ROLE OF ENDOCRINE MECHANISMS IN THE DEVELOPMENT OF ATHEROSCLEROSIS

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After prolonged administration of hydrocortisone to rabbits a relative adrenal insufficiency develops. As a result, 12-14 days after administration of the hormone ceases the permeability of the blood vessels is increased; in the presence of lipemia and if an excess of glucocorticoids in the body leads to the formation of atherogenic lipoproteins, lipids are deposited in the aorta.

A model of neurogenic atherosclerosis not requiring administration of cholesterol to animals, but by overstress of the nervous system was obtained previously by the writers [9-11] and it was postulated that neurogenic atherosclerosis develops through disturbances of hormonal regulation and of metabolism produced by stress and overstress of the apparatus of the emotions [12]. Hypofunction of the thyroid gland and gonads is known to promote the development of atherosclerosis [18, 19]. The role of glucocorticoids (the content of which rises sharply in emotional excitation) in atherogenesis is not sufficiently clear. Data on adrenocortical function in atherosclerosis likewise are conflicting [1, 3-5, 8, 14, 20].

The object of the present investigation was to study the effect of glucocorticoids on atherogenesis under the conditions of normal and depressed function of the thyroid gland and the gonads.

EXPERIMENTAL METHOD

Experiments were carried out on 55 female rabbits weighing initially 2,500-2,800 g. Microcrystalline hydrocortisone acetate (Richter, Hungary) was injected subcutaneously into intact and castrated animals in a dose of 0.3 mg/kg body weight daily for 6 months. Thyroidectomized rabbits received the same doses of hydrocortisone subcutaneously for 2 months. Every month the β-lipoproteins were analyzed and the absolute and relative content of the following components were determined: cholesterol (by the Liebermann–Burchard reaction), phospholipids (by the Fiske–Subbarow method), triglycerides (by the method of Carlson and Wadstrom [13]), and proteins (by Lowry's method [17]). The β-lipoproteins were precipitated from the blood serum by the method of Burstein and Sammaille in the modification of Klimov and Lovyagina [2]. The serum was first freed from chylomicrons by centrifugation for 10 min at 10,000 rpm. Lipids were extracted from the β-lipoproteins by Folch's method [16]. The animals were sacrified 12-14 days after administration of the hormone ceased. Before and 12-14 days after administration of the hormone ceased (before sacrifice) the blood corticosteroids were determined. At the same times the permeability of the vascular wall was studied in some animals by the method of Oivin and Monakova [6]. After sacrifice the intravital penetration of trypan blue into the wall of the aorta was studied by Petrov's method [7]. The adrenals also were weighed, and in 22 of the 26 animals a macroscopic investigation was made of the aorta after staining in toto with Sudan III. Parts of the aorta with pathological changes were examined under the microscope after sections cut on a freezing microtome had been stained with Sudan III and the nuclei counterstained with hematoxylin.

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TABLE 1. Composition of Blood β-lipoproteins of Rabbits Receiving Hydrocortisone (M ± m)

Group of experiments	Cholesterol		Phospholipids		Triglycerides		Protein	
	mg %	%	mg %	%	mg %	%	mg %	%
Control (n = 29)	32,3±2,2	16,8±1,2	33,8±3,0	17,4±1,15	65,5±3,9	34,0±1,78	61,4±4,1	31,7±1,35
Injection of hydrocortisone:								
into intact rabbits (n = 7)	37,8±5,0	9,85±1,5	85±8,0	21,6±0,56	188±25,5	46,4±2,1	82,5±5,5	22,15±2,3
P	>0,5	<0,001	<0,001	<0,002	<0,001	<0,001	<0,002	<0,001
Thyroidectomized rabbits	42,8±4,31	10,0±0,74	57,3±6,2	13,45±1,0	218±27,6	48,4±1,85	120,4±12,2	27,9±0,91
P	<0,05	<0,001	<0,001	<0,05	<0,001	<0,001	<0,001	<0,05
Castrated rabbits	98,6±20,3	11,6±2,03	184,1±21,2	20,3±1,78	482±85,6	50,2±2,67	151,5±7,61	17,9±1,83
P	<0,001	<0,05	<0,001	>0,05	<0,001	<0,001	<0,001	<0,001

Note: P denotes significance of difference between mean values and corresponding control figure.

Fig. 1. Deposition of lipids in intima of thoracic portion of aorta (lipids colored black) and thrombus formation at site of deposition of lipids in castrated rabbit 14 days after stopping administration of hydrocortisone. Sudan III–hematoxylin, 160 ×.

EXPERIMENTAL RESULTS

It is clear from Table 1 that an excess of glucocorticoids led to a disturbance of the composition of the β-lipoproteins (an increase in the absolute contents of cholesterol, phospholipids, triglycerides, and protein).

The most marked increase was observed in the absolute content of triglycerides and phospholipids, with the result that the relative percentages of the components of the protein-lipid complex were disturbed. Lipoproteins with a higher relative content of triglycerides and with a lower content of protein were formed (Tanle 1). Lipoproteins loaded with triglycerides are atherogenic and can be included in the type 4 of Fredrickson's classification [15]. Changes in the composition of β-lipoproteins in the castrated and thyroidectomized animals were more marked than in intact animals receiving the same doses of the hormone.

Macroscopic and microscopic investigation of the aorta of 22 animals revealed deposition of lipids in 10 cases (in 3 of 7 intact animals, in 3 of 7 thyroidectomized rabbits, and in 4 of 8 castrated animals receiving the hormone), and in 6 of these cases microthrombi were formed at the sites of the lipidosis (Fig. 1). Lipidosis was observed in the ascending, thoracic, and abdominal portions of the aorta but microthrombi were seen only in the thoracic and abdominal portions. Microscopically lipids were detected as dust-like deposits and droplets of various sizes in the endothelium, the subendothelial layer, and to some extent in the tunica media of the aorta. Before administration of the hormone was discontinued the blood corticosteroid concentration was $18.8 \pm 0.91\ \mu g\%$ while 12-14 days after its discontinuation the level fell to $6.12 \pm 0.54\ \mu g\%$ ($P < 0.001$). At the same time the vascular permeability was increased to 1.5-3 min, or by about 3-4 times compared with its value during administration of the hormone (8-10 min). Increased permeability of the vascular

wall after discontinuation of the hormone was also confirmed by the appearance of focal penetration of the dye through all layers of the intima and the subendothelial layer and into the media in the thoracic and abdominal portions of the aorta whereas in the control animals only small amounts of the dye were concentrated in the intima. The weight of the adrenals at the end of the experiment in the intact rabbits receiving hydrocortisone was 240 ± 27.2 mg while in the thyroidectomized animals it was 130±12.9 mg (912± 56.2 mg in the control animals).

It can be concluded from the results of this investigation that during prolonged administration of hydrocortisone atrophy of the adrenals develops and relative glucocorticoid insufficiency arises. As a result, 12-14 days after administration of the hormone ceases the vascular permeability is increased, and if lipemia and atherogenic lipoproteins are present this leads to deposition of lipids in the aorta. It can be postulated that one mechanism of the development of neurogenic atherosclerosis is a periodic relative adrenal insufficiency due to overstrain of the apparatus of the emotions and of the pituitary-adrenocortical system.

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