS

Progressive starvation results in atrophy of villi and crypts. The intestinal lumen of starved rats is lined by short and blunt villi. The luminal surface of well-fed adult rat IEC showed well-developed MV (about 17 MV per unit area) (Fig. 1a), however, in starved rats long and thin cylindrical MV were observed (about 27 MV per unit area) on the cell surface. The surface area of these MV was found to the 2.6-fold higher in 6-day-starved intestine compared to well-fed intestine (Fig. 1b). By day 8, MV were less in number per unit area compared to day 6 starvation and MV were shed-off from IEC. On re-feeding the 7 day fasted rats, the MV tended to occupy almost the same area as in controls by 6 h of re-feeding (Fig. 1c).

The activity of alkaline phosphatase in the BBM fraction was found to be 14-fold higher than that of total homogenate (Table I). In negatively stained BBM preparations, the membranes appeared as vesicles; mitochondria, nucleus, lysosome and Golgi bodies were absent. However, some contamination of endoplasmic reticulum was found, which was less than 10%.

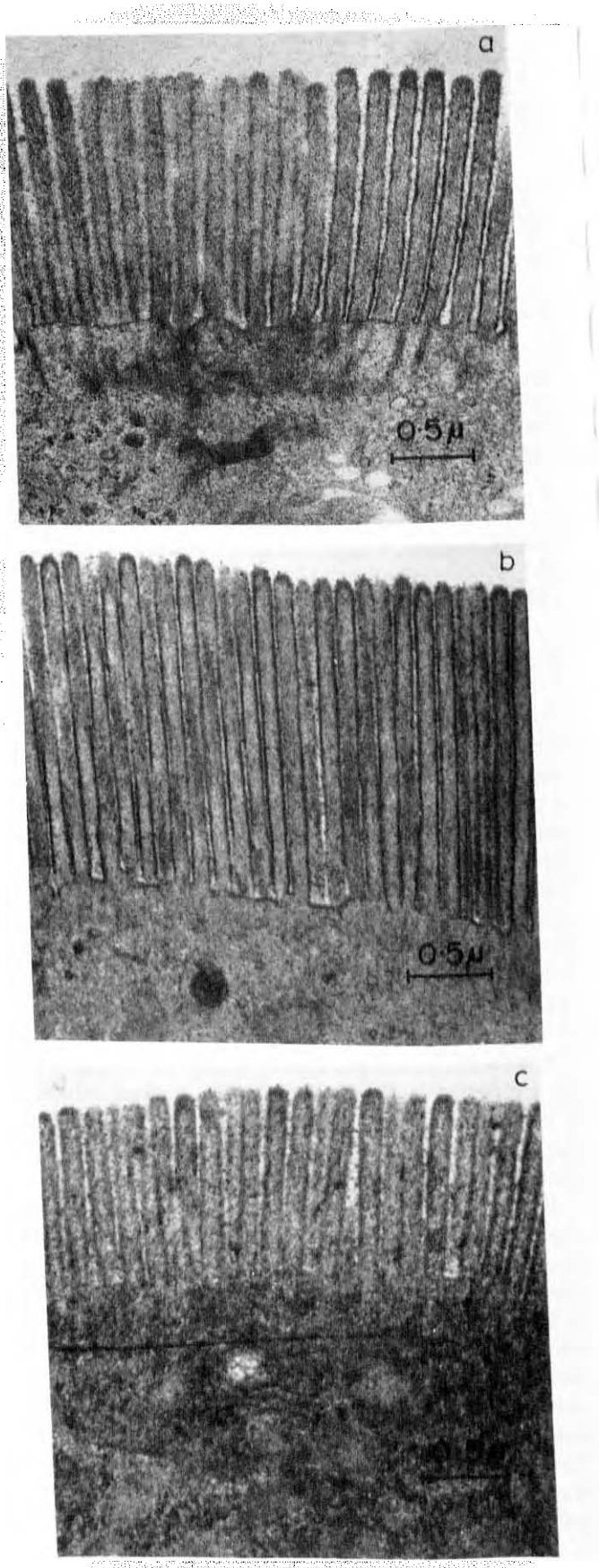

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Fig. 1. Electron micrographs of a portion of intestinal epithelial cells showing distribution of microvilli (MV) on the luminal surface of the cell. (a) In a well-fed rat, the MV are short and less in number per unit area. (b) In a 6 day starved rat, the MV are narrow, long and slender in shape. (c) In a rat re-fed 6 h after 7 days starvation, the MV are short and broad and are comparable to the well-fed rat. Magnification ×24,500.

Table I

Specific activity of alkaline phosphatase in the homogenate and brush border membranes of rat intestine

Experimental condition	Homogenate	Membrane fraction	Increased activity (fold) in membrane fraction
Well-fed	0.293	3.97	13.5
3-days starved	0.324	5.0	15.5
6-days starved	0.293	4.05	13.8
3 h re-fed after 7 days of starvation	0.312	5.0	16.0

Enzyme activity is expressed in μmol/mg protein/min.

Pyrene has been used to study the fluidity of BBM. Fig. 2 shows the effect of membrane protein concentration on the eximer/monomer emission intensity. The ratio increased with increasing concentration of the membrane protein and reached a saturation point at 250–300 μg protein. The eximer/monomer ratio at saturation point is given in Table II. The eximer formation was more prominent in starved rat membranes and reached a maximum at day 6 of starvation (Fig. 3a). On re-feeding, this ratio (470 nm/372 nm) went up after 2 h and then came down to close to the control level after 12 h (Fig. 3b). The whole set of experiments were repeated at least 6 times and found to be consistent.

The D-glucose transport levels in well-fed, fasted (day 1 to day 7) and re-fed rats are shown in Table II. Transport was increased during starvation, reaching a maximum on day 6 (about 2-fold higher compared to well-fed rats, Fig. 3a). On re-feeding 7-day-fasted rats, D-glucose transport increased within 4 h (2.5-fold to control) and then decreased (Fig. 3b). Fig. 4 shows the effect of Na^+ on D-glucose trnsport; it is about 6-fold higher to that of transport in the absence of Na^+. However, with or without Na^+, the ratio of D-glucose transport in starved to well-fed rats remains the same. Similarly with ethanol, although there is a reduction in glucose uptake in controls and starved rats, nevertheless, there is greater uptake in the starved rats (data not shown). Transport at low temperature (4°C) was only 0.125-times that of transport at 25°C, and transport at 25°C was found to be higher than that of transport at 37°C. However, the pattern of increase and decrease in transport of D-glucose due to temperature remained the same in starved and well-fed rats.

Table II

Fluidity of brush border membranes and D-glucose transport through intestinal epithelial cell membranes during starved and re-fed conditions

Experimental condition	Fluidity (eximer/monomer ratio)	D-glucose transport (pmol/mg protein ± S.D.)
Well-fed	0.220 ± 0.003	258 ± 22
1 day starved	0.242 ± 0.007	390 ± 28
2 days starved	0.263 ± 0.003	459 ± 100
3 days starved	0.262 ± 0.005	457 ± 105
4 days starved	0.267 ± 0.025	402 ± 104
5 days starved	0.285 ± 0.013	457 ± 109
6 days starved	0.307 ± 0.015	544 ± 109
7 days starved	0.290 ± 0.026	482 ± 110
2 h re-fed*	0.326 ± 0.026	570 ± 114
4 h re-fed*	0.308 ± 0.025	632 ± 120
8 h re-fed*	0.255 ± 0.015	432 ± 108
12 h re-fed*	0.235 ± 0.012	348 ± 70

*after 7 days of starvation

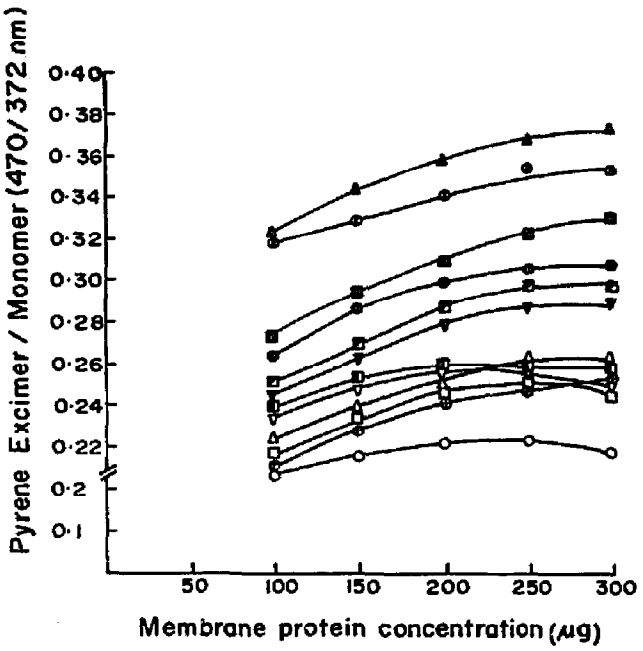

Fig. 2. Eximer/monomer intensity ratio of pyrene (4 μM) incorporated into brush border membranes of intestinal epithelial cells from well-fed, starved and re-fed conditions, as a function of membrane protein concentration. Well-fed (○); starved for 1 (□), 2 (△), 3 (▣), 4 (▽), 5 (◩), 6 (●) or 7 (■) days; re-fed for 2 (▲), 4 (⊗), 6 (▼) or 12 (⊕) h.

4. DISCUSSION

Glucose transport plays a pivotal role in providing energy for cellular growth and other vital functions. Various chemical factors such as insulin-like growth factor [12], epidermal growth factor [13], transforming growth factor [14], and hormones such as insulin [15], estradiol [16,17] and FSH [18] stimulate glucose transport. Recently Schron [19] has demonstrated that pH change modulates brush border folate transport. Treves et al. [11] have shown a high rate of metabolic transport in young rat intestine when compared to the adult rat. Presence of Na^+ and change in temperature also have predominant effects on D-glucose transport through IEC membranes [20]. Semi-starvation induced a significant increase in total D-glucose transport [20–22]. However, no details are available on D-glucose transport during advanced starvation (up to 7 days) and re-feed-

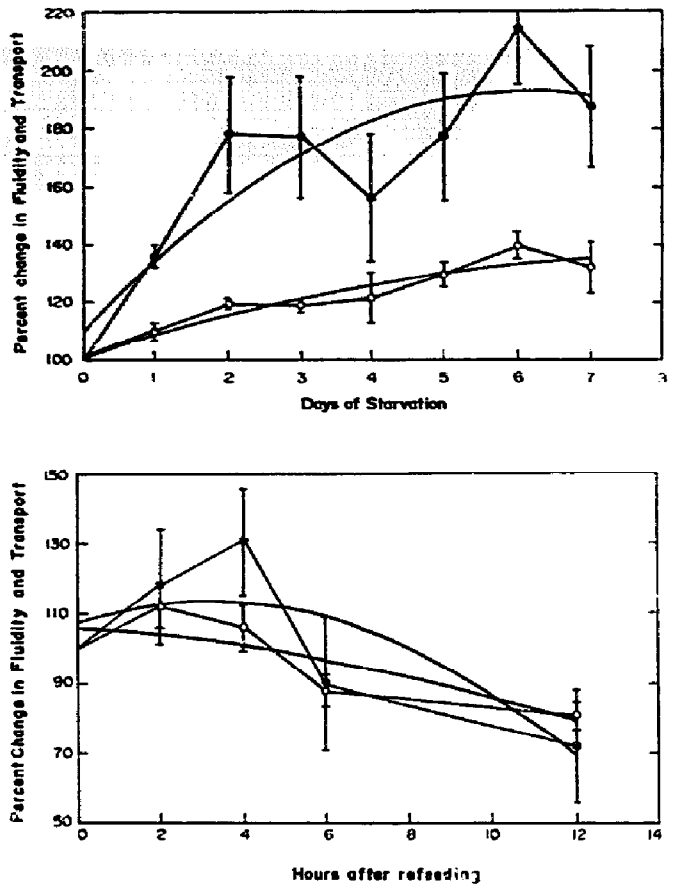

Fig. 3. (a) Changes in D-glucose transport and membrane fluidity of well-fed rats and starved rats. The values of the well-fed rats are taken as 100%. (b) Changes in D-glucose transport and membrane fluidity under re-feeding conditions. 7 day starvation values are taken as 100%. ○, fluidity; ●, transport.

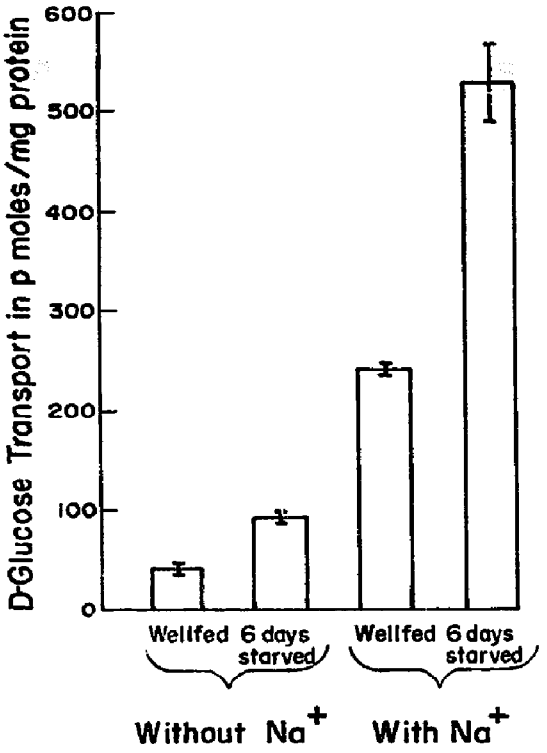

Fig. 4. Histogram showing D-glucose transport in the presence and absence of Na^+ through intestinal epithelial cell membranes from control and 6-day-starved rats.

ing conditions. The present study shows the enhancement of the D-glucose transport during progressive starvation which reached its peak value on day 6 of starvation.

It is quite understandable that during starvation there is a need for large amounts of energy for vital cellular processes. During starvation elevated levels of gluconeogenic enzymes which in turn break down stored glycogen [4,7], increase lypolysis and ketogenesis to provide more glucose to the system via the gluconeogenic pathway. However, the mechanism underlying the increased uptake of glucose during starved conditions is not well understood. The present study sheds some light on the mechanisms of higher uptake of glucose during starvation conditions. This could be accomplished either by an increase in the absorptive area or by a change in the BBM so as to facilitate increased transport of D-glucose. In the present morphometric study on MV of IEC, it was found that by day 6 starvation, the MV had become very slender and were present in greater number per unit area (the surface area of MV increased by a factor of 2.6). Similarly, in the membrane fluidity study, it was found that the membranes became more fluid during progressive starvation; the maximum fluidity of these membranes was recorded on day 6 of starvation.

Membrane fluidity is affected during various physiological conditions such as ageing [23] and cell division [24] and following treatment with various chemicals such as *n*-aliphatic alcohol [25], testosterone [26] and estradiol [27]. Many possible mechanisms have been described [28] in the literature by which membrane fluidity might be enhanced. For example, unsaturation of phospholipid fatty acyl groups [29], phospholipid methylation [30] and cholesterol depletion [31]. Membrane fluidity is known to affect the permeability to metabolites such as D-glucose [22,32] and acetate [33]. Recently Dudeja et al. [34,35] have shown that chemically induced fluidity in BBM affects the uptake of D-glucose and L-glutamate. In the present study we have shown that starvation brings fluidity changes in the BBM which in turn facilitates enhanced D-glucose transport across IEC membranes.

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REFERENCES

[1] Shekspheare, P., Srivastava, L.M. and Hubscher, G. (1969) Biochem. J. 111, 63–67.

[2] Heitman, D.W., Pool, T.B. and Cameron, I.C. (1980) J. Anat. 180, 603–615.
[3] Sugden, M.C. and Holness, M.J. (1990) Biochem. J. 268, 77–81.
[4] Holness, M.J. and Sugden, M.C. (1990) Biochem. Soc. Trans. 18, 591–592.
[5] Kenny, A.J. and Booth, A.G. (1978) Essays Biochem. 14, 1–43.
[6] Altman, G.G. (1972) Am. J. Anat. 133, 391–400.
[7] Lata, S., Srivastava, L.M. and Gupta, P.D. (1978) Ind. J. Exp. Biol. 16, 461–464.
[8] Kessler, M., Acuto, O., Storelli, C., Murev, H., Muller, M. and Semenza, B. (1978) Biochim. Biophys. Acta 506, 136–154.
[9] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–273.
[10] Thempi Dorai, D. and Bachhawat, B.K. (1977) J. Neurochem. 29, 503–512.
[11] Treves, C., Favilli, F., Stio, M., Iantomasi, T. and Venunzii, M.T. (1990) Mech. Ageing Dev. 52, 263–276.
[12] Mita, M., Borland, K., Price, J.M. and Hall, P.F. (1985) Endocrinology 116, 987–992.
[13] Bames, D. and Colowich, S.P. (1976) J. Cell Physiol. 89, 633–639.
[14] Inman, W.H. and Colowich, S.P. (1985) Proc. Natl. Acad. Sci. USA 82, 1346–1349.
[15] Czech, M. (1980) Diabetes 29, 389–409.
[16] Smith, D.E. and Gorski, J. (1960) J. Biol. Chem. 243, 4169–4174.
[17] Srivastava, L.M., Datta, L. and Gupta, P.D. (1978) J. Steroid Biochem. 9, 838.
[18] Hall, P.L. and Mita, M. (1984) Biol. Reprod. 31, 863–869.
[19] Schron, C.M. (1990) J. Memb. Biol. 118, 259–267.
[20] Brot-Laroche, E., Serreno, M.A., Delhomme, B. and Alvarado, F. (1986) J. Biol. Chem. 261, 6168–6176.
[21] Debnam, E.S. and Levin, R.J. (1975) J. Physiol. 252, 681–700.
[22] Brot-Laroche, E., Dao, M.T., Alcalde, A.I., Dulhomme, B., Triadon, N. and Alvarado, F. (1988) Proc. Natl. Acad. Sci. USA 85, 6370–6373.
[23] Brasitus, T.A., Yeh, K.Y., Holt, P.R. and Schachter, D. (1984) Biochim. Biophys. Acta 778, 341–348.
[24] Cheng, S. and Levy, D. (1979) Arch. Biochem. Biophys. 196, 424–429.
[25] Fernandez, Y.J., Biogegrain, R.M., Cambon Gros, C.D., Mitjavila, S.E. (1984) Biochim. Biophys. Acta 770, 171–177.
[26] Recio, M.N., del Hoyo, N., Carmena, M.J. and Perez Albarsanz, M.A. (1985) Int. J. Biochem. 17, 1129–1132.
[27] Reddy, A.G., Shivaji, S. and Gupta, P.D. (1989) J. Steroid Biochem. 33, 1229–1233.
[28] Jain, M.K. (1983) in: Membrane Fluidity in Biology, vol. 1 (R.C. Aloia ed.) Academic Press, New York, pp. 1–37.
[29] King, M.E. and Spector, A.A. (1978) J. Biol. Chem. 253, 6493–6501.
[30] Mirata, P., Strittmatter, W.J. and Axelrod, J. (1979) Proc. Natl. Acad. Sci. USA 79, 368–372.
[31] Dave, J.R. and Witorsch, R.J. (1984) Biochim. Biophys. Acta 772, 321–327.
[32] Conard Kessel, W.J., Beck, C.A. and Harner, W.C. (1988) Mol. Cell. Endocrinol. 57, 125–131.
[33] Recio, M.N., del Hoyo, N. and Perez Albarsenz, M.A. (1988) Biochem. Int. 16, 1–13.
[34] Dudeja, P.K., Harig, J.M., Wali, R.K., Knaup, S.M., Ramaswamy, K. and Brasitus, T.S. (1991) Arch. Biochem. Biophys. 284, 338–345.
[35] Dudeja, P.K., Wali, R.K., Harig, J.M. and Brasitus, T.A. (1991) Am. J. Physiol. 260, 586–594.