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Age-related changes in enzyme activities, protein content and lipid composition of rat kidney brush-border membrane

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Age-related changes in enzyme activities, protein electrophoretic patterns and lipid composition of kidney-brush-border membranes were studied in 10–20- and 30-month-old male and female Wistar rats. Polyacrylamide gel electrophoresis of membrane proteins revealed very little changes with increasing age, whether males or females were considered. The K_m of three hydrolases – maltase, L-aminopeptidase and alkaline phosphatase – were not affected by age while the V_{max} of maltase and alkaline phosphatase, but not of L-aminopeptidase, decreased from 10 to 30 months. The phospholipid to protein ratio which remained constant between 10 and 20 months, rose in both sexes from 20 to 30 months. In males, the cholesterol content of the membrane increased faster than that of phospholipid and the cholesterol over phospholipid ratio was then greater at 30 months than at 10 months, while in females this ratio remained unchanged in the course of aging. The fatty acid composition of the brush-border remained more or less constant with age in female rat whereas in the male, a 10% decrease in the proportion of arachidonic acid from 10 to 30 months was responsible for a lower unsaturation index.

Introduction

Aging more or less affects the lipid composition of cell membranes as well as the activity of their enzymes. Grinna and Barber [1], and O'Bryan and Lowenstein [2] reported that in rat kidney, the specific activities of some membrane-bound hydrolases decreased between 6 and 24 months and that this was due to a drop in the V_{max} without alteration of the K_m of the enzymes. These results have recently been extended by Reiss and Sacktor [3] who showed that the native maltase of rat kidney brush-border membrane and its highly purified form exhibited a lower V_{max} and a constant K_m in 25-month-old animals when compared with those of 6-months-old animals. This was due to a modification in the relative proportion of active and inactive enzyme forms present in the membrane of old rats [4]. However, not all brush-border enzymes appeared to be modified with age. O'Bryan and Lowenstein [2] did not find significant differences in the activity of alkaline phosphatase in 6- and 24-month-old male Charles River rats, although in the same preparations the maltase activity was diminished. This raised the questions whether some renal enzymes escape the aging process and remain unaltered, or whether their reduction in activity is delayed when compared with others such as maltase. According to the latter hypothesis, it would be expected that a change in alkaline phosphatase activity would appear later in the life of the animal. To investigate this point, we therefore compared the changes in the kinetic parameters of maltase, alkaline phosphatase and

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; L-aminopeptidase, leucine-aminopeptidase.

L-aminopeptidase from kidney membranes of 10–20- and 30-month-old Wistar rats.

The lipid composition of biological membranes is also supposed to change with age. The cholesterol to phospholipid ratio increased during senescence in most tissues including kidney and liver cell membranes [5,6] and heart and liver mitochondrial membranes [7–9]. This was due either to an increase in the cholesterol content per mg protein and a small change in total phospholipid, or to a decrease in the phospholipid content of the membrane, the cholesterol per mg protein remaining constant. The fatty acid composition of membranes was also found to vary as the animal became older, despite few changes in the proportion of each class of phospholipid. This would cause a modification in the fluidity of the membrane and in the mobility of its constituents, including possible mobile carriers [6]. Unfortunately, studies relating to lipid modifications of kidney membranes were performed on microsomal fractions [1,5] which included brush-border, basolateral and intracellular membranes, and it was not clear whether the aging process affected all or some of the membranes or merely modified the proportion of each constituent of the microsomal fraction; since the different cell membranes have different lipid compositions, a change in their proportion would cause differences in the total lipid composition of the final pellet. To avoid this difficulty, we investigated the lipid composition of highly enriched brush-border membranes isolated from rat kidneys. We found that some changes in cholesterol, total phospholipids and fatty acid content of these membranes did indeed occur with age and that these modifications were quite different in male and female rats.

Materials and Methods

Animals. 10–20- and 30-month-old male and female Wistar rats (WAG/Rij) – a strain known for its very low age-associated pathological changes in kidney and bladder [10] – were purchased from the Institute for Experimental Gerontology of the Organization for Health Research, TNO, Rijswijk, The Netherlands. The survival curve established earlier by Bureck [11] for a parental colony, indicated that the female population entered its phase of senescence – corresponding to the first mortalities – at the age of 18–20 months, and that the 50% survival age was 30 months. The life-span of males was shorter: the bend of the survival curve was apparent at 16 months and the 50% survival age was 24 months. Nevertheless, in the present study, we decided to compare male and female rats of the same chronological ages even though their biological ages differed. Female body weights were around 200 g, while those of the males were 300–340 g.

Isolation of brush-border membranes. Rat renal brush-border fraction was prepared by the calcium precipitation method. Before killing, animals were fasted overnight with free access to water. Two to eight kidneys – depending on the number of determinations to be done – were pooled together in each preparation, and treated as described by Evers et al. [12], with slight modifications [13]. The final pellet was rinsed three times to eliminate the calcium content thus avoiding activation of phospholipase, and resuspended in 100 mM mannitol/20 mM Hepes/Tris buffer (pH 7.4). The whole process was conducted at 4°C and part of this membrane fraction was kept in liquid nitrogen for analyses not done on the day of preparation.

Enzyme assays. Specific activities of maltase, L-aminopeptidase and alkaline phosphatase were all measured in homogenate and brush-border fractions to calculate the enrichment of each preparation. Additional kinetic experiments were performed for the same enzymes on the brush-border fraction. Maltase activities were assayed by the method of Dahlquist [14]. The glucose liberated from maltose was measured as described by Bergmeyer [15]. Alkaline phosphatase activity was determined at room temperature by the procedure of Hübscher and West [16] using *p*-nitrophenyl phosphate as substrate. Leucine-aminopeptidase activity was measured as proposed by Kramers and Robinson [17]. The K_m and V_{max} of each enzyme were calculated from the Lineweaver plot of the data.

Protein electrophoresis. Polyacrylamide gel electrophoresis of the brush-border membrane proteins was carried out on the preparation obtained from the 10–20- and 30-month-old male and female rats [18]. Aliquots of membrane pro-

teins (2 mg/ml) were dissolved in an equal volume of the following buffer: 2.5% sodium dodecyl sulfate (SDS)/25% glycerol/10% mercaptoethanol by incubation for 5 min at 100°C. About 10 μg of the peptide chains obtained were resolved by electrophoresis in 12% polyacrylamide running gel at pH 8.8 after stacking at pH 7.5 in 3% polyacrylamide gels. The gels and the electrode buffer (Tris-glycine (pH 8.2)) contained 0.1% SDS. The separation was performed at room temperature for 3 h with a 25 mA current using a Bio-Rad Model 500/200 power supply. Gels were stained with the silver-stain technique as described by Oakley et al. [19] and scanned by laser densitometry (LKB 2202 Ultrascan). Calibration of the gels for molecular mass (in KDa) was done using known standard proteins: phosphorylase *b* (94.0), bovine serum albumin (67.0), ovalbumin (43.0), carbonic anhydrase (30.0), soybean trypsin inhibitor (20.1) and α-lactalbumin (14.4).

Lipid determination. Total phospholipids were extracted from the brush-border fraction by the method of Rose and Ocklander [20]. The solvent system was water/isopropanol/chloroform (0.5 : 2.75 : 1.75, v/v); phosphorus of the mineralized extract (HCl and $HClO_4$, 170°C) was measured as proposed by Rouser [21]. Total cholesterol was extracted (1 vol. H_2O/2 vol. petroleum ether) from saponified brush-border membranes (alcoholic potassium hydroxide 2 M, 80°C, 2 h), cleared by the precipitation procedure of Sperry and Webb [22] (digitonin 0.5% overnight), and measured by the method of Bhandaru et al. [23] (acetic acid/1 $mg \cdot ml^{-1}$ orthophthaldialdehyde /H_2SO_4, 1 : 1 : 1, v/v).

The fatty acid composition of brush-border membrane lipids extracted by the Bligh and Dyer's technique [24] (water/methanolchloroform (0.8 : 2 : 1, v/v) and water/chloroform (1 : 1, v/v)) was determined by gas chromatography. Fatty acids were methylated with 2.5% H_2SO_4 in methanol (60°C, 1 h) and chromatographed on an OV-1 2‰ capillary column which was temperature-programmed (180–240°C, 2 deg. C/min). The carrier gas was helium at a pressure of 0.85 kg/cm^2. Fatty acids were identified by comparison with methyl ester standards. A known amount of tricosanoic acid (23 : 0) was also added in the brush-border fraction at the beginning of the analysis as an internal standard, in order to calculate the molar concentration of each of the detected fatty acids. The recorded areas from the flame ionization detection were corrected by using the effective number of carbons as defined by David [25]. Results are given in percent of total molar content of the whole fatty acid content.

Enzyme activities, phospholipids and cholesterol in the brush-border membrane are expressed per mg proteins, which were assayed by Bradford's technique [26]. Sex and age differences were statistically analyzed by Wilcoxon's non-parametric tests and significance was set at $p < 0.05$. Means are expressed with their standard errors.

Results

Enzyme activities

The specific activities of maltase, L-aminopeptidase and alkaline phosphatase in homogenates of renal cortex and in the brush-border membrane fraction are given in Table I. In both males and females, the specific activities of maltase and alkaline phosphatase decreased in homogenates from 10 to 30 months while that of L-aminopeptidase did not change significantly. This pattern was qualitatively reproduced in the brush-border membrane and the enrichment factors of these three marker enzymes were very similar at 10–20 and 30 months, suggesting that the obtained membranes represented the same highly purified brush-border fraction. In addition, the protein recoveries of this latter fraction, expressed in percent of crude homogenate, were not different in the 10–20- and 30-month-old animals: 3.05 ± 0.14 ($n = 11$), 3.21 ± 0.12 ($n = 6$), 2.92 ± 0.29 ($n = 6$), respectively for male, and 2.69 ± 0.07 ($n = 14$), 2.60 ± 0.11 ($n = 8$), 2.48 ± 0.13 ($n = 5$) for female rats.

The results of the kinetic analysis of alkaline phosphatase, maltase and L-aminopeptidase activities performed on the brush-border membranes are depicted in Fig. 1. The apparent K_m values of the three enzymes were not affected by aging in either male or female. It should be noted that the leucine-aminopeptidase K_m was lower in males than in females at all ages, and that no sex difference was apparent in the K_m of alkaline phosphatase or maltase.

TABLE I

SPECIFIC ACTIVITIES OF ALKALINE PHOSPHATASE, L-AMINOPEPTIDASE AND MALTASE IN CRUDE HOMOGENATE AND BRUSH-BORDER MEMBRANE FRACTIONS FROM 10-, 20- AND 30-MONTH-OLD MALE AND FEMALE WISTAR RAT KIDNEYS

Specific activities are expressed as μmol/mg protein per min. Values are means ± S.E. of (n) number of preparations.

	Specific activities						Enrichment factor: brush-border fraction/homogenate		
	Homogenate			Brush-border fraction					
Male	10 months (n = 11)	20 months (n = 6)	30 months (n = 6)	10 months (n = 11)	20 months (n = 6)	30 months (n = 6)	10 months	20 months	30 months
Alkaline phosphatase	0.102 ± 0.003	0.094 ± 0.001	0.090 ± 0.002	0.99 ± 0.01	0.96 ± 0.01	0.72 ± 0.01	×10.0 ± 0.5	×10.3 ± 0.6	×8.0 ± 0.4
L-Aminopeptidase	39.7 ± 0.7	36.1 ± 0.5	37.7 ± 1.8	436 ± 22	358 ± 7	426 ± 20	×11.0 ± 0.5	×10.0 ± 0.3	×11.7 ± 0.4
Maltase	0.146 ± 0.003	0.127 ± 0.004	0.122 ± 0.009	1.80 ± 0.06	1.67 ± 0.06	1.52 ± 0.12	×12.4 ± 0.5	×13.2 ± 0.5	×12.7 ± 0.9
Female	10 months (n = 14)	20 months (n = 8)	30 months (n = 5)	10 months (n = 14)	20 months (n = 8)	30 months (n = 5)	10 months	20 months	30 months
Alkaline phosphatase	0.099 ± 0.002	0.088 ± 0.003	0.082 ± 0.001	1.34 ± 0.07	1.14 ± 0.02	0.97 ± 0.05	×13.4 ± 0.5	×12.9 ± 0.4	×11.8 ± 0.5
L-Aminopeptidase	31.7 ± 0.8	36.2 ± 1.5	32.0 ± 1.4	423 ± 22	419 ± 27	410 ± 38	×13.6 ± 0.9	×11.8 ± 0.5	×13.7 ± 1.3
Maltase	0.089 ± 0.003	0.094 ± 0.004	0.071 ± 0.002	1.21 ± 0.04	1.11 ± 0.06	0.89 ± 0.05	×13.6 ± 0.3	×11.9 ± 0.5	×13.1 ± 1.0

The V_{max} of maltase showed a 14% decrease from 10 to 30 months in males and a 20% in females. The amplitude of this V_{max} decrease was comparable in the two sexes, despite the large difference in the absolute values. The maximal activities of alkaline phosphatase also decreased by 28% from 10 to 30 months in females while a 26% drop was observed only from 20 to 30 months in males. No sex difference was observed in the value of the L-aminopeptidase V_{max} and these V_{max} values were not significantly different when 10- and 30-month-old animals were compared. A small decrease appeared in males at the intermediate age of 20 months.

SDS-Polyacrylamide gel electrophoresis

Representative electrophoretic protein subunit patterns obtained from solubilized membrane preparation of 10–20- and 30-month-old male and female rats are shown in Fig. 2. Visual examination of the gels revealed the presence of at least 40 protein bands. No marked age or sex differences in the distribution of proteins with molecular weight were observed, with the exception of the staining intensity of the 14.0 kDa band. Scanning of the gels confirmed this lack of modification with age of nearly all the bands, and indicated that the intensity of the 14.0 kDa band decreased by 31% between 10 and 30 months in males and by 32% in females. Because the electrophoretic pattern of brush-border, lysosomal, mitochondrial and endoplasmic membranes are very different [27], the observed similarity of protein patterns indicates that any small contamination of our brush-border preparation by intracellular membrane should be identical at every age and thus the reported changes in enzyme activities or lipid composition would not result from difference in the proportion of the extracted membranes.

Lipid composition

The total amount of cholesterol present in the brush-border membrane of the male kidney was significantly lower than that of the female (Fig. 3). When expressed per mg protein, cholesterol increased in males by 11% from 10 to 20 months and by 17% from 20 to 30 months. In females, the proportion of cholesterol in the brush-border was

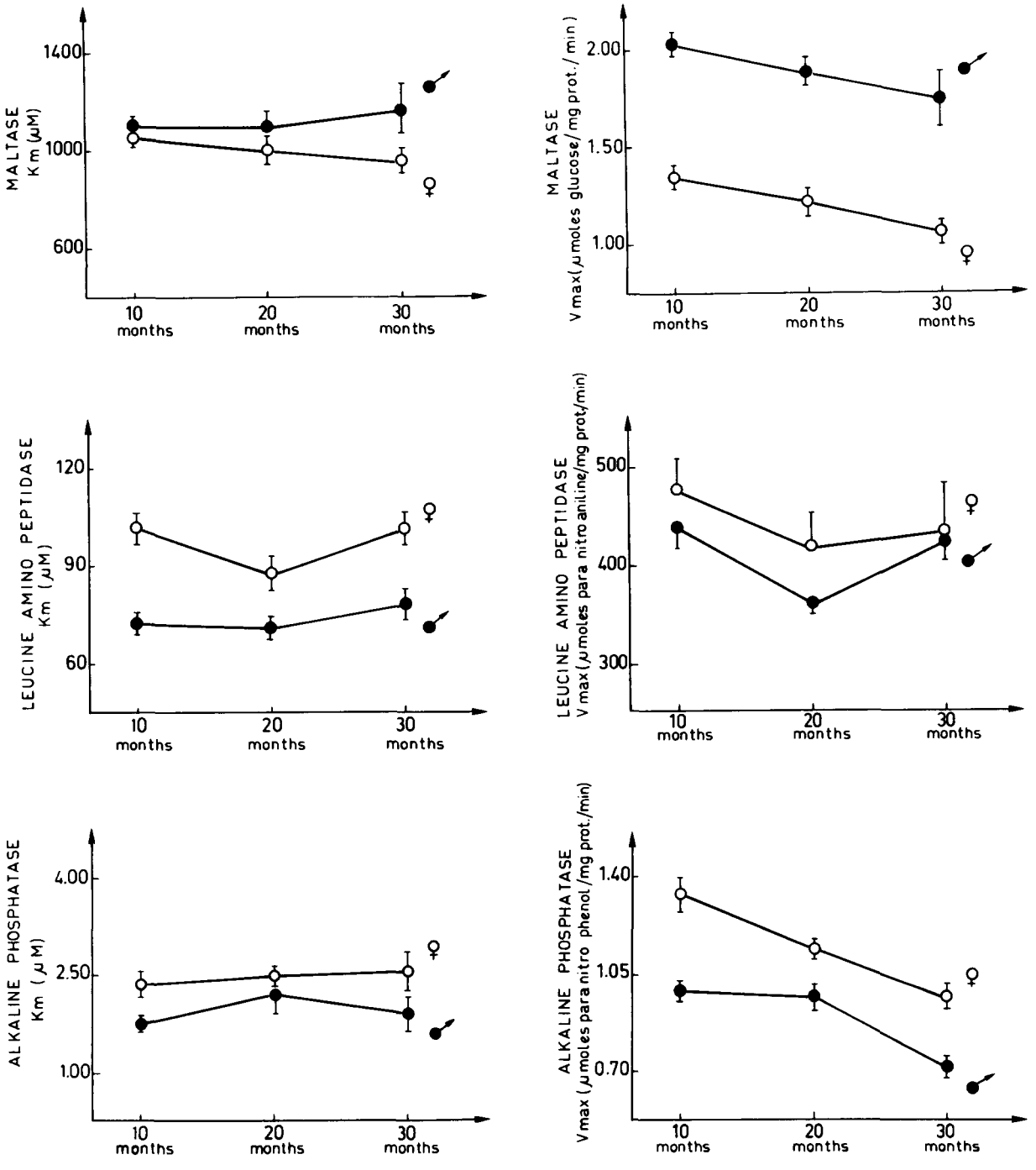

Fig. 1. V_{max} and K_m of alkaline phosphatase, L-aminopeptidase and maltase present on the kidney brush-border membranes isolated from 10–20- and 30-month-old male (●) and female (○) Wistar rats. Each point represents mean ± S.E.

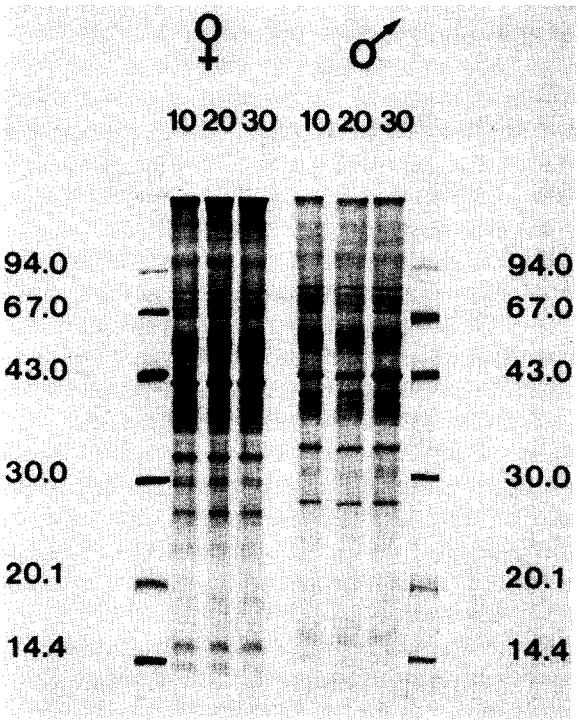

Fig. 2. SDS-polyacrylamide gel electrophoresis of kidney brush-border membrane proteins from 10–20- and 30-month-old male and female Wistar rats. The gels were stained with the silver stain technique. The left and right tracks are those of the molecular weight markers in kDa: phosphorylase *b* (94.0), bovine serum albumin (67.0), ovalbumin (43.0), carbonic anhydrase (30.0), soybean trypsin inhibitor (20.1) and α-lactalbumin (14.1).

unchanged from 10 to 20 months but increased thereafter by 22%. The total phospholipid contents of these kidney membranes were very similar in 10- and 20-month-old males and females, and increased slightly between 20 and 30 months (Fig. 3). As a consequence, the cholesterol to phospholipid molar ratio was unchanged with age in the female (Fig. 3) whereas it rose in the male by 9% from 10 to 20 months and by 10% from 20 to 30 months. At this age, the ratios were no longer statistically different in males and females.

Analysis of the fatty acid composition of the brush-border membrane revealed few age differences (Table II). In males, no change was observed between 10 and 20 months. In turn, considering the 10- and 30-month-old rats, the proportion of palmitic acid (16 : 0) increased by

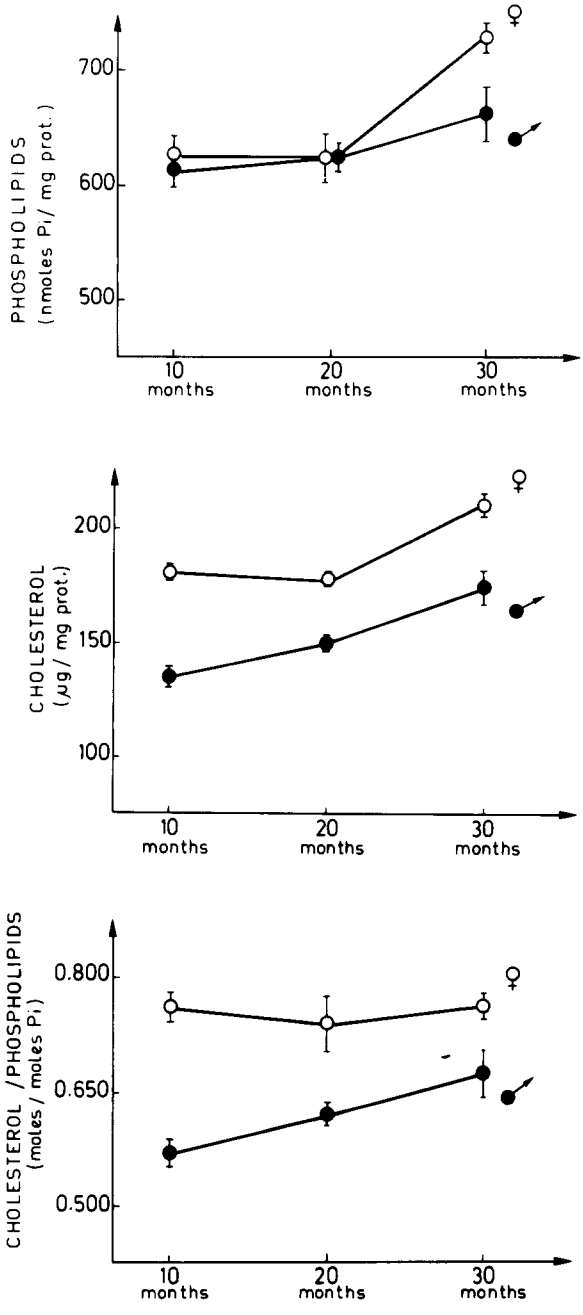

Fig. 3. Total phospholipid and cholesterol contents of kidney brush-border membranes isolated from 10–20- and 30-month-old male (●) and female (○) Wistar rats. Lipid contents are expressed per mg of membrane proteins. Each point represents mean ± S.E.

TABLE II

FATTY ACID COMPOSITION OF KIDNEY BRUSH-BORDER MEMBRANES ISOLATED FROM 10–20- AND 30-MONTH-OLD MALE AND FEMALE WISTAR RATS

Fatty acid are expressed as percent of total molar content of the whole fatty acid content. The unsaturation index is the sum of the molar percent of each fatty acid multiplied by the number of its unsaturations. Values are means ±S.E. of (n) number of preparations.

	Male			Female		
	10 months (n = 11)	20 months (n = 6)	30 months (n = 6)	10 months (n = 8)	20 months (n = 5)	30 months (n = 3)
14:0	1.1 ±0.2	1.4 ±0.4	1.0 ±0.2	1.1 ±0.3	1.1 ±0.2	0.7 ±0.1
15:0	3.7 ±0.3	3.9 ±0.4	3.4 ±0.6	2.4 ±0.3 [c]	2.6 ±0.1	1.7 ±0.4 [c]
16:0	18.2 ±0.4	18.7 +0.6	20.1 ±0.3 [b]	21.3 ±0.6 [c]	21.8 ±0.3	21.3 ±1.3 [c]
16:1	0.6 ±0.2	0.9 ±0.2	0.4 ±0.0	0.6 ±0.1	1.1 ±0.1	0.3 ±0.0
16:2	0.7 ±0.1	0.7 ±0.2	1.1 ±0.2	0.6 ±0.0	0.5 ±0.0	0.6 ±0.1
17:0	3.3 ±0.2	3.1 ±0.2	3.9 ±0.2	2.3 ±0.2 [c]	2.6 ±0.0	2.0 ±0.4
18:0	19.3 ±0.3	19.4 ±0.4	17.3 ±0.4 [b]	20.1 ±0.6	19.2 ±0.3	20.5 ±0.8 [c]
18:1	7.7 ±0.2	8.1 ±0.2	8.4 ±0.2	7.0 ±0.1	7.6 ±0.2	9.2 ±0.2 [b]
18:2	6.5 ±0.1	6.7 ±0.2	6.7 ±0.2	5.5 ±0.3	6.7 ±0.3	6.2 ±0.5
20:0	0.3 ±0.0	0.4 ±0.0	0.4 ±0.0	0.5 ±0.1	0.4 ±0.0	0.5 ±0.0
20:1	0.3 ±0.1	0.5 ±0.2	0.3 ±0.0	0.4 ±0.1	0.3 ±0.0	0.3 ±0.1
20:3	0.4 ±0.1	0.4 ±0.0	0.6 ±0.0	0.7 ±0.0	0.5 ±0.0	0.6 ±0.0
20:4	22.3 ±0.4	21.1 ±0.8	20.2 ±0.7 [a]	23.1 ±0.6	21.1 ±0.2	21.6 ±0.8
22:0	0.5 ±0.1	0.7 ±0.1	0.6 ±0.0	0.9 ±0.1	1.3 ±0.0	0.8 ±0.1
22:1	0.7 ±0.1	0.9 ±0.2	0.4 ±0.0	0.6 ±0.1	0.5 ±0.1	0.6 ±0.2
22:5	1.1 ±0.1	1.3 ±0.2	1.0 ±0.1	1.0 ±0.1	1.0 ±0.0	1.1 ±0.1
22:6	2.5 ±0.3	3.1 ±0.5	2.4 ±0.1	2.5 ±0.2	2.3 ±0.0	2.6 ±0.1
24:0	2.9 ±0.1	2.8 ±0.1	2.6 ±0.1	3.1 ±0.2	3.5 ±0.1	3.0 ±0.4
24:1	3.2 ±0.2	2.7 ±0.2	3.2 ±0.3	2.3 ±0.4	2.1 ±0.3	2.2 ±0.4
26:0	4.6 ±0.6	3.0 ±0.9	5.6 ±1.2	3.6 ±0.5	3.4 ±0.7	3.5 ±1.7
26:1	0.4 ±0.0	0.5 ±0.1	0.5 ±0.0	0.7 ±0.2	0.6 ±0.1	0.5 ±0.1
Unsaturation index	1.39±0.02	1.37±0.02	1.30±0.03 [a]	1.38±0.02	1.31±0.02	1.35±0.04

[a] Different from 10 months.
[b] Different from 10 and 20 months.
[c] Female different from male.

10% and that of stearic (18 : 0) and arachidonic (20 : 4) acids decreased by 10 and 9%, respectively. In females, such differences were not apparent and only a 17% increase in oleic acid (18 : 1) between 20 and 30 months was significant. Male and female brush-border membranes from 10-month-old rats differed in their pentadecanoic (15 : 0), heptadecanoic (17 : 0) and palmitic (16 : 0) acid compositions; at 30 months, the differences were still present in the penta- and heptadecanoic acids and also in stearic (18 : 0) acid. The unsaturation index – calculated as the sum of the molar percent of each fatty acid multiplied by the number of its unsaturations [28] – slightly decreased with age in males and was unchanged in females (Table II). No statistical sex difference in the unsaturation index was recorded in these experiments.

Discussion

The present results indicate that the K_m of three hydrolases localized at the surface of the kidney brush-border membrane – maltase, L-aminopeptidase and alkaline phosphatase – are not affected by age but that the maximum activity (V_{max}) is modified in certain enzymes. For maltase, a decrease in V_{max} without alteration of the K_m was already reported [2,3], but the magnitude of

the change slightly differed from one study to another (33% for Reiss and Sacktor [3], 40–50% for O'Bryan and Lowenstein [2] and 14–20% in the present work). This diminished V_{max} is mainly linked to a conformational alteration of some of the molecules which become inactive, and not to a modification of the lipid environment or to a sharp decrease of the enzyme units present in the membrane [3,4].

As regards alkaline phosphatase, O'Bryan and Lowenstein [2] did not find a significant change in its V_{max} in 6- and 24-month-old male Charles River rats. By comparing animals of 10, 20 and 30 months, we also showed that, in males there is no change in the V_{max} of alkaline phosphatase until 20 months, but that a decrease in activity of the enzyme appears later, between 20 and 30 months. In contrast, in females, the change is already apparent after 10 months. This suggests that there is different timing in the age-related change of enzyme activities, depending on the sex of the animals. Moreover, age alteration of enzyme V_{max} does not seem to be common to all brush-border hydrolases. Despite a slight decrease between 10 and 20 months, there was no difference in the V_{max} of the leucine-aminopeptidase in the 10- and 30-month-old male or female rat kidneys. The presence of altered and unaltered enzymes in the same tissue of old rats recall similar observations made on muscle and liver [29–32]. It suggests that enzyme aging is not controlled by a common mechanism affecting all the membrane-bound hydrolases in the same way or to the same degree, and that the finding of Reiss and Sacktor [4] of age-modification in the relative proportion of active and inactive enzyme would probably not apply to the leucine-aminopeptidase.

The results on the lipid composition of the brush-border membrane also emphasized the need to study quite old animals to detect age-related alterations and showed the sex-dependence of these aging processes. From 10 to 20 months, the phospholipid to protein ratio of both male and female remained similar, an increase in this ratio only occurring from 20 to 30 months. That is, the brush-border membrane became more lipidic per unit area, either due to an increase in phospholipid or to a decrease in the total amount of proteins in the brush-border. In contrast to this similarity of the phospholipid to protein ratio in male and female, the change in the total cholesterol to protein ratio was sex-dependent. In the male, the cholesterol content of the membrane – much lower than that of the female in adults – increased from 10 to 20 and from 20 to 30 months while in the female the rise in the cholesterol to membrane protein ratio appeared only from 20 to 30 months. This was largely due to an increase in free cholesterol since the proportion of cholesterol ester we measured in these membranes was less than 8% of the total extracted cholesterol (not reported in this paper). The consequence of a parallel increase in cholesterol and phospholipid per mg protein in female rats was a constant cholesterol to phospholipid ratio from 10 to 30 months. In the male, as the increase of cholesterol is greater than that of phospholipid, the molar ratio of cholesterol to phospholipid increased with age, attaining a value at 30 months not statistically different from that of the female. According to the hypothesis that more cholesterol in a biological membrane reduces its fluidity [28], our data suggest that the viscosity of the male brush-border membrane rose with age, and that of the female remained elevated throughout life.

Not only the cholesterol content of the membrane but also its fatty acid composition is reported to vary in senescent animals. A theory has been proposed that lipid peroxidation increases with age – due to an increased production of superoxide or to an inhibition of physiological repair mechanisms of oxidated lipids, or both – and the proportion of unsaturated to saturated fatty acid therefore decreases in old membranes [6,9,33]. This has been recorded at least for mitochondria and for some plasma membranes [6–8,34]. In rat kidneys, the observations made by Grinna [1,5] on the microsomal membrane fraction from male rats, suggested that the same process takes place between 6 and 24 months. However, the gas chromatography analysis of brush-border fractions from 10–20- and 30-month-old female rats failed to demonstrate any change other than a small decrease in the stearic acid (18 : 0) between 20 and 30 months, i.e., the unsaturation index was not modified during aging. In males, the unsaturation index of the whole fatty acids slightly decreased in the 30-month-old membrane, but this

was mostly, if not exclusively, due to the 9% drop in the amount of arachidonic acid. This could suggest either that the peroxidation of arachidonic acid alone is increased with age, or that its metabolism is modified in senescent rats. It is also noteworthy that owing to the sex difference in life-span of the WAG/Rij strain, the changes in fatty acid composition reported in male could occur later in the life of female.

In conclusion, the present data indicate that the lipid composition of highly purified brush-border membrane from female Wistar rats is largely unchanged from 10 to 30 months, while in the male an age-dependent increase in the cholesterol to phospholipid ratio is accompanied by a slight modification in the fatty acid composition of the membrane. No simple relationship between enzyme changes and lipid composition of the brush-border was found when comparing adult with senescent or female with male rats. This apparent indifference of the microvillar hydrolases to their lipid environment is probably due to the fact that their hydrophobic anchor represents a relatively small proportion of the total polypeptide chain which is apparently independent of the catalytic site of the enzyme [35]. Furthermore, each of the hydrolases studied showed an individual aging behavior varying from a continuous decrease in the V_{max} to a constant value over the 10 to 30 months. These observations should be compared with the age modifications in transport properties recently reported in rat brush-border membranes [36,37].

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