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.

Beaton, J. R., Szlavko, A. J. & Stevenson, J. A. F. (1966). *Canad. J. Physiol. Pharmacol.* **44**, 701.

Cahill, G. F., jun., Pawan, G. L. S. & Chalmers, T. M. (1961). *Endocrinology*, **69**, 648.

Chalmers, T. M., Pawan, G. L. S. & Kekwick, A. (1960). *Lancet*, **ii**, 6.

Chalmers, T. M., Kekwick, A., Pawan, G. L. S. & Smith, I. (1958). *Lancet*, **i**, 866.

Dole, V. P. & Meinertz, H. (1960). *J. biol. Chem.* **235**, 2595.

Kekwick, A. & Pawan, G. L. S. (1963). *Metabolism*, **12**, 222.

Kekwick, A. & Pawan, G. L. S. (1965). *Ann. N.Y. Acad. Sci.*, **131**, 519.

Kekwick, A. & Pawan, G. L. S. (1967). *Metabolism*, **16**, 787.

Kekwick, A. & Pawan, G. L. S. (1968). *Lancet*, **ii**, 198.

Pawan, G. L. S. (1958). *Biochem. J.* **68**, 33P.

Metabolism of Vitamin D₃ in Man

By E. BARBARA MAWER and S. W. STANBURY.
(Division of Metabolism, Department of Medicine,
University of Manchester)

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falls very slowly (Warkany, Guest & Grabill, 1942) with a half-life of up to six months (G. A. Lumb & S. W. Stanbury, unpublished work).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).

This work was supported by a grant from the Medical Research Council.

Avioli, L. V., Lee, S. W., McDonald, J. E., Lund, J. & DeLuca, H. F. (1967). *J. clin. Invest.* **46**, 983.

Thompson, G. R., Lewis, B. & Booth, C. C. (1966). *J. clin. Invest.* **45**, 94.

Warkany, J., Guest, G. M. & Grabill, F. J. (1942). *J. Lab. clin. Med.* **27**, 557.