(concentration test, aspartate transaminase content), liver function (alanine transaminase content, alkaline phosphatase content), blood (Hb, haematocrit, haemolysis, white blood cells, coagulation, platelet adhesiveness), post-mortem examinations and body fat composition, no indications were obtained that a 12-week feeding period with high amounts of hydrogenated fats of extremely divergent compositions induces abnormal changes in rats.

REFERENCES

de Iongh, H. & Beerthuis, R. K. (1965). *Bibliotheca Nutritio Dieta*, Fasc. no. 7, p. 137.
Gottenbos, J. J. & Thomasson, H. J. (1965). *Bibliotheca Nutritio Dieta*, Fasc. no. 7, p. 110.
Hellem, A. J., Ödegaard, A. E. & Skålhegg, B. A. (1963). *Thromb. Diath. haemorrh.* **10**, 61.
La Due, J. S., Wróblewski, F. & Karmen, A. (1954). *Science, N.Y.*, **120**, 497.
Thomasson, H. J. & Gottenbos, J. J. (1957). *Verh. K. vlaam. Acad. Geneesk. Belg.* **19**, 369.
Wróblewski, F. & La Due, J. S. (1956). *Proc. Soc. exp. Biol. Med.* **91**, 569.

Heat–induced changes during processing and use of edible fats

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Many recorded studies have combined to confirm that oxidized, heated fats, when given to test animals, produce toxic signs ranging from growth retardation to death. There is general agreement that the toxic components are concentrated in the fraction of the fat which does not form a urea adduct, but there remains considerable uncertainty of the chemical nature of the toxic substances. That this should be so is in no way surprising, since the chemical processes involved are extraordinarily complex, and the problems of separation and isolation of the various chemical entities almost unsurmountable.

These chemical processes may be generally described as cyclization and polymerization, and the ease with which they proceed increases with the degree of unsaturation of the fat. Fats which are commonly used for edible purposes do not readily undergo these reactions; and a criticism which may be made of a number of recorded nutritional and toxicological studies is that to provide sufficient material for feeding experiments oils have been treated under drastic conditions far removed from those encountered in normal use, or that more highly unsaturated oils such as linseed and tung oil, not commonly regarded as edible oils, have been used.

Our interest lay in the extent to which the reactions of cyclization and polymerization occurred during the refining of glyceride oils for edible purposes. Subsequently our investigations were extended to oils used in deep frying, since this operation is probably the most drastic treatment to which an edible oil is subjected. These latter studies are in their early stages, and represent no more than a preliminary survey to provide the basis for a systematic investigation of such factors as the influence of frying conditions on fat deterioration, and the extent to which changes in the bulk fat are reflected in the fat content of fried foods.

Our work began with cottonseed oil (CSO), chosen because it combines a high degree of unsaturation with good oxidative stability, and for this reason is used in

Table 1. *Composition of urea non-adduct (expressed as % of whole oil)*

	Refined deodorized CSO	Fried GNO	Fried CSO(1)	Fried CSO(2)	Fried CSO(3)	Oil extracted from batter
Total UNA	1·3	8·6	7·4	10·1	5·0	5·0
Spot A	0·5	1·2	1·4	1·7	1·5	0·4
Spot B	0·2	0·8	0·6	0·7	0·9	0·6
Spot C	0·5	4·6	4·0	6·4	2·2	2·9
Spots C_1+C_{1A}	0·2	0·7	0·9	1·8	0·3	0·8
Spots C_2-C_4	0·3	3·0	2·3	3·3	1·5	1·7
% FFA in whole oil	0·1	0·5	0·7	0·8	0·4	0·3

FFA, free fatty acids; UNA, urea non-adduct; CSO, cottonseed oil; GNO, groundnut oil. The batter referred to in column 7 was removed from fish fried in CSO (3).

very large quantities in a variety of edible products, as well as for domestic and commercial deep frying. The primary concentration of the polymer fraction was achieved by urea segregation after saponification of the oil. The urea non-adduct (UNA) in the form of methyl esters was resolved by thin-layer chromatography on silica gel into three zones, termed spots A, B and C in order of decreasing R_F value (Pl. 1). The material comprising these zones was isolated by solvent extraction of the appropriate area of silica, and the most diffuse zone C rechromatographed on silica gel (Pl. 2).

Qualitatively similar chromatograms were obtained from the UNA from refined, deodorized CSO and from oils used in deep frying.

The weight distribution between zones was determined by chromatography on thick (1 mm) layers of silica gel, and weighing of the isolated zones; approximately 250 mg of UNA was chromatographed on four plates, and corresponding zones were combined for weighing. Shown in Table 1 are results for a typical sample of refined and deodorized CSO, and four randomly selected samples of oil used for deep frying. For reasons which will emerge later, combined figures are quoted for spots C_1+C_{1A}, and spots C_2-C_4.

The R_F value of spot A corresponded to that for normal fatty acid methyl ester. The gas–liquid chromatogram (polyester column) of spot A from deodorized CSO showed the presence of only palmitate, stearate, oleate and linoleate; that of spot A from CSO(3) showed peaks with R_t corresponding to palmitate and linoleate, as well as a broad peak presumed due to several unresolved components, with R_t between that of palmitate and linoleate. The identity of these components has not yet been established.

Spot B appears to be due to dimer. The R_F value corresponds to that of a synthetic dimer of the 'dehydro dimer' type (see Fig. 1) formed by dimerization of myristate, and also of the dimer fraction obtained after thermal polymerization of methyl linoleate (Fig. 2). The molecular weight of spot B from deodorized CSO was 650 (C_{18} dimer=*c.* 596). The ultraviolet spectrum of spot B showed a maximum in the 230 nm region; calculated conjugated diene contents for the oil samples referred to earlier (Table 1) are shown in Table 2.

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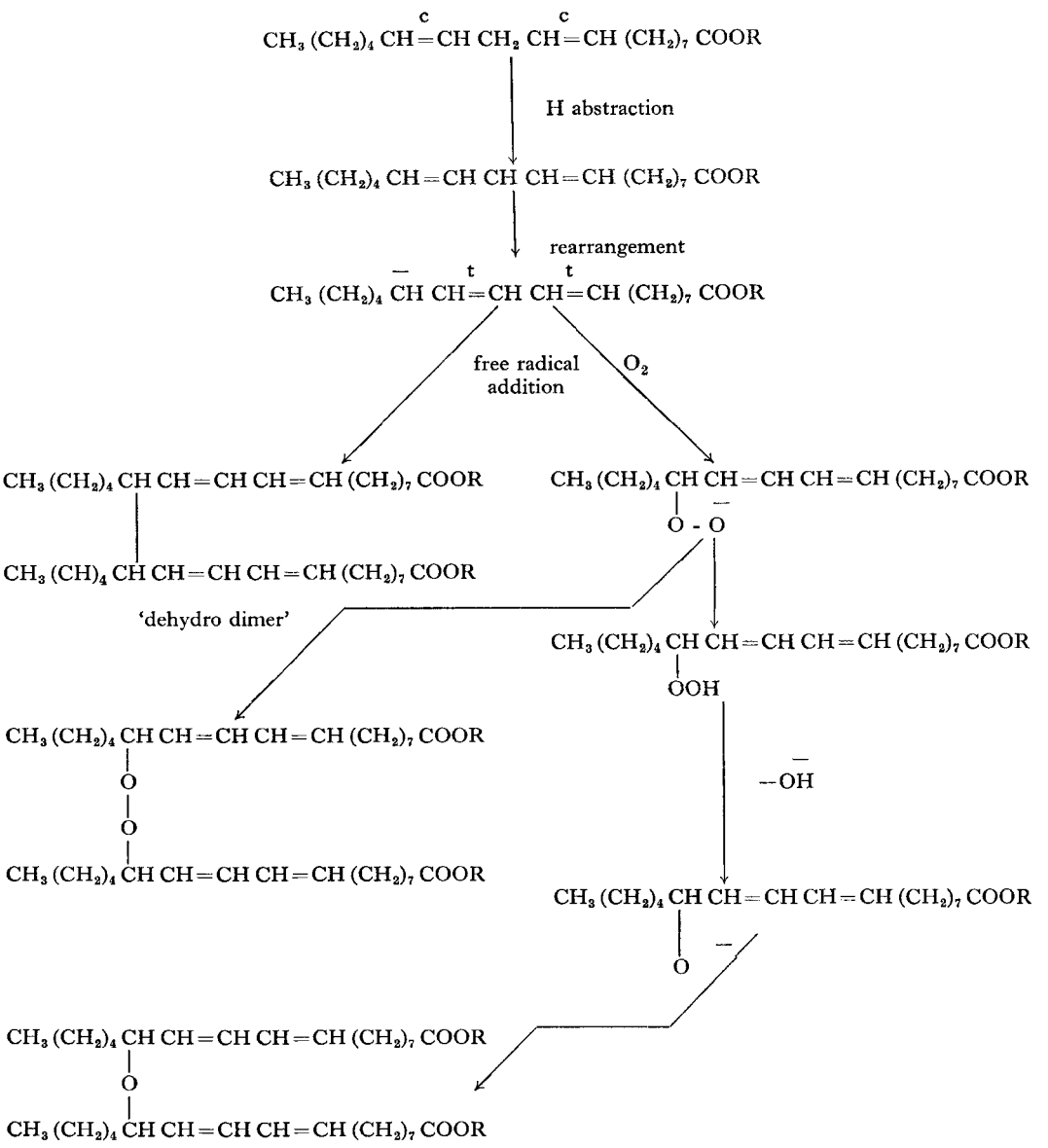

Fig. 1. Dimerization of linoleate by free radical mechanisms.

The major features of note in the infrared spectrums (Fig. 3) were the absence of absorption in the hydroxyl region (*c.* 3μm), and the appearance of bands at 10·3 μm (isolated *trans* double bond), 10·51 μm (*cis-trans* conjugation), and 10·12 μm (*trans-trans* conjugation) the latter being considerably enhanced in the spectrum of spot B from the fried CSO(2). If the hydroxyl region is excluded, the similarity of this spectrum to that of *trans-trans* methyl linoleate hydroperoxide reported by Banks, Fazakerley, Keay & Smith (1961) is most striking.

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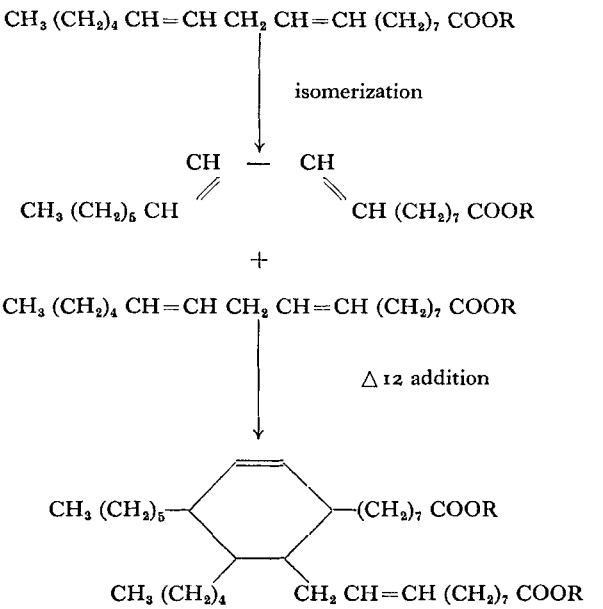

Fig. 2. Dimerization of linoleate by Diels–Alder addition.

The molecular weight (665) of the combined spots C_1 and C_{1A} again suggested dimer. Their individual u.v. and i.r. spectrums were almost identical, and very similar to the spectrums of spot B; conjugation was predominantly *trans-trans*, and the overall content less than spot B (Table 2).

By contrast, spots C_2, C_{2A}, C_3 and C_4 were all hydroxylated. On the evidence of molecular weight measurements, i.r. spectrums, and R_F values on TLC, C_2 and C_{2A} appear to be, respectively, monohydroxy stearate and monohydroxy oleate. The i.r. spectrum of C_3 was similar to that of a sterol ester; the presence of a hydroxyl group, suggested by the R_F value, was confirmed in the i.r. spectrum. These facts are explained on the assumption that C_3 is the sterol ester of a hydroxy acid. Spot C_4, clearly a mixture of several components, and as yet only cursorily examined, had a similar R_F value to dihydroxystearate.

Our interest at the present time centres on the nature of the spots of high molecular weight, B, C_1 and C_{1A}. Whatever may be the mechanism of polymerization, a natural oil would be expected to give rise to a mixture of dimers. Clear evidence

Table 2. *Conjugated diene contents* (%) *of spots B, and* C_1+C_{1A}

	Refined deodorized CSO	Fried GNO	Fried CSO(2)	Fried CSO(3)	Oil extracted from batter
Spot B	19·9	28·2	86·4	39·3	43·2
Spots C_1+C_{1A}	8·6	9·7	39·8	16·9	19·5

CSO, cottonseed oil; GNO, groundnut oil. The batter referred to in column 6 was removed from fish fried in CSO(3).

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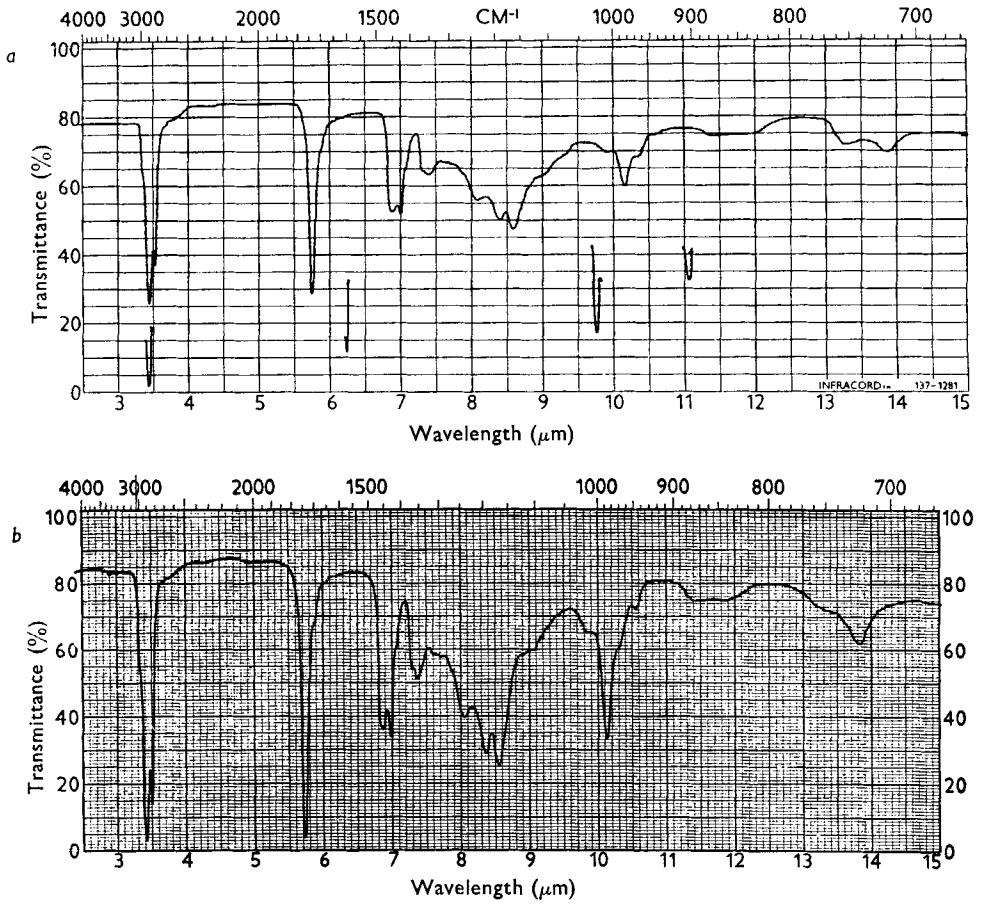

Fig. 3. Infrared spectra of spot B. *a*, spot B from UNA of deodorized CSO; *b*, spot B from UNA of CSO(2).

that spot B from deodorized CSO was a complex mixture came from the chromatogram (Pl. 1), and more especially from re-examination of spot B by thin-layer chromatography on silica impregnated with silver nitrate, an absorbent which resolves primarily according to degree of unsaturation, but to some extent also on position and configuration of double bonds. The chromatogram showed continuous streaking from base-line to solvent front, but at least six dark zones were discernible. After hydrogenation in the presence of Adams platinum oxide catalyst, the absorptions in the 10·0–10·5 μm region of the i.r. spectrum disappeared, and chromatography on silica–silver nitrate showed only a single spot at about R_F 0·5.

The presence of *trans-trans* diene conjugation, and particularly the high levels found in the frying oil samples can best be explained on the assumption that dimerization occurs by an oxidative free radical pathway. Fig. 1 depicts a simplified scheme for the formation of three dimer types from linoleate. As there is no evidence in the i.r. spectrums for the presence of oxygen links, the likelihood is that spot B contained dimers of the carbon–carbon linked 'dehydro dimer' type. A similar explanation can

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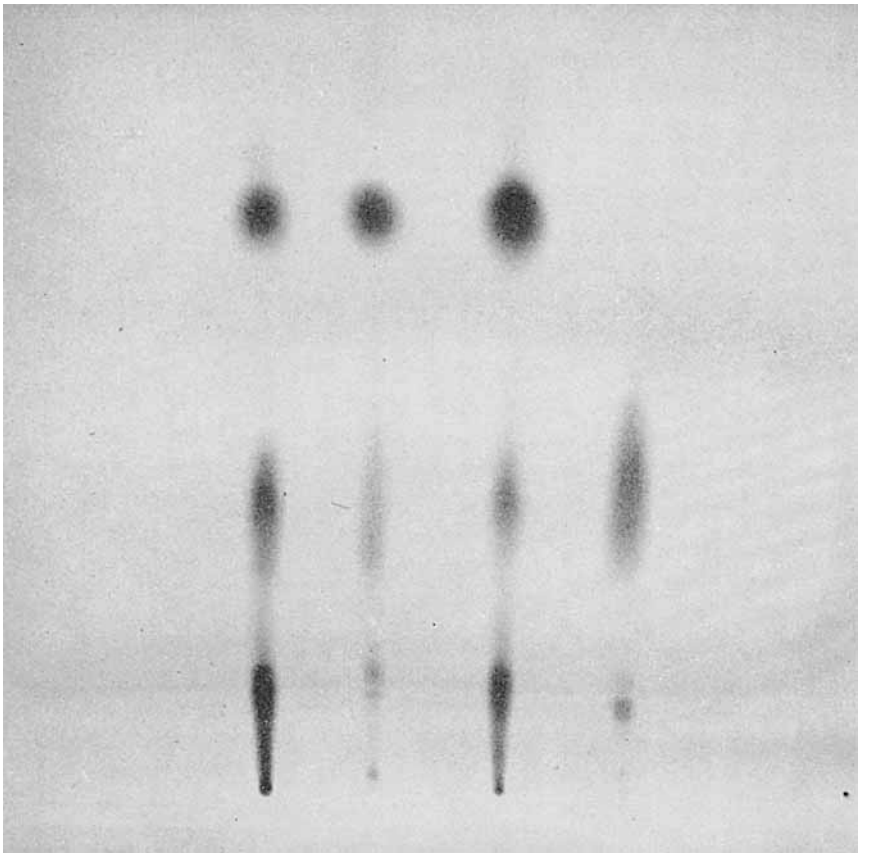

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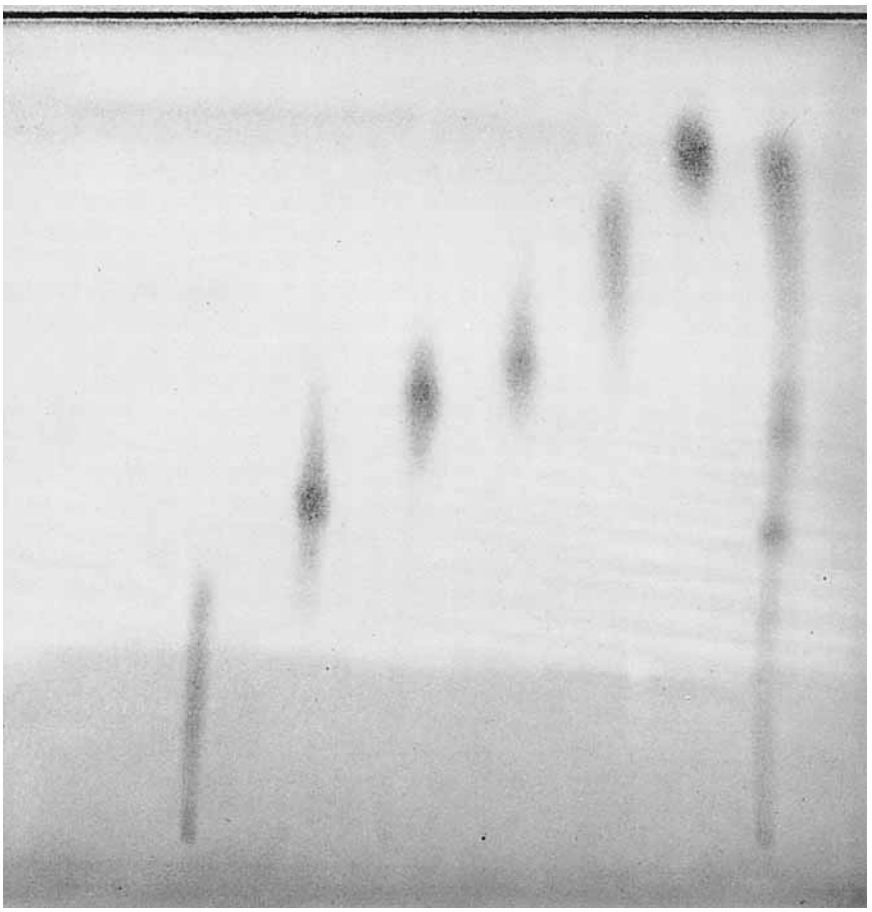

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be advanced for the presence of *trans-trans* conjugation in spots C_1 and C_{1A}, although this mechanism does not afford a reason for the difference in R_F value between spots B and C_1, C_{1A}.

The alternative explanation, that the dimeric material is formed by a Diels–Alder addition (Fig. 2) appears to be excluded on several counts. If dimerization occurred by this mechanism, absorption due to conjugated dienes would not be expected, and indeed the dimeric fraction from co-polymerization of 9, 11 and 9, 12 methyl octadecadienoate at 300° in an inert atmosphere (which might be expected to cause polymerization by a Diels–Alder mechanism) showed only a single band for *trans*-absorption at 10·3 μm. Moreover, we were unable to aromatize spot B from deodorized CSO either by the bromination–dehydrobromination procedure of Clingman, Rivett & Sutton (1954) or by heating to 275° with palladium-on-charcoal catalyst, a method used by Paschke, Peterson & Wheeler (1964) to demonstrate the presence of a cyclohexene structure in a Diels–Alder type dimer.

Finally, chromatograms of the UNA of crude groundnut oil, and groundnut oil in the various stages of refining, all showed the presence of spot B. The maximum temperature of 180° reached in the refining sequence is lower than that required for Diels–Alder addition of fatty acids, and dimer formation is much more likely to have occurred by an oxidative mechanism.

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REFERENCES

Banks, A., Fazakerley, S., Keay, J. N. & Smith, J. G. M. (1961). *J. Sci. Fd Agric.* **12**, 724.
Clingman, A. L., Rivett, D. E. A. & Sutton, D. A. (1954). *J. chem. Soc.* p. 1088.
Paschke, R. F., Peterson, L. E. & Wheeler, D. H. (1964). *J. Am. Oil Chem. Soc.* **41**, 723.

EXPLANATION OF PLATES

Pl. 1. Thin-layer chromatogram of spot B. (1) UNA of fried cottonseed oil. (2) Mixture of methyl linoleate and thermal dimer of methyl linoleate. (3) UNA of refined and deodorized cottonseed oil. (4) Synthetic 'dehydro dimer' from methyl myristate.

Adsorbent: silica gel G (Merck). Developing solvent: benzene, chloroform (1:1). Development in 'S' tank; chromatogram overrun for 15 min.

Pl. 2. Thin-layer chromatogram of spot C. (1–6) Chromatograms of isolated components of spot C, spots C_4, C_3, C_{2A}, C_2, C_{1A}, C_1. (7) Spot C

Adsorbent: silica gel G (Merck). Developing solvent: benzene/di-isopropyl ether (70:30). Development in 'S' tank; chromatogram overrun for 15 min.

The effect of lipid peroxides on the biochemical constituents of the cell

By P. J. O'Brien and A. C. Frazer, *Department of Medical Biochemistry and Pharmacology, University of Birmingham*

Many food lipids can oxidize rapidly in the presence of oxygen or peroxides and catalysts, such as ionizing radiations, light, lipoxidases, or metals. Antioxidants in