causes some loss of the phosphatide phosphorus, and chromatograms treated accordingly are therefore unsuitable for quantitative analysis.

(2). Immersing the chromatograms in an aqueous solution of an ultraviolet absorbing organic compound, such as 2,4-dinitrophenylhydrazine, p-nitrophenylhydrazine, and phenylhydrazine, (in 0.15 % solutions in $2N$ HCl). These become adsorbed on the lipid spots and increase their ultraviolet absorption, within 1/2 h, 1 h, and overnight respectively. The chromatograms are then successively washed with $2N$ HCl (10 min), running tap water (1 min), and finally dried. The adsorption treatment causes no loss of the phosphatide phosphorus, and the chromatograms are therefore suitable for purposes of quantitative analysis.

The dry chromatograms are imposed on a Kodak document paper (Duostat Rapid Reflex 23) and pressed between two thin plates of glass. Ultraviolet light (from a low pressure mercury resonance tube "Hanovia Chromatolite") is shone through for 5–10 sec, at a distance of 10 cm. It is found necessary to lower the fore-end of this lamp by 2 cm to shine a light of uniform intensity along the chromatograms. The prints shown in Fig. 1 are typical. The total lipid phosphorus load is indicated on each chromatogram after analysis of an identical but untreated chromatogram in the case of the first treatment, and a duplicate in the case of the second.

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Relative response of fatty acid methyl esters on the flame ionization detector

Considerable discussion has dealt with the relative response of the flame ionization detector for different organic substances. It was mentioned first by McWilliam and Dewar[1] that for hydrocarbons, the relative molar responses seem to be directly proportional to the carbon number; detailed data on this subject were presented at the 1961 International Gas Chromatography Symposium, in East Lansing, Mich.[2]. However, only very few values are available regarding substituted organic compounds[3].

Farquhar *et al.*[4] reported in 1959 that when a beta-ray (Argon) ionization detector is used, for higher fatty acid methyl esters, the peak area per cent can be taken with good approximation as concentration by weight of the individual components. We could not, however, find any published data on the relative response of a flame ionization detector for fatty acid methyl esters. Therefore, we carried out some inves-

tigations with two standard mixtures of known concentration obtained from the Applied Science Laboratories, Inc., University Park, Pa. The samples were analyzed under isothermal conditions, at 190°, on a 60 m long open tubular (Golay) column with 0.50 mm internal diameter, coated on the inside wall with butanediol succinate liquid phase: 0.1 μl liquid samples were injected directly into the carrier gas using Hamilton syringes of 0.5 μl capacity and the entire gas flow (without any split) was conducted into the column. The peak area values were evaluated with a commercial integrator.

As shown in columns 2–4 of Table I, the agreement between nominal concentration and peak area per cent is very good particularly if one keeps in mind that the samples also contained some impurities originating in the "pure" fatty acid methyl esters used for sample preparation. Our results demonstrate that with the flame

TABLE I

Components methyl esters of:	Concentration wt-%	Peak area %	Deviation %	Response per equal weight[a]	Relative molar response[a]	Calculated relative molar response[b]	Relative deviation %
Mixture I							
Caprylic acid	1.56	1.53	—0.03	96.8	56.6	58.0	—2.3
Capric acid	3.00	3.07	+0.07	101.0	69.6	68.5	+1.6
Lauric acid	5.96	6.01	+0.05	99.5	79.0	79.0	—
Myristic acid	11.96	11.66	—0.30	96.2	86.2	89.5	—3.6
Palmitic acid	19.42	19.68	+0.26	100.0	100.0	100.0	—
Stearic acid	24.91	25.21	+0.30	100.0	110.3	110.5	—0.2
Arachidic acid	33.19	32.84	—1.16	97.6	117.9	121.0	—2.5
Mixture II							
Myristic acid	11.83	12.23	—0.40	100.5	89.9	89.5	+0.4
Palmitic acid	23.62	23.59	—0.03	100.0	100.0	100.0	—
Palmitoleic acid	6.84	6.85	+0.01	100.3	101.9	100.0	+1.9
Stearic acid	13.09	13.43	+0.34	102.7	113.4	110.5	+2.6
Oleic acid	44.62	43.90	—0.72	98.5	107.9	110.5	—2.3

[a] Relative to methyl palmitate (= 100).
[b] Calculated from equation (1).

ionization detector, the relative peak area values can be taken as concentration by weight or in other words, that the relative response per equal weight of the detector for these substances is very close to unity (the fifth column of the table). It can also be seen that the difference between saturated and unsaturated fatty acids in this respect is very small.

Since in our previous work[2,3] we demonstrated that the relative *molar* response of various homologous series is linearly proportional to the carbon number of the molecules, we also calculated these values for the fatty acid methyl esters (sixth column of the table). For this calculation, the molar response of methyl palmitate was arbitrary taken as 100. The values obtained are plotted in the Fig. 1. It can be seen that these points form with good approximation a straight line which was obtained by connecting the points of methyl laurate and palmitate from the analysis
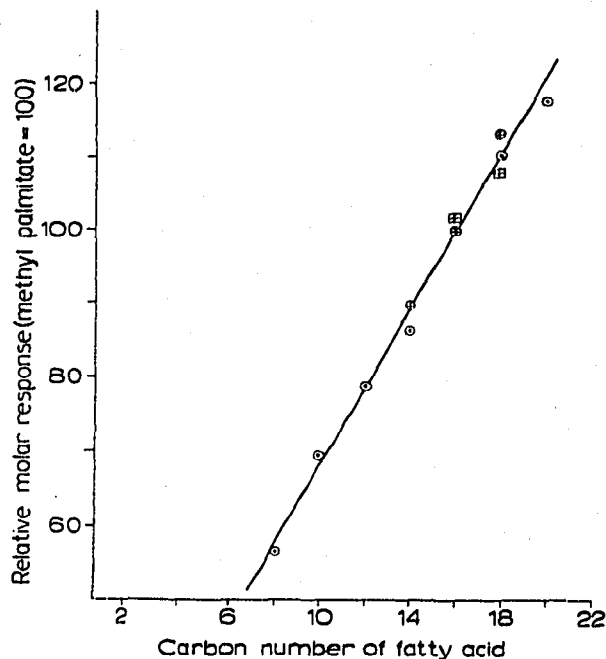
Fig. 1. Relative molar response of fatty acid methyl esters *vs.* carbon number of the molecules. ☉ = Mixture No. I; ⊕ = Mixture No. II (saturates); ⊞ = Mixture No. II (unsaturates).

of the first mixture; this straight line corresponds—in the given system—to the following equation:

$$R_{Mol} = 5.25\ C_A + 16 \tag{1}$$

where R_{Mol} is the relative molar response and C_A the carbon number of the respective fatty acids; R_{Mol} for methyl palmitate is equal to 100. The seventh column of the table gives the relative molar responses calculated from this equation and the eighth column, the relative deviation of the actual values from these data. As a conclusion, one can say that the relative molar response of the higher fatty acid methyl esters follow the same relationship as was demonstrated previously for other homologous series: it is linearly proportional to the carbon number.

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