

## Effects of Different Doses of Estrogen on the Aortic Wall of Young Immature Rabbits\*

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Received October 25, 1969

### *Über die Wirkung unterschiedlicher Oestradiol-Dosen auf die Aortenwand junger, noch nicht geschlechtsreifer Kaninchen*

*Zusammenfassung.* 77 jugendliche Neuseeland-Kaninchen beiderlei Geschlechtes wurden mit verschiedenen Dosen von Oestradiol behandelt und mit 72 unbehandelten Kontrolltieren vergleichend daraufhin geprüft, welche Veränderungen an der Aortenwand entstanden waren. Die Tiere waren noch nicht geschlechtsreif. Die Oestradiol-Medikation wurde über Zeitspannen von 8—22 Tagen vorgenommen. Es fanden sich uncharakteristische Veränderungen, Mitosen und circumscripate Proliferationen der Aortenmedia. Echte skleratheromatöse Bilder wurden nicht gesehen. Dagegen schien die Hormonmedikation zur Entwicklung von Remissionen beitragen zu können. Die Untersuchungen wurden mittels einfacher lichtmikroskopischer Arbeiten vorgenommen; ihre Ergebnisse sollen durch histoenzymatische Kontrollen ergänzt werden. Es scheint, daß die morphologische Reaktion der Aortenmedia bei den verschiedenen Dosen der Hormongaben eine unterschiedliche ist. Die Frage, ob durch die genannte Methode gezeigt werden kann, daß sich Prozesse im Sinne der Entwicklung einer spontanen Arteriosklerose durch eine Hormonbehandlung mitigieren oder gar zur Remission bringen lassen, kann noch nicht schlüssig beantwortet werden.

*Summary.* The object was to study with a light microscope the morphological changes in the aorta and the effects on spontaneous aortic arteriosclerosis produced by administering different doses of estrogens (estradiol) to animals. Seventy-seven immature New Zealand rabbits were treated and compared with 72 controls. None showed fat content in the aortic wall. The estradiol was given for a short period of 8—9 days or for 22 days. Nearly identical morphological effects were noted in all animals receiving estrogen. The reversal effect on arteriosclerosis was noted only in the group treated for the short period with a dosage of 9.7 mg/kg. Those animals given smaller or larger doses or treated for a longer period showed no reversal effect. This indicates that the reversal effect is the product of metabolic changes not understood at this time. Further studies, including enzymatic studies, must be performed before we can discover what metabolic processes are responsible for the favorable arteriosclerotic reversal effect.

Since the ovarian transplant studies by Knauer (1900) and Halban (1900) of rabbit and guinea pig females we know that ovaries are organs causing a growth of the entire genital organs. Halban (1905) observed that the ovaries produce a substance which influences also the growth of the mammary glands. After these experimental studies many others followed. Since Stockard and Papanicolaou (1917), Allen and Doisy (1923, 1925), Zondek and Aschheim (1927), Laqueur and Coll. (1927), and many others, we know that there are chemically

\* Aided by Grant of Veterans Administration Medical Research Appropriation 360161.101 (016).

many different estrogens with variable different hormonal effects. The literature of these numerous investigations is summarized also by Burrows (1949) and by Diczfalusy and Lauritzen (1961).

Today we know that estrogens influence in animals of both sexes the genital organs and additionally also the entire body, producing general effects. After the classical work of Katz, Stamler (1953), Pick (1958); and papers by Lindsay (1946), Chaikoff, Lindsay, Lorenz, Entenman (1948); Horlick and Katz (1948); and Pick (1952) we know that estrogens in small doses may prevent or minimize coronary arteriosclerosis in chicks; however, large doses aggravate arteriosclerotic processes in these animals.

In my own experimental studies in rabbits, we have observed that estrogens on the aortic wall have produced an increase of cellular activity, further an increase of the ground substance (matrix) and have produced a denser collagenous tissue of the entire vessel wall. This was observed after a short time of treatment (9 days) with a total dose of 10 mgs. The animals given this treatment showed less spontaneous aortic arteriosclerosis than the control animals, thereby indicating that this treatment produces a reversal effect. We have assumed that the above mentioned doses of estrogen have produced a stronger wall and a favorable, healing effect on spontaneous aortic arteriosclerosis. Gostimirovich (1968). The cellular morphological changes in the aortic wall are changes due to general estrogen effects in the entire body of different animals, which were first described in mammals by Bullough (1946). According to many biochemical studies a further general effect of estrogens should be mentioned here. Estrogens are involved in many different enzymatic processes *in vitro* and *vivo*. So are estrogens involved in genital organs in the female, in the placenta and in other tissues including the arterial wall in different aged animals and in the arteriosclerotic vessel wall in humans Barrow and Chow (1959). The extensive literature of these enzymatic studies is summarized in many papers: Golden and Sevringhaus (1938), Marrian (1959), and Diczfalusy and Lauritzen (1961). Estrogens involved in different metabolic changes in proteins, fats, and carbohydrates are complicated and extensive further studies will elucidate their actions.

My own studies of estrogen in rabbits and its effect on the aortic spontaneous arteriosclerosis, have led me to further examine the effects of estrogen.

The scope of this publication is to submit the results of influencing the morphology and the reversal effect on spontaneous aortic arteriosclerosis after a shorter or longer time of estrogen treatment using different estrogen doses in immature young rabbits.

### Material and Methods

One hundred and forty nine young immature rabbits, of both sexes, (0.75—1.2 kg) were fed with normal commercial pellets with ordinary drinking water. Of these 77 (45 male and 32 female) were treated with a watery solution of estradiol (progynon) and 78 rabbits were controls (44 males and 28 females). Estradiol was given in different doses and for a different time of treatment as follows: 25 animals were given 0.5 cc for 8 days with a total dosage of 4.0 mg (3.9 mg/kg). 15 animals were given 1.0—3.0 cc (5×) for 9 days with a total dosage of 10.0 mg (9.7 mg/kg). 20 animals were given 5.0 cc (4×) in 9 days with a total dosage of 20.0 mg (19.5 mg/kg). 17 animals were given 5.0 cc (9×) in 22 days with a total dosage of 45.0 mg (43.87 mg/kg). From 72 controls, 40 were non-treated, 32 treated for 9 or 22 days with the same amount and frequency of saline solution as estradiol animals.

The effect of estradiol on the aorta, was studied on the entire aorta, especially in the region of the arch, where spontaneous arteriosclerosis occurs most frequently. The immaturity of the animals was proven in each case by examination of the gonads. The material for histological examination was fixed in Bouins solution. For fat content, unfixed or briefly fixed material was used. Metachromasia was studied with Toluidine Blue or Colloidal Iron stain. Acid and neutral mucopolysaccharides were studied with Alcian Blue, with and without P.A.S. stain (A.F.I.P. Manual 1960). The elastica fibers were studied with Verhoff's, the reticular fibers with Wilder's stain. For differentiation of cells Weigert Iron Hematoxylin, P.T.A.H. and Mallory stain were chosen. The connective tissue was studied in Mallory and Van Gieson's stain. The judgment of spontaneous arteriosclerosis was based on the occurrence of metaplastic endothelium or proliferation of intima and on changes in media and the splitting of elastica fibers. Larger area of fibrosis and the occurrence of metaplasia (cartilage or bone formation) are all changes in the media. The judgment in every case was made on light microscope.

### Results

In this morphological study of the aortic wall of young immature rabbits, of both sexes, in our controls individual variations were again observed in respect to cell activity, distribution of the ground substance and development of connective tissue fibers. In estrogen animals, the susceptibility to different doses of estrogen was also observed. The controls and the estrogen animals have not shown any fat content in the aortic wall. Different doses of estrogen have however shown in most cases somewhat different effects on the components of the aortic wall and distinct different effects on the frequency on spontaneous arteriosclerosis.

In a short time of treatment (8—9 days) our observations of the morphological content of the aortic wall and of the occurrence of spontaneous arteriosclerosis were as follows:

Twenty-five animals treated for 8 days with a total dosage of 4.0 mg have shown consistent changes in varying degree of an increased cellular activity, an increase of the matrix, and an increase of the collagenous tissue. The occurrence of spontaneous arteriosclerosis was  $56.00 \pm 9.89\%$ .

Fifteen animals treated for 9 days with a total dosage of 10.0 mg have shown distinct estrogen effects; on the cellular activity, on the matrix and the collagenous fibers, which have been previously described. (1962, 1968). The spontaneous arteriosclerosis in the aortic wall of these animals was found  $6.67 \pm 6.48\%$ , significantly lower than in control animals.

Twenty animals which were treated for 9 days with a total dosage of 20.0 mg showed in most cases identical effect in the aortic wall like other animal groups treated with doses mentioned above. The frequency of spontaneous arteriosclerosis of these animals was the highest; it was  $75.00 \pm 9.64\%$ .

The last group of our animals were 17 animals which were treated for a longer time (22 days) with a total dosage of 45.0 mg. In this group of animals the aortic wall showed changes somewhat different than in group of animals treated only 8—9 days. The cellular elements showed proliferation and quite frequently disturbed metaphases exhibited aberrant chromosomes like the estrogen effects observed in vitro by von Mollendorf (1941). The matrix and the collagenous fibers were less prominent than in animals treated for a shorter time only with estrogens. The spontaneous arteriosclerosis of this group was  $70.59 \pm 11.05\%$ .



Fig. 1. Media of an aortic wall of an untreated control animal (120 I). A normal late metaphase is seen. (Weigert-Hematoxylin Iron stain, Enl. 1260)

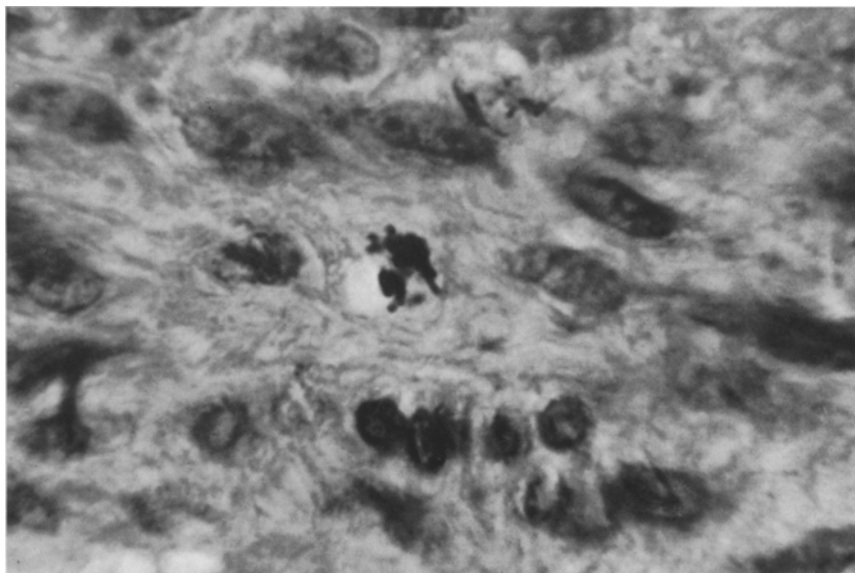


Fig. 2. Media of an estrogen animal treated with a watery solution of estradiol for 22 days with a total dosage of 45 mg. A late metaphase with aberrant chromosomes in a disorderly arrangement is seen. (Weigert-Hematoxylin Iron stain, Enl. 1260)

The morphology of the aortic wall by different doses of estrogens is altered. The changes in a short time of treatment are nearly identical. On the other hand a reversal effect was present only with a certain total dose. Doses larger or smaller did not have this effect.

Our results indicate that specific doses of estrogens produce changes of the morphology of the aortic wall with an additional reversal effect by metabolic means, not understood at the present time. Further studies, including enzymatic studies, will be needed to discover precisely what metabolic means are responsible for the favorable arteriosclerotic reversal effect.

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