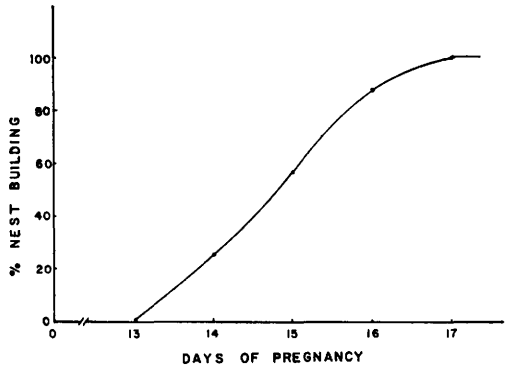

FIG. 1. Percentage of rabbits that built a maternal-nest when castrated between days 13 to 17 of gestation.

This is indicative of the existence of a critical or minimum period for the action of the hormones involved. This finding offers a useful technic for designing treatment schedules in an attempt experimentally to induce maternal-nest building in the castrated female rabbit with various combinations of ovarian steroids and other hormones.

Summary. A critical period of exposure to certain hormones appears to be necessary for maternal-nest building since no nest building occurs in animals castrated before day 14 of gestation. It appears that ovarian steroids must act for a period of 16 or 17 days for the occurrence of maternal-nest building.

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Conversion of Lecithin to Lysolecithin as a Source of Fatty Acids in Incubated Plasma or Serum. (27847)

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Gurd(1) has pointed out the variable results reported for phospholipid breakdown on incubation of plasma. Dole(2) found that the extractable acidity increased at a rate of 35 μeq per liter per hour upon incubation of plasma at 37°C, "presumably by enzymatic hydrolysis of esterified fatty acids." We have observed that this rate of release of fatty acids is adequately accounted for by the conversion of lecithin to lysolecithin. Our evidence is presented here.

Methods. The conversion of lecithin to lysolecithin in plasma or serum upon incubation at 38°C was measured by quantitative silicic acid paper chromatography as previously reported(3). Lipid extracts (Bloor) of plasma or serum kept at 38°C were made after 10 minutes (zero time reference), 4 hours and 20 hours. Values reported are those of single aliquots of lipid extracts applied to the paper chromatograms. The initial lysolecithin values for the normal serums are not reported as true normal values since the serums had been kept frozen for a variable period before being utilized in this experiment(3). The normal serums of section B of the Table are approximately 4 μg higher in lysolecithin P content than when originally analyzed.

Results. Section A of the table records

TABLE I. Concentration of Phospholipids in Various Sera or Plasmas before and after Incubation.

	Type of serum or plasma	Pancreatic lecithinase A (units/ml)	Incubation time in hr and phospholipid concentration (μg P/ml)											
			Lysolecithin (LLe)			Lecithin (Le)			LLe + Le			Sphingomyelin		
			0	4	20	0	4	20	0	4	20	0	4	20
A.	Normal, not fasting	—	11	17	23	58	55	40	69	71	63	20	20	19
	Plasma (EDTA)	—	11	16	26	59	55	44	70	72	70	21	21	23
	" (heparin)	—	10	15	25	59	55	46	69	69	71	20	21	22
	" (oxalate)	—	10	15	22	57	51	42	67	66	64	20	20	21
B.	Normal, fasting Serum 1	9	12	20	—	70	61	—	82	81	—	24	24	—
	" 2	7	13	24	—	93	83	—	106	107	—	32	32	—
	" 3	9	12	20	—	77	71	—	89	91	—	23	25	—
	" 4	5	12	18	—	63	56	—	75	74	—	25	24	—
	Pancreatitis, not fasting Serum 1	34	4	9	—	47	41	—	51	50	—	18	18	—
	" 2	72	5	10	—	50	42	—	55	52	—	17	16	—
	" 3	80	3	9	—	60	51	—	63	60	—	12	15	—
	" 4	450	3	13	—	78	65	—	81	78	—	21	21	—
	" 5	136	2	9	—	64	55	—	66	64	—	17	17	—
C.	Normal, not fasting Plasma (citrate)													
	Preheparin	—	10	13	—	55	47	—	65	60	—	18	18	—
	Postheparin, 10′	—	7	15	—	54	40	—	61	55	—	18	16	—
	Postheparin, 20′	—	6	16	—	53	40	—	59	56	—	15	—	—

one of 3 separate studies in which the incubation of serum or plasma was continued for 20 hours. The conversion of lecithin to lysolecithin was similar for serum or plasma obtained with various anticoagulants, being approximately 5 μg P per ml in 4 hours and 15 μg P per ml in 20 hours. There was no change in sphingomyelin nor in total lipid phosphorus.

This conversion is further verified in section B where 4 frozen serums from normal persons and 5 fresh serums from patients with acute pancreatitis were treated similarly with similar results, the conversion varying between 5 and 11 μg P per ml. The lack of a difference between the normal and pancreatitis sera is noteworthy in view of the considerable amounts of pancreatic lecithinase A (4) present in 4 of the pancreatitis sera. While there has been one report(5) of an increase in serum lysolecithin in acute pancreatitis, our data incidentally confirm the converse observation of Marinetti(6) that there is an initially decreased concentration of lysolecithin.

A single study of the effect of incubating plasma drawn before and after intravenous heparin (5000 units) is shown in section C. The results are no different. As similar qualitative study in another fasting subject was carried out, plasma being drawn before and 18 minutes after heparin. Lipid extracts of each plasma taken initially and after incubations for 1, 2, 4, 11 and 23 hours were chromatogrammed on silicic acid impregnated paper for inspection of the phospholipids, and on thin-layer silica gel(7) utilizing 2 solvent systems for inspection of the neutral lipids. The phospholipid chromatogram confirmed the increase in lysolecithin seen in section C of the Table. Conversion of cephalin (phosphatidylethanolamine) to the lyso compound was not evident after spraying with ninhydrin. The preheparin plasma showed a decrease in free cholesterol but no detectable increase in fatty acids. The postheparin plasma showed an extensive and progressive loss of triglyceride, and a similarly extensive and progressive increase in diglycerides and free fatty acids, but no detectable monoglycerides. The free cholesterol again decreased as before.

Comments. These data support the view that fatty acids released in plasma may be derived from degradation of phospholipids as well as triglycerides(1). The amount of fatty acid released as reported by Dole would require that 4.4 μg of lecithin P per ml be converted to lysolecithin P in 4 hours. The conversion reported herein exceeds this amount slightly; however, an allowance must be made for the conversion of free cholesterol to esterified cholesterol which was not measured.

The conversion of lecithin to lysolecithin on incubating serum is apparently not related to the amount of pancreatic lecithinase A present. Also there seemed to be no difference between pre- and postheparin plasma. Nevertheless the implication of some type of phospholipase A action led us to vary the incubation constituents resulting in the demonstration of a postheparin thermolabile phospholipase A particularly active against egg phosphatidylethanolamine, whose activity is augmented by the presence of protein(8). While we only observed the conversion of lecithin to lysolecithin on incubating serum alone for as long as 20 hours, we have become aware of other conditions under which lecithin may be converted to diglyceride by a phospholipase D produced by a common bacterial contaminant.

Summary. Incubation of human serum or plasma at 38°C for 4 hours results in conversion of approximately 10% of the lecithin to lysolecithin. Serum from patients with acute pancreatitis and postheparin plasma was no different than normal serum or plasma in this respect.

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In vitro Instability of a Commonly Used Tricarbocyanine Dye. (27848)

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The use of a tricarbocyanine dye (Cardio Green,® Hynson, Westcott, & Dunning) as the indicator for dye dilution measurement of cardiac function has become standard in many laboratories. One of the principal advantages of this dye is its rapid disappearance from the circulation(1), thus permitting serial determination of cardiac output at relatively short intervals. Such serial use of this dye presupposes that it remains stable *in vitro* throughout the study. Fox *et al.*(2) and Miller *et al.*(3) have reported such stability for this dye. However, we were unable to substantiate these findings, and the following study was instituted.

Methods. This study was divided into 2 parts: (1) The stability of Cardio Green dye in the manufacturer's aqueous solvent, and (2) the stability of Cardio Green dye in whole blood.

(1) The stability of Cardio Green dye for 8 hours in the manufacturer's aqueous solvent was investigated from 2 standpoints. The first phase was initiated to see if the wave length of maximum absorbance remained constant, and the second phase to examine the absorbance stability of the dye at a fixed wave length of 775 mμ.

At hourly intervals, an aliquot of the stock dye solution (0.25%, 2.5 mg/cc) was diluted