This was shown by chromatography of the triethylamine salts of phosphatidic acid on silicic acid-impregnated glass-fibre paper, with the solvent system chloroform-methanol-0.73M-ammonia soln. (200:45:6, by vol.). When the phosphate group was removed with L- α -phosphatidate phosphohydrolase (EC 3.1.3.4) (Weiss, Kennedy & Kiyasu, 1960), diglyceride accounted for 85% of the phosphatidate hydrolysed. The remainder was monoglyceride (Hill, Husbands & Lands, 1968). A twofold purification of the enzyme was achieved by ultrasonic treatment in 0.01% deoxycholate. Further purification was not successful. The apparent K_m for L-glycerol 3-phosphate was $50\mu\text{M}$. This is similar to the value calculated from the results of Tzur, Tal & Shapiro (1964) for rat liver, but is lower than the value for either the yeast enzyme (Kuhn & Lynen, 1965) or the *E. coli* enzyme (Ailhaud & Vagelos, 1966).

The enzyme can be protected from inhibition by the thiol-binding agent *N*-ethylmaleimide (NEM) by prior incubation with acyl-CoA. The amount of thiol group so protected was determined by using [^{14}C]NEM. When 42% protection of the enzyme activity was achieved by incubation of the enzyme with palmitoyl-CoA before the addition of NEM, the amount of thiol group so protected was determined to be 0.1 $\mu\text{mole/mg}$. of enzyme protein. This was calculated from the difference in binding of [^{14}C]NEM to the protected enzyme in the presence and absence of L-glycerol 3-phosphate. These thiol groups are essential for the activity of the enzyme. There relationship to the active site of the enzyme is very close; we were unable to separate the thiol groups from the enzyme activity.

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Gas-Liquid Chromatography of Mono-glycerides and Diglycerides

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Qualitative parameters defining the gas-liquid-chromatographic behaviour of trimethylsilyl (TMS) ethers of mono- and di-glycerides are largely lacking in the literature (see Kuksis, 1967). We therefore extended our studies of factors affecting the separation of triglycerides on SE-30 and QF-1 (Watts & Dils, 1968) to the TMS ethers of a wide range of mono- ($\text{C}_2\text{--C}_{18}$) and di-glycerides ($\text{C}_4\text{--C}_{36}$).

Both gas-liquid and thin-layer chromatography were used to determine completeness of silylation, and conditions that have been used (Tallent & Kleiman, 1968) were shown to yield partially silylated products.

Isothermal analysis showed that on SE-30 or QF-1, log(retention volume) was directly proportional to carbon-atom number and inversely proportional to absolute temperature. However, the TMS ethers of lower monoglycerides ($\text{C}_2\text{--C}_6$) and diglycerides ($\text{C}_4\text{--C}_{12}$) deviated from these relationships. Since no single temperature could be employed to analyse the full range of either mono- or diglyceride TMS ethers, temperature-programmed analysis was used. The temperature-programmed conditions that enabled triglycerides to be separated (Watts & Dils, 1968) gave good separation of both mono- and di-glyceride TMS ethers. Relative elution temperatures (T_{RE}) were therefore used to compare the chromatographic properties of different glyceride classes. We defined the carbon equivalent of a TMS group ($\Delta\text{C}_{\text{TMS}}$) as the difference in carbon-atom number (per TMS group) between a mono- or di-glyceride TMS ether and the triglyceride with the same T_{RE} under the same conditions of temperature programming. Though the TMS group is equivalent to about 5.3 carbon atoms, the apparent increase in molecular weight, as indicated by $\Delta\text{C}_{\text{TMS}}$, was found to be only 1.7–2.5 for monoglycerides, and varied from –2.0 to 4.8 for diglycerides. This indicates specific interactions between these solute molecules and column components.

To compare the behaviour of free diglycerides, we defined the carbon equivalent of hydroxyl group ($\Delta\text{C}_{\text{OH}}$) in an analogous manner to the definition of $\Delta\text{C}_{\text{TMS}}$. Values of $\Delta\text{C}_{\text{OH}}$ (3.1–4.4) showed that free and silylated diglycerides are eluted at the same T_{RE} . Thus the usefulness of silylation for diglycerides appears to be solely in minimizing adsorption on to column walls or supports (or both).

By using weight and molar correction factors, the composition of the TMS ethers of diglycerides

derived from egg and ox brain lecithins was determined.

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The Incorporation of [¹⁴C]Palmitate into Glycerides and Phospholipids of Liver Homogenates from Fed and Starved Rats

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The incorporation of labelled palmitate into glycerides and total phospholipids of rat liver homogenates was measured in the presence of optimum amounts of cofactors and albumin (Smith & Hübscher, 1966; Smith, Sedgwick, Brindley & Hübscher, 1967). Adult female rats, fed *ad lib.* or starved for 36–40 hr., were used in these experiments.

The ratio of labelled glycerides to labelled phospholipids changed significantly in starvation (0.82 in fed and 3.0 in starved animals). Over 80% of the label incorporated into total phospholipids was found in phosphatidate. When calculated per total liver, the incorporation of palmitate into glycerides was slightly increased in starvation, but the labelling of phosphatidate was decreased to 34% of the control value. The sum of the label in glycerides and phosphatidate decreased to 75% of the fed control.

The latter decrease might be explained by a decreased phosphatidate formation on subcellular particles. When a total particulate fraction prepared from liver homogenates was assayed as above, the rate of incorporation of palmitate into phosphatidate, which was the major lipid fraction labelled, was decreased in starved animals to 68% of the control value. This decrease was of the same order as that of total proteins (64% of fed control).

The increased glyceride/phospholipid ratio observed with homogenates from starved animals might be due to an increased phosphatidate phosphohydrolase activity. The phosphohydrolase participating in glyceride biosynthesis is known to be present in the particle-free supernatant (Smith *et al.* 1967). This enzyme was assayed by using membrane-bound biosynthetically produced phosphatidate as substrate. In starvation, the activity per total liver was increased to 190% and the specific activity to 320% of the corresponding values of fed control animals. As phosphatidate phosphohydrolase activity was found to be rate-limiting in homogenates, the increase of its activity in starva-

tion explains the higher amounts of palmitate incorporated into glycerides in spite of a decreased phosphatidate formation.

The increase of phosphatidate phosphohydrolase activity in starvation is probably related to the increased flux of free fatty acids into liver (Olivecrona, 1962) and increased glyceride content observed in livers of starved animals (Mayes, 1962). Our results do not explain the changes in fatty acid esterification in starvation found in liver slices (Vavrečka, Poledne & Petrásek, 1967) and perfused liver (Mayes & Felts, 1967).

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Extraction and Assay of the Fat-Mobilizing Substance from Urine of Normal Adult Human Subjects in Various Physiological States

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A fat-mobilizing substance (FMS) has been detected in urine of man and some other mammals during conditions of active fat catabolism (Chalmers, Kekwick, Pawan & Smith, 1958; Beaton, Szlavko & Stevenson, 1966). This material produces marked effects on fat metabolism, body weight and energy loss in certain animal species (Kekwick & Pawan, 1963, 1965) and in human subjects (Kekwick & Pawan, 1968). It has been shown to increase lipolysis in adipose tissue both *in vivo* and *in vitro* (Chalmers, Kekwick & Pawan, 1960; Cahill, Pawan & Chalmers, 1961). This communication reports results of a study of the relative activity of FMS extracted from urine of normal subjects.

Urine collections were obtained from the following groups: (1) 72 normal adults (48 men, 24 women; aged 18–72, mean age 38 years; all within 85–115% of ideal body weight) before, during, and after a 36 hr. fast; (2) 20 normal pregnant women (mean age 29 years) eating *ad lib.*, at different stages of pregnancy; (3) 18 athletic men (mean age 27 years) before and after periods of exercise (1000 yards swim or 3 mile run, in 25 min.). FMS was extracted from each urine specimen as outlined by Kekwick & Pawan (1967), special precautions being taken, to avoid loss from inactivation, and because of the