ever, similar bile duct hyperplasia was not observed in cirrhotic rabbits receiving only CCl_4 in this laboratory. Estrogen has been reported to cause a decrease in growth, decrease in body weight of adult animals, increase in basal metabolic rate, hair loss, testicular atrophy, infertility, and hyperplasia of pituitary, thyroid and adrenal(3-7). In view of the well-established effect of estrogens on growth processes and excretion of estrogens in the bile, one might expect hyperestrogenemia to cause changes in bile duct epithelium. However, due to the body-wide endocrinopathy produced by estrogens one can only speculate as to whether the bile duct changes are due to a direct toxic effect of the estrogen, which seems most plausible, or to some other endocrine abnormality.

Summary. Administration of diethylstilbestrol to intact rabbits, or to rabbits made cirrhotic with carbon tetrachloride, produced in all animals a marked bile duct proliferation and in one animal changes suggestive of neoplasia of the bile ducts. The possibility that hyperestrogenism may be responsible for the increased incidence of carcinoma of the liver in cases of liver cirrhosis is suggested.

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An Improvement in Quantitative Separation of Phospholipids with Silicic Acid Impregnated Filter Paper. (26159)

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Recent developments in filter paper chromatography and the perfection of micro technics of analysis for specific substances have made it possible to quantitate compounds separated on paper. We have developed a simple method for quantitating minute amounts of the phospholipids, lecithin, lysolecithin, and sphingomyelin, following chromatographic separation on silicic acid impregnated filter paper, which uses the solvent systems and identification technics developed by Marinetti and coworkers(1,2). However we found that some of the lipid material from serum extracts, which was allowed to dry at point of application, became bound to the silicic acid at the origin and did not move with the chromatographic solvents employed. In addition to staining with Rhodamine G, it gave a positive test for choline. We avoided loss of this material, which was principally lecithin, by a technic we call "running start" chromatography. The technic is applicable to phospholipid analyses in tissue extracts as well as various biological fluids.

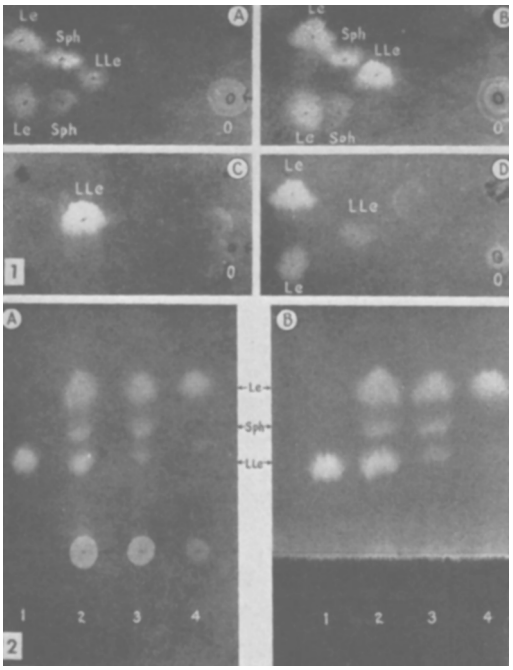

FIG. 1. Two dimensional separation of phospholipids on silicic acid paper. Applications of phospholipids in each of 4 chromatograms were: A, an extract of .02 ml serum containing 525 mg/100 ml phospholipids; B, same as A with 20 μg each of lecithin and lysolecithin added; C, 40 μg lysolecithin; D, 40 μg lecithin (relatively pure with slight contamination by lysolecithin). First separation (right to left) was with diisobutylketone : acetic acid : water 40 : 30 : 7 (v/v) for 11 hr at 20°C. After drying the paper, second separation (bottom to top) was with n-butyl ether : acetic acid : chloroform : water 40 : 35 : 6 : 5 (v/v) for 6 hr at 20°C. 0 = point of original application of samples. Direction of first solvent flow was right to left. Separated components after each solvent separation indicated by: Le, lecithin; LLe, lysolecithin; Sph, sphingomyelin. (Photographs of Rhodamine G stained chromatograms taken under ultraviolet illumination.)

FIG. 2. Contrast between "running start" and standard procedure for separating phospholipids on silicic acid impregnated paper, as exemplified with an extract of blood serum. Solvent used was diisobutylketone : acetic acid : water 40 : 30 : 7 (v/v). Chromatogram A (standard procedure) was run for 19½ hr, and B ("running start" procedure) for 16 hr at 11°C. Both stained with Rhodamine G and photographed under ultraviolet illumination. Point of application in B is approximately 2.0 cm lower than that in A. Applications at origins in both A and B were: 1, 40 μg of lysolecithin; 2, serum extract enriched with 20 μg each of lecithin and lysolecithin; 3, serum extract alone; 4, 40 μg of lecithin (relatively pure but with slight contamination by lysolecithin).

Methods. Whatman #1 filter paper was impregnated with silicic acid as originally described(1) in the cases of the chromatograms of Fig. 1 and 2, and as later modified (2) in the case of the data of Table I. An aliquot of an alcohol:ether 3:1 (v/v) extract of the material to be analyzed was evaporated and redissolved in isoamylalcohol:benzene 1:1 (v/v). A suitable proportion of this solution was applied to the origin. Diisobutylketone:acetic acid:water 40:30:7 (v/v) was our usual solvent. The chromatograms of Fig. 1 and 2 were developed in 6" diameter jars and those for the data of Table I in a Kurtz-Miramon chromatographic apparatus, both not lined. Rhodamine G rather than 6G was used for staining the developed chromatograms. Elution of the phospholipid was accomplished with 1N HCl in methanol(2). The individual phospholipid spots were cut out and extracted in a 15 x 150 mm test tube with 3 successive volumes of 2 ml 1N HCl in methanol, bringing the solvent to the boiling point for 10-15 seconds. Each 2 ml extract was successively evaporated in a second 15 x 150 mm test tube placed in a steam heated water bath. The small residual volume was removed by placing the tube in a heating block at 130°C for 30 minutes. Lipid phosphorous was measured by a modification of the method of Beveridge and Johnson(3) in which volumes were reduced by one-tenth. One μg phosphorous yielded an absorbancy of 0.173 in 1 cm cells. Silicate from the paper has not interfered with the determination.

Results. In one-dimensional runs with pure

TABLE I. Recovery of Lecithin at Origin after Phospholipid Chromatography on Silicic Acid Paper.*

Lecithin applied, μg P	Recovery in separate runs, %	Lecithin applied, μg P	Recovery in separate runs, %
.24	21	1.20	19, 10, 12
.48	31	2.40	8
.60	12	3.60	6
.72	26	4.80	6
.96	25, 10, 14	6.00	8†

* Mixture of 3 phospholipids as described in the text were applied in a volume of 10 μl or by repeated additions to a total volume of 50 μl. Areas of application were essentially equal. Chromatograms were run for 16 hr at 17°C. After chromatography the amount of phosphorus remaining at the origin was measured and is reported as percentage of lecithin phosphorus applied.

† Overloaded origin spot. Value a little high.

lecithin, sphingomyelin, and lysolecithin, only lysolecithin moved completely from the point of application. In 2-dimensional runs with different solvent systems, lecithin and sphingomyelin left origin spots at both first and second separations (Fig. 1). The relatively pure lecithin (dipalmitoleyl-glycerylphosphorylcholine) and lysolecithin (β-monostearoyl-glyceryl-phosphorylcholine) were gifts of Dr. D. J. Hanahan. The faintness of the sphingomyelin spots indicated that only slight amounts were bound at the origin. This was found to be 2% in one analysis in which 1.20 μg sphingomyelin phosphorous were applied. However the intensity of the lecithin origin spots warranted special analysis of the effect of varying the amounts of phospholipid applied.

Table I gives percentage recovery of lecithin at the origin in runs in which varying amounts of lecithin were applied in a mixture containing equal amounts of pure lecithin, sphingomyelin, and lysolecithin phosphorus (P). At least 10%, and as much as 30% of the lecithin was left at the origin when 1.20 μg of lecithin P (29 μg lecithin) or less were applied to the paper. Approximately 7% was left at the origin when 2.40 μg lecithin P (58 μg lecithin) or more were applied.

A means of avoiding this loss at the origin is provided by the technic of "running start" chromatography. Since on regular Whatman #1 filter paper, with the solvents used, all lipid material travels with or close to the solvent front, papers have been prepared so the application of material is made on an unimpregnated area of the filter paper. The applied material travels 2.0-2.5 cm before reaching the silicic acid impregnated portion where separation of the individual phospholipids begins. No Rhodamine G staining material is left at the origin, and separations of individual compounds are favorably affected in shape, so delineation of the boundaries of the spots is more definite. Fig. 2 contrasts the "running start" method with the standard application to silicic acid impregnated paper. The component traveling with an R_f between lecithin and lysolecithin is sphingomyelin. It gives a positive choline test and has the same R_f as pure sphingomyelin prepared from egg yolk by the method of Rhodes and Lea(4). Our recoveries of lecithin have improved 7-10% by the use of "running start" chromatography with loadings up to 5 μg of lecithin P. Repeated applications of extracts may be made, as the variation in the area of loading is not critical. Our recoveries of lysolecithin and sphingomyelin have been essentially the same with or without the "running start" procedure. The technic may have application in other areas where fixation of compounds may occur.

Summary. A technic is described for avoiding loss of lecithin at point of origin during quantitative separation of phospholipids on silicic acid impregnated paper.

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Effect of Drugs on Cx-Protein Responses in the Rabbit.* (26160)

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Availability of appropriate blocking agents commonly provides a valuable tool in studies of sites of action of drugs and sequences of biochemical and physiological reactions. Studies directed toward an elucidation of the role of C-reactive protein, which appears during inflammatory episodes, would be aided if

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