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Short Communications

The Occurrence of Cephalin, Sphingomyelin, and Lysolecithin in Bile

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Since Thudichum studied the phospholipids in bile as early as 1862[1], lecithin has been believed to be the only phospholipid present in bile [2,3]. However, in 1958 Nakayama and Johnston [4] fractionated biliary phospholipids with silicic acid column chromatography and presented data showing the constant presence of several phospholipid peaks other than that corresponding to lecithin. Furthermore, the complicated pattern of fatty acids of biliary phospholipids obtained by gas chromatography suggests the complexity of phospholipids composition in bile [5]. These findings prompted us to identify the phospholipid components in bile.

The bile from rat and rabbit given ^{32}P-orthophosphate intraperitoneally was extracted with chloroform-methanol 2:1 mixture followed by partition with physiological saline solution essentially based on the technique of Folch *et al* [6]. The labelled biliary phospholipids were purified by silicic acid column chromatography [7]. The methanol fraction containing the phospholipids was concentrated and subjected to paper chromatography together with ^{32}P-labelled liver phospholipids using the solvent system of di*iso*butylketone, acetic acid, and water (40:25:5) [8] followed by autoradiography (Fig. 1). The autoradiogram of rat liver phospholipids (Ia) indicated the presence of six phospholipid components which were earlier identified by Marinetti *et al.*[9] as A-unidentified phospholipid, B-lysolecithin, C-sphingomyelin, D-lecithin, E-cephalin, F-unidentified phospholipid, as shown in Fig. 1. The autoradiogram of biliary phospholipids from rabbit (IIa) and from rat revealed the presence of lysolecithin, sphingomyelin, lecithin and cephalin in bile of the two species studied.

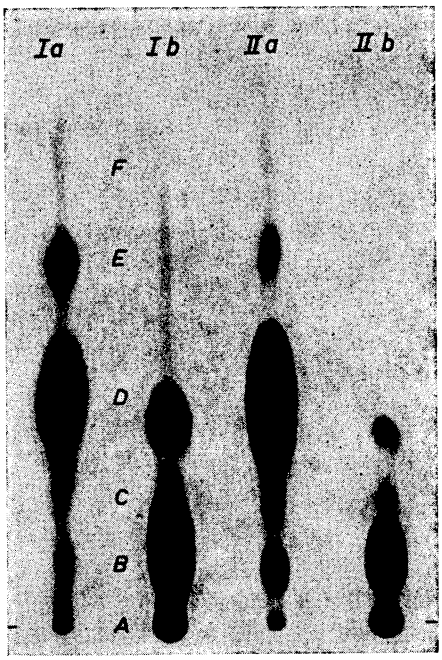

Fig. 1. Autoradiogram of ^{32}P-labelled rabbit liver and bile phospholipids. Ia-liver phospholipids, Ib-liver lecithin hydrolysate with snake venom *(Crotalus adamanteus)*, IIa-bile phospholipids, IIb-its hydrolysate with snake venom. Silicic acid impregnated Whatman No. 3 paper, ascending, solvent system-di*iso*-butylketone: acetic acid:water 40:25:5. A-unidentified phospholipid, B-lysolecithin, C-sphingomyelin, D-lecithin, E-cephalin, and F-unidentified phospholipid.

The further evidence for the presence of lysolecithin in bile was provided using the combination of snake venom and autoradiography. A part of the labelled phospholipids was hydrolyzed with snake venom *(Crotalus adamanteus)* [10] and subjected to paper chromatography together with the original phospholipids. After hydrolysis with snake venom of the pure liver lecithin obtained by preparative paper chromatography, the lecithin spot was diminished greatly and transformed into a lysolecithin spot (Ib). The lecithin spot of biliary phospholipids (IIa) was diminished greatly after similar hydrolysis and transformed into a lysolecithin spot, thus confirming the presence of lysolecithin in bile (IIb).

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Separation of Nucleic Acids and Protein by Electrophoresis Combined with Counter Flow

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A good separation of proteins and nucleic acids from cell extracts could be achieved by electrophoresis in long columns stabilised with ethanolised cellulose [1]. The length of the path needed to affect such a separation was 300—320 cm. The diameters of the columns was 3.5 cm, and in a few cases 8.0 cm (unpublished). It became apparent that some material contained in the protein fractions was lost in the large amounts of cellulose (*e. g.* the neurotoxin from *Bordetella pertussis*).

The experiments to be described aim at a separation of the nucleic acids from the proteins in small columns as a preliminary to a further separation of these compound groups by means of chromatography. It was also desirable to let the proteins be in contact with as little cellulose as possible. Very small columns are also desirable for the separation of the components in biologically active nucleoproteins.

From these considerations we have tried to replace the length of the migration path by a steady flow of buffer against the direction of the electrophoretically migrating compounds. For this purpose we have used jacketed and cooled columns, with a length and diameter of 45 and 3.5 cm, respectively. We have also been working with 35 × 1 cm columns, about which will be reported later on. The packing, cooling, continuous buffer exchange and the buffer composition were exactly the same as described before [1]. The end of the side tube was, as usual, closed by a tightly fitting, thick dialysis membrane. Micropumps were constructed with capacities suitable for pumping the buffer in the direction opposite to the electrophoretic migration of the polyelectrolytes to be separated.

In the present paper we report experiments (*cf.* Table 1) where suction was simply applied to the buffer above the stabilised part of the column, with the purpose of finding the rate of flow that would permit the nucleic acid to migrate towards the anode at reduced speed, and drive the