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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

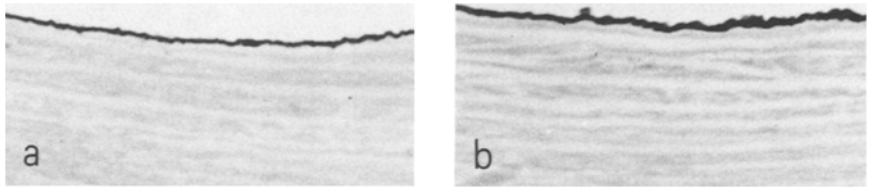

Figure 1. *a* Thoracic aorta of absolute control rat. The cell coat is revealed by the Con-A reaction in the form of a dark and continuous thin layer, covering the luminal surface of the endothelium. *b* Thoracic aorta of ovariectomized rat. Note the thick and granular reaction product at the luminal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

intervals, according to the standard model (a 2-sec 35-ml puff/min)[23]. The smoking machine was similar to that described by Simani et al.[24]. Sham smokers were placed in the smoking machine cylinders for the same period of time as the smokers, however, they were only exposed to air.

After light ether anesthesia, the rats were sacrificed by aldehyde perfusion of the left ventricle[25], 5 min after smoking the last cigarette in the case of smokers and 5 min after being submitted to the stress of the smoking machine for sham smokers. The thoracic aorta was dissected, divided into 3 portions (3 mm each) and prepared for the concanavalin A-peroxidase treatment according to the method of Bernhard and Avrameas[22].

The thickness of the concanavalin-A reaction product was evaluated using unstained semi-thin sections (1 μm). The surface of 3 transversal aortic sections were photographed at a magnification of ×50. From a photograph magnified ×375, 10 measurements were obtained at regular intervals with the aid of a Zeiss OpMi-1 stereomicroscope equipped with an 80 unit micrometer. 150 measurements were made for each group. All of the data were analyzed using Student's t-test.

Results. The reaction between the glycoproteins of the cell coat and concanavalin A was observed by light microscopy in the form of a dark, brownish product. In control rats a continuous layer (1.41±0.37 μm) covered the luminal surface of the endothelium (fig. 1a). Transmission electron microscopy revealed a dense reaction product which is located at the external portion of the plasma membrane. Plasmalemmal vesicles were also labelled (fig. 2a).

Sham ovariectomized (1.39±0.27 μm) and sham smoker-sham ovariectomized (1.47±0.35 μm) animals have a cell coat thickness similar to that of control rats. Sham smokers showed a decreased cell coat thickness (1.25±0.21 μm) but not significantly different from controls. A decrease in cell coat thickness was observed in smokers (1.23±0.16 μm) and in smokers-sham ovariectomized (1.23±0.16 μm). However, these results were also not significant. Ovariectomized (1.81±0.37 μm) and sham smoker-ovariectomized animals (1.87±0.51 μm) revealed a significant increase in cell coat thickness when compared to controls (fig. 1b). Furthermore, transmission electron microscopy showed that this thick and dense reaction was flocculent (fig. 2b). Smoker-ovariectomized animals (1.28±0.27 μm) revealed that combined treatment decreased cell coat thickness, but not significantly. These results are summarized in the table.

Discussion. These experiments showed that the endothelial cell coat from rat thoracic aorta is modified by arteriosclerotic risk factors. Ovariectomy increased the thickness of the cell coat as revealed by concanavalin A (Con-A). Cigarette smoke in itself slightly decreased the thickness of the reaction product (not statistically significant) but when combined with ovariectomy, a significant contrary effect was produced. In fact, smoker-ovariectomized animals showed that the increase of cell coat thickness after ovariectomy was inhibited by cigarette smoke. The mechanism of action of these 2 factors on the cell coat remains obscure. However, it is known that estrogens[26,27] and cigarette smoke[28] can influence the metabolism and turnover of glycoproteins.

Several studies have been done regarding variations of the endothelial cell coat with risk factors other than ovariectomy and cigarette smoke. Depending on the histochemical method used, hypercholesterolemia initially produced contrary effects: Con-A showed an increase in cell coat thickness[29,30], while ruthenium red (R.R.) showed a decrease[31]. R.R. revealed that cell coat thickness decreased with hypertension[11], whereas experiments in which aging was studied with Con-A, showed a gradual increase[29]. The different molecules labelled by R.R. and Con-A may explain these conflicting results. R.R. is a low molecular weight polycation which binds electrostatically with polyanions (hyaluronic acid, chondroitin sulfate) and sialoglycoproteins[13], whereas Con-A is a lectin with 4 active sites which react with glycoproteins carrying the terminal non-reducing α-D-glycopyranosyl, α-D-mannopyranosyl or β-D-fructo-

Concanavalin A reaction thickness at the luminal surface of rat thoracic aorta as measured by light microscopy

Experimental group	Con-A reaction thickness (μm ± SD)
Control	1.41±0.37
Smoker	1.23±0.16
Sham smoker	1.25±0.21
Ovariectomized	1.81±0.37*
Sham ovariectomized	1.39±0.27
Smoker - ovariectomized	1.28±0.27
Sham smoker - ovariectomized	1.87±0.51*
Smoker - sham ovariectomized	1.23±0.16
Sham smoker - sham ovariectomized	1.47±0.35

*Statistically significant difference from absolute control ($p < 0.05$).

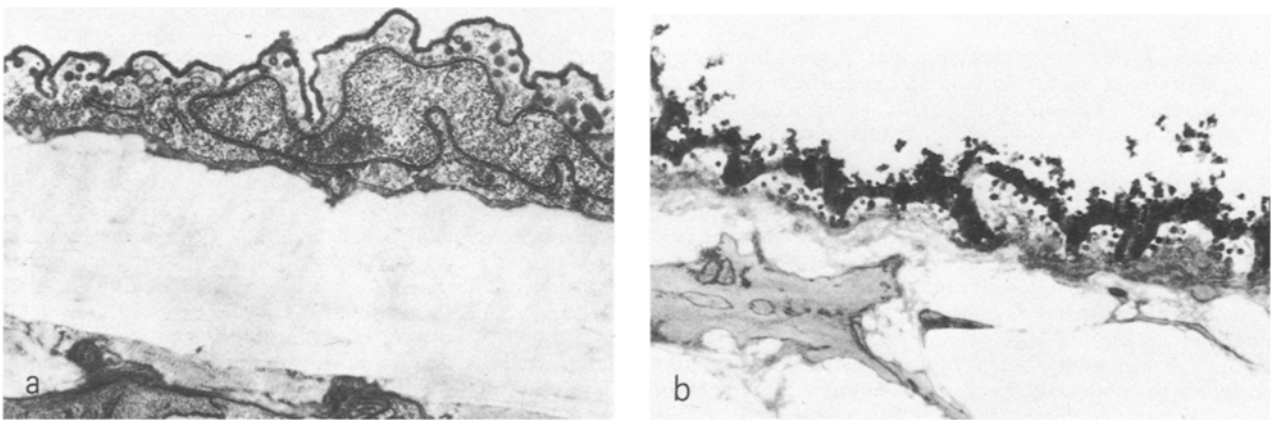

Figure 2. *a* Thoracic aorta of absolute control rat. A thin layer of the reaction product is covering the luminal surface of the endothelial cell. Plasmalemmal vesicles near the lumen are also labeled. TEM, ×15,500. *b* Thoracic aorta of ovariectomized rat. The cell coat is different from control. Note the increased thickness and the flocculent appearance of the reaction product. TEM, ×11,600.

furanosyl residues[32]. It is believed that R.R. and Con-A do not specifically identify the same macromolecules[33].

In our experiments, changes observed in the thickness of the cell coat as visualized by Con-A permitted us to conclude that ovariectomy and cigarette smoke associated with ovariectomy modified glycoproteins on the surface of the thoracic aorta. We were unable to determine whether these changes at the molecular level were of a qualitative or quantitative nature. Also, we cannot be sure that cigarette smoke in itself did not affect to cell coat. These cell surface modifications are probably very important for explaining the increase in permeability during the initial stages of experimental arteriosclerosis since the integrity of the endothelial cell plasma membrane is directly dependent on the quantity and quality of structural glycoproteins in the cell coat[34].

Furthermore, these findings are important because they emphasize that hormonal factors can be related to arteriosclerosis and this may explain the higher rate of the disease in men.

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Cyst formation in metanephric organ culture induced by cis-dichlorodiammineplatinum (II)

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Summary. A new experimental model of renal tubular cyst formation has been developed in metanephric organ culture. The addition of cis-dichlorodiammineplatinum (II), 50 μg/ml, to culture medium induces cystic changes during in vitro nephrogenesis. The model has particular utility in the study of basic mechanisms underlying renal tubular cystic changes, as well as the mechanisms by which nephrotoxins may mediate renal tubular injury.

The pathogenesis of human renal polycystic disease remains unknown despite numerous experimental studies in animal models[3,4]. We have recently described a mouse metanephric organ culture system for the study of normal renal development in which organotypic tubulogenesis and glomerular epithelial formation occur without capillary ingrowth or the presence of endothelial or mesangial elements[5]. Drawing on a recent report of renal cyst formation induced in adult rats by the i.v. administration of cis-dichlorodiammineplatinum (II) (CP)[6], we have utilized CP to induce cystic changes in the serum-free organ culture model. We have thus developed an experimental model in which renal polycystic changes occur during in vitro nephrogenesis without vascularization, glomerular filtration, or tubular urine formation.

Materials and methods. Our method of whole metanephric organ culture has been described in detail[5]. Pregnant Swiss-Webster albino mice are sacrificed by cervical dislocation at 13±0.4 days gestation. Under aseptic conditions fetal metanephric tissue is microdissected from embryos and transferred to a 0.8 μm Millipore filter sitting atop a Trowell double-welled organ culture assembly. Culture medium, which consists of equal volumes of Dulbecco's modified essential media and Ham's F-12 medium, supplemented with insulin (5 μg/ml), PGE-1 (25 ng/ml), T_3 (3.2 pg/ml), hydrocortisone (5 μg/ml), and transferrin (5 μg/