Fig. 1. The migration of serum albumin heated with congo red at (*p*H 6·4) The photograph was taken using a red filter to remove the red colour of congo on the strip

phoresis. But according to our unpublished experiments, it remains clear and migrates when it is heat-treated in alkaline solution. We now find that albumin also remains clear and migrates when it is heat-treated in the presence of congo red. The aqueous solution of congo red is alkaline (about *p*H 10). But albumin also remains clear and migrates when it is heat-treated with congo red acidified to *p*H 6·4, whereas it coagulates and does not migrate when it is heat-treated without congo red at the same *p*H.

In our experiments albumin was prepared by isolation from beef serum using ammonium sulphate[7]. The precipitate was dialysed to remove the salts, the albumin content (2·2 per cent) was determined refractometrically and the *p*H (5·8) electrometrically. To 100 mg congo red in aqueous solution was added 6·3 ml. of 0·1 N sulphuric acid and the volume made up to 50 ml. with water. In two test-tubes were put 1 ml. (22 mg) of serum albumin; to one was added 5 ml. of acidified congo red solution and to the other 4·9 ml. of water and 0·1 ml. of 0·01 N sodium hydroxide to adjust the *p*H to 6·4. The two tubes were then heated in a boiling-water bath for 15 min and cooled. The mixture containing congo red was clear while the other was coagulated. The *p*H value of the dye-containing mixture was 6·4 after heating. Paper electrophoresis of the dye-containing mixture, using an L.K.B. type *B* apparatus with barbitone–sodium barbitone buffer, *p*H 8·6, 225 V and ionic strength 0·1, was carried out for 7 h. The strips were then dyed with bromo-phenol blue, washed and dried. Fig. 1 shows the migration of albumin heated with congo red at *p*H 6·4. The experiments show that congo red prevents coagulation of serum albumin at *p*H 6·4 and permits paper-electrophoretic migration of the protein heat-denatured in this way.

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Biliary Phospholipids in Various Species

While carrying out investigations on the fatty acids of bile and plasma lecithins differences were noted in the overall biliary phospholipid pattern in man, dog, rat and ox.

Bile lipids were extracted, separated and analysed by previously described methods[1]. Purity after silicic acid column chromatography was checked by thin-layer[2] and paper chromatography[3]. Phospholipid standards were prepared from rat liver and red blood cells according to the methods of Glenn *et al.*[4] and Spitzer and Balint[5].

Lipid extraction with chloroform–methanol[6] or by refluxing with hot ethanol–ether yielded similar results.

The distribution of phospholipids obtained is shown in Table 1. Compounds noted as trace amounts could only be detected by thin-layer chromatography. Besides the phospholipids indicated, trace amounts of what appeared to be cardiolipin and phosphatidic acids were also detected in the four species examined. The final identification of all compounds was based on comparison with standards using thin layer or paper chromatography. The phosphorus-to-ester ratio determined on the lecithins of the species examined gave an average value of 1 : 1·88 (range 1 : 1·71–1 : 2·10).

The results obtained in this work confirm those of Turner[8], Isaksson[9] and Nakayama and Blomstrand[10] that the major phospholipid of bile is lecithin. The detection of phosphatidyl ethanolamine and lysolecithin in human bile also confirms the work of others[8–10].

Isaksson[9], in his work on bile from man, dog and ox, concluded that lecithin was the only phospholipid present. The results obtained in this work indicate a more complex phospholipid pattern. The detection of phosphatidyl inositol and sphingomyelin by Nakayama and Blomstrand[10] in human bile could not be confirmed.

Table 1. Average Percentage Distribution of Bile Phospholipids

Compound	Man (10*)	Rat (4)	Dog (3)	Ox (2)
Phosphatidyl ethanolamine	3·6	6·0	*N.D.*†	1·1
Phosphatidyl serine	*N.D.*	*N.D.*	1·8	1·2
Phosphatidyl inositol	*N.D.*	Trace	Trace	Trace
Lecithin	92·2	92·2	95·8	89·8
Sphingomyelin	*N.D.*	Trace	2·3	*N.D.*
Lysolecithin	3·9	Trace	*N.D.*	7·8

* No. of animals.
† *N.D.* not detected.

The phosphorus-to-ester ratio of less than 1 : 2 obtained for lecithin is consistent with the reported presence of choline plasmalogens[10]. Attempts at a similar elucidation of the phosphatidyl ethanolamine and phosphatidyl serine proved unsuccessful, due to bile pigments in these fractions interfering with the hydroxylamine reaction in determining ester linkages.

The results presented indicate that there are species differences in the phospholipid composition of bile. In all species examined, lecithin was the major phospholipid amounting to 90 per cent or more of the total. It is difficult to assign any significance to the differences noted at this time. This project was supported by grant *AM*-07563-02 from the National Institutes of Health, U.S. Public Health Service.

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