very high proteolytic activity or the presence of one or more proteases, specific for phosphoproteins, in this preparation, which are not present in NM of rat liver and of Zajdela's hepatoma.

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EFFECT OF OVARIECTOMY ON FATTY ACID COMPOSITION OF PHOSPHOLIPIDS IN JAVA MACAQUES WITH CHOLESTASIS

Yu. A. Bogdarin and N. P. Goncharov

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Steroid hormones of the estrogen group are involved in regulation of the metabolism of biliary lipids, including cholesterol and bile acids [14], through regulation of activity of the corresponding enzyme systems, in the formation of protein components of lipoproteins [12], and also regulation of the fatty acid composition of different classes of lipids [9]. There is now much biochemical evidence on some aspects of regulation of lipid metabolism by estrogens in cholestasis — an important factor in the development of hepatocholecystitis. In particular, estrogens stimulate esterification of cholesterol by the hepatocytes, which causes a decrease in bile acid synthesis such as is observed during pregnancy and following administration of estrogenic hormones, often giving rise to cholestasis [11]. Meanwhile, the role of estrogens in the process of incorporation of their most important components (bile acids) of the biliary lipids, participating in the formation of biliary micelles and vesicles in cholestasis, has received little study.

The aim of this investigation was to study the effect of ovariectomy on formation of the composition of lipids in the bile and on the phospholipid level, with analysis of the spectra of their component fatty acids in the organs and tissues of the enterohepatic system in Java macaques with experimental cholestasis.

Laboratory of Biochemistry, Research Institute of Pediatrics, Ministry of Health of the RSFSR, Gor'kii. Laboratory of Experimental Endocrinology, Institute of Experimental Pathology and Therapy, Academy of Medical Sciences of the USSR, Sukhumi.

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TABLE 1. Biliary Lipid Content and Lithogenic Index of Bile of Experimental Java Macaques ($M \pm m$)

Exptl. conditions	Biliary lipids, mN			Lithogenic index
	Bile acids	Cholesterol	phospholipids	
Intact animals (n = 2)	192,7±30,7	2,15±0,17	16,15±0,35	0,14±0,02
Cholestasis (n = 3)	142,1±26,4	5,77±0,61*	9,99±0,93*	0,50±0,04*
Cholestasis (n = 3)	254,9±36,5	1,7±0,23	9,89±0,29*	0,09±0,02

Legend. Here and in Table 2: *$p < 0.05$ compared with results for intact animals.

TABLE 2. Phospholipid Content in Organs and Tissues of Enterohepatic System of Java Macaques during Experiments ($M \pm m$)

Exptl. conditions	IFB, mM	Entero-cytes	OFB, mM	Hepatocytes, mg/g	Bile, mM	Chyme, mM
Intact animals (n = 2)	1,13±0,05	23,15±0,44	1,17±0,08	24,84±0,65	16,15±0,35	1,64±0,10
Cholesterol (n = 3)	1,13±0,22	16,10±1,97	1,15±0,19	19,53±3,05	9,99±0,93*	0,97±0,11*
Cholestasis + ovariectomy (n = 3)	0,84±0,12	17,16±1,60	0,93±0,12	17,47±0,86*	9,89±1,17*	1,14±0,11

EXPERIMENTAL METHOD

The investigation was conducted on eight Java macaques from the Sukhumi nursery, weighing 2.8-5 kg. The control group consisted of two animals. Measured constriction (by 50%) of the lumen of the bile duct in the region of the duodenum was carried out on six monkeys, as the result of which the animals developed a noncalculous form of hepatocholecystitis [2]; in addition, bilateral ovariectomy was performed on three animals of this group. The operations were performed after premedication (1% trimeperidine, intramuscularly, 1 ml) and under anesthesia (2% pentobarbital solution, 1.5 to 4 ml intravenously). The monkeys were kept on a standard laboratory diet. The animals were killed 60 days after the operations by injection of 2% pentobarbital solution (from 5.5 to 8 ml). The contents were collected from the gall bladder, blood flowing into (IFB) and out of the intestine (OFB) was collected, the liver was removed and hepatocytes isolated from it [4], and the intestine was removed to separate the mucosa from its upper third. The composition of the biliary lipids was determined in the above-mentioned six biological materials: bile acids [3], phospholipids, and cholesterol [7]. Biliary lipids were fractionated in thin layers of silica-gel LS 5/40 + 13% plaster of Paris (Czechoslovakia), on glass plates (18 × 35 cm) in appropriate systems of organic solvents. The lipid fractions were analyzed densitometrically on an IFO-451 microphotometer. Quantities of the separated fractions were calculated against calibration curves, plotted on the basis of corresponding standard preparations. The fatty-acid composition of total phospholipids (the start bands during fractionation of neutral lipids) of the above organs and tissues was studied by gas–liquid chromatography [1]. Student's t test was used for statistical analysis.

EXPERIMENTAL RESULTS

Comparison of the lipid parameters of the macaques with the noncalculous form of hepatocholecystitis revealed considerable differences in the cystic bile in the animals after ovariectomy (Table 1). Calculations in a triangular system of coordinates [5] revealed an increase in the cholesterol saturation index of the bile (the lithogenic index) in mature animals with hepatocholecystitis. Under these conditions cholesterol is retained in the bile in the soluble state, for the level of total bile acids is preserved while the cholesterol concentration is raised and the phospholipid level of the bile lowered. Meanwhile, in the monkeys after ovariectomy, the lithogenic index did not differ from that in intact animals. No change was found in the levels of cholesterol and bile acids in the bile from this group of animals, but the phospholipid concentration was simultaneously reduced. In these two groups of animals differences were found in the levels of the bile acids in the bile ($p \sim 0.05$), confirming the role of ovarian hormones in the regulation of bile acid biosynthesis in the hepatocytes [12, 13].

The results given in Table 2 show that ovariectomy had no specific effect on phospholipid levels in organs and tissues of the enterohepatic system in hepatocholecystitis. Accordingly, particular attention had to be paid to the study of the composition of the phospholipid fractions and also the fatty acid spectrum of their components.

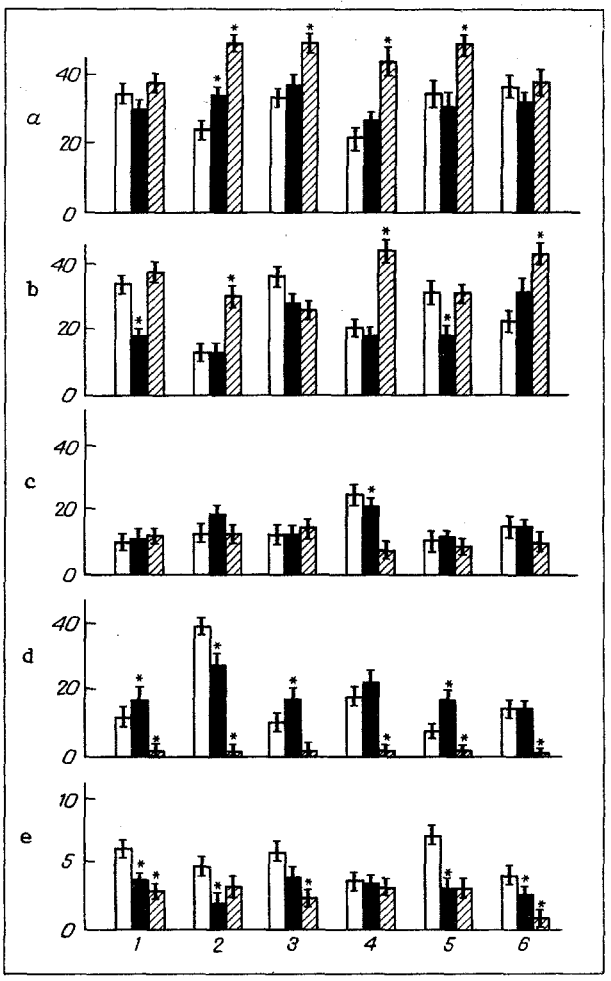

Fig. 1. Composition of principal fatty acids (in %) of phospholipids of organs and tissues of enterohepatic system in Java macaques with noncalculous form of hepatocholecystitis and undergoing ovariectomy. Ordinate for acids: a) 16:0, b) 18:0, c) 18:1, d) 18:2, e) 20:4. Unshaded columns indicate control (intact animals), black columns — cholestasis, causing noncalculous form of hepatocholecystitis; obliquely shaded columns show hepatocholecystitis in ovariectomized animals. 1) Chyme, 2) bile, 3) IFB, 4) enterocytes, 5) OFB, 6) hepatocytes. $*p < 0.05$ indicates significant difference from data for intact animals.

In man, monkeys, and certain other mammals more than 90% of the biliary phospholipids consists of the phosphatidylcholine (PCh) fraction. The principal components of PCh are palmitic (16:0), oleic (18:1), and linoleic (18:2) acids. The significance of this fatty acid composition in the biliary PCh is not known, but it is specific and it differs, in particular, from the set of fatty acids in PCh of the hepatocytes, in which the main components are stearic (18:0) and arachidonic (20:4) acids [6]. The fatty-acid composition of the biliary phospholipids of intact Java macaques which we found (Fig. 1) corresponds on the whole to the set of bile acids of human biliary phospholipids, and differs in having a lower percentage of the 16:0 acid (about 41% in man) and a higher percentage of the 18:2 acid (33% in man).

The development of hepatocholecystitis in macaques is accompanied by an increase in the fraction of the 18:2 acid in the composition of phospholipids of the chyme, IFB, and OFB, with a simultaneous decrease in its relative content in the bile and with maintenance of the normal level in phospholipids of enterocytes and hepatocytes (Fig. 1). The content of the 20:4 acid remained unchanged in phospholipids of the hepatocytes, but its level in phospholipids of the chyme, bile, and OFB was reduced. Preservation of this very important structural and energy-yielding material, namely essential fatty acids, in the liver and intestinal cells is evidently necessary for the body under these pathological conditions.

Bilateral ovariectomy, performed at the same time as occlusion of the bile duct, had a considerable effect on incorporation of the principal fatty acids and, in particular, of the 18:2 acid, into the composition of phospholipids of the organs and tissues of the enterohepatic system. The content of saturated acids 16:0 and 18:0 was increased in the bile, blood, and enterocytes, and that of the 18:0 acid in the hepatocytes. Their incorporation into phospholipids of the chyme remained unchanged. Meanwhile, the fraction of the 18:2 acid in the composition of the phospholipids of all the objects studied was considerably reduced compared with the corresponding data both in the group of intact animals and in the group of monkeys not subjected to ovariectomy besides cholestasis. Meanwhile, in phospholipids of the chyme, blood, and hepatocytes the relative content of the 20:4 acid was reduced. However, its levels did not differ in animals with experimental cholestasis whether the gonads were present or not (Fig. 1).

Thus, gonadectomy on female Java macaques, accompanied by the development of a noncalculous form of hepatocholecystitis led to blocking of incorporation of the essential acid 18:2 into the composition of the phospholipids of organs and tissues containing the enterohepatic circulation and metabolism of biliary lipids. This fact is evidence of the specific action of estrogenic steroid hormones on linoleic acid metabolism and, in particular, on the regulation of its incorporation into phospholipids. This sex-related difference in essential fatty acid metabolism [9] in the period of sex cycles or pregnancy [12], is evidently not accidental. It is evident that estrogens, by their influence on activity of liver microsomal Δ^6-desaturase [8], thereby regulating the formation of the 20:4 acid from the essential 18:2 acid, are involved in the redistribution of incorporation of the 18:2 acid into different classes of biliary lipids. As a result, in ovariectomized macaques the positive correlation of the cholesterol saturation index of the bile with the relative content of the 18:2 acid is changed, whereas this correlation remains with the level of the 16:2 acid, the characteristic feature of sexually mature individuals [11]. In the noncalculous form of hepatocholecystitis new correlations are formed between biliary lipids, which contain predominantly saturated and monoenic acids (18:1) in their composition. Changes of this kind are evidence that the ovariectomized monkey can compensate for the induced deficiency of the 18:2 acid by preservation of synthesis of unsaturated acids 18:1 and 20:4 and their incorporation into lipids, taking place when a high level of bile acids is preserved in the bile and in the absence of lithogenicity of the bile in monkeys with the noncalculous form of hepatocholecystitis. Bilateral ovariectomy, performed on the macaques simultaneously with measured occlusion of the bile duct, in the region of the duodenum, inducing a noncalculous form of hepatocholecystitis in the monkeys, did not alter the lithogenic index of the bile. A specific fatty acid composition of the phospholipids of organs and tissues of the enterohepatic system is formed in gonadectomized monkeys with hepatocholecystitis, with predominance of palmitic, stearic, and oleic acids, while the previous level of arachidonic acid is preserved, and the essential linoleic acid is present only in trace amounts.

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