cause of encephalomalacia, one would expect the disease to occur in the hen as well as in the young chicken. However, since the proportion of "22:5" in the cerebella of hens is much less than that of the cerebella of chicks fed linoleic acid, it is possible that the peroxidation of this fatty acid or similar fatty acids is responsible for the development of encephalomalacia. Since dietary linoleic acid increased "20:3" in the cerebella we assume that this "20:3" belongs to the linoleic family in regard to the position of doubled bonds. Such a 20:3 acid has been found in liver tissue (27) and probably exists in other tissues as well.

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• *Letter to the Editor*

The Use of Iodine Vapor as a General Detecting Agent in the Thin Layer Chromatography of Lipids[1]

A SULPHURIC ACID SPRAY, followed by heating, is commonly recommended as a detection system of general utility in thin-layer chromatography. Although highly sensitive and capable of giving additional information through the development of colors peculiar to certain compounds or lipid classes, the corrosive nature of the spray makes the plates difficult to handle and not readily photographed when a permanent record is desired.

In this laboratory, iodine vapor has found favor as a general detecting agent for the following reasons: it is sensitive; corrosive residues do not remain on the plates; the plates can be photographed readily in color or in black and white, and additional information can be gained from the behavior of iodine in the spots.

Limits of sensitivity were established with the following compounds: inositol phosphate, glycerol phosphate, tripalmitin, triolein, egg lecithin, dipalmitoyl lecithin, β-sitosterol, and a corn oil sterol fraction. The compounds were spotted on plates using a concentration range of 50 to 1 μg. The plates were set in covered chromatography jars containing iodine crystals in a glass dish. An exposure time of 60 min was found to be sufficient when the temperature in the laboratory was between 70 and 80F; color formation starts within 5 sec of exposure. The plates were then removed and the location of the colored spots fixed by scraping around their periphery with a sharp pencil. All the above compounds could be detected at the 1 μg level. When 1 μg spots of each were placed on the origin and the plate developed in hexane:ether:acetic acid 90:10:1, only the sugar phosphates could not be located readily at the 1 μg level.

The observation that the brown iodine color left certain spots more readily than others suggested the possibility of classifying compounds on this basis. The data obtained with 5, 10, and 20 μg spots exposed to iodine vapor for 40 min are summarized in Table I.

TABLE I

Compound	Color after removal from vapor				
	1 min	1 hr	2 hr	4 hr	6 hr
Glycerol	M-Y[a]	V-P-Y	V-P-Y	V-P-Y	V-P-Y
Glycerol phosphate	M-Y[a]	P-Y	V-P-Y	V-P-Y	V-P-Y
Inositol	M-B[a]	V-P-Y	C	C	C
Inositol phosphate	P-B[a]	V-P-Y	C	C	C
Tripalmitin	Y	P-Y	P-Y	V-P-Y	V-P-Y
Triolein	V-D-B	D-B	D-B	D-B	D-B
Trilinolein	V-D-B	V-D-B	V-D-B	V-D-B	V-D-B
66% Tripalmitin 33% triolein	D-B	B-Y	M-B-Y	S-Y	S-Y
33% Tripalmitin 66% Triolein	D-B	B	B	B	B
66% Tripalmitin 33% Trilinolein	D-B	D-B	D-B	D-B	D-B
Dipalmitoyl kephalin	M-Y	M-Y	S-Y	S-Y	S-Y
Dipalmitoyl lecithin	D-Y	M-Y	M-Y	M-Y	M-Y
Egg lecithin	V-D-B	D-B	D-B	D-B	D-B
Lyso egg lecithin	M-Y	M-Y	B	B	B
β-sitosterol	S-Y	S-Y	S-B	S-B	S-B
Corn oil sterol fraction	V-D-B	V-D-B	V-D-B	V-D-B	V-D-B

B = brown; Y = yellow; D = dark; S = strong; M = medium; P = pale; V = very pale; C = colorless.
[a] Spot had pale white center surrounded by brown ring.

Sugars and sugar phosphates tended to lose their color quickly, and saturated fats were observed to lose color more rapidly than unsaturated fats. This characteristic served to differentiate between the triglyceride mixtures. The well-known ability of lecithin to bind iodine was demonstrated by the retention of color by both the saturated and unsaturated lecithins. At no time, however, was the dipalmitoyl lecithin as deeply colored as the egg lecithin. As expected, lyso egg lecithin behaved in an intermediate manner. Dipalmitoyl kephalin resembled tripalmitin rather than dipalmitoyl lecithin. The plant sterols tested developed a permanent dark brown color. The β-sitosterol spots, however, did not become brown until 2 hr after removal from the iodine vapor.

Bromine vapor was tried as a possible substitute for iodine vapor and proved unsatisfactory. A 4% aqueous solution of Thiodene, as a spray, was also tested as a supplement to iodine vapor. It decreased rather than increased the sensitivity and is not recommended as an adjunct to iodine vapor.

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