EFFECT OF PHOSPHOLIPID, STEARIC ACID AND CHOLESTEROL ON RNA SYNTHESIS IN RAT UTERINE NUCLEUS

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Lipid is an essential component and plays many physiological important roles in living system. It has been cleared that lipid affects on various enzymic reactions. Recently, our results showed that phosphatidylethanolamine, which is an important phospholipid in rat uterus, increased rat uterine RNA polymerase activity (Aizawa and Nishigori, 1967). This present paper further investigated whether RNA synthesis in rat uterine nucleus, which is a fundamental factor for a dynamic behavior in living system, is affected or not by crude lipid, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, stearic acid and cholesterol in vitro. It was found that RNA synthesis in uterine nucleus was increased by the addition of phosphatidylethanolamine, phosphatidylinositol or crude lipid, but was inhibited by that of stearic acid or cholesterol. Phosphatidylcholine hardly increased RNA synthesis in rat uterine nucleus. However, these lipids had no effect on RNA synthesis in estradiol-17β-treated rat uterine nucleus. These present results suggested that lipid plays a role in RNA synthesis in uterine nucleus.

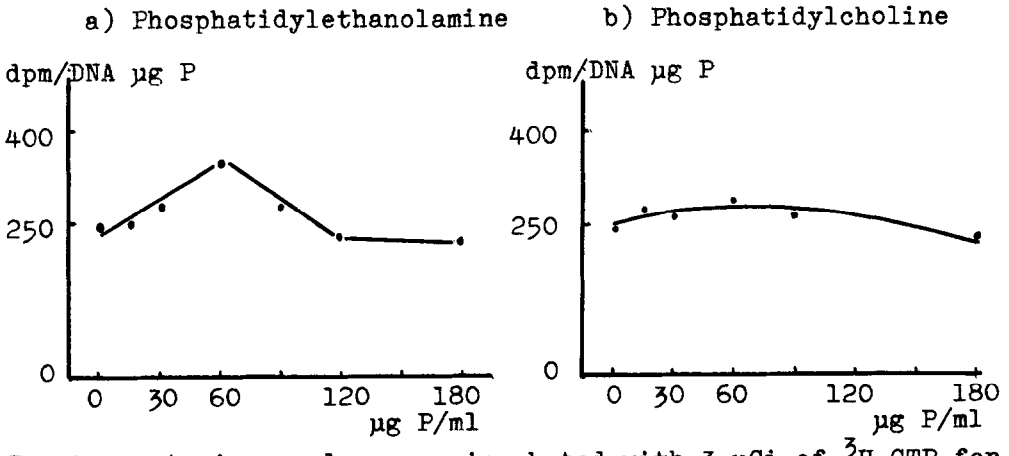
EXPERIMENTAL METHODS : Wistar strain immature rats (21 to 22 days old) or ovariectomized rats were submitted to experiments, The assay procedure of rat uterine RNA polymerase activity was

due to the method described in previous paper (Aizawa and Nishigori, 1967) that is similar to the method of Gorski (1964). The labeled nucleotides used were ^{3}H-CTP or ^{3}H-ATP (respectively, 1.20 Ci/mmole, 4.15 Ci/mmole, Schwarz BioResearch, Inc.). Phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol were obtained from immature female rats liver by the method of Hanahan et al. (1957). Crude lipid used in these experiments was extracted from rats uteri with ethanol, chloroform-ethanol (1:1) and ether. Lipid was suspended in the assay medium and RNA polymerase activity was determined. The incorporation of ^{3}H-CTP or ^{3}H-ATP into the acid-insoluble and defatted residue was assayed in either of two ways. In one procedure, the residue was dissolved in 1.0 ml of Hyamine and then counted in a liquid scintillation spectrometer. Results are expressed dpm per uterus after the data were corrected for unincubated control values. In the other procedure, the acid-insoluble and defatted residue was incubated at 37° for 18 hrs. with 1.0 ml of 0.3N KOH. After the incubation was over, 0.5 ml of the supernatant was transferred into a vial and dried. One ml of Hyamine and 10 ml of scintillation solution (5 g of 2,5-diphenyl-oxazole plus 0.3 g of 1,4-bis-2-(methyl-5-phenyloxazolyl)benzene per liter of toluene) were added to the sample and its radioactivity was counted in a liquid scintillation spectrometer. On the other hand, in order to determine the amount of DNA content, 0.3 ml of the supernatant was acidified with 5% $HClO_4$ to precipitate DNA. The precipitate was ashed with 0.2 ml of 70% $HClO_4$ and amount of phosphorus was determined by the method of Chen et al. (1956). Results were expressed in dpm per μg (P) of DNA present.

RESULTS : RNA polymerase activity in immature or ovariectomiz-

ed rat uterine nucleus was examined by the addition of lipid to the assay medium. Fig. 1-a shows the effect of phosphatidylethanolamine concentration on RNA synthesis, and it was recognized that the optimal dose for RNA synthesis was 60 μg (P) of phosphatidylethanolamine. The effect of phosphatidylcholine concentration was presented in Fig. 1-b, and it was observed that the addition of phosphatidylcholine had hardly effect on RNA synthesis. In this report, for the assay of the effect of phospholipid on RNA synthesis, 60 μg (P) of phosphatidylethanolamine, phosphatidylcholine or phosphatidylinositol were added to the incubation medium. Table 1 shows that the addition of phosphatidylethanolamine increased the incorporation of ^{3}H-CTP into RNA by from 35 to 41% of control, and when phosphatidylinositol was added to the assay medium, the incorporation of ^{3}H-CTP into RNA

Fig. 1 : EFFECT OF PHOSPHATIDYLETHANOLAMINE AND PHOSPHATIDYLCHOLINE CONCENTRATION ON RNA SYNTHESIS IN UTERINE NUCLEUS



Immature uterine nucleus was incubated with 3 μCi of ^{3}H-CTP for 15 minutes at 37°. Phosphatidylethanolamine or phosphatidylcholine was added at the levels indicated. Each point represents an average of two preparations.

TABLE I

EFFECT OF LIPID ON THE RNA SYNTHESIS IN RAT UTERINE NUCLEUS

	Control	Ph-Et (60 µg P)	Change (%)
Exp. 1*	178, 173	277, 215	+ 41
Exp. 2*	168, 157	240, 218	+ 41
Exp. 3*	238, 250	320, 338	+ 35
Exp. 4**	327, 331	699, 518, 715	+ 96
	Control	Ph-Ch (60 µg P)	
Exp. 1*	131, 120	145, 129	
Exp. 2*	183, 206	190, 188	
Exp. 3*	207, 203	225, 218	
	Control	Ph-Ins (60 µg P)	
Exp. 1*	169, 155	253, 215	+ 44
Exp. 2*	260, 228	304, 309	+ 26
Exp. 3*	190, 188, 174	274, 267, 271	+ 47
	Control	Stearic acid (5 µmoles)	
Exp. 1*	212, 210	164, 147	- 26
Exp. 2**	1211, 908	586, 573, 579	- 45
	Control	Cholesterol (2 µmoles)	
Exp. 1**	856, 857	690, 675, 594	- 24
	Control	Crude lipid	
Exp. 1***	2037, 1661	2677, 2614 (150 µg P)	+ 43
Exp. 2***	2530, 2470	5959, 4600 (250 µg P)	+112

* Uterine nucleus from immature rat was incubated with 3 µCi of ^{3}H-CTP for 15 minutes at 37° and the results were expressed dpm per µg of (P) in DNA.

** Uterine nucleus from immature rat was incubated with 3 µCi of ^{3}H-ATP for 15 minutes at 37° and the results were expressed dpm per uterus.

***Uterine nucleus from ovariectomized rat (before 3 weeks, operated) was incubated with 3 µCi of ^{3}H-CTP for 15 minutes at 37° and the results were expressed dpm per uterus.

Ph-Et : Phosphatidylethanolamine Ph-Ch : Phosphatidylcholine
Ph-Ins : Phosphatidylinositol

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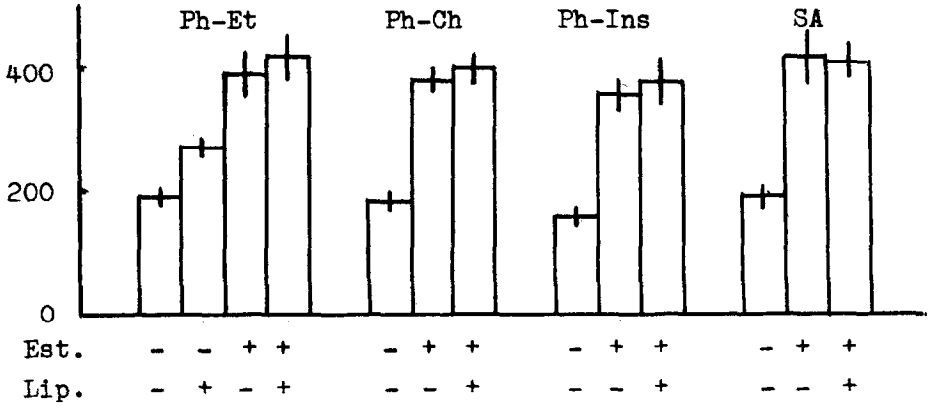
was increased by from 26 to 47% of control. However, RNA synthesis was hardly increased by the addition of phosphatidylcho-

line. In Table 1, the results for the effect of stearic acid, cholesterol or crude lipid on RNA synthesis were also presented. When 5 μmoles of stearic acid were added to the assay medium, the incorporation of ^{3}H-CTP into RNA was inhibited by 26% of control. RNA polymerse activity was examined in the assay medium containing ^{3}H-ATP as labeled nucleotide and it was found that phosphatidylethanolamine increased the incorporation of ^{3}H-ATP into RNA by about two times of control, and that when 5 μmoles of stearic acid or 2 μmoles of cholesterol were added to the medium, the incorporation of ^{3}H-ATP into RNA was respectively inhibited by 45% or 24% of control. As shown in

Fig. 2 : EFFECT OF LIPID ON RNA SYNTHESIS IN UTERINE NUCLEUS FROM ESTRADIOL-17β-TREATED RAT



Est. : Immature female rats received subcutaneous injections of 10 μg of estradiol-17β dissolved in 0.1 ml of sesame oil, and after 4 hrs the animals were used.
Lip. : Sixty μg (P) of phosphatidylethanolamine (Ph-Et), phosphatidylcholine (Ph-Ch) or phosphatidylinositol (Ph-Ins), or 5 μmoles of stearic acid were added to 1 ml of the assay medium.
Uterine nucleus was incubated with 3 μCi of ^{3}H-CTP for 15 minutes at 37° and the values were dpm per μg (P) of DNA.
Bars represent the means and standard errors of four values.

these results, it was found that RNA synthesis differed according to the kind of lipid. The addition of crude lipid from uterus also increased the incorporation of ^{3}H-CTP into RNA.

Gorski (1964) reported that rat uterine RNA polymerase activity was increased by the administration of estradiol-17β. Fig. 2 presents the effect of phospholipid or stearic acid on RNA polymerase activity in uterine nucleus from estradiol-17β-treated rat. It was indicated that phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol or stearic acid had hardly effect on RNA polymerase activity in estrogen-treated uterine nucleus.

DISCUSSION : Administration of estradiol-17β to castrated rats rapidly enhanced the phospholipid synthesis in uterus (Aizawa and Mueller, 1961), and moreover it was found that the nuclear phospholipid was increased by the hormone treatment (Gorski and Nicolette, 1963). On the other hand, many investigators have been examining the effect of estrogen on RNA synthesis in uterus and suggest that these effects may involve RNA polymerase (Gorski, 1964), inactivation of repressors (Talwar et al., 1964), chromatin template activity (Barker and Warren, 1966), nuclear membrane permeability (Szego, 1965; Means and Hamilton, 1966). Our present report examined the relationship between RNA synthesis and lipid in rat uterus indicated that lipid had effects on the RNA synthesis in uterine nucleus. It is particular interest that phosphatidylethanolamine and phosphatidylinositol increased RNA synthesis in uterine nucleus from control animal though its addition could not increase polymerase activity in control to a level equal to that of the estrogen-treated animals. Our previous paper (1967) demonstrated that RNA synthesis in the ovariectomized rat uterine nucleus was increased by phosphatidyl-

ethanolamine and that the increase of RNA synthesis caused by it was not recognized in the cases which the incubation for the assay was carried out at 4^{o} or ^{3}H-CTP was only present in the incubation medium as nucleotide.

On the influence of fatty acids, it is interesting report, reviewed by Nieman (1954), that the trace amounts of fatty acid inhibit the growth of microorganism. Our findings, the inhibitive effect of stearic acid, was an interesting fact.

These present results suggested that lipids may play roles in RNA synthesis in rat uterine nucleus in some ways, and these subjects are currently investigating.

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