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BILE SECRETORY FUNCTION OF THE LIVER IN BIRDS OF DIFFERENT AGES WITH EXPERIMENTAL ATHEROSCLEROSIS

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UDC 616.13-004.6-092.9-07:616.36-008.6-072.7

The bile-secretory function of the liver under normal conditions and in experimental atherosclerosis produced by administration of cholesterol was studied in experiments on young (3-4 months old) and adult (30-36 months old) hens of the Russian White breed. During natural aging a decrease in the total and free cholesterol concentrations in the blood serum and in the bile-secretory function of the liver was observed. These indices were raised during administration of cholesterol and atherosclerotic changes developed in the aorta. The severity of these changes compared with normal was greater in the adult than in the young experimental birds.

KEY WORDS: experimental atherosclerosis; age; bile acids; cholesterol; liver.

Disturbances of the metabolism, structure, and function of the liver play an important role in the pathogenesis of atherosclerosis [2, 8, 11].

Changes in the bile-secretory function of the liver were studied in birds of different ages under normal conditions and with experimental atherosclerosis caused by chronic cholesterol loading.

EXPERIMENTAL METHOD

Experiments were carried out in the fall and winter on hens of the Russian White breed of two ages: 3-4 months (the hens begin to lay at the age of 5-6 months) and 30-36 months (laying, which had ceased because of

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Gorev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 9, pp. 1038-1041, September, 1976. Original article submitted March 26, 1976.

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TABLE 1. Secretion and Chemical Composition of Bile from Young Hens under Normal Conditions and after Administration of Cholesterol for 2 Months (M ± m)

Hours of secretion	Control (n = 6)			Experiment (n = 8)		
	Volume of bile, ml	Cholesterol, mg	Bile acids, mg	Volume of bile, ml	Cholesterol, mg	Bile acids, mg
1-st	1,00±0,07	2,00±0,34	8,09±0,92	1,91±0,20 $P<0,01$	2,86±0,25	10,79±0,94
2-nd	0,81±0,05	1,46±0,23	6,46±1,15	1,88±0,09 $P<0,001$	2,68±0,18 $P<0,01$	10,56±0,56 $P<0,5$
3-nd	0,76±0,03	1,51±0,10	5,24±0,78	1,77±0,15 $P<0,001$	2,39±0,24 $P<0,01$	8,89±1,31
4-th	0,72±0,06	1,46±0,11	3,88±0,62	1,93±0,21 $P<0,001$	2,47±0,29 $P<0,05$	8,29±0,86 $P<0,01$
Total for 4 h	3,30±0,11	6,44±0,50	21,16±1,11	7,51±0,43 $P<0,001$	10,42±0,48 $P<0,001$	38,54±2,80 $P<0,01$
Total for 4 h calculated per kg body weight	4,66±0,27	6,83±0,32	30,08±2,58	5,66±0,27 $P<0,05$	8,02±0,40 $P<0,05$	29,01±1,74

Legend. Here and in Table 2 P denotes significance of difference between control and experiment.

TABLE 2. Secretion and Chemical Composition of Bile from Adult Hens under Normal Conditions and after Administration of Cholesterol for 2 Months (M ± m)

Hours of secretion	Control (n = 10)			Experiment (n = 7)		
	Volume of bile, ml	Cholesterol, mg	Bile acids, mg	Volume of bile, ml	Cholesterol, mg	Bile acids, mg
1-st	0,91±0,11	1,66±0.28	4,45±0,81 $P_1<0,01$	1,81±0,09 $P<0,001$	2,44±0,09 $P<0,05$	14,03±1,25 $P<0,001$
2-nd	0,68±0,09	1,17±0,19	4,03±0,90	1,38±0,21 $P_2<0,05$	2,00±0,26 $P<0,05$	9,49±1,86 $P<0,05$
3-nd	0,62±0,10	1,09±0,17 $P_1<0,05$	2,96±0,80 $P_1<0,1$	1,18±0,12 $P_2<0,01$ $P<0,01$	1,66±0,21	7,59±1,31 $P<0,05$
4-th	0,55±0,09	0,97±0,23 $P_1<0,1$	2,88±0,36	1,27±0,11 $P<0,01$ $P_2<0,04$	1,71±0,12 $P<0,05$	6,70±1,13 $P<0,05$
Total for 4 h	2,77±0,28 $P_1<0,1$	4,91±0,65 $P_1<0,1$	14,64±2,05 $P_1<0,02$	5,69±0,39 $P<0,001$ $P_2<0,01$	7,83±0,57 $P<0,05$ $P_2<0,01$	37,82±5,29 $P<0,01$
Total for 4 h calculated per kg body weight	1,39±0,22 $P_1<0,001$	2,65±0,34 $P_1<0,001$	7,90±1,04 $P_1<0,01$	4,51±0,20 $P<0,001$ $P_2<0,01$	6,28±0,37 $P<0,001$ $P_2<0,01$	30,17±3,87 $P<0,001$

Legend. P_1) Significance of age differences in normal state; P_2) significance of age differences in atherosclerosis.

the age of the birds and the time of year, was not artificially stimulated). Experimental atherosclerosis was produced in 8 of the 14 birds of the 1st and 7 of the 17 birds of the 2nd age group by giving cholesterol by mouth daily for 2 months as a 50% emulsion in sunflower oil in a dose of 2 g of the preparation/kg body weight. The rest of the hens were kept on a normal diet. The blood concentrations of total, free, and esterified cholesterol of all the birds were determined [16]. In an acute experiment a fistula was formed in the gall bladder [5] and the bile ducts were simultaneously ligated. Samples of bile were taken every hour for 4 h. Cholesterol [6] and bile acids [4] were determined in the bile. At the end of the experiment all the birds were killed by exsanguination. The aorta was removed, opened longitudinally, stained in toto with Sudan III-IV solution [9], and the degree of severity of the atherosclerotic lesions was determined by direct planimetry [1]. All the numerical data were subjected to statistical analysis [7].

EXPERIMENTAL RESULTS AND DISCUSSION

The experiments showed that normally the blood levels of total (127 ± 9 mg %) and free (50 ± 3 mg %) cholesterol were higher in the young birds than in the adults (102 ± 6 and 32 ±3 mg %, respectively; $P < 0.05$).

Keeping the birds on an atherogenic diet led to a marked increase in the blood cholesterol level, later in the adults than in the young hens. At the end of the experiments, for instance, the concentrations of total (209 ± 31 mg %) and free (91 ± 10 mg %) cholesterol were lower in the young hens than in the adults (408 ± 52 and 143 ± 17 mg %, respectively; $P < 0.02$).

Data showing the volume of bile secreted and the concentrations of cholesterol and bile acids in it for the young and adult hens under normal conditions and with atherosclerosis respectively are given in Tables 1 and 2. Clearly more bile was secreted by the normal young birds than by the normal adult hens and it contained more cholesterol and bile acids. This is clearly revealed by calculating these indices per unit of body weight.

Administration of cholesterol was accompanied by a marked increase in the volume of bile secreted by all the experimental hens and in the quantity of cholesterol and bile acids in it. The quantity of bile acids secreted during the experiment, expressed per kg body weight, was about the same for the young experimental birds as for the adults. Meanwhile, the bile of the adult hens contained less cholesterol than that of the young hens.

Macroscopic sudanophilic changes in the intima of the aorta were found in the control group only in the hens of the older age group. They were found in 8 of the 10 birds of this group, in which they occupied an average of 1.9% of the area of the intima of the aorta and they corresponded in shape and localization to spontaneous lesions in the aorta of birds described by other workers [15, 18]. In the experimental group atherosclerotic lesions were found in both young and adult birds. Of the 8 young hens investigated, plaques were seen macroscopically in only 3. They appeared as whitish elevations above the intima and were situated chiefly in the arch and thoracic part of the aorta, where they occupied an area of 1 to 12% (mean 2.4%) and stained red with Sudan III-IV solution. In the experimental birds of the older age group atherosclerotic lesions were found more often (in 6 of 7 cases) and they occupied a larger area of the intima of the aorta (from 7 to 80%, mean 23.4%) than in the young birds.

Other workers have also observed a decrease in the blood cholesterol concentration [10], a decrease in the rate of synthesis of cholesterol and bile acids in the liver tissue, and a decrease in their excretion [13, 14, 17] in birds with age.

The increase in the bile-secretory function of the liver and in the excretion of bile acids and sterols with the feces during alimentary cholesterol loading [12, 13] are probably a compensatory reaction to the excessive intake. The lower blood cholesterol level in the young experimental hens than in the adults is in harmony with its greater excretion with the bile in the former. Meanwhile the quantity of bile acids excreted with the bile was about the same in the young and adult hens with experimental atherosclerosis. It can be postulated that the absence of a parallel between the degree of hypercholesteremia and the quantity of bile acids excreted with the bile is connected to some extent with their increased reabsorption in the intestine of the birds of the older age group.

Although the increased bile secretion in atherosclerosis is interpreted as a compensatory reaction of the body, it must not be forgotten that with an excessive intake of cholesterol with the diet the increased secretion of bile could result in better absorption of cholesterol in the intestine and it could therefore be a contributory factor to the genesis of the hypercholesteremia and atherosclerosis. Divergence of the changes in the blood cholesterol concentration and in the bile-secretory function of the liver during physiological aging (a decrease) and during the development of experimental atherosclerosis (an increase) will be noted. This fact confirms the view of Gorev et al. [3] that age changes and atherosclerotic changes in the organism are not identical.

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GLUCOCORTICOID FUNCTION AND CYTOPLASMIC DEHYDROGENASE ACTIVITY OF THE ADRENALS FOLLOWING MECHANICAL AND TOXIC INJURY TO THE LIVER

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UDC 616.36-001+616.36-099]-092.9-07:616.453-008.6-072.7

A decrease in the rate of corticosterone synthesis by adrenal slices and a decrease in cytoplasmic dehydrogenase activity of the adrenal cells were observed in male rats 48 h after partial hepatectomy, when the level of steroid metabolism in the liver was low, compared with the situation in animals undergoing a mock operation. The changes found are the result of depression of central mechanisms of stress as a result of the lowered steroid metabolism. Intraperitoneal injection of CCl_4 into rats in a dose of 0.1 ml/100 g body weight at these same times led to a marked increase in steroid production in the adrenal tissue and activation of NAD-dependent cytoplasmic dehydrogenases. The role of toxic damage to the glands in the changes in the functional state of the adrenocortical cells is discussed.

KEY WORDS: CCl_4; partial hepatectomy; adrenals; dehydrogenases; corticosteroids.

Acute and chronic liver diseases are accompanied by changes in adrenal function [6, 7]. However, the problem of whether these changes are the results of indirect inhibition of the adrenal glands through the depression of steroid metabolism or the result of direct toxic injury to the adrenal cortex still remains unsolved [9].

In this investigation a comparative analysis was made of the steroid producing function of the adrenals and of certain mechanisms of its energy provision in animals with mechanical and toxic liver damage.

EXPERIMENTAL METHOD

Experiments were carried out on four groups of noninbred male albino rats weighing 150-200 g. Under ether anesthesia laparotomy was performed on the animals of group 1, partial hepatectomy by the method of Higgins and Anderson on the rats of group 2, the rats of group 3 received an intraperitoneal injection of CCl_4 in a dose of 0.1 ml/100 body weight, and the rats of group 4 acted as the control. All manipulations were carried out between 9 and 11 a.m. The animals were decapitated 48 h later. The adrenals were removed, freed from connective tissue in the cold, cut into four parts, and incubated in a Warburg apparatus in Krebs–Ringer-phosphate buffer with 20 mM glucose at 37°C in an atmosphere of O_2. The concentration of 11-hydroxycorticosteroids (11-HCS) in the plasma and incubation samples was determined fluorometrically [12]. The trans-

Central Research Laboratory, Novosibirsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 9, pp. 1041-1043, September, 1976. Original article submitted March 15, 1976.

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