vaginas grew maximally in castrated male mice with ovarian transplants and minimally in intact males with similar grafts. Growth was intermediate in castrated or intact males without ovarian grafts. 2. Vaginal grafts showed one or 2 estrous phases in castrated males with ovarian grafts between 4 and 5 months after grafting but otherwise remained in late proestrus. Such activity did not occur in castrated males without ovarian transplants or in intact males with or without ovarian transplants, vaginal grafts in these hosts remaining in diestrus.

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Time Response of Lipid Changes in Cockerels after Single Dose of Estradiol Cyclopentylpropionate. (25933)

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Estrogen-induced hyperlipemia and atherosclerosis in cockerels has been described by several investigators(1,2,3,4). More recently Caldwell and Suydam(5) have studied atherogenic effect of estradiol cyclopentylpropionate (ECP) in young birds and noted significant changes in concentration of serum lipids and polysaccharides. Purpose was to study the effect of a single dose of ECP on the lipid pattern in 3-week-old cockerels. Cholesterol and phospholipids of serum, liver and aorta were determined daily, during a 7-day period, following a dose of 5 mg of ECP, to evaluate time response of cockerels to this estrogen.

Methods. Cockerels of White-Rock Broiler strain used for this experiment were obtained from a commercial hatchery when 8 days old. They were kept in thermostatic brooders and received a routine chicken starter with tap water as desired. Hormone was injected when birds were 3 weeks old. Estradiol cyclopentylpropionate (Upjohn Co.) was given intramuscularly as a single dose of 5 mg, dissolved in 0.2 ml of corn oil/bird. Beginning the next day after injection and every day for 7 days, a group of cockerels was anaesthetized, killed by decapitation and the blood collected. Untreated birds, killed on various days of this 7-day period, were considered as controls. Livers and aortas were dissected and weighed. Serum, livers and aortas were studied for lipid content. Total cholesterol was determined following the procedure of Abell *et al.*(6). For study of lipid phosphate the samples were extracted with alcohol-ether and analysis carried out following the method of Zilversmit and Davis(7). Mean values for treated and control groups were compared by the Wilcoxon Rank Test and significance levels were expressed in per cent(8).

Results. Body weight of cockerels was 226 g (200-252) at day of injection, and 387 g (343-452) at end of experiment. Injection was well tolerated and no inhibition of appetite or growth resulted. The results of study of lipids in serum, liver and aorta are presented in Tables I and II. Early and significant rise of both cholesterol and phospholipids was noted in serum and in liver after ECP. Changes in aorta were slower to appear, but significant increase of both studied lipids occurred between the 4th and 7th days, after injection of hormone.

Discussion. Significant increase of lipids

TABLE I. Response of Cockerels to 5 mg of ECP. Serum, Liver and Aorta Cholesterol. Mean ± S.E. No. of birds in parentheses.

Days after ECP	Total cholesterol Serum	Liver	Aorta
	(mg %)		
	Controls		
	165 ± 4 (30)	206 ± 4 (12)	194 ± 3 (10)
	ECP treated		
1	245 ± 7 (16)	*292 ± 5 (16)	192 ± 8 (10)
2	*363 ± 6 (16)	*310 ± 4 (16)	194 ± 5 (10)
3	*450 ± 12 (16)	*335 ± 8 (16)	250 ± 15 (9)
4	*542 ± 19 (16)	*371 ± 11 (16)	241 ± 17 (9)
5	*550 ± 25 (16)	*392 ± 11 (16)	*278 ± 11 (10)
6	*529 ± 22 (16)	*348 ± 9 (16)	*336 ± 13 (10)
7	*476 ± 24 (16)	*356 ± 10 (16)	*376 ± 14 (10)

* Significance level, treated *vs* control, less than 1%.

in serum, liver and aorta of young cockerels was found after a single dose of an estrogen. Rate of change was different for various tissues studied. Rise of serum and liver lipids was noted as early as 24 hours after administration of ECP. Alterations in arterial phospholipids and cholesterol developed slowly. Time element in induction of changes in the hepatic lipogenesis and cholesterogenesis was studied previously and it was found, for example, that significant changes in lipid metabolism occurred as early as 30 minutes after injection of a hormone(9). The relative rapidity in alteration of lipogenic capacity of the liver is associated with high sensitivity of this organ to changes in the level of circulating fats. The possibility exists that fatty acid synthesis in the liver is under homeostatic regulation(10). Rate of increase of serum phospholipids was not parallel with changes in liver. It is known that plasma phospholipids, being only the choline containing type, are quite different from hepatic phospholipids(11). These 2 groups of lipids may, therefore, play different roles in fat metabolism and may react in a different way to estrogenic stimulation. Changes in aortic phospholipids did not follow the rapid rise of serum phospholipids. It is apparent that phospholipids in aorta may be synthesized *in situ* and are not necessarily deposited from plasma (12). Rate of serum and arterial phospholipid turnover may be, therefore, influenced in different manner by hormones.

Several aspects of mobilization and biosynthesis of cholesterol and phospholipids remain unexplained. It is, however, evident that some hormones may influence these processes. Estradiol cyclopentylpropionate is a potent and rapidly acting stimulant of lipid metabolism under described experimental conditions. Exact function of estrogens in metabolism of lipids is not well known. This hormone may modify the activity of enzyme systems involved in absorption and incorporation of exogenous fats into tissue lipids. It may also play an important role in postabsorption phase of lipid synthesis in the tissue and in mobilization of endogenous fats. Possible effect of estrogens on cellular permeability and on transport of lipids may also be considered.

Summary. Effect of a single dose of 5 mg of estradiol cyclopentylpropionate on lipid metabolism in 3-week-old cockerels was

TABLE II. Response of Cockerels to 5 mg of ECP. Phospholipids of serum, liver and aorta. Mean ± S.E. No. of birds in parentheses.

Days after ECP	Phospholipids Serum	Liver	Aorta
	(mg %)		
	Control		
	286 ± 30 (30)	2076 ± 18 (19)	704 ± 14 (10)
	ECP treated		
1	*749 ± 24 (16)	*2757 ± 91 (14)	803 ± 45 (8)
2	*1467 ± 54 (10)	*2820 ± 70 (14)	740 ± 42 (8)
3	*1825 ± 73 (16)	*2850 ± 58 (16)	766 ± 97 (8)
4	*1941 ± 47 (16)	*3064 ± 32 (16)	*1013 ±146 (8)
5	*2131 ± 99 (16)	*3091 ± 70 (16)	*1060 ± 97 (8)
6	*2378 ±135 (16)	*2922 ± 63 (16)	890 ± 75 (8)
7	*1497 ±107 (16)	*2899 ± 31 (16)	869 ± 73 (10)

* Significance level, treated *vs* control, less than 1%.

studied. Cholesterol and phospholipids of serum, liver and aorta were determined daily during 7 days following injection of hormone, to evaluate time response of birds to this estrogen. Early and significant rise of serum and liver cholesterol and phospholipids was observed. Increase of aortic lipids was slower to appear, but significant changes in these parameters were also noted.

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Non-Enzymatic Lysis of Fibrin *s* by Bile.* (25934)

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The fibrin plate was devised by Astrup and Mullertz(1) to serve as a simple substrate for detecting fibrinolytic activity. Lassen(2) demonstrated that heating the fibrin to 86°C for 30 minutes destroyed adsorbed plasminogen. Lysis of unheated but not heated fibrin is interpreted to indicate the presence of plasminogen activators whereas lysis of both types of plate indicates that a proteolytic enzyme is present. Using fibrin plates, Albrechtsen *et al.*(3) found plasminogen activator in many body fluids. We have been unable to find a prior study of fibrinolytic activity in bile, although Albrechtsen(4) and Todd(5) investigated plasminogen activator in body tissues and reported its absence in liver. Consequently, bile was tested on the fibrin plate and appeared to contain a heat-resistant activator. Further investigation, however, showed that the observed lysis was not due to plasminogen activation, but rather was due to simple solution of fibrin *s*(6), formed in the absence of calcium. Fibrin *i*, formed in presence of calcium, was not dissolved by bile, but was susceptible to true plasminogen activators.

Materials and methods. Human or canine bile was either tested within 2 hours of collection or frozen at −20°C for not longer than 2 weeks. Na dehydrocholate (Decholin, Endo), Na taurocholate, (Delta Chemical Works) and Na glycocholate, (Delta and Mallinckrodt Chemical Works) were used as supplied by the manufacturers. *Fibrin plates* (1.2): 0.4 g of bovine fibrinogen (Fraction I, Armour Laboratories) was dissolved in 100 ml of 0.25 M borate-saline buffer, pH 7.4(7), and filtered through Whatman #1 filter paper with suction. Eight ml was pipetted into 100 mm Kimax flat bottomed Petri dishes and clotted with 0.1 ml thrombin (Upjohn, Inc., 50 u/ml in 0.9% saline). Clots were allowed

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