nary starvation alone (control group 2) did not increase the total content of cytochrome P-450, it is possible that only certain forms of cytochrome P-450, converting TA, and induced under its influence, and the concentration of the remaining forms was reduced. Meanwhile, participation of cytochrome P-450-independent microsomal oxygenase in TA metabolism is now accepted [4].

The preliminary rise of the cytochrome b_5 level under the influence of TA is very interesting. These data correlate with hyperplasia of the smooth endoplasmic reticulum. An increased cytochrome b_5 concentration also has been found in galactosamine-induced hepatitis [1]. The cause of the opposite changes in concentrations of cytochrome P-450 and b_5 in acute parenchymatous lesions of the liver, still awaits explanation.

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EFFECT OF HYDROCORTISONE ON THE LIPID COMPOSITION OF THE RAT LIVER NUCLEAR MATRIX

É. S. Gevorkyan, Zh. V. Yavroyan, and G. A. Panosyan

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KEY WORDS: nuclear matrix; hydrocortisone; phospholipids; neutral lipids

It is now generally agreed that the nuclear matrix can play an important structural and functional role in cellular activity. Proteins of the nuclear matrix can take part in regulation and transcription processes and in establishment of the complex superstructure of chromatin [8, 11]. Although lipids are present in the matrix in small quantities, they may also possibly play a role in these processes, especially because lipid components of other nuclear structures, according to some workers [6], carry a definite functional load. Despite the ever-increasing interest of research workers in problems of the nuclear matrix, its lipid composition has been inadequately studied.

The aim of this investigation was to study the phospholipid and neutral lipid composition of the nuclear matrix of rat liver under the influence of hydrocortisone, in an attempt to shed some light on the role of the lipids of nuclear structures in hormonal regulation of the genome.

Biology Faculty, Erevan University. Institute of Experimental Biology, Academy of Sciences of the Armenian SSR, Erevan.

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TABLE 1. Content of Components in Nuclear Matrix Preparations from Rat Liver

Component	Content		Percent of total content in nuclei
	mg/g tissue	%	
Protein	0,840±0,066	76,36±5,95	9,41±0,74
DNA	0,081±0,005	7,27±0,42	3,29±0,19
RNA	0,142±0,011	12,72±0,99	23,89±1,88
Phospholipids	0,021±0,001	1,98±0,12	1,67±0,11
Neutral lipids	0,018±0,002	1,63±0,15	2,11±0,26

Legend. Mean results of five series of experiments are given.

TABLE 2. Content of Total Phospholipid and Its Individual Fractions in Control and under Influence of Hydrocortisone

Phospholipids	Control		Hydrocortisone	
	μg/mg protein	%	μg/mg protein	%
Total	40,27±2,45	100	60,79±1,58	100
Including				
phosphatidylcholine	8,14±0,68	20,2	6,72±0,41	11,1
phosphatidylethanolamine	10,17±0,29	25,3	15,98±0,42	26,3
sphingomyelin	15,88±0,31	39,7	29,43±0,45	48,4
cardiolipin	5,98±0,21	14,8	8,64±0,34	14,2

EXPERIMENTAL METHOD

Noninbred male and female rats were used. Hydrocortisone (Sigma, USA) was injected intraperitoneally in a dose of 5 mg/100 g. The animals were decapitated 4 h after injection of the hormone. The nuclear matrix was separated from the rat liver cells by the method in [9]. Protein was determined by Lowry's method [12], DNA as in [10], and RNA as in [14]; lipids were extracted by Kargapolov's method [5]. Phospholipids and neutral lipids were separated by thin-layer chromatography on Silica-gel L plates (12 × 25 cm^2) from Chemapol, Czechoslovakia), by the use of appropriate systems of solvents: chloroform–methanol–water (65:25:4) for phospholipids, petroleum benzene–diethyl ether–formic acid (80:20:2) for neutral lipids. Standard lipids were used for qualitative analysis: phosphatidylcholine, sphingomyelin, cardiolipin, cholesterol (all from Sigma). Phospholipids were determined quantitatively as inorganic phosphorus [8] and neutral lipids by the method in [15].

A characteristic feature of methods used to isolate the nuclear matrix is a combination of consecutive salt extraction of isolated nuclei with treatment by detergent, yielding preparations with similar morphological and biochemical parameters despite possible modifications of the solutions used and the order of their application [2, 9]. These preparations are characterized by a high protein content (up to 80% or more), a moderate content of nucleic acids (up to 15-20%), and very small quantities of lipids, especially phospholipids (1-2%) [9]. Subsequent careful treatment of the nuclear matrix preparations with nucleases enables a a structure with a much lower nucleic acid content to be isolated by nucleases, namely the "nuclear protein matrix." The latter, by the composition of its phospholipids, is almost indistinguishable from preparations of the nuclear matrix [9], and accordingly, in order to study lipids of the matrix, we isolated the preparations without the last stage of nuclease treatment.

EXPERIMENTAL RESULTS

The results for the biochemical composition of the matrix (Table 1) agree with data in the literature [2, 9], although in these investigations no neutral lipids were present in the composition of the matrix. The reason for this was evidently that no comprehensive investigation of lipids of the matrix was carried out. The presence of appreciable amounts of neutral lipids in the nuclear matrix was noted in one study, but without any special analysis of them [1].

Fractionation of phospholipids of the matrix revealed four individual fractions with the characteristic relative percentages of these preparations (Table 2): 40% of total phospholipid was represented by sphingomyelin, only 20% by phosphatidylcholine, the main nuclear phospholipid. The presence of an appreciable amount (15%) of cardiolipin in the preparations, also characteristic of the internal structures of the nucleus, will be noted [2]. Treatment with hydrocortisone led to a marked increase (by 50%) in the total phospholipid content in the nuclear matrix, with a varied effect on the content of the individual phospholipid fractions (Table 2). For instance, there was an almost twofold increase in phospholipid in the sphingomyelin fraction, whereas the phosphatidylcholine content was actually reduced. As a result, the relative percentage of sphingomyelin increased, and this was accompanied by a decrease in the phosphatidylcholine fraction (Table 2). The relative percengages of phosphatidylethanolamine and cardiolipin were almost unchanged by the action of hydrocortisone.

We know that a high sphingomyelin concentration in the nuclear matrix can be attributed to the possible role of this phospholipid in the initiation of DNA replication [1]. An increase

TABLE 3. Content of Neutral Lipids in Control and under Influence of Hydrocortisone

Neutral lipids	Control		Hydrocortisone	
	μg/mg protein	%	μg/mg protein	%
Total neutral lipid	33,31±2,92	100	25,72±1,72	100
Including cholesterol	12,09±1,01	36,3	14,95±0,86	58,1
free fatty acids	7,93±0,67	23,8	5,14±0,47	19,9
triglycerides	9,66±0,82	29,0	3,81±0,28	14,9
cholesterol esters	3,63±0,31	10,9	1,82±0,14	7,1

in its relative concentration may lead to destabilization of the structure of DNA and loosening of the superstructure of chromatin, thus facilitating the increase in its template activity under the influence of the steroid. The opposite nature of the changes in the phosphatidylcholine and sphingomyelin content, which is also observed in the phospholipids of chromatin [4], can be explained by hormonal potentiation of transfer of phosphorylcholine from the phosphatidylcholine molecule to the sphingomyelin molecule, which other workers have postulated [13].

Cholesterol and its esters accounted for more than 45% of the neutral lipid composition of its nuclear matrix (Table 3). The relatively high triglyceride content and the absence of mono- and diglycerides are evidently a feature of the lipid composition of the nuclear matrix, which distinguishes it from the neutral lipid composition of the nuclei and their membranes, where more than 75% is accounted for by cholesterol and by free fatty acids [16]. Treatment with hydrocortisone reduced the neutral lipid content of the matrix by almost 25%, mainly due to a sharp fall in the level of triglycerides and cholesterol esters (Table 3). A marked decrease in the content of free fatty acids, evidence of inhibition of lipid deacylation processes and also an increase in the free cholesterol fraction, evidently due to conversion of cholesterol esters into free cholesterol, also were observed. On the whole, the decrease in the neutral lipid content of the matrix under the influence of the steroid correlated with the rise in the level of its phospholipids.

These results, and also data obtained previously on changes in the phospholipid composition of the nuclear membranes [3] and chromatin [4] of rat liver under the influence of hydrocortisone, show that lipids isolated in the chromatin and nuclear matrix fractions are in all probability not the result of contamination of the preparations with nuclear membranes during isolation, but are components of these intranuclear structures. This view is supported by evidence both of their unique composition, which differs from that of nuclear membranes, and the opposite direction of the changes in the principal nuclear phospholipid, namely phosphatidylcholine, the fraction of which increases in nuclear membranes under the influence of the steroid [3] but decreases in preparations of chromatin [4] and the nuclear matrix (Table 2).

Under the influence of hydrocortisone the composition of phospholipids and neutral lipids of the nuclear matrix thus undergoes appreciable changes, evidence of the influence of the steroid hormone on lipid metabolism of intranuclear structures. Meanwhile these changes may be connected with processes of specific activation of the genome by the steroid, if the possibility is accepted that the nuclear matrix may contain acceptor sites, specifically binding receptors of the hormone, as has been shown in the case of estrogens and androgens [7].

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EFFECT OF ACTH AND HYDROCORTISONE ON Ca^{++}-ATPase ACTIVITY OF THE SARCOPLASMIC RETICULUM OF SKELETAL MUSCLE

V. I. Korkach and A. N. Fedorov

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The neuromuscular system and the pituitary–adrenocortical system are functionally interconnected. This connection is expressed in the fact that muscular activity modifies activity of the pituitary–adrenal system, and the hormones of the latter take part in adaptive reactions of the body to muscular activity [1, 2, 4]. ACTH and hydrocortisone have been shown to act through metabolic processes in the muscles, leading to changes in their contractile activity [3-6].

The Ca pump of the sarcoplasmic reticulum (SR), which regulates the intracellular Ca^{++} distribution, plays an important role in the mechanisms of development of muscular contraction. Meanwhile the mechanism of action of the adenohypophyseal and adrenocortical hormones on the work of this transport system of the myocytes has not yet been studied.

The aim of this investigation was to study the effect of ACTH and hydrocortisone on Ca^{++}-ATPase activity of SR of skeletal muscles.

TABLE 1. Ca^{++}-ATPase Activity (in μmoles P_i/min/mg protein) of SR Isolated from Gastrocnemius Muscles of Rats 1 h after Intraperitoneal Injection of ACTH (1 unit/100 g) and Hydrocortisone (5 mg/100g) (M ± m, n = 6)

Experimental conditions	ATPase activity of SR		
	total	basal	Ca^{++}-ATPase
Control	6,3±0,7	3,3±0,6	3,0±0,3
ACTH	8,5±0,1*	2,1±0,2	6,4±0,2*
Control	4,9±0,6	2,4±0,3	2,4±0,3
Hydrocortisone	8,9±0,8*	5,9±1,0*	3,0±0,4

Legend. Here and in Tables 2 and 3; *p < 0.05 compared with control.

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