J. M., and Hunter, R. B., *British Med. J.*, 1956, v1, 448.

15. Canal, N., Garattini, S., and Tessari, L., *Boll. Soc. it. Biol. sper.*, 1956, v32, 1.

16. Foà, P. P., Weinstein, H. R., and Smith, J. A., *Am. J. Physiol.*, 1949, v157, 197.

17. Nelson, N., *J. Biol. Chem.*, 1944, v153, 375.

18. Bratton, A. C., and Marshall, E. K., Jr., *ibid.*, 1939, v128, 537.

19. Gomori, G., In Ferner, H., *Das Inselsystem des Pankreas*. Stuttgart: G. Thieme, 1952.

20. Wrenshall, G. A., and Best, C. H., *Canadian Med. Assn. J.*, 1956, v74, 968.

21. Franke, H., and Fuchs, J., *Deut. med. Wschr.*, 1955, v80, 1449.

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Plasma Phospholipide Synthesis in the Eviscerated Rabbit.* (22812)

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Fishler *et al.*(1) studied the synthesis of plasma phospholipides in fasting hepatectomized dogs with P^{32} and Goldman *et al.*(2) repeated this study with C^{14}-labelled palmitic acid. From this work it was concluded that the liver is the major source of plasma phospholipides in the fasting dog. Plasma phospholipides of rats in the post-absorptive state were also found to originate in the liver(3). On the other hand, Ranney *et al.*(4) demonstrated that, in the chicken, extrahepatic tissues contribute phospholipides to the plasma.

The following experiments were conducted to determine the site(s) of plasma phospholipide synthesis in the rabbit.

Methods. Normal white rabbits maintained on a diet of 100 g Purina rabbit chow per day, and fasted 24 hours prior to the experimental period, were sedated with intraperitoneally administered urethane (750 mg/kg) and anesthetized 30 minutes later by intravenously administered sodium pentobarbital (15 mg/kg). The gastrointestinal tract and spleen, and in some instances the kidneys, were removed through a median abdominal incision. The liver was left *in situ* isolated from the hepatic artery and portal vein. Before closing the incision, bandages wet with warm saline were placed in the abdominal cavity. Eviscerations were generally completed within 10 to 20 minutes, following which the rabbits were kept warm by application of external heat. Immediately after evisceration, rabbits received an intravenous dose of 100 mg glucose (10% in saline), followed by 100 mg/kg at 30 minute intervals through the remainder of the experiment(5). Control animals were sham operated, some receiving glucose, others not. Rabbits were injected intravenously with 0.5 to 1.0 mc of labelled phosphate and sacrificed four hours later by intravenous administration of sodium pentobarbital. Two series of experiments were performed. In series I plasma and tissues were extracted with alcohol-ether, petroleum ether and analyzed as previously described

TABLE I. Tissue Phospholipide Concentrations.

Tissue	Sham operated (mg P/g)		Eviscerated (mg P/g)	
Series I*				
Liver	1.36‖	± .02§	1.11‖	± .02
Kidney	1.11	± .03	1.12	± .01
Muscle	.283	± .010	.272	± .009
Lung	1.13	± .01	1.11	± .05
Plasma (4 hr)	.0359	± .0065	.0273	± .0050
Series II†				
Liver	1.20‖	± .06	.995‖	± .012
Kidney	1.02	± .07	1.03	± .11
Muscle	.310	± .022	.311	± .24
Lung	.990	± .008	.944	± .033
Plasma‡ (0 hr)	.0416	± .0052	.0494	± .0156
(4 hr)	.0481‖	± .0076	.0332‖	± .0089

* 5 sham-operated, 6 eviscerated rabbits.
† 4 " , 4 " " .
‡ 9 " , 9 " " .

§ Stand. error $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

‖ Diff. significant at 5% level.

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TABLE II. Specific Activity* of Tissue Phospholipides.

Tissue	Sham operated	Eviscerated
Series I†		
Liver	14.9‖ ± 2.1¶	.104‖± .02¶
Kidney	33.2‖ ± 3.7	58.1‖ ± 2.8
Muscle	.480 ± .063	.746 ± .121
Lung	8.07 ± 1.00	13.8 ± 1.0
Plasma	3.21‖ ± .65	1.22‖ ± .28
Series II‡		
Liver	12.9‖ ± 1.6	.120‖± .049
Kidney	29.8 ± 2.2	44.6 ± 5.7
Muscle	.707 ± .159	1.34 ± .28
Lung	9.97 ± 1.16	11.3 ± 2.0
Plasma§	3.36‖ ± .36	1.29‖ ± .26

* Specific activity = $\frac{\%\text{ injected } P^{32}}{\text{g lipide phosphorus}}$.

† 5 sham-operated, 6 eviscerated rabbits.
‡ 4 " , 4 " "
§ 9 " , 9 " "
‖ Diff. significant at 5% level.
¶ Stand. error.

(6,7). In series II, experimental procedures were modified as follows: a) zero time (t = 0) blood samples were taken for phospholipide analysis; b) plasma acid soluble specific activities were determined on trichloroacetic acid supernatants 2 and 4 hours after administration of P^{32}; c) plasma and tissues were extracted by the chloroform-methanol procedure of Folch *et al.*(8). Tables I and II as well as further unpublished observations indicate that the two extraction methods gave comparable results for specific activities even where the concentrations differed somewhat.

Results. No significant differences in concentration of lipide phosphorus of kidney, muscle, and lung were observed between normal and eviscerated animals (Table I). Only liver showed a decrease of 17-18% in the eviscerates. Although there was no difference in plasma lipide phosphorus concentration between sham operated and eviscerated animals of series I, inspection of series II would appear to show a decrease in the eviscerates. However, the more critical comparison of zero time and 4 hour plasma phospholipide levels in each eviscerated rabbit failed to show a significant drop in concentration.

Table II summarizes the specific activity data. In control rabbits, the specific activity of liver, kidney and lung exceeded the specific activity of plasma phospholipides. Muscle, however, showed a specific activity less than that of plasma excluding this tissue as a likely source of plasma phospholipides. In eviscerated rabbits liver specific activities were 7 to 9% of the control values, evidence that liver circulation was almost completely absent. The mean plasma phospholipide specific activities in series I and II were 8-12 times as great as those observed in liver. It is apparent that labelled phospholipides from sources other than liver found their way into plasma. Since the specific activities of kidney and lung phospholipides exceeded those of plasma phospholipides, these tissues could contribute phospholipides to the plasma pool.

In an attempt to localize further the possible sites of plasma phospholipide synthesis 9 rabbits were eviscerated: in 5 animals the kidneys were left in (eviscerated controls) and in 4 they were removed (eviscerated nephrectomized). Specific activities of plasma and liver phospholipides in eviscerated controls were 1.07 ± 0.11 and 0.071 ± 0.028, respectively, which agree with the animals of Table II. In the nephrectomized animals, however, the plasma phospholipide specific activity had decreased to 0.462 ± 0.016. It is thus evident that plasma phospholipide specific activity is reduced to ⅓ of normal upon removal of liver, intestine and spleen and that by removal of kidneys it is further reduced to about 1/10th of normal. That the small amount of labelled phospholipide appearing in plasma of eviscerated nephrectomized animals is not derived from liver is shown by the fact that the specific activity of plasma phospholipide of individual animals was 10-100 times that of liver.

In series I, kidney phospholipides showed a significantly higher specific activity in eviscerates than in controls. In order to explain this difference and the similar tendency towards higher specific activity in muscle and lung, ratios of tissue phospholipide specific activity to 4 hour plasma acid soluble phosphate specific activity were determined in series II. These ratios represent an index of the rate of synthesis of phospholipide from their phosphate precursors. No evidence of altered tissue phospholipide synthesis was

found in eviscerated rabbits. It appears, therefore, that the tendency towards higher specific activity in eviscerated groups is related to decrease in total phosphate pool and a subsequent increase in specific activity of phospholipide precursors.

Summary. In the rabbit, plasma phospholipide synthesis continued at a reduced rate in absence of liver, intestine and spleen. Removal of kidney further reduced plasma phospholipide synthesis. The rate of synthesis of kidney, lung or muscle phospholipides from phosphate precursors does not appear to be altered by evisceration.

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1. Fishler, M. C., Entenman, C., Montgomery, M. L., and Chaikoff, I. L., *J. Biol. Chem.*, 1943, v150, 47.

2. Goldman, D. S., Chaikoff, I. L., Reinhardt, W. O., Entenman, C., and Dauben, W. G., *ibid.*, 1950, v184, 727.

3. Zilversmit, D. B., Bollman, J. L., *Arch. Biochem. Biophys.*, 1956, v63, 64.

4. Ranney, R. E., Chaikoff, I. L., and Entenman, C., *Am. J. Physiol.*, 1951, v165, 596.

5. Bergman, H. C., and Drury, D. R., PROC. SOC. EXP. BIOL. AND MED., 1937, v37, 414.

6. Zilversmit, D. B., Shore, M. L., Ackerman, R. F., *Circulation*, 1954, v9, 581.

7. King, E. J., *Biochem. J.*, 1932, v26, 292.

8. Folch, J., Ascoli, I., Lees, M., Meath, J. A., LeBaron, F. N., *J. Biol. Chem.*, 1951, v191, 833.

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Metabolic Conversion of Primidone (Mysoline) to Phenobarbital.* (22813)

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5-Ethyldihydro - 5 - phenyl - 4.6-(1*H*,5*H*)-pyrimidinedione (primidone, Mysoline), a drug used in the treatment of epilepsy, differs structurally from phenobarbital only in that the former has a methylene group in the 2-position and the latter a carbonyl group. The only metabolic reaction that primidone has heretofore been reported to undergo is conversion to 2-ethyl-2-phenyl malonamide(1). Another reaction that suggested itself as a possibility is oxidation of the methylene group in primidone to yield phenobarbital as a product. The present investigation has shown that this reaction does indeed occur, and this report concerns a study of this metabolic conversion and its significance in therapeutics.

Methods. Administration of primidone. Crystalline primidone supplied through the kindness of Dr. A. Stanley Cook, Ayerst Laboratories, N. Y. City, was used in experiments on dogs. It was administered in gelatin capsules by mouth. The patients received commercial tablets. *Lack of contamination of primidone with phenobarbital.* A sample of crystalline primidone was partitioned between ether and water in a countercurrent design calculated to effect 99% separation of primidone and phenobarbital. Spectrophotometric examination of the fractions that would have contained phenobarbital demonstrated that the sample of primidone could have contained no more than 0.01% of phenobarbital. Information obtained from the manufacturer indicates that the method of synthesis excludes the possibility of any initial contamination with phenobarbital. *Spectrophotometric measurements* were made with a Beckman DU ultraviolet spectrophotometer. *Isolation of phenobarbital and p-hydroxyphenobarbital from urine.* The procedure described by Butler(2) was used. All samples of urine were subjected to acid hydrolysis. *Determination of phenobarbital in plasma.* For dog plasma the method of Butler *et al.*(3) was used. Unchanged primidone does not interfere to a significant extent with this method. A con-

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