enzyme activity should be taken into consideration, these results collectively suggest that erythrocytes in the peripheral circulation lose at least 50% of their SOD activity during the process of aging, starting from reticulocytes to the presumably oldest cells. Such a change of erythrocyte SOD activity appears to be a function only of the age of the cells themselves, and probably is not a reflection of the donor's age. Although this was not specifically determined in the present study, a lack of correlation found in other work between the erythrocyte SOD activity and the age of human subjects may serve as supporting evidence[16-18].

The biological significance of increased reticulocyte SOD activity still remains to be elucidated, but some possibilities may be suggested. Compared with mature erythrocytes, reticulocytes, which contain mitochondria, are known to engage in an active aerobic metabolism, for example involving the tricarboxylic acid cycle, with a markedly higher oxygen consumption[19]. Consequently, a higher SOD activity may be required in reticulocytes than in mature cells in order to scavenge possibly increased generation of superoxide anions, and thus effectively to maintain an oxidative metabolism. A number of erythrocyte enzymes are found to decrease with increased age of cells, i.e., higher activities are associated with younger cells[20-24]. It appears that a gradual lowering of various enzyme levels is a finding commonly associated with aging of erythrocytes. Alternatively, therefore, reticulocyte SOD may simply conform to this physiological pattern of senescence.

Correlation of elevated SOD activity of reticulocytes with their cellular functions has not been specifically evaluated. According to Walls et al.[25], young erythrocytes of humans are shown to be able to protect themselves effectively against thyroxine-peroxide induced hemolysis, while older cells exhibited less protection. Although their sensitivity was discussed primarily in relation to decreased glucose-6-phosphate dehydrogenase activity in older cells, there is a possibility that reduced SOD activity may also be a contributing factor to the above reaction.

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Metabolism of resorcinol and salicylate in *Aspergillus niger*

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Summary. In *A. niger*, resorcinol and salicylate are catabolized to β-ketoadipate, following ortho ring fission. The enzymes involved in the degradation are repressed in the presence of a primary substrate like glucose.

The degradation of resorcinol and salicylate has been studied extensively in bacteria[2-4], but comparatively little is known about the metabolism of these aromatic compounds in yeasts and fungi. However, Neujahr and coworkers have studied the resorcinol and phenol metabolism in *Trichosporon cutaneum* and *Candida tropicalis*[5,6]. *A. niger* is known to degrade various aromatic compounds[7,8]. Degradation of mandelic acid and benzoic acid in this organism occurs by the protocatechuate pathway while salicylate is catabolized by the β-ketoadipate pathway[9,10]. The objective of this study was to investigate the mode of degradation of resorcinol in this mould and compare it with that operative in bacteria.

A. niger was grown in shake cultures at 28±1 °C using resorcinol (0.5% W/V), salicylate (0.75%) and glucose (2% W/V) as carbon sources in a synthetic medium[11]. Oxygen uptake was measured using a washed cell suspension, as described earlier[10]. After oxygen consumption has

ceased, cells were removed and β-ketoadipate was determined in the supernatants by the Rothera color reaction[12]. The supernatant culture fluid was acidified to pH 2 with HCl and extracted with diethylether. The organic layer was evaporated to a small volume and the metabolites were separated by ion exchange chromatography on a Dowex 5OW−X4 (100/120 mesh) column (100×1.2 cm)[13]. The fractions with separated metabolites were evaporated to small volume and spotted on 0.25 mm thick silica gel-G plates. The chromatograms were developed with solvent (benzene-methanol-acetic acid; 45:8:4). Compounds were located by using UV-light as well as by spraying with the mixture of equal parts of 0.3% solutions of $FeCl_3$ and $K_3Fe(CN)_6$. The R_f-values of the developed spots were compared with authentic samples. UV-spectra were determined on a Zeiss DMR 21 recording spectrophotometer. The preparation of cell free extracts and assay methods for salicylate hydroxylase and catechol 1,2 oxygenase were the same as those described earlier[10]. All the enzymes except maleyl acetate reductase were partially purified by $(NH_4)_2SO_4$ fractionation followed by dialysis and gel filtration through sephadex G-200 columns (30×2.2 cm). All the steps in the purification procedure were carried out at 0–5 °C (table 1). Resorcinol hydroxylase activity was measured by following the oxidation of NADPH at 340 nm in a reaction mixture containing in μmoles: Tris-SO_4, NADPH 0.5, resorcinol 0.5, FAD 20 nmoles and appropriate amount of enzyme protein to final volume of 3 ml. The reaction was started by the addition of resorcinol. 1 unit of enzyme was defined as the amount of enzyme that catalyses the disappearance of 0.5 μmoles of NADPH/min, in presence of resorcinol. The activity of maleyl acetate reductase was checked by the method described by Gall et al.[14]. Protein was determined by the method of Lowry et al.[15].

Table 1. Purification of salicylate and resorcinol-degrading enzymes in *A. niger*

Step	Catechol 1,2** oxygenase Sp.act.	Fold	Salicylate hydroxylase Sp.act.	Fold	Resorcinol hydroxylase Sp.act.	Fold
Crude extract	0.21	1	0.26	1	0.30	1
Protamine sulphate	0.33	1.5	0.34	1.3	0.41	1.36
$(NH_4)_2SO_4$ fraction*	0.95	4.5	1.35	4.8	1.5	5.0
Sephadex G-200 eluate	3.83	18.2	5.14	19.7	9.1	30.3

*45–65% $(NH_4)_2SO_4$ saturation was used for catechol 1,2 oxygenase and salicylate hydroxylase whereas a saturation of 40–70% was used for resorcinol hydroxylase. **From salicylate grown *A. niger*.

Table 2. Respiratory activities; QO_2 of whole cells of *A. niger* grown on different sources of carbon

Substrate used in respirometric measurements	QO_2 of cells grown on Glucose	Salicylate	Resorcinol
Glucose	53	38	48
Benzoate	27	31	28
p-(OH) benzoate	31	25	28
Protocatechuate	38	44	26
Salicylate	42	100	29
Catechol	30	91	50
Resorcinol	30	51	80
Hydroxyquinol	ND	38	62

ND, not detectable; QO_2; expressed as $\mu l0_2$ consumed/h/mg dry wt of cells.

Table 3. Specific activities of enzymes of resorcinol and salicylate degradation in cell free extracts of *A. niger* grown on different carbon sources

Enzyme	Carbon source Glucose	Resorcinol	Salicylate
Catechol 1,2 oxygenase	ND	0.26±0.009	0.21±0.007 (3.81)*
Salicylate hydroxylase	ND	0.11±0.004	0.26±0.015 (5.14)
Resorcinol hydroxylase	0.17±0.006	0.31±0.009 (9.1)	0.13±0.007
Maleylacetate reductase	ND	0.65±0.012	ND

ND, not detectable. Values are means±SE of 5 determinations. *Values in brackets are specific activities of partially purified enzymes.

Results and discussions. Oxygen uptake rates of glucose-grown cells on salicylate, resorcinol, catechol and hydroxyquinol were lower as compared to those observed in cells adapted to either salicylate or resorcinol (table 2). Cells grown on salicylate oxidized salicylate and catechol more rapidly than any other aromatics. Similarly, resorcinol grown culture exhibited higher oxygen uptake rates on resorcinol, catechol and hydroxyquinol. When *A. niger* was grown in mineral salt medium containing resorcinol as carbon source, only one metabolite besides the substrate could be isolated and identified as hydroxyquinol (as discussed under Methods). Absorption spectra of this metabolite showed a peak at 259 nm region. This spectral behavior is characteristic of hydroxyquinol after autooxidation[16]. When partially purified catechol 1,2 oxygenase from *A. niger* was incubated with hydroxyquinol, only 1 peak at 243 nm could be seen, which disappeared at pH 3 and reappeared on neutralization. These spectral characteristics are consistent with the formation of maleyl acetate[14]. Detection of β-ketoadipate in the supernatant after incubation of cells with resorcinol or hydroxyquinol, suggested that resorcinol is converted to β-ketoadipate through hydroxyquinol and maleylacetate as the intermediates. The degradation of salicylate in this mould is also by the β-ketoadipate pathway[10]. It was also observed that the activities of resorcinol hydroxylase and maleylacetate reductase in resorcinol-grown cultures were higher than in salicylate-grown cultures, whereas salicylate-grown cells showed higher activities of salicylate hydroxylase, but the activity of catechol 1,2 oxygenase in both the cultures was more or less same (table 3). However, the glucose-grown culture did not show a significant level of activity of any of the above enzymes. Our results suggest that resorcinol degradation differs from salicylate degradation in *A. niger*, though the mode of ring fission remains the same. In *Pseudomonas putida*, resorcinol is converted to hydroxyquinol, which is further catabolized via either ortho or meta cleavage[2]. In *A. niger*, resorcinol is converted to hydroxyquinol, followed by ortho ring fission which forms maleylacetate, which in turn is converted to β-ketoadipate. A similar pathway for degradation of resorcinol in *T. cutaneum* has been proposed by Gall and Neujahr[14]. Glucose is known to cause a catabolite repression in a variety of bacteria and yeasts[17]. Moreover, glucose is also known to inhibit the induction of phenol degradation in cultures of *T. cutaneum*[18]. Hence, it was not surprising to observe that glucose-grown cultures of *A. niger* did not show significant activities of any salicylate and resorcinol degrading enzymes.

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Endothelial cell coat modifications in rat thoracic aorta. Effect of ovariectomy and cigarette smoke[1]

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Summary. The effects of acute cigarette smoking and bilateral overiectomy on the thickness of rat aortic cell coat (Con A) were investigated. Ovariectomized rats showed a significant increase in the thickness of the cell coat. When cigarette smoking was combined with ovariectomy the thickness of the reaction product was similar to controls. Cigarette smoke without ovariectomy resulted in a decreased thickness, but these changes were not significant.

The pathogenesis of arteriosclerosis remains obscure in spite of all the experimental data accumulated. A recent hypothesis suggests a direct relationship between endothelial cell injury and the initiation of arteriosclerotic disease[3-6]. An increase in endothelial permeability is one of the initial alterations observed during the reproduction of experimental arteriosclerosis regardless of the model used: hypertension, hypoxia, immune complexes, carbon monoxide, cholesterol[7,8] and cigarette smoke[9,10]. Certain authors attribute these changes in permeability to quantitative or qualitative modifications of the cell coat[6,8,11,12]. The glycoproteins of the cell coat described by Luft[13] constitute a structural element essential for the integrity of the endothelial cell[14]. In addition to its anti-thrombotic properties, assured by the presence of heparan sulfate[14], the cell coat plays an important role in the mechanism of molecular selectivity[15,16].

In this investigation we attempt to better define the possible relationship between the cell coat and certain risk factors for arteriosclerosis, using the combination of cigarette smoke and bilateral ovariectomy. Both factors are closely related to this pathologic phenomenon[17-20]. Recent studies showed that cigarette smoke[9,10] produced, in rat thoracic aorta, a subendothelial enlargement induced by the presence of large vesicular structures and an increased number of monohistiocytic cells. Bilateral ovariectomy resulted in this phenomenon, but to a lesser degree[21]. This intimal thickening, a morphological expression of an increased permeability, could be closely associated with a modification of the cell coat in pre-arteriosclerotic changes.

Experiments were carried out using the technique of Bernhard and Avrameas[22] (concanavalin A-peroxidase) to reveal the cell coat of rat thoracic aorta.

Materials and methods. In this study 45 female Sprague-Dawley rats, weighing 200–225 g at their arrival, were used. Each animal was housed in an individual cage. The animals were divided into 9 groups of 5 rats: absolute controls; ovariectomized; sham ovariectomized; smokers; sham smokers; ovariectomized-smokers; ovariectomized-sham smokers; sham ovariectomized-smokers and sham ovariectomized-sham smokers. After Nembutal anesthesia (50 mg/kg) a dorsal bilateral ovariectomy was performed in the 3 ovariectomized groups. The 3 groups of sham ovariectomized animals were subjected to cutaneous and muscular incisions only. Animals were sacrificed 10 weeks after the surgical procedure. The 3 groups of smokers were exposed to cigarette smoke (2 cigarettes containing 25 mg tar and 1.5 mg nicotine) for a period of 8 min each, at 5-min

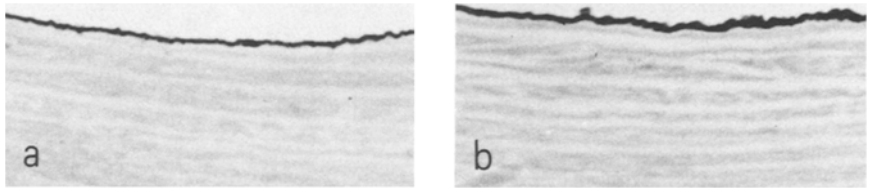

Figure 1. *a* Thoracic aorta of absolute control rat. The cell coat is revealed by the Con-A reaction in the form of a dark and continuous thin layer, covering the luminal surface of the endothelium. *b* Thoracic aorta of ovariectomized rat. Note the thick and granular reaction product at the luminal surface, as compared to absolute control. LM, × 375.