Aortic Acid Mucopolysaccharides: Changes During Pregnancy, Enovid-Treatment, and Hypercholesteremia in Rabbits[1] (35501)

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(Introduced by C. B. Taylor)

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It is found by Likar *et al.* (1) that the concentration of nonsulfated acid mucopolysaccharides (AMPS) in the inner third of aortic wall varies with the estrual cycle in the female cattle. This concentration is highest in proestrus and estrus (proliferative period) and lowest in metestrus and diestrus (progestational period). Our previous histochemical studies of aortas from pregnant women and rabbits also revealed a remarkable decrease of aortic acid mucopolysaccharides (AMPS) throughout the gestation period (2, 3).

Contrary to diminution of aortic acid mucopolysaccharides, Gillman and Pillay (4), based on 220 determinations in control, pregnant, and parturient women, showed that a significant increase in serum mucoprotein occurred by the second trimester and was maintained throughout the rest of pregnancy. Moreover, Danforth and Buckingham (5) could not find any change of hexosamine content of the human cervix during different stages of gestation. In order to test the validity of the histochemical methods for demonstrating tissue AMPS employed in our previous studies (2, 3), quantitative chemical analysis of the AMPS content of the same aortas, including aortas from a group of hypercholesteremic rabbits, was carried out.

Materials and Methods. Thirty-six adult New Zealand white female rabbits, housed in individual cages in a well-ventilated animal facility, were divided into four groups: Group I, six nonpregnant controls, Group II, 12 pregnant rabbits. Four of them were sacrificed on 7, 10, 15, and 21 days of gestation, respectively, and the rest of eight animals were sacrificed at term (28 to 30 days). Group III, 10 Enovid-treated rabbits. Each animal received a daily dose of 0.2 mg of Enovid or norethynodrel–mestranol, in sesame oil intramuscularly for 6 days and was sacrificed on day 7. A single dose of 0.2 mg of Enovid prevents ovulation in 100% of the rabbits. The effect is evident within 6 hr and lasts for at least 24 hr (3). The above animals were fed, *ad libitum*, Rockland rabbit pellets (Monmouth, Ill). Group IV, 8 cholesterol-fed rabbits. The cholesterol diet was prepared each time by dissolving 500 g of crystalline cholesterol in 500 ml of heated corn oil and mixing it thoroughly with 25 kg of Rockland rabbit pellets.

All rabbits were virgin does when received at the age of 2 to 3 months and were 8 to 10 months old at the time of sacrifice. At sacrifice, blood was drawn from the central artery of the ear for serum cholesterol and phospholipid determinations, and the animals were then killed by intravenous pentobarbital injection. The entire aorta was removed and cleaned of adventitia and surrounding adipose tissue. The histological changes of these aortas, except that of Group IV rabbits, has been studied and reported previously (3). The total acid mucopolysaccharides were isolated from the aorta by the procedure of Bollet (6). The uronic acid content of the isolated aortic acid mucopolysaccharides was determined by the carbazole method of Dische (7). Serum cholesterol levels were done by the Zak *et al.* (8) method modified for automatic analysis. Serum phospholipids were determined by the method of Goodwin *et al.* (9).

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TABLE I. Serum Cholesterol and Phospholipid Levels and Aortic Uronic Acid Content of Four Groups of Rabbits: Controls, Pregnant, Enovid-Treated, and Cholesterol-fed.

Group	No.	Serum cholesterol (mg/100 ml)	Serum phospholipids (mg/100 ml)	Aortic uronic acid (mg/g of defatted dry tissue)
I. Control	6	64 ± 25[a]	88 ± 21	3.55 ± 0.42
II. Pregnant before term	4	21 ± 6[b]	41 ± 11[b]	3.62 ± 0.45
at term	8	12 ± 2[b]	29 ± 7[b]	3.49 ± 0.28
pooled	12	14 ± 3[b]	32 ± 9[b]	3.52 ± 0.32
III. Enovid-treated	10	58 ± 20	73 ± 12	3.74 ± 0.47
IV. Cholesterol-fed	8	1033 ±208[b]	260 ± 73[b]	4.17 ± 0.33[b]

[a] Mean ± standard deviation.

[b] Significant difference from the control ($p < 0.01$ by t test).

Results and Discussion. The average weight of the rabbits at the time of sacrifice was 3750 g. The average weight of the pregnant rabbits at term (4213 g) was heavier than that of the nonpregnant rabbits (3630 g). This excessive body weight gain is believed to be contributed by fetuses, enlarged uterus, and body water retention.

The results of serum lipids and aortic uronic acid content are shown in Table I. The marked elevation of serum cholesterol and phospholipid levels in the cholesterol-fed rabbits was expected. However, the significant lowering of serum cholesterol and phospholipid in pregnant rabbits to one-third or less of the normal values was surprising. In 1946, Popjak (10) already noticed this phenomenon and concluded from his studies that this hypolipedemia was not due to increased demands of the fetus *in utero* but rather an increased lipid deposition in tissues, especially in the placenta. Enovid in this short treatment did not alter the serum lipids.

The aortic uronic acid content was expressed as milligrams of uronic acid per gram of defatted dry tissue. Uronic acid represents about one-fourth of the weight of chondroitin sulfate. Thus the total weight of aortic acid mucopolysaccharides can be estimated by multiplying the uronic acid content by 4. The mean control value would be approximately 14 mg/g of defatted dry tissue.

Although previous histochemical studies of the same materials revealed a marked reduction of alcian blue positive material in the aorta of both pregnant (either before or at term) and Enovid-treated rabbits (3), the chemical analysis of these aortas showed no significant change of the nondialyzable uronic acid content from that of the control (Table I). In our recent study of 60 castrated female rabbits either treated with estrogen, progesterone, weekly alteration of estrogen, and progesterone or corn oil, only a slight decrease of hyaluronic acid and chondroitin sulfate-C was found in the aorta of estrogen-treated rabbits (11). Such small changes of aortic AMPS content should not account for the reduction in alcian blue positive materials by histochemical studies.

A possible explanation for the decreased stain is the changes of the quality, not quantity, of the aortic AMPS under the hormonal influence. Increase of smooth muscle, fragmentation of reticulum fibers and loss of corrugation of the internal elastic membrane were found in the aorta during pregnancy (3). Increase in water content and decrease in hydroxyproline content and dissociation of the collagen bundles into their fibrillar components were found in human cervix during pregnancy and labor (5, 12), which may also be true in the aorta. There would be a change in the relationship between aortic AMPS and the surrounding fibers, cells, and other components of the ground substance. The polarity, charges, and configuration of aortic AMPS might be altered and affect

their affinity for the staining chemicals. Thus, a false reduction in quantity was observed.

The histochemical studies of the aorta of cholesterol-fed rabbits (Group IV) showed an increase of both sulfated and nonsulfated AMPS in the atheromatous plaques which was reported previously (13). Chemical analysis also revealed a significant increase of nondialyzable aortic uronic acid content (Table I), and correlates quite well with the histochemical methods. Increase of AMPS is believed to be a phenomenon of nonspecific mesenchymal reaction to various stimulants and plays an important role in the pathogenesis of atherosclerosis (14). Thus, the quantitative change of AMPS in atherosclerotic aortas is different from the qualitative change in aortas from pregnant or Enovid-treated rabbits.

Summary. Thirty-six adult female rabbits were divided into four groups: controls, pregnant, Enovid-treated, and cholesterol-fed. Hypolipidemia was noted in pregnant rabbits and hyperlipidemia in cholesterol-fed rabbits. The nondialyzable aortic uronic contents of pregnant and Enovid-treated rabbits were not different from those of the controls, although previous histochemical studies on the same aortas revealed a marked reduction of alcian blue positive materials in the aortas of both pregnant and Enovid-treated rabbits. This false reduction may be the result of qualitative changes of aortic AMPS during pregnancy; these changes are very different from the quantitative increases found in atherosclerotic aortas. The latter showed no loss of stain with alcian blue dye.

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