derived from egg and ox brain lecithins was determined.

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The Incorporation of [14 C]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

occasional presence of inhibitors and antagonists. The FMS extracts were assayed for activity by measuring the percentage increase in free fatty acids (method of Dole & Meinertz, 1960) and 'ketones' (method of Pawan, 1958) in the blood plasma of groups of mice, 6 hr. after an injection of 5% of the 24 hr. extract/mouse. Control plasma samples were simultaneously obtained from groups of litter-mate animals similarly injected with aq. 0.9% sodium chloride solution.

In normal individuals (group 1) FMS activity of the urine extracts increased progressively with time during the 36 hr. fast, and decreased relatively quickly when refeeding commenced. Urinary FMS contents after the 36 hr. fast appeared to be unrelated to age, up to age 40 years, but in general, older individuals above 40 years excreted less FMS in their urine. The urine of pregnant women eating *ad lib.* contained practically no FMS activity, regardless of duration of pregnancy. Strenuous physical exercise in men was associated with a relatively high output of urinary FMS. In all groups, however, there was a large inter-individual variation in activity of FMS in the urine.

The urine specimens from colleagues, students and other individuals are gratefully acknowledged.

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Metabolism of Vitamin D₃ in Man

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When treatment with vitamin D is stopped after a period of high dosage, the concentration of biologically assayable activity in plasma falls very slowly (Warkany, Guest & Grabill, 1942) with a half-life of up to six months (G. A. Lumb & S. W. Stanbury, unpublished work). It was surprising therefore when studies made after administration of isotopically labelled vitamin D revealed a serum

half-life measured in hours (26–54 hr.) (Thompson, Lewis & Booth, 1966; Avioli, Lee, McDonald, Lund & DeLuca, 1967). In the present study the period of measurement of serum half-life of vitamin D₃ (cholecalciferol) was extended beyond the three or four days investigated by these workers.

Results have been obtained from four subjects to date and in each the decay curve of lipid-soluble serum radioactivity is biphasic. The first phase, which occupies 3–4 days, has a half-life of up to 48 hr.; the second-phase half-life can be measured in weeks, and radioactivity could still be detected in serum after 3 months.

The tracer dose, 8 μ C (110 μ g.) of [4-¹⁴C]cholecalciferol or 18 μ C (216 μ g.) of [1,2-³H₂]cholecalciferol, was administered as an intravenous infusion after dispersal in 'Intralipid', a stabilized lipid emulsion. Serum samples were extracted and fractionated on silicic acid columns by using a modification of the method of Avioli *et al.* (1967). Two major fractions were obtained; one corresponded to cholecalciferol and the other contained more polar metabolites of the vitamin.

Radioactivity-time plots of these fractions showed that whereas labelled cholecalciferol decayed with a similar biphasic pattern to that of the total radioactivity, the radioactivity of the polar-metabolite fraction increased from zero, reached a maximum within 7–14 days and then decayed with a long half-life.

The proportion of radioactivity in the polar fraction exceeded that in the cholecalciferol after 3–11 days, and remained in excess during the decay phase.

These results indicate that a single tracer dose of vitamin D is retained in circulation, largely in the form of more polar metabolites, for much longer than was previously supposed. Work is now in progress to determine whether the biological activity is associated solely with vitamin D or also with the polar fraction, as reported by Avioli *et al.* (1967).

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