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Estrogen Effects on the Aortic Wall in Young Immature Rabbits*

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Oestrogenwirkungen auf die Aortenwand bei jungen geschlechtsunreifen Kaninchen

Zusammenfassung. Es wurde die Oestrogenwirkung auf die Aortenwand jugendlicher geschlechtsunreifer neuseeländischer Kaninchen beiderlei Geschlechtes histologisch untersucht. Dabei wurden kleine Dosen und eine jeweils kurze Behandlungsdauer gewählt. Durch ausgedehnte Kontrollen wurde gezeigt, daß individuelle Verschiedenheiten sowohl im histologischen Bau der Aortenwand als auch bezüglich der Reagibilität der Tiere auf die gesetzten Hormonwirkungen bestanden. Die Oestrogenmedikation zeitigte eine sog. gesteigerte Aktivität der Aortenwandzellen, eine Verbreiterung der kollagenen Fibrillen und eine quantitative Vermehrung der Grundsubstanz. Unter dem Einfluß der Oestrogenmedikation wurde die Aortenwand dicker. Die mit Oestrogen behandelten Kaninchen zeigten einen signifikant niedrigeren Prozentsatz sog. spontaner Arteriosklerose als die Kontrolltiere.

Summary. The effect of estrogen (estradiol) on the aortic wall of immature young (New Zealand) rabbits of both sexes was studied with the light microscope. The hormone was given in small doses for short periods. Comparative studies of the aortas of control animals showed the known individual variations in the histology of the vessel wall. The studies of the estrogen-treated animals revealed the well-known variable response to hormonal effects. Estrogen in experimental animals induced progressive changes: cellular hyperactivity with proliferation, an increase in the amount of ground substance, and an enlargement of collagenous fibers. These changes were essentially those known as extra-genital estrogen effects observed in many other animal species. Estrogen changed the aortic wall making it stronger. The estrogen animals had a significantly lower percentage of spontaneous arteriosclerosis than did the control animals.

Since the publications of Katz, Stamler (1953), and Pick (1958) it has been known that estrogens have an influence on coronary arteriosclerosis in chicks. Larger doses are capable of inducing (Lindsay, 1946); (Chaikoff, Lindsay, Lorenz, Entenman, 1948); (Horlick and Katz, 1948), and smaller doses may prevent or minimize the lesions (Pick, 1952). Of some importance is the observation, in fowl, that the reversal of arteriosclerosis is seen in the coronary arteries, but not in the aorta (Katz and Stamler, 1953). Histological studies of the effects of estrogen upon the aorta of birds have shown only an increase in basophilia and macrophages. Histological and histochemical studies of the coronary arteries of the estrogen birds have not resulted in any morphological evidence for the understanding of estrogen-induced anti-atherogenesis (Hoj-

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MAN, PELLEGRINO, IRALDIA, MALINOW, PICK, STAMLER and KATZ, 1959). It was concluded, therefore, that the mechanism of action of the estrogens was through the alteration of lipid metabolisms by favorably changing the ratio of cholesterol and phospholipids (STAMLER, PICK and KATZ, 1956).

After the observation that estrogen has a favorable effect on arteriosclerosis, other different animals and humans were studied for these anti-arteriosclerotic or anti-atherosclerotic effects. There are many summarized papers and monographs in the literature dealing with these different problems (PINCUS, 1959; MALINOW, 1959; MARMORSTON, 1960; DISZFAULUSY and LAURITZEN, 1961; STAMLER, 1963).

A careful study of published data relating to the effects of estrogens in rabbits reveals that these data are not always in agreement. There are papers reporting that an anti-arteriosclerotic effect on coronary and aortic lesions was not seen (STAMLER, PICK and KATZ, 1956), while other reports indicate that a favorable estrogen effect had been observed in the coronary as well as the aortic lesions (CHARKRAVATI and MUKEREJI, 1956). There are reports indicating that cholesterol fed rabbits treated with estrogen have shown less sudanophilic material in the aortic wall than the controls (CONSTANTINIDES and GUTMAN-AUERSBERG, 1960). Favorable athero-preventive effects after estrogen treatments have been observed in the aortas of non-castrated female rabbits, and unfavorable effects in castrated females and intact males (BRUGER, WRIGHT and WILAND, 1943). Further, there is our own report which showed that estrogen increased the ground substance and cellular activity, and produced stronger, denser collagenous fibers (GOSTIMIROVICH, 1962). Rats, too, have shown favorable anti-arteriosclerotic effects on the aorta (MALINOW and PELLEGRINO, 1958) and on coronary arteries (M. MOSKOWITZ, A. MOSKOWITZ, BRADFORD, WISSLER, 1958) after estrogen treatments.

The purpose of this publication is to present detailed data and micrographs of our own investigation. Before doing so, however, one interesting observation should be mentioned — all sesame oil controls have shown an increase of the ground substance. This observation led us to study different fat effects with different iodine numbers and their effects on arteriosclerosis of the aortic wall and smaller vessels in rabbits. The results of these studies will follow.

Material and Methods

Eighty-three young immature rabbits, both sexes (0.75—1.2 kg) fed with normal commercial pellets and ordinary drinking water were selected. Of these, 32 were treated with Estradiol (Progynon-Schering) and 51 were controls. The Estradiol was given subcutaneously in a watery solution to 15 of the animals, 1—3 mg, for 9 days for a total of 10 mg, and Estradiol Benzoate was given subcutaneously in a sesame oil solution to 17 animals, 0.2 mg, 4 times in 8 days for a total of 0.8 mg. Of the 51 controls, 20 were not treated, 15 were treated with sterile water, and 16 with sesame oil, all in the same amount and frequency as the Estradiol animals. The effects of Estradiol on the aortas in the region of the arches, and on the genital organs were studied. Immaturity of the animals was proven in each case by histological examination of the gonads. The material for histological examination was fixed in Bouin solution. For fat content, unfixed or briefly fixed material was selected. Metachromasia was studied with Toluidine Blue or Colloid-Iron stain. Acid and neutral mucopolysaccharides was studied with Alcian Blue, with and without PAS stain (AFIP Manual, 1960). The

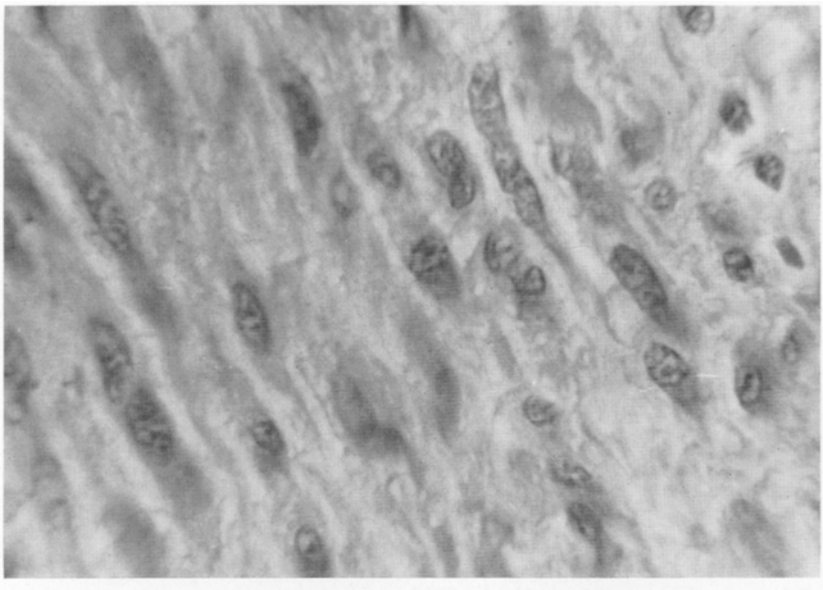

Fig. 2

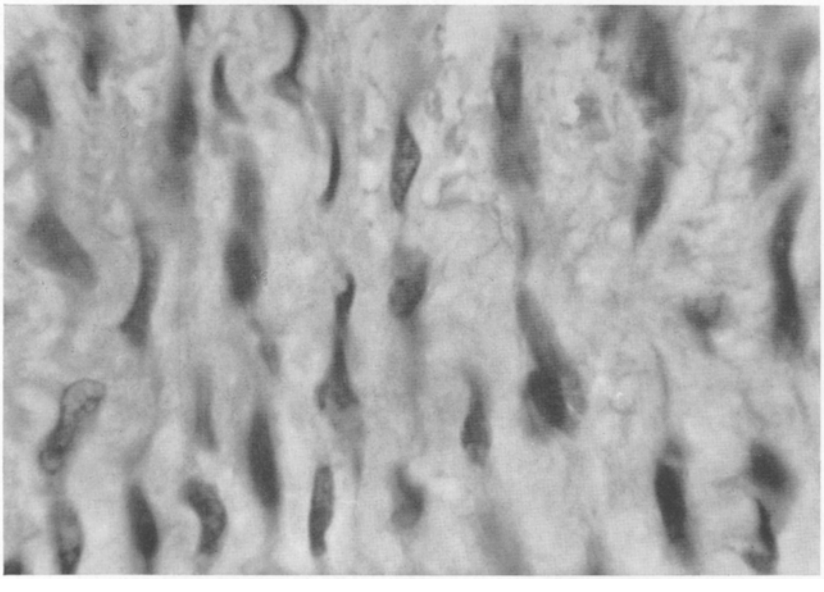

Fig. 1

Fig. 1. Media of an aortic wall of an untreated control animal (110.I). The muscular nuclei are different shaped with a less prominent chromatin pattern. The matrix is coarse granular. H.E. stain. (Enl. 970×)

Fig. 2. Media of an estrogen animal treated with a watery solution of estradiol for 9 days with a total dosage of 10 mg (461. VI) showing a marked chromatin pattern of the muscular nuclei. The ground substance is predominantly fine granular threaded. H.E. stain. (Enl. 970×)

elastica fibers were studied with VERHOEFF'S, the reticular fibers with WILDER'S stain. For differentiation of cells of the vessel wall, Weigert Iron Hematoxylin, P.T.A.H. and Mallory stain were chosen. The connective tissue was studied in Mallory and Van Gieson stain. The judgment of spontaneous arteriosclerosis was based on the occurrence of metaplastic endothelium or proliferation of intima, and on changes in media. The splitting of elastica fibers, larger areas of hyalinization, necrotic areas, with or without calcification, the occurrence of larger areas of fibrosis and the occurrence of metaplasia (Cartilage or bone formation) are all changes in the media. The judgment in every case was microscopically. Only a light microscope was used.

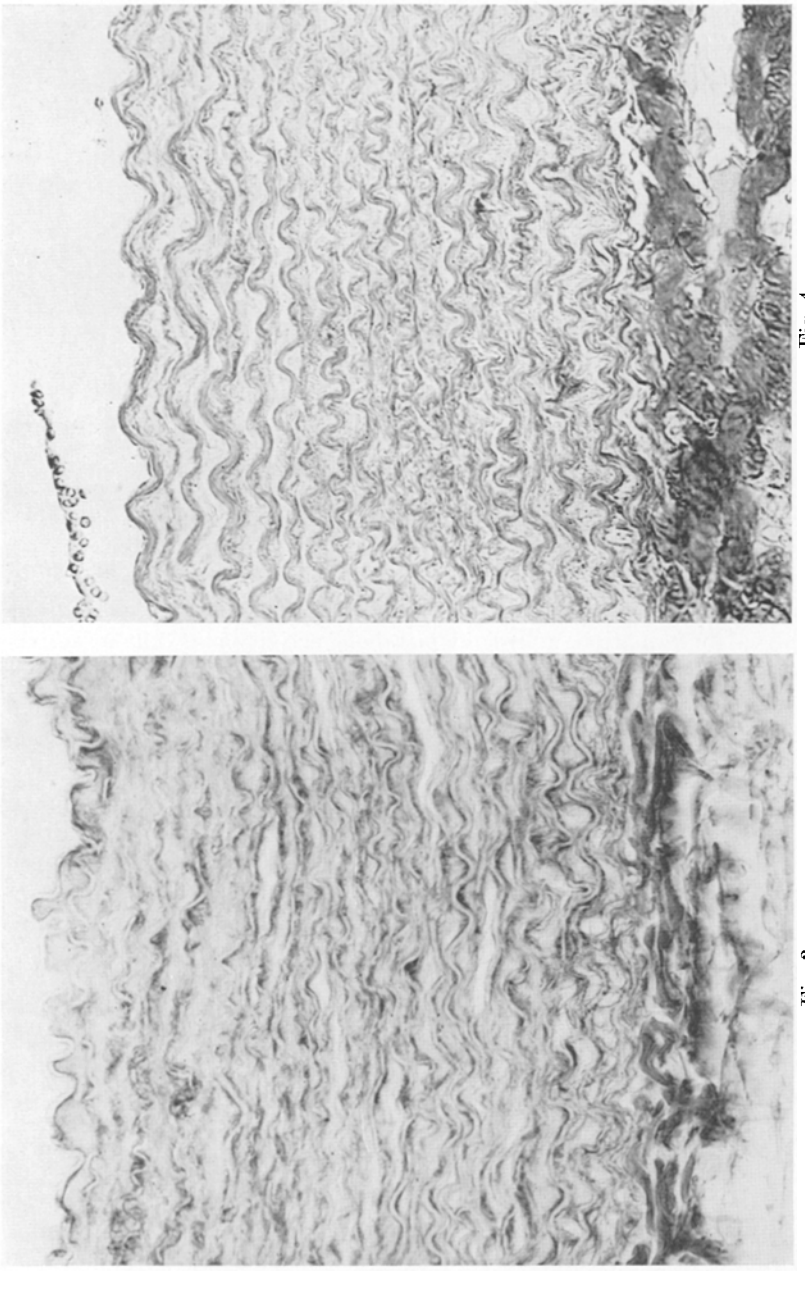

Fig. 3

Fig. 4

Fig. 3. Adventitia of an untreated control (112. I) showing collagenous fibers. Colloidal iron stain. (Enl. 489×)

Fig. 4. Adventitia of an estrogen animal treated with a watery solution of estradiol for 9 days for a total dosage of 10 mg (456. VI) showing an increase and dense collagenous fibers. Colloidal iron stain. (Enl. 480×)

Results

Individual variations in different experimental animals is known. In our study, too, variations in the aortic wall of our control animals were seen with respect to distribution of ground substance, cellular activity, development of the collagenous and elastica fibers, and the susceptibility to estrogens. After estrogen

treatment, consistent changes in varying degrees were seen in our immature animals as follows:

1. Estradiol affects the ground substance.

Morphologically, the substance is fine or coarse granular threaded. The control animals showed, predominantly, coarse granular features. Chemically, the ground substance is predominantly composed of acid and, to a lesser degree, neutral mucopolysaccharides.

Estradiol increased the total ground substance. Morphologically, the estrogen animals presented a predominantly fine granular threaded appearance.

2. Estradiol affects the cellular elements of the entire aortic wall.

The majority of the nuclei in the aortic wall are smooth muscle nuclei. The muscle nuclei show features characteristic of the active and inactive phases. The active nuclei are round, ovoid, with prominent chromatin patterns and the inactive nuclei are spindle shaped with a more homogeneous dark stained chromatin (HENNENBERG, 1901). Intermediate nuclei between these two forms are also present. Different phases of mitosis represent cellular activity. A prominent swelling of one part or end of the nucleus may represent an amitotic cell division in the absence of other signs of cell division (BENNINGHOFF, 1922). An indentation of a spindle shaped nucleus with prominent chromatin, with or without a small vacuole-like formation, represents metabolic activity plus cellular activity (FRANCILLON, 1926—1927). Other cells of the aortic wall of mesenchymal origin are fibroblasts, macrophages, mesenchymal cells, and "transitional cells" (BENNINGHOFF, 1930), all of which are more prominently seen in estrogen treated animals.

All cellular elements of the aortic wall of estrogen animals appear numerically increased, and show, morphologically, more prominent chromatin patterns and features of activity with more frequent mitotic features than the controls.

3. Estradiol affects the collagenous fibers.

The collagenous fibers of the entire aortic wall of estrogen animals are coarser and denser than in the controls. This was clearly demonstrated with special stains.

The aortas of the estrogen animals have shown less spontaneous arteriosclerosis than the controls. The spontaneous arteriosclerosis in controls was $42.06 \pm 6.98\%$, and in estrogen animals $6.25 \pm 4.28\%$. The difference is highly significant. Estradiol has produced, in a high percentage of our immature rabbits, a reversal effect on spontaneous aortic arteriosclerosis.

Morphological findings in the aortic wall of our immature rabbits subjected to the above treatment are essentially the same, but to a far less degree, as those seen in the genital or target organs of estrogen.

Estrogen affects the extra-genital tissue in different animal species (BURROWS, 1949). It is known that estrogen causes an increase of the matrix of connective tissue (KOCH, 1950—1951), an increase of different cells with mitotic cell division in the entire body except for the brain and striated muscle (BULLOUGH, 1946), and dense collagenous fibers of most of the connective tissue of the body (BURROWS, 1949).

The stress of the blood flow regulates the morphology of the vessel wall. The estrogens altered the plasticity of the vessel wall, causing a stronger ground substance, stronger fibers of the connective tissue, and an increase of mesenchymal cell proliferation. These changes are synergic to normal repair.

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