PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **183,** 169–176 (1986)

Hydrolase Activities Increase in the Rat Aorta with Growth and Aging but Not in Liver and Kidney[1] (42401)

RONALD A. MARKLE

Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011

Abstract. We examined specific activities (based on DNA) of six glycosidases and cathepsin C in aorta, kidney, and liver from male rats of 2, 6, 10, and 14 months of age. The premise was that assessing cellular catabolism of arterial and nonvascular tissues over age might more fully clarify the impact of age (and growth) alone upon vascular wall metabolism. All aortic glycosidases increased significantly ($P < 0.05$) over the holding period as follows: neutral α-glucosidase, up 93%; β-galactosidase, up 102%; *N*-acetyl-β-glucosaminidase, up 119%; α-mannosidase, up 77%; β-glucuronidase, up 65%; acid α-glucosidase, up 95%. Cathepsin C specific activity was unchanged as was aortic DNA content; total protein content increased 136%. In the kidney, all glycosidase specific activities declined over age with decreases ranging 39–55%; cathepsin C was unchanged. In the liver, neutral α-glucosidase increased 12%, acid α-glucosidase was unchanged, and the four remaining glycosidases decreased an average of 5–35% by 14 months of age. Liver cathepsin C decreased 44% over this period. Thus, enhancement of hydrolase baseline activities prevails during growth and aging in rat aortic tissue whereas hydrolases of kidney and liver tissues generally decline.

In 1974, de Duve (1) proposed a model for transformation of aortic smooth muscle to foamy cells, attributing a central role to a functional deficiency of lysosomal cholesterol esterase. Subsequently, a variety of studies were undertaken to assess lysosomal responses to various manipulable atherogenic risk factors in several animal models. For example, diabetic rats demonstrated depressed aortic hydrolase activities which insulin treatment restored to nearly normal (2). Two-kidney Goldblatt hypertensive rats showed marked elevations of aortic hydrolases which reverted to control levels or less with removal of the renal artery clip (3). When rats were regularly swum, significant elevations of aortic hydrolases resulted, compared to sedentary controls (4). Thyroidectomized rats had depressed aortic hydrolase activities which were restored to control levels or higher by T_3 treatment (5). Finally, dietary hypercholesterolemia in rabbits (6) and monkeys (7, 8) induced lipid accumulations and biochemical alterations in aortic smooth muscle cell lysosomes.

Among the preceding manipulated risk factors, diabetes, hypertension, and hypercholesterolemia are noted as age-related risk factors in humans (9). That is, the risk of vessel disease is greater with increasing age in individuals with diabetes, hypertension, or hypercholesterolemia. In contrast to many risk factors, age cannot be manipulated, yet epidemiological studies have identified age as a risk factor for vessel disease (9). Ultimately, the impact of the risk factor upon cellular metabolism of the blood vessel wall must be assessed (4). Thus, we examined the underlying influence of animal age, a nonmanipulable risk factor, on aortic hydrolase activities in rats. A preliminary report of this work has been presented (10).

Materials and Methods. Four groups of 15 male Sprague–Dawley rats (Hilltop Lab Animals, Inc.), 5–6 weeks old at the outset (weight, 150–200 g), were housed on campus and maintained to the ages of 2, 6, 10, and 14 months. Chow (5001, Ralston Purina Co.) and water were provided *ad libitum.* The rats were exsanguinated under light ether anesthesia, the aortic segment between the left subclavian and celiac arteries was removed, and intima-media strips were prepared over ice by the peeling technique of Wolinsky and Daly (11). Each aortic datum represents aortic strips from two or three rats pooled in 3.0 or 4.5 ml, respectively, of an ice-cold 0.25 *M* sucrose solution

[1] Supported by University Organized Research of Northern Arizona University.

0037-9727/86 $1.50

containing 1.0 m*M* EDTA. Similarly, the right kidney and anterior lobule of the right lobe of the liver were removed for comparisons to aortic tissue. Each of these organs was homogenized in 10 ml of sucrose–EDTA solution. All tissues were homogenized with a motor-driven Teflon pestle in a Potter tissue homogenizer at 4°C. DNA analyses were done on fresh 1.5-ml aliquots from each homogenate; remaining homogenates were frozen at −70°C for later enzyme and protein assays.

Enzyme assays.[2] Glycosidases and cathepsin C were assayed fluorometrically as described by Wolinsky *et al.* (2) with the following incubation conditions:

Neutral α-glucosidase: 1 m*M* 4-methylumbelliferyl-α-D-glycopyranoside, 50 m*M* Na phosphate buffer, pH 6.9, and 0.1% Triton X-100.

N-Acetyl-β-glucosaminidase: 0.25 m*M* 4-methylumbelliferyl-2-acetamido-deoxy-β-D-glucopyranoside, 50 m*M* Na citrate buffer, pH 4.4, and 0.1% Triton X-100.

β-Galactosidase: 0.25 m*M* 4-methylumbelliferyl-β-D-galactopyranoside, 50 m*M* Na citrate buffer, pH 3.6, 5 m*M* $MgCl_2$, and 0.1% Triton X-100.

α-Mannosidase: 1 m*M* 4-methylumbelliferyl-α-D-mannopyranoside, 50 m*M* Na acetate buffer, pH 4.6, and 0.1% Triton X-100.

Acid α-glucosidase: 1 m*M* 4-methylumbelliferyl-α-D-glucopyranoside, 50 m*M* Na acetate buffer, pH 4.8, and 0.1% Triton X-100.

β-Glucuronidase: 0.25 m*M* 4-methylumbelliferyl-β-D-glucuronide trihydrate, 50 m*M* Na acetate buffer, pH 4.8, and 0.1% Triton X-100.

Cathepsin C: 0.5 m*M* glycyl-L-phenylalanyl-β-naphthylamide, 50 m*M* Na acetate buffer, pH 4.2, 40 m*M* NaCl, 5 m*M* dithiothreitol, and 0.1% Triton X-100.

Incubation mixtures consisted of 0.1 ml of appropriately diluted homogenate to which 0.1 ml of substrate mixture was added. After incubating at 37°C for 20–60 min, the reactions were stopped and fluorescence of free 4-methylumbelliferone or β-naphthylamine was developed by adding 2 ml of stopping solution (50 m*M* glycine with 5 m*M* tetrasodium EDTA, adjusted to pH 10.4 with NaOH). Under the incubation conditions used, enzyme activities were linear as a function of time and protein or DNA concentration. Enzyme activities are expressed as milliunits of activity, with 1 mU defined as 1 nmole of substrate hydrolyzed per minute at 37°C.

Analytical procedures. DNA was measured by the method of Burton (13) using 2-deoxy-D-ribose and calf thymus DNA as standards. Tissue protein was determined by the Bio-Rad protein assay (Richmond, Calif.) with bovine serum albumin as the standard (14).

Materials. All chemicals were of analytical grade. The 4-methylumbelliferyl glycoside substrates were purchased from Koch Light Laboratories, Ltd., through Research Products International Corp. (Mount Prospect, Ill.). Glycyl-L-phenylalanyl-β-naphthylamide, fluorescent substrate standards (4-methylumbelliferone and β-naphthylamine), EDTA, Triton X-100, and dithiothreitol were purchased from Sigma Chemical Company. Other reagents were purchased from Fisher Scientific Company.

Statistical analyses. One-way analysis of variance and determination of confidence intervals between group means were performed for the animal body weights, DNA, and total protein contents and enzyme data of each organ with age as the single variable. Subsequently, multiple regression analysis was used to test if aortic enzyme activities were predictable from animal age and liver and kidney enzyme activities. When this three-variable model was significant, we next performed a partial *F* test to determine if liver and kidney enzyme activities contributed significantly in the three-variable analysis. In all statistical tests, a confidence level of 95% or higher ($P < 0.05$ or less) was deemed significant (15).

Results. *Characteristics of the animal groups.* Table I summarizes the final body weights, DNA, and total protein contents of the pooled aortic specimens, liver samples, and right kidneys. The period of rapid growth between 2 and 6 months was reflected by a significant increase of mean body weight which essentially leveled off thereafter. Because of premature deaths of two rats in the 10-month group and one in the 14-month group, three aortic preparations contained two aortas rather

[2] Trivial names for enzymes are used throughout. Nomenclature recommended by the International Union of Biochemistry may be found elsewhere (12).

TABLE I. CHARACTERISTICS OF RATS

	Group age in months[a]			
	2 (15)	6 (15)	10 (13)	14 (14)
Final body weight (g)	376 ± 44	593 ± 50[b]	569 ± 43[b]	600 ± 54[b]
Pooled aortic DNA (μg)	145 ± 13	163 ± 13	157 ± 14	160 ± 8
Kidney DNA (mg)	2.17 ± 0.15	2.83 ± 0.23	3.68 ± 0.39[b,c]	3.97 ± 0.58[b–d]
Liver lobule DNA (mg)	1.78 ± 0.18	2.05 ± 0.55	2.73 ± 0.41[b,c]	2.54 ± 0.73[b,c]
Pooled aortic protein (mg)	1.85 ± 0.60	3.86 ± 0.61[b]	2.37 ± 1.61	4.36 ± 0.75[b,d]
Kidney protein (mg)	159.5 ± 44.6	163.5 ± 26.1	174.1 ± 11.1	184.4 ± 13.7[b]
Liver lobule protein (mg)	225.3 ± 38.4	276.6 ± 45.2[b]	241.4 ± 44.1	253.1 ± 44.1

Note. The right kidney and anterior lobule of the right lobe of the liver were used.
[a] Results are expressed as means ± SD. Numbers in parentheses = number of rats.
[b] $P < 0.05$ compared to 2-month group.
[c] $P < 0.05$ compared to 6-month group.
[d] $P < 0.05$ compared to 10-month group.

than the usual pool of three aortas. Hence, DNA contents of the "two aorta" pooled samples were normalized prior to determining the group means. Insignificant differences of DNA content between the pooled, anatomically defined aortic specimens were found over the age groups. The liver and kidney DNA contents increased significantly by 10 months of age and again in the kidney at 14 months (although this increase was barely significant at $P = 0.05$).

Mean protein contents of pooled aortic specimens increased significantly from 2 to 6 months and again from 10 to 14 months of age. The apparent decline seen between 6 and 10 months, however, was not statistically significant. Mean protein contents of the kidney tissues demonstrated a progressive rise across the age groups with the increase by 14 months being significantly higher compared to the 2-month-old group. Liver samples reflected a significant increase of protein contents from 2 to 6 months; protein contents at 10 and 14 months were intermediate to and not significantly different from the 2- and 6-month levels. Thus, protein contents of aortic and kidney tissues significantly increased from the youngest to the oldest ages examined. Aortic and liver protein contents significantly increased during the period of most rapid weight gain (2 to 6 months), after which liver protein levels fell slightly for the remainder of the holding period.

Biochemical findings. Mean hydrolase specific activities, expressed as milliunits of enzyme activity per milligram of DNA, for the aortic specimens are summarized in Table II. Every glycosidase significantly increased be-

TABLE II. AORTIC HYDROLASE SPECIFIC ACTIVITIES PER UNIT DNA IN RATS DURING GROWTH AND AGING

	Group age in months[a]			
Enzyme	2 (5)	6 (5)	10 (5)	14 (5)
Neutral α-glucosidase	18.7 ± 4.9	29.2 ± 3.5[b]	26.0 ± 7.0[b]	36.0 ± 3.0[b–d]
β-Galactosidase	12.3 ± 2.3	16.9 ± 1.3[b]	15.0 ± 2.6[b]	24.8 ± 1.2[b–d]
N-Acetyl-β-glucosaminidase	64.6 ± 22.0	100.4 ± 12.6[b]	84.6 ± 21.8[b]	141.3 ± 13.9[b–d]
α-Mannosidase	9.7 ± 3.6	13.1 ± 1.2[b]	10.7 ± 2.7	17.2 ± 1.9[b–d]
β-Glucuronidase	10.5 ± 3.0	13.6 ± 1.3[b]	12.5 ± 2.7	17.3 ± 2.2[b–d]
Acid α-glucosidase	1.70 ± 0.37	2.96 ± 0.38[b]	2.66 ± 0.32[b]	3.32 ± 0.11[b–d]
Cathepsin C	20.6 ± 7.0	23.7 ± 5.6	21.3 ± 3.6	21.6 ± 2.1

[a] Results are expressed in milliunits of enzyme activity/mg DNA, means ± SD. 1 mU of activity is defined as 1 nmole of substrate hydrolyzed per minute at 37°C. Number in parentheses = number of pooled aortic specimens.
[b] $P < 0.05$ compared to 2-month group.
[c] $P < 0.05$ compared to 6-month group.
[d] $P < 0.05$ compared to 10-month group.

tween 2 and 6 months of age with increases ranging from 30% for β-glucuronidase to 74% for acid α-glucosidase. Further significant increases through 14 months of age were measured for each glycosidase as compared to 6 months of age and ranged from 12% for acid α-glucosidase to 47% for β-galactosidase. In marked contrast to the glycosidases, specific activities of the protease cathepsin C did not change in aortic specimens over the ages examined.

Table III lists aortic hydrolase mean specific activities expressed per milligram of protein. As aortic protein content increased or decreased between age groups (Table I), reciprocal changes were found for all hydrolases. For example, as protein content increased from 2 to 6 months of age, mean specific activities for all hydrolases decreased, four significantly. As protein content declined between 6 and 10 months of age, all enzyme specific activities increased, six significantly. Finally, as protein content increased to its highest level between 10 to 14 months of age, all hydrolase mean specific activities decreased again, four significantly. Thus, when aortic hydrolase specific activities were expressed per unit of protein, the data were inversely associated to changes of aortic protein content.

Mean hydrolase specific activities of the right kidney are summarized in Table IV. From 2 to 6 months no changes were noted except a significant decrease for *N*-acetyl-β-glucosaminidase. However, by 10 months every mean glycosidase specific activity had declined significantly and further decreases were evident to 14 months of age. Overall, decreases from 2 to 14 months ranged from 39% for β-glucuronidase to 55% for α-mannosidase. Cathepsin C showed no significant changes between nor over all age groups for the kidney.

Table V lists the mean hydrolase specific activities measured from liver specimens. The results were less consistent over the age groups than data for aortic and kidney tissues. In general, significant declines were found at 10 months of age (exceptions are β-galactosidase and *N*-acetyl-β-glucosaminidase). From 10 to 14 months significant increases were found for neutral α-glucosidase, β-glucuronidase, and acid α-glucosidase; the other hydrolase activities were essentially unchanged from the 10-month levels.

Prior to performing multiple regression analyses, the liver and kidney enzyme data for the rats were pooled, corresponding to the pooling of the aortic specimens. A model with three variables was tested in which aortic enzyme activity was examined for dependence upon animal age and liver and kidney enzyme activities. In all multiple regression analyses, a significant F ratio was found for each glycosidase but not for the protease, cathepsin C. Subsequent examination of the t ratio for each variable (animal age and liver and kidney enzyme activities) of a significant F test indicated that aortic enzyme activities correlated significantly to age only. The one exception to this was the significant reciprocal correlation of increasing aortic β-glucuronidase activities to

TABLE III. AORTIC HYDROLASE SPECIFIC ACTIVITIES PER UNIT PROTEIN IN RATS DURING GROWTH AND AGING

Enzyme	Group age in months[a]			
	2 (5)	6 (5)	10 (5)	14 (5)
Neutral α-glucosidase	33.12 ± 2.75	27.46 ± 2.22[b]	38.42 ± 1.75[c]	30.19 ± 7.37[d]
β-Galactosidase	22.77 ± 5.45	15.90 ± 0.90[b]	23.82 ± 2.45[c]	20.84 ± 4.65
N-Acetyl-β-glucosaminidase	113.32 ± 22.00	94.59 ± 10.19	132.44 ± 10.44[c]	118.00 ± 24.39
α-Mannosidase	16.50 ± 3.64	12.38 ± 1.04	15.89 ± 1.32	14.32 ± 2.37
β-Glucuronidase	18.59 ± 2.25	12.87 ± 1.01[b]	18.74 ± 1.74[c]	14.36 ± 2.54[b,d]
Acid α-glucosidase	3.20 ± 0.65	2.78 ± 0.42	3.99 ± 0.64[c]	2.79 ± 0.66[d]
Cathepsin C	35.97 ± 5.16	22.39 ± 5.52[b]	33.06 ± 10.42[c]	18.42 ± 5.82[b,d]

[a] Results are expressed in microunits of enzyme activity/mg protein, means ± SD. 1 μU of activity is defined as 1 pmole of substrate hydrolyzed per minute at 37°C. Number in parentheses = number of pooled aortic specimens.
[b] $P < 0.05$ compared to 2-month group.
[c] $P < 0.05$ compared to 6-month group.
[d] $P < 0.05$ compared to 10-month group.

TABLE IV. KIDNEY HYDROLASE ACTIVITIES IN RATS DURING GROWTH AND AGING

Enzyme	Group age in months[a]			
	2 (15)	6 (15)	10 (13)	14 (14)
Neutral α-glucosidase	258 ± 53	260 ± 47	164 ± 38[b,c]	148 ± 48[b,c]
β-Galactosidase	411 ± 62	406 ± 94	221 ± 39[b,c]	189 ± 40[b,c]
N-Acetyl-β-glucosaminidase	996 ± 158	905 ± 185[b]	508 ± 83[b,c]	487 ± 111[b,c]
α-Mannosidase	227 ± 41	221 ± 47	113 ± 22[b,c]	102 ± 19[b,c]
β-Glucuronidase	125 ± 25	135 ± 29	79 ± 11[b,c]	76 ± 18[b,c]
Acid α-glucosidase	136 ± 33	125 ± 23	75 ± 17[b,c]	64 ± 24[b,c]
Cathepsin C	608 ± 134	690 ± 171	689 ± 111	592 ± 82

Note. The right kidney was used.

[a] Results are expressed in milliunits of enzyme activity/mg DNA, means ± SD. 1 mU of activity is defined as 1 nmole of substrate hydrolyzed per minute at 37°C. Number in parentheses = number of rats.

[b] $P < 0.05$ compared to 2-month group.

[c] $P < 0.05$ compared to 6-month group.

the declining β-glucuronidase activities of the kidneys over age. Lastly, a partial *F* test was performed to assure that age, and not liver nor kidney enzyme activities, was truly the variable correlated with the aortic findings. For each enzyme, the *F* test of the joint contribution of liver and kidney enzyme activities to the three-variable model was found to be insignificant, thus supporting the model that aortic enzyme activities was correlated to animal age, but not liver nor kidney enzyme activities.

Discussion. Underlying this study is our belief that assessment of baseline metabolic activity of vascular smooth muscle cells, particularly catabolic activity, is critical prior to understanding influx–efflux imbalances of intracellular materials as may be implicated in atherogenesis (1). Manipulable risk factors and clinical interventions may affect this balance to the detriment or benefit of the arterial wall, respectively (16). Within this framework we have examined the influence of the non-manipulable clinical risk factor of age on aortic catabolic hydrolase activities. For strictly comparative purposes, liver and kidney tissues were also assessed. We would remind the reader that the rat is generally considered to be resistant to naturally occurring and experimentally induced atherosclerosis and thus a poor model for human atherosclerosis (17). The data and discussion of the present study are solely intended to examine the potential impact of animal age, as one recognized risk factor, upon aortic smooth muscle and non-

TABLE V. LIVER HYDROLASE ACTIVITIES IN AGING RATS

Enzyme	Group age in months[a]			
	2 (15)	6 (15)	10 (13)	14 (14)
Neutral α-glucosidase	195 ± 38	232 ± 46[b]	182 ± 35[c]	219 ± 53[d]
β-Galactosidase	185 ± 45	163 ± 31	140 ± 47	161 ± 44
N-Acetyl-β-glucosaminidase	433 ± 96	432 ± 92	366 ± 101	375 ± 87
α-Mannosidase	154 ± 27	170 ± 35	94 ± 17[b,c]	100 ± 31[b,c]
β-Glucuronidase	695 ± 115	664 ± 149	510 ± 141[b,c]	661 ± 177[d]
Acid α-glucosidase	16.0 ± 2.7	20.1 ± 4.7[b]	9.9 ± 2.3[b,c]	16.2 ± 3.3[c,d]
Cathepsin C	5795 ± 663	6247 ± 1955	2853 ± 858[b,c]	3242 ± 719[b,c]

Note. The anterior lobule of the right lobe of the liver was used.

[a] Results are expressed in milliunits of enzyme activity/mg DNA, means ± SD. 1 mU of activity is defined as 1 nmole of substrate hydrolyzed per minute at 37°C. Number in parentheses = number of rats.

[b] $P < 0.05$ compared to 2-month group.

[c] $P < 0.05$ compared to 6-month group.

[d] $P < 0.05$ compared to 10-month group.

vascular tissue hydrolase activities in this mammalian species, thus complementing numerous earlier studies of other risk factors and these same rat tissues (2–5, 18).

Across the age groups of our study, body weights and DNA contents of the pooled aortic specimens are consistent with Sprague–Dawley rats of comparable ages reported by others (2–4, 19). Relative to the control data of Wolinsky and co-workers (2–5, 18) for aortic, kidney, and liver enzyme activities, our data are lower but well within the same orders of magnitude reported for each tissue. However, in comparing the levels of aortic hydrolase activities to those in kidney and liver tissues, the enzyme activities of the aorta, on a per cell basis (DNA), are one order of magnitude lower than those of the kidney and liver tissues, which is also in agreement with the data of Wolinsky and co-workers (4, 5, 18). We have expressed our enzyme-specific activities per unit of tissue DNA to allow direct comparisons of our data to earlier studies of rat aortic hydrolases and risk factors (2–5, 18) and because the hydrolases and DNA studied are constituents of the intracellular compartment of the vascular smooth muscle cells in these peeled aortic preparations (devoid of endothelium and adventitia). Although we did not specifically determine aortic cell numbers in this study, we believe that the unchanged aortic DNA content across our age groups is reflective of a relatively stable number of aortic smooth muscle cells, whereas the kidney and liver DNA contents reflect animal growth over the ages studied.

We have observed consistent increases ranging from 65 to 119% in glycosidase activities, yet no change of cathepsin C protease activity in aortic preparations from rats 2 to 14 months of age. Similarly, Kritchevsky (19) has reported that aortic lipolytic activity of Sprague–Dawley rats increases with age up to 24 months. Even though cholesterol ester synthesis also rose through this period, he further reported that the ratio of cholesteryl oleate synthesis to hydrolysis in the aorta fell from 1.95 at 2 months of age to 0.31 by 24 months, and suggested this may contribute to the atherosclerosis-resistant nature of the rat. The pattern of our data for aortic glycosidases is consistent with Kritchevsky's lipase findings, suggesting that increases (or at least no decline, as with cathepsin C) of aortic lysosomal hydrolases are a natural concurrence of increasing age in arterial tissue of rats. The fact that vascular lysosomes can adapt to changing physiological states has been clearly and repeatedly shown with experimental diabetes and hypertension (2, 3), physical activity (4), altered thyroid states (5), and dietary hypercholesterolemia (6–8). It now appears that in the rat, lysosomal glycosidase activities increase with age, as does lipase activity (19), but protease activity, as indicated by cathepsin C, remains unchanged.

Although blood pressure measurements were not made in the present study, the possibility must be raised that our observed increases of aortic enzyme activities reflect blood pressure increases reported to occur in male rats during growth and aging (20). Wolinsky (20) has shown that between 21 and 31 weeks of age, even normal rats experience significant age-related increases of blood pressure. Subsequent studies by Wolinsky and co-workers demonstrated that elevations of blood pressure could prompt enhanced lysosomal enzyme activities in the rat aorta (3).

When aortic hydrolase specific activities were calculated on the basis of tissue protein, the data fluctuated inversely between age groups compared to increases or decreases of protein content. We believe one must interpret these data with caution since this basis for expressing enzyme specific activities (Bio-Rad protein) in Table III was itself changing with animal growth and aging. More importantly, the data do not clarify what percentage of total aortic protein was measured by the Bio-Rad assay. For the same aortic segment as we studied, Wolinsky (20) reported a significant increase of noncollagenous, alkali-soluble protein in normotensive male rats between 21 to 31 weeks of age. We did not, however, determine noncollagenous, alkali-soluble protein in this study, which would have estimated intracellular protein content (20).

Our findings that kidney and liver glycosidases exhibit general declines with age after the first 6 months of growth suggest that the arterial wall may be presented with an increased circulating glycoside substrate load, and in turn, vascular lysosomes adapt their baseline hydrolytic activities to maintain new higher activity levels in the rat. However,

without determinations of endogenous glycoside loads and clearance rates, linking aortic glycosidase activity levels to those of nonvascular organs is speculative.

Possible correlation between aortic and nonvascular tissue hydrolase activities has been examined in relation to circulating low-density lipoprotein (LDL). Reports by Wolinsky *et al.* (18) and Katz-Feigenbaum *et al.* (5) have indicated that LDL clearance from the circulation of rats is more closely linked to lysosomal acid cholesterol esterase (ACE) activity of liver and kidney than aorta. Changes of ACE specific activity in these nonvascular organs reflected the fractional catabolic rate (clearance) of LDL in diabetic, exercised, and hypothyroid rats, both in direction and degree of change. These authors concluded that the catabolic capacity of the aorta may be a minor factor in total body LDL metabolism, and does not follow the same pattern of changes found in kidney and liver. Yet according to Wolinsky, the catabolic capacity of arterial tissue certainly plays a pivotal role for maintaining an influx–efflux balance between the arterial wall and circulating macromolecules, especially those containing lipid (16).

Bonner *et al.* (21) reported that aortic lysosomal enzyme activities of species resistant to experimental atherosclerosis (rat and guinea pig) have baseline levels two to four times higher than susceptible species, such as the rabbit and pig. They suggested that the aortas of resistant species are better adapted to resist the pathophysiological changes occurring with the early experimental fatty lesion. Although we did not specifically assess lipase activity, the findings of Kritchevsky (19) and Bonner *et al.* (21) complement the conspicuous absence of any age-related declines of aortic hydrolase activities in the present study. Collectively, these studies demonstrate that over increasing ages the rat aorta experiences intrinsically enhanced cholesteryl esterase (19) and glycosidase activities with no diminution of cathepsin C protease activity.

In summary, rat aortic glycosidase activities increased between 2 and 14 months of age while cathepsin C activity was unaltered. For example, we observed age-related elevations of specific activities ranging from a 65% increase for β-glucuronidase to a 119% increase for *N*-acetyl-β-glucosaminidase. In contrast, whole kidney glycosidase activities declined between 39 and 55%; cathepsin C was unchanged. Liver tissue enzyme activities varied between a 12% increase (neutral α-glucosidase) to a 35% decline (α-mannosidase) over the 14-month period. Multiple regression analyses indicated that elevations of aortic glycosidase activities correlated with animal age, not kidney nor liver enzyme activities. In addition to lipase studies by others (19), our findings indicate that the intrinsic catabolic state of the rat aorta does not decline with growth and aging.

We thank Dr. G. Bell for statistical advice, Ms. Virginia Coryell and Debby Pallett for data processing, Dr. T. M. Hollis of The Pennsylvania State University for use of his fluorometer, and Ms. Pat Broyles for typing this manuscript.

1. de Duve C. The participation of lysosomes in the transformation of smooth muscle cells to foamy cells in the aorta of cholesterol-fed rabbits. Acta Cardiol (Suppl) **20**:9–25, 1974.
2. Wolinsky H, Goldfischer S, Capron L, Capron F, Coltoff-Schiller B, Kasak LE. Hydrolase activities in the rat aorta. I. Effects of diabetes mellitus and insulin treatment. Circ Res **42**:821–831, 1978.
3. Wolinsky H, Capron L, Goldfischer S, Capron F, Coltoff-Schiller B, Kasak LE. Hydrolase activities in the rat aorta. II. Effects of hypertension alone and in combination with diabetes mellitus. Circ Res **42**:831–839, 1978.
4. Wolinsky H, Goldfischer S, Katz D, Markle R, Gidez L, Wassertheil-Smoller S, Coltoff-Schiller B. Hydrolase activities in the rat aorta. III. Effects of regular swimming activity and its cessation. Circ Res **45**:546–553, 1979.
5. Katz-Feigenbaum D, Braun L, Wolinsky H. Hydrolase activities in the rat aorta. V. Comparison to activities in liver and kidney after thyroidectomy and relation to dynamic clearance of circulating low density lipoproteins. Circ Res **49**:733–741, 1981.
6. Haley NJ, Shio H, Fowler S. Characterization of lipid-laden aortic cells from cholesterol-fed rabbits. I. Resolution of aortic cell populations by Metrizamide density gradient centrifugation. Lab Invest **37**:287–396, 1977.
7. Goldfischer S, Schiller B, Wolinsky H. Lipid accumulation in smooth muscle cell lysosomes in primate atherosclerosis. Amer J Pathol **78**:497–504, 1975.
8. Wolinsky H, Goldfischer S, Daly MM, Kasak LE, Coltoff-Schiller B. Arterial lysosomes and connective tissue in primate atherosclerosis and hypertension. Circ Res **36**:553–561, 1975.
9. Bierman EL, Ross R. Aging and atherosclerosis. In:

Paoletti R, Gotto AM Jr, eds. Atherosclerosis Reviews. New York, Raven Press, Vol 2:pp79–111, 1977.
10. Markle RA. Aortic hydrolase activities in rats of varying age. Fed Proc **42**:807, 1983.
11. Wolinsky H, Daly MM. A method for the isolation of intima-media samples from arteries. Proc Soc Exp Biol Med **135**:364–368, 1970.
12. Florkin M, Stolz EH. Comprehensive Biochemistry, 3rd ed. New York: American Elsevier, 1973.
13. Burton K. A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J **62**:315–323, 1956.
14. Bio-Rad Protein Assay, Bulletin 1069. Richmond, Calif., Bio-Rad Laboratories, 1982.
15. Snedecor GW, Cochran WG. Statistical Methods, 7th ed. Ames, Iowa State Univ. Press, 1980.
16. Wolinsky H. Special article. A proposal linking clearance of circulating lipoproteins to tissue metabolic activity as a basis for understanding atherogenesis. Circ Res **47**:301–311, 1980.
17. Jokinen MP, Clarkson TB, Pritchard RW. Recent advances in molecular pathology. Animal models in atherosclerosis research. Exp Mol Pathol **42**:1–28, 1985.
18. Wolinsky H, Katz D, Markle R, Mills J, Brem S, Wassertheil-Smoller S. Hydrolase activities in the rat aorta. IV. Relation between clearance rates of circulating ^{125}I-labeled low-density lipoproteins and levels of tissue hydrolase activity. Circ Res **47**:433–442, 1980.
19. Kritchevsky D. Age-related changes in lipid metabolism. Proc Soc Exp Biol Med **165**:193–199, 1980.
20. Wolinsky H. Effects of hypertension and its reversal on the thoracic aorta of male and female rats. Circ Res **28**:622–637, 1971.
21. Bonner MJ, Miller BF, Kothari HV. Lysosomal enzymes in aortas of species susceptible and resistant to atherosclerosis. Proc Soc Exp Biol Med **139**:1359–1362, 1972.

Received July 26, 1985. P.S.E.B.M. 1986, Vol. 183.
Accepted June 24, 1986.