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Lipid Composition of the Plasma Membrane Isolated from Normal and Precancerous Rat Bladder Epithelium

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Summary. Four-week-old Wistar strain rats were given 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) solution for 12 weeks and used as a precancerous model. Sixteen-week-old rats of the same strain were used as controls. Plasma membranes (PMs) were prepared from normal and precancerous bladder epithelium. The lipid content and the fatty acid compositions of total lipids, phospholipids, and their subclasses were analysed. The following observations were made: 1) Precancerous PMs had longer chains composed of more unsaturated fatty acids than the normal ones. 2) There were high amounts of arachidonic acid in rat bladder epithelium, and it accounted for more than fifty percent of phosphatidylethanolamine. PMs of precancerous epithelium had more arachidonic acid than those of the normal epithelium. These findings may be useful for the early detection of precancerous epithelium.

Key words: Plasma membrane, Lipids, Bladder, Carcinogenesis, N-butyl-N-(4-hydroxybutyl)nitrosamine.

In spite of successful treatment of a superficial vesical tumour, there is a definite tendency for new neoplasms to develop elsewhere in the bladder. It is thought that with a tumor elsewhere in the bladder epithelial cells have an increased susceptibility to neoplastic change. It is therefore important to detect epithelia with early cancerous changes. Many investigations have been considered as markers for bladder tumor by morphological and/or biochemical techniques, but there remains no reliable marker to predict precancerous epithelia.

Many variations in the PM structure during carcinogenesis of rat bladder epithelia have been reported. Increased concanavalin A agglutinability [10], heterogenous localization of alkaline phosphatase and 5′-nucleotidase [14], and increased membrane potential [8] have been observed. However, there have been no reports of lipid changes in the PMs during carcinogenesis of rat urinary bladder. Fatty acid alterations in the PMs during rat bladder carcinogenesis by BBN were investigated in this study.

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Materials and Methods

Animals. Four-week-old male Wistar strain rats were given water containing 0.05% BBN for twelve weeks to produce precancerous bladder epithelia. Sixteen-week-old male rats of the same strain were used for the normal control group. They were killed by decapitation.

Chemicals. BBN was obtained from Nakarai Chemical Co., Kyoto, Japan and given as a 0.05% solution in water. Fluorescein mercuric acetate (FMA) was from Sigma Biochemical Co., London, England. All other reagents acquired were of the analytical grade.

Isolation of PMs from Rat Bladder Epithelia

PMs of the rat bladder epithelial cells were prepared according to the method of Hicks and Ketterer [7], modified as follows. The scrapings of 10 bladder epithelia treated with FMA were suspended in 5 ml of 0.02 M Tris-HCl, pH 8.0 and homogenized by 15–20 strokes of the tight B-type pestle with a Dounce homogenizer (Kontes, New Jersey Vinland). Homogenates were mixed with an equal volume of 60% (w/v) sucrose and layered over 12 ml of 45% (v/w) sucrose and then centrifuged for 45 min at 4,000 g (average). The supernatant was diluted to 70 ml with distilled water and centrifuged for 1.5 hr at 100,000 g. The resulting pellet was suspended in 2 ml of 17.5% sucrose and layered over discontinuous density gradients each consisting of 1.5 ml of 20, 25, 30, 35, 40 and 45% (w/v) sucrose and centrifuged for 6 hrs at 70,000 g (average). The 30, 35 and 40% sucrose layers were collected, diluted to 35 ml with distilled water, and then centrifuged for 1.5 hr at 77,500 g. The pellet was used for PM fraction.

Lipid Analyses and Protein Determination

The total lipids of PMs were extracted according to the method of Bligh-Dyer [3]. The phospholipids were separated from this extract by thin-layer chromatography which was developed with the solvent

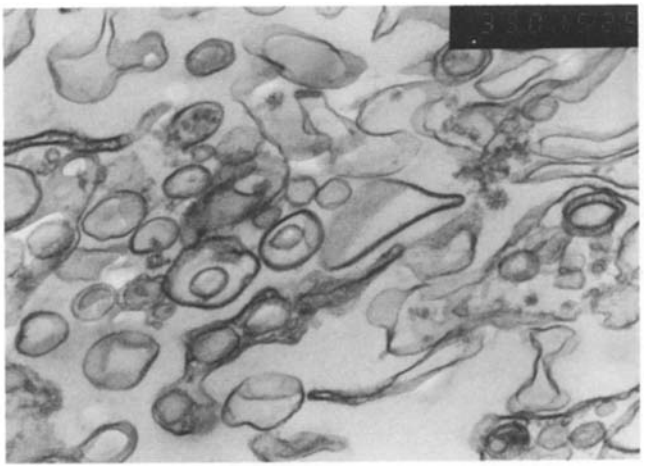


Fig. 1. Electron micrograph of the plasma membrane fraction prepared from epithelia of normal rat bladders. Asymmetrically thickened membranes are shown and have no structures within them

Table 1. Contents of protein and fatty acids in the plasma membranes of epithelia obtained from normal and precancerous rat bladders

	Normal[a]	Precancerous[a]
Total Proteins (μg)	258	497
Total Fatty Acids (μg)	44.7	113
$\frac{\text{Total Fatty Acids}}{\text{Total Protein}}$ (%)	17.3	22.5

[a] Results were obtained from 10 rats

system of petroleum ether-diethyl ether-acetic acid (100:12:1, by vol.), and fractionated into phospholipid subclasses according to Skipski et al. [12]. Each spot was visualized by iodine vapor, scraped into tubes and extracted by chloroform-methanol (2:1) for fatty acid analysis. The preparations for gas chromatography has been reported previously [15]. The amount of total fatty acid was calculated by the sum of the areas of each peak with pentadecanoic acid used as an internal standard.

Protein content was determined according to the method of Lowry as modified by Bensadoun and Weinstein [2].

Results

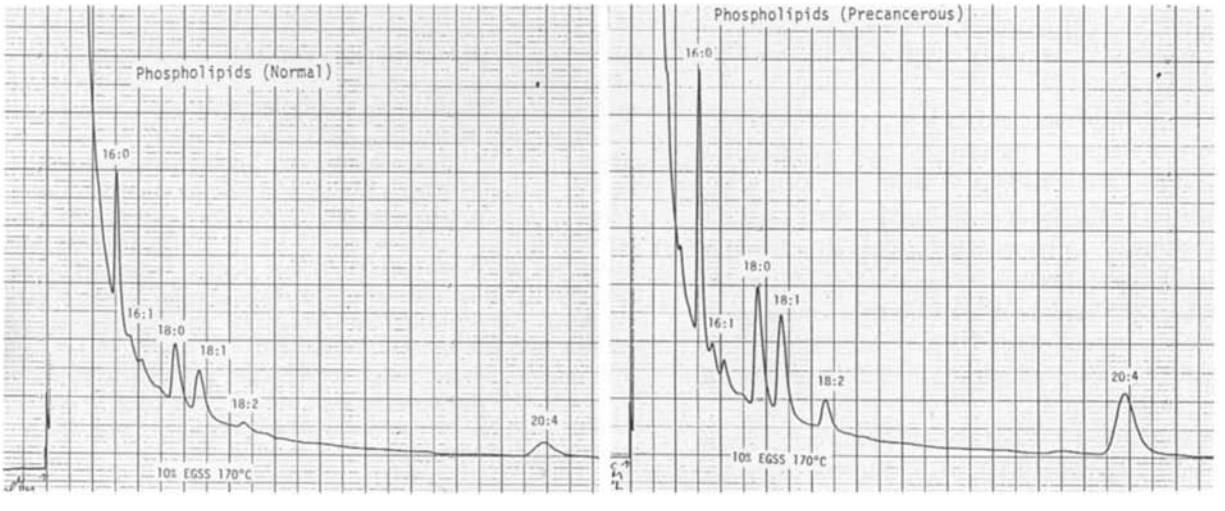
The precancerous rats weighed 320 to 480 g (n = 10), (mean weight 394 g). Normal rats weighed 300 to 460 g (n = 10), (mean weight 393 g).

Figure 1 shows an electron microphotograph of the PMs isolated from bladder epithelia of normal rats. Membranes were asymmetrically thickened, and there were no intramembraneous structures usually seen in mitochondria, lysosomes, secretory vesicles and other organella.

Table 1 compares contents of protein and total fatty acids in the PMs isolated from normal rat bladder epithelia with those from precancerous epithelia. The amounts of protein and fatty acids in precancerous PMs were about 2 and 2.5 times larger respectively than those in normal ones, indicating the hyperplastic changing of precancerous rat bladder epithelia.

Figures 2, 3 and 4 show gas chromatograms of fatty acids in phospholipids, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) of the PMs isolated from normal and precancerous bladder epithelia.

Table 2 shows major fatty acid compositions in phospholipids, PC and PE of the PMs from normal and precancerous



a b

Fig. 2a, b. Gas chromatograms of fatty acid analysis on the phospholipid of normal (**a**) and precancerous (**b**) plasma membranes. Major fatty acids are shown as abbreviations of 16:0 (palmitic acid), 16:1 (palmitolecic acid), 18:0 (stearic acid), 18:1 (oleic acid), 18:2 (linoleic acid), and 20:4 (arachidonic acid). The column was packed with 10% EGSS and column temperature was 170 °C

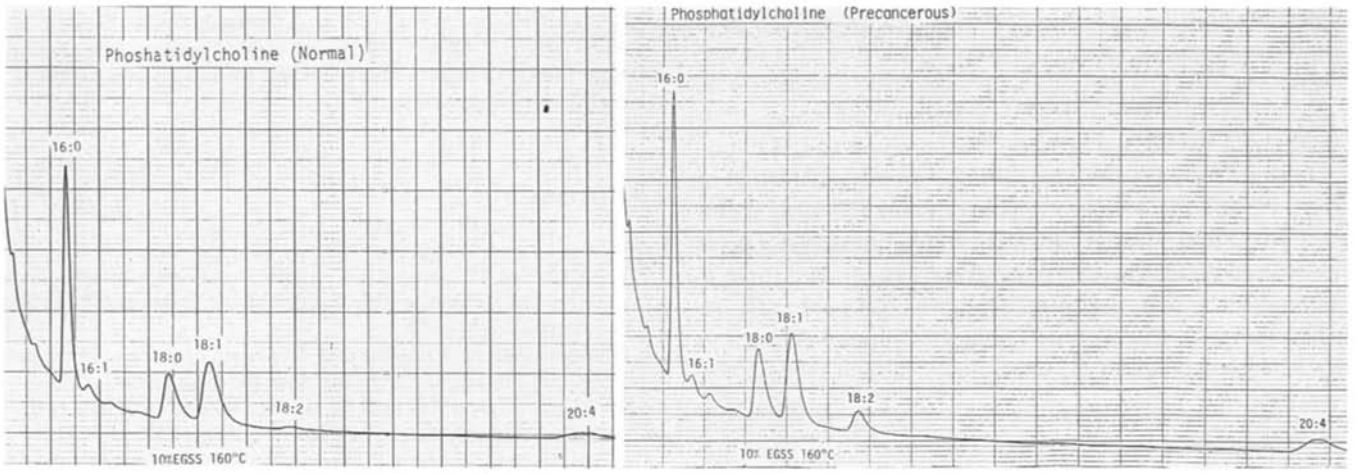


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Fig. 3a, b. Gas chromatograms of fatty acid analysis on the phosphatidylcholine of normal (a) and precancerous (b) plasma membranes. Abbreviations are the same as Fig. 2. Column temperature was 160 °C

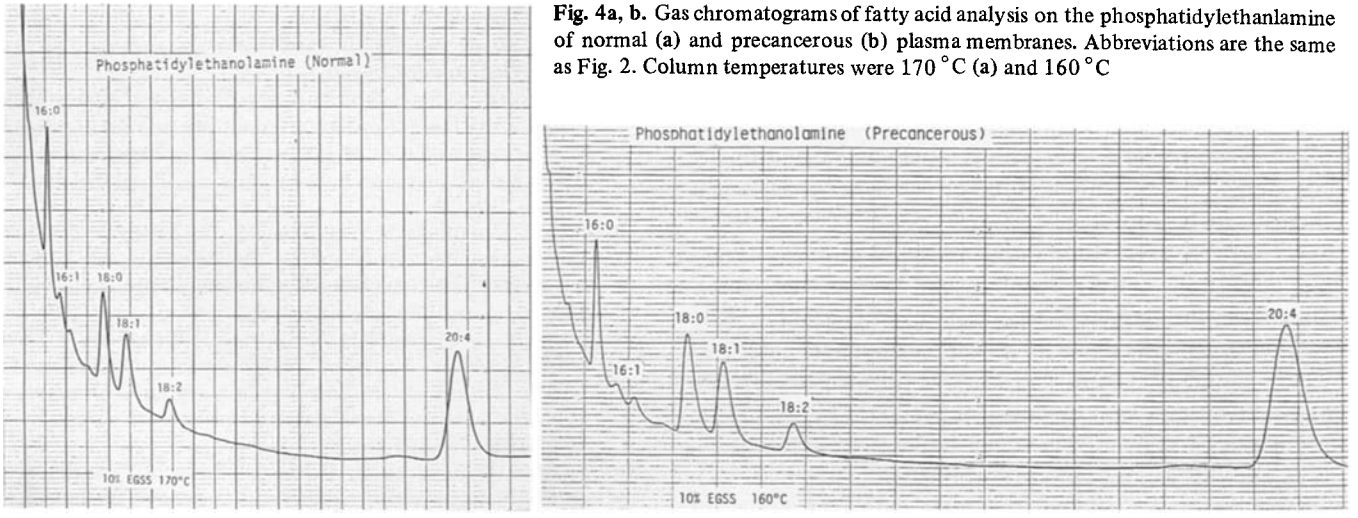

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Fig. 4a, b. Gas chromatograms of fatty acid analysis on the phosphatidylethanlamine of normal (a) and precancerous (b) plasma membranes. Abbreviations are the same as Fig. 2. Column temperatures were 170 °C (a) and 160 °C

Table 2. Major fatty acid composition of the plasma membranes of epithelia obtained from normal and precancerous rat bladders

fatty acid	Phospholipids		Phosphatidylcholine		Phosphatidylethanolamine	
	Normal	Precancerous	Normal	Precancerous	Normal	Precancerous
16:0	28.1 ± 1.4[a]	23.7 ± 2.5	44.1 ± 2.0	38.0 ± 1.7	14.4 ± 3.5	7.7 ± 1.0
			$P < 0.02$[b]		$P < 0.05$	
16:1	3.1 ± 0.8	1.8 ± 0.3	3.1 ± 1.1	2.5 ± 0.6	2.8 ± 1.7	0.7 ± 0.2
18:0	22.0 ± 0.4	19.2 ± 3.0	18.3 ± 2.0	16.2 ± 0.6	16.5 ± 4.7	10.9 ± 0.2
18:1	18.4 ± 2.6	18.0 ± 1.6	26.3 ± 3.3	27.0 ± 1.9	12.3 ± 2.1	10.1 ± 0.4
18:2	3.8 ± 0.3	7.2 ± 1.2	3.0 ± 1.9	7.4 ± 0.6	4.0 ± 1.8	5.1 ± 0.1
	$P < 0.02$		$P < 0.05$			
20:4	24.6 ± 3.5	37.8 ± 5.0	4.7 ± 1.4	9.0 ± 3.3	50.0 ± 8.8	65.1 ± 0.6
	$P < 0.05$				$P < 0.05$	
unsaturated[c] / saturated	0.997 ± 0.065	1.357 ± 0.275	0.602 ± 0.021	0.847 ± 0.076	2.360 ± 0.818	4.390 ± 0.035
			$P < 0.01$		$P < 0.02$	

[a] weight percentages of total fatty acids (mean ± S.D)
[b] Student's test (experimental number = 3)
[c] unsaturated to saturated fatty acids ratio (mean ± S.D.)
Fatty acid abbreviations are shown in the legend of Fig. 2

bladder epithelia. The phospholipids, linoleic acids (18:2) and arachidonic acids (20:4) were more abundant in precancerous PMs than in normal ones. Linoleic acid in precancerous PMs was also increased compared with normal PMs in PC, and arachidonic acid in PE was also elevated in precancerous PMs. On the other hand, palmitic acid (16:0) of both PC and PE were less in precancerous PMs than in normal ones. The ratios of unsaturated to saturated fatty acids of PC and PE in precancerous PMs were larger than those in normal ones, and a similar trend was observed with the phospholipids. These results indicated that precancerous PMs had longer and more unsaturated fatty acids than normal PMs.

Discussion

This is the first report focusing on variations of the fatty acids which constitute phospholipids, major PM components, during rat bladder carcinogenesis. We think that the fatty acids in PMs are possible markers for precancerous bladder epithelia.

During carcinogenesis of rat bladder by BBN, epithelia first undergo simple hyperplasia, followed by nodular or papillary hyperplasia, progressing to papilloma, and finally carcinoma [9, 13]. Only nodular or papillary hyperplasia is thought to be a precancerous epithelium, because simple hyperplasia is a reversible stage and can be returned to normal epithelium if BBN is withdrawn [9]. We observed differences in the fatty acid compositions of phospholipids in PMs between normal and precancerous rat bladder epithelia which have both simple and nodular (or papillary) hyperplasia. If we take only nodular (or papillary) hyperplasia as precancerous sample, there may be larger differences between normal and precancerous epithelia.

The unsaturated acyl chains (oleate and palmitoleate, for example) produced membranes of a fluid state [11] and increased permeability [5, 6]. We found that the acyl chains of phospholipids of precancerous bladder epithelia were more unsaturated than those of the normal ones. Therefore, precancerous bladder epithelia may be more permeable than normal epithelia and, as a result, irritable molecules in urine may penetrate submucosa and induce symptoms of cystitis such as uregency, frequency, and strangury. However, increased permeability may be beneficial to the intravesical instillation therapy of anticancer agents, which will penetrate precancerous epithelial cells more easily than normal cells.

Noteworthy in this work was the observation that rat bladder epithelia had high amounts of arachidonic acid which accounted for more than fifty percent of PE. And, precancerous epithelia had more arachidonic acid than normal ones. These observations may be closely related to the fact that bladder epithelial cells are able to produce prostaglandins [1, 4]. But, whether precancerous bladder epithelial cells can produce them or not is unknown. We are attempting to examine this question.

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