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This Week's Citation Classic

Dawson R M C. A hydrolytic procedure for the identification and estimation of individual phospholipids in biological samples. *Biochem. J.* **75**:45-53, 1960.

The author describes a simplified, single-assay method using paper chromatography after mild alkaline hydrolysis for the rapid quantitative determination of the individual phospholipids in a small sample of biological material. [The *SCI*® indicates that this paper has been cited 225 times since 1961.]

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"It is surprising that this paper should have proved of such value since it reports modifications, albeit substantial, of a method for investigating phospholipid structure published in principle six years earlier.[1] At that time there existed no method of resolving the individual phospholipids present in a small sample of tissue. Although the solvent fractionation techniques evolved by the pioneering work of J. Folch had indicated the complex nature of the kephalins, this procedure could not be scaled down to yield a worthwhile separation when only a few milligrams of phospholipid phosphorus was available.

"We began to think of the possibility of isolating identifiable fragments of the phospholipids after they had been subjected to various degradative procedures. The final breakthrough came as a result of wasting time. In my laboratory in the Biochemistry Department at Oxford, I was, at that period, helped by an attractive female assistant who acted as a magnet for the other young scientists around. Consequently, at midmorning an extensive break was taken, with coffee drinking and the exchange of much gossip and scientific chit-chat. During one such session, we discussed the observations of the late Professor Baer and Dr. Kates showing that the deacylation of synthetic phosphatidylcholine by methanolic alkali was complete long before any liberation of choline. Would this work for the other deacylated phosphoglycerides and produce recognizable fragments? Experiments quickly showed this was so and that the deacylated parent structure was left essentially intact producing 'glycerylphosphoryl' derivatives, which could be adequately separated by paperchromatographic techniques.

"This principle proved extremely useful for accurately measuring the specific radioactivities of the diacylphosphoglycerides. Eventually, it was developed into a complete analytical technique, and extended so that those phospholipids not rendered water-soluble by the alkali treatment could be examined. This involved much trial and error research, although I believe the principle of using a simple, low molecular weight ester for rapid neutralization of the alkaline digests before examining the deacylated plasmalogens was novel. By far the most effective of these esters was ethylformate. How this came into our chemical stores is a mystery, but it is perhaps not without significance that ethylformate is used in the manufacture of artificial rum and that Christmas parties were an annual event in the laboratory.

"After five years it was decided to write the accumulated experience in collaboration with my colleagues Norma Hemington and James Davenport, who had played a substantial part in the development of the method. Subsequently, the technique has been modified by many workers, but it is gratifying to see many papers appearing even today in which unidentified phospholipids and glycolipids resolved as spots by T.L.C. have had their structures investigated using the same principle of successive selective degradations."

REFERENCE

1. **Dawson R M C.** The measurement of 32p labeling of individual kephalins and lecithin in a small sample of tissue. *Biochim. Biophys. Acta* **14**:374-9, 1954.