turbid again on removing the ammonia by dialysis. A translucent emulsion of fraction I also becomes transparent when ammonia was added, but it soon became very turbid again, and on standing a white precipitate formed with a clear supernatant which became turbid on dialysis. The white precipitate contained inorganic phosphate and lipids.

SUMMARY

1. When a turbid water emulsion of inositol phosphatide fraction (Folch's fraction I) or phosphatidylserine (Folch's fraction III) from brain tissues is added to a solution of ethylenediaminetetra-acetate at pH 10, the solution quickly becomes transparent and remains so even after removal by dialysis of the reagents added. Such a phenomenon is not seen with phosphatidylethanolamine (Folch's fraction V) or with a brain-'lecithin' fraction.

2. The clear solutions become turbid on freezing and thawing, and on the addition of chloroform, ether or neutral salts.

3. Aqueous ammonia also has a clearing action at higher pH values, but the resulting clear solutions were far less stable than those obtained by the use of ethylenediaminetetra-acetate.

We wish to express our indebtedness to Mrs R. Irie and Miss M. Iwanaga, of the Division of Chemistry in this Institute, for the elementary analyses. This work was supported by a Grant for Fundamental Research obtained from the Ministry of Education.

REFERENCES

Amelung, D. & Böhm, P (1954). *Hoppe-Seyl. Z.* **298**, 199.
Folch, J. (1942). *J. biol. Chem.* **146**, 35.
Hawke, J. C. & Lea, C. H. (1953). *Biochem. J.* **54**, 479.
Kimura, Y. & Nagai, Y. (1960). *Biochem. J.* **77**, 3.
Nagai, Y. & Kimura, Y. (1958). *Nature, Lond.*, **181**, 1730.
Thierfelder, H. & Klenk, E. (1930). *Die Chemie der Cerebroside und Phosphatide*, pp. 177–8. Berlin: Springer Verlag.

Biochem. J. (1960) **77**, 3

Infrared Spectra of Brain Phosphatidylserine

BY Y. KIMURA AND Y. NAGAI
Department of Biochemical Cell Research, Institute for Infectious Diseases, University of Tokyo, Shiba, Minato-Ku, Tokyo, Japan

(*Received* 19 *October* 1959)

Infrared spectroscopy has been applied to many complex lipids, but the infrared spectrum of phosphatidylserine seems not to have been published. Marinetti, Erbland & Stotz (1958) mention that a sodium salt of brain phosphatidylserine has more absorption at 6·1 μ and less at 2·9 μ than has the free acid form.

MATERIALS AND METHODS

Phosphatidylserine. This was prepared as described by Kimura & Nagai (1960).

Metal-free phosphatidylserine. This was prepared according to the procedure of Folch (1948).

Monosodium phosphatidylserine. In this the atomic ratio Na:P was 1:1; it was prepared by the addition, with cooling and stirring, of the calculated amount of CO_2-free NaOH (0·01 N) to a suspension (2%, w/v) of the metal-free phosphatidylserine in CO_2-free water. The solution was then freeze-dried.

Disodium phosphatidylserine. In this the atomic ratio Na:P was 2:1 and it was prepared similarly.

Infrared spectroscopy. All infrared spectra were obtained by the KBr-disk technique. A Hitachi Model EPI-2 double-beam automatic-recording spectrophotometer (Hitachi Ltd., Tokyo, Japan) with an NaCl prism was used. The same infrared spectral patterns were obtained in a KBr disk, in chloroform solution and in Nujol film. When phosphatidylserine was dialysed against water for 9 days, its ash content was decreased and, in parallel with this, a similar spectral pattern to that of the metal-free form was obtained.

RESULTS AND DISCUSSION

The infrared spectra of the four forms of phosphatidylserine are shown in Fig. 1. All forms possess the following bands which can be correlated with specific molecular groups: 1750–1740 cm.$^{-1}$, C=O ester, stretching; 1475 cm.$^{-1}$ and 1375 cm.$^{-1}$, CH deformation; 1235 cm.$^{-1}$, P=O free, stretching; and 1050 cm.$^{-1}$, P—O—C stretching. Four absorption bands (1655 cm.$^{-1}$, 1525 cm.$^{-1}$, 1420 cm.$^{-1}$ and 1175 cm.$^{-1}$) are affected by the form of the material and thus must be attributed to the acidic (—CO_2H, —$\overset{\|}{P}$—OH) and basic (—NH_2) groups of phosphatidylserine.

The 1655 cm.$^{-1}$ absorption, a characteristic of the original phosphatidylserine, vanishes in the metal-free form but reappears in the mono- and di-sodium forms, although the bands become broader

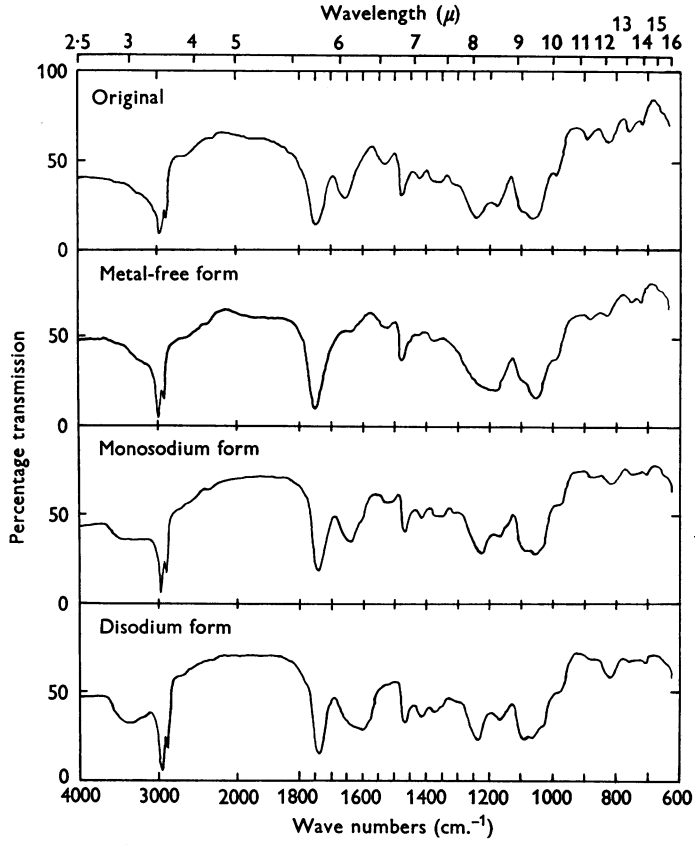

Fig. 1. Infrared-absorption spectra (KBr) of four different forms of phosphatidylserine.

and the absorption maxima shift to lower frequencies of 1645 and 1600 cm.$^{-1}$ respectively. The 1655 cm.$^{-1}$ band is always accompanied by a weaker band at 1420 cm.$^{-1}$ and the two bands are therefore assumed to be the symmetrical and anti-symmetrical vibrations of the ionized carboxyl group (Colthup, 1950; Fuson, Josien & Powell, 1952; Sutherland, 1952; Koegel, McCallum, Greenstein, Winitz & Birnbaum, 1957; Bellamy, 1958). Presumably the un-ionized carboxyl group absorbs at 1750 cm.$^{-1}$, since this band is more intense in the metal-free form.

In the original and monosodium forms, the band of the ionized carboxyl group is outside the range of 1610–1550 cm.$^{-1}$ which it occupies in simpler carboxylic and amino acids (Colthup, 1950; Fuson *et al.* 1952; Koegel *et al.* 1957; Bellamy, 1958). The reason for this is not known, but it is not surprising in view of the more complex structure of phosphatidylserine.

SUMMARY

1. Infrared spectra are reported for four forms of phosphatidylserine, namely the original preparations, metal-free, mono- and di-sodium derivatives, the last two denoting that the atomic ratios Na:P in the molecules are 1:1 and 2:1 respectively.

The authors wish to express their gratitude to Dr H. Watanabe, Department of Chemistry, for his helpful advice. The authors are also very much indebted to Professor T. Yamakawa, Department of Chemistry, for his useful advice.

REFERENCES

Bellamy, L. J. (1958). *The Infrared Spectra of Complex Molecules*, 2nd ed., pp. 174–5, 240–2. London: Methuen and Co. Ltd.

Colthup, N. B. (1950). *J. opt. Soc. Amer.* **40**, 397.

Folch, J. (1948). *J. biol. Chem.* **174**, 439.

Fuson, N., Josien, M. & Powell, R. L. (1952). *J. Amer. chem. Soc.* **74**, 1.

Kimura, Y. & Nagai, Y. (1960). *Biochem. J.* **77**, 1.

Koegel, R. J., McCallum, R. A., Greenstein, J. P., Winitz, M. & Birnbaum, S. M. (1957). *Ann. N.Y. Acad. Sci.* **69**, 94.

Marinetti, G. V., Erbland, J. & Stotz, E. (1958). *Biochim. biophys. Acta*, **30**, 41.

Sutherland, G. B. B. M. (1952). *Advanc. Protein Chem.* **7**, 291.