two-thirds of the plasma cholesterol is esterified with fatty acids. The plasma free cholesterol is exchangeable with free cholesterol in many tissues, including erythrocytes, liver, intestinal wall, skin and arteries. Since the rate of equilibration between plasma and tissue cholesterol differs in different tissues, there are several exchangeable pools of cholesterol within the whole body. There is also a net transfer of cholesterol from the plasma into the liver, followed by excretion into the duodenum via the bile, either without metabolic change or after conversion into bile acids. The cholesterol excreted into the duodenum is partially reabsorbed, the unabsorbed fraction leaving the body in the faeces. Cholesterol in the plasma also exchanges with the pools of cholesterol from which steroid hormones are formed in the gonads and adrenal cortex. There is therefore a continual net loss of cholesterol from the plasma owing to faecal excretion and conversion into bile acids and steroid hormones. This loss is replaced by absorption of dietary cholesterol and by endogenous synthesis.

Cholesterol ingested in the food mixes with that excreted into the duodenum and is partially absorbed from the jejunum via the lymphatic system after incorporation into chylomicra, largely in esterified form. Chylomicron cholesterol is removed by the liver and hydrolysed enzymically to free cholesterol. The free cholesterol is then incorporated into lipoproteins and released into the circulation.

The plasma cholesterol of endogenous origin is formed mainly in the liver, but some is also contributed by other tissues, particularly the small intestine. In man, the newly formed cholesterol is incorporated into lipoprotein and released into the circulation, where it is esterified with long-chain fatty acids by a plasma enzyme (lecithin-cholesterol acyltransferase), which transfers the fatty acid residue on C-2 of the plasma lecithin to plasma free cholesterol. In keeping with this mode of synthesis, in human plasma the composition of the fatty acids of the cholesteryl esters in all lipoprotein fractions is similar to that of the fatty acids attached to C-2 of lecithin and is different from that of the cholesteryl esters of human liver. In rats, lecithin-cholesterol acyltransferase is present in plasma and is probably responsible for the formation of the cholesteryl esters in the high-density lipoproteins, but the cholesteryl esters in the very-low-density lipoproteins of rat plasma are believed to be formed in the liver and small intestine.

The plasma cholesteryl esters are hydrolysed enzymically in the liver, the resulting free cholesterol following the same fate as that resulting from hepatic synthesis and from the hydrolysis of chylomicron cholesteryl esters.

From this simplified description it will be seen that the body contains a mass of exchangeable

cholesterol in both free and esterified forms, and that randomization of cholesterol throughout the whole mass takes place by two processes: direct exchange of free cholesterol between plasma and the various tissue pools, and indirect exchange due to hydrolysis of esterified cholesterol in the liver, followed by re-esterification. In man, re-esterification of the plasma cholesterol takes place entirely in the plasma, as shown below:

(FC = free cholesterol; EC = esterified cholesterol)

In rats, the plasma cholesteryl esters are formed partly in the liver and partly in the plasma and intestine. In man, the fractional rate of turnover of the whole mass of exchangeable cholesterol is 1–2%/day (equivalent to the removal and replacement of about 1 g of exchangeable cholesterol/day) and the fractional rate of turnover of the pool of plasma cholesteryl esters is about 2%/h.

Nutritional and Endocrine Factors Influencing Plasma Triglyceride Metabolism

By BARRY LEWIS. (*Departments of Chemical Pathology, Royal Postgraduate Medical School and St. Mark's Hospital, London W12 0HS, U.K.*)

Patients with atherosclerotic heart disease are metabolically heterogeneous; amongst their commonest abnormalities is endogenous hypertriglyceridaemia (Carlson, 1960). This form of hyperlipidaemia usually is manifested as an increase in the group of very-low-density lipoproteins in plasma, though other dyslipoproteinaemias may occur. The mechanisms of hypertriglyceridaemia are poorly understood, and are discussed in relation to conditions frequently associated with it.

1. About 50% of hypertriglyceridaemic patients show a greater than normal rise of plasma triglyceride on isocaloric high-carbohydrate diets; this response, carbohydrate inducibility, is a graded phenomenon (Glueck, Levy & Fredrickson, 1969). Carbohydrate restriction decreases plasma lipids in this group. Insulin response to oral glucose is high in some patients but is not consistently abnormal (Glueck *et al.* 1969). In the rat, insulin may increase hepatic secretion of triglyceride into plasma, but more certainly enhances both major stages of triglyceride removal from the circulation, inducing

lipoprotein lipase in adipose tissue (Salaman & Robinson, 1966) and promoting fatty acid re-esterification. Insulin decreased human plasma triglyceride concentrations in acute experiments (Jones & Arky, 1965); in the rat glucose administration has the same effect, probably by increasing triglyceride outflow (Baker, 1968). These findings cannot necessarily be extrapolated to long-term effects; plasma insulin and triglyceride concentrations have been reported to show positive correlations in carbohydrate-induced patients with coronary disease (Farquhar, Frank, Gross & Reaven, 1966) and in population surveys (Abrams, Jarrett, Keen, Boyns & Crossland, 1969).

In normal subjects, using the intravenous fat-tolerance test, we find decreased clearance of triglyceride from plasma when hypertriglyceridaemia is induced by high carbohydrate intake (Lewis, Mancini, Mattock, & Niteckis, 1970); this is accompanied by decrease of plasma post-heparin lipolytic activity by 50%, but it is uncertain whether a change of this extent is functionally important. Insulin response and glucose tolerance are enhanced in some subjects but are unchanged in others, in whom triglyceride concentrations rise.

Endogenous hypertriglyceridaemia may well result from more than one mechanism, but decreased triglyceride clearance from plasma is commonly demonstrable by the fat-tolerance test (Boberg, Carlson & Hallberg, 1969).

2. A large majority of hypertriglyceridaemic patients are obese, and decreases in weight lower plasma triglyceride concentrations which appear to be related both to decreased energy intake and adipose-tissue mass. Yet experimental over-feeding of normal men may but does not necessarily produce hypertriglyceridaemia; in our studies energy surplus appears less important than diet composition, and triglyceride clearance too is little changed by overfeeding with a diet of normal composition (Lewis *et al.* 1970; B. Lewis, M. Mancini, I. Mervart & M. Mattock, unpublished work). Hyperinsulinaemia is common in obesity and may influence plasma triglyceride turnover.

A moderate positive correlation exists between adipose-tissue mass and free fatty acid turnover in obesity (Nestel & Whyte, 1968). The latter is one determinant of hepatic triglyceride secretion (Gidez, Roheim & Eder, 1962). Proportionality between free fatty acid turnover and triglyceride concentration occurs in some situations, e.g. catecholamine administration (Carlson, Boberg & Högstedt, 1965), but is not invariable: in lipotrophy, virtual absence of adipose tissue is associated with low or normal free fatty acid concentrations and hypertriglyceridaemia. In protein-calorie malnutrition, despite high free fatty acid turnover, plasma triglyceride is low, possibly owing

to apoprotein deficiency (Lewis, Wittman, Krut, Hansen & Brock, 1966).

3. In uncontrolled diabetes plasma lipid concentrations may be normal, moderately elevated or, occasionally, extremely high. Control of diabetes, whether by carbohydrate restriction alone or with insulin, decreases triglyceride concentrations (Sterky, Larsson & Persson, 1963). Plasma triglyceride concentrations are higher in patients with vascular complications than those without.

Free fatty acid turnover is increased in most diabetics; it is decreased by carbohydrate- and calorie-restriction or by insulin, and is weakly correlated with plasma triglyceride concentration (B. Lewis, M. Mancini, C. Lowy, M. Mattock & I. Mervart, unpublished work). Diabetic dogs show decreased hepatic incorporation of free fatty acids into plasma triglyceride, implying that their hyperlipidaemia results from impaired removal (Basso & Havel, 1970). The fat-tolerance test often shows initially low but increasing triglyceride clearance from plasma when diabetics are controlled by diet or insulin (B. Lewis, M. Mancini, C. Lowy, M. Mattock & I. Mervart, unpublished work).

Contrasting with the decrease in free fatty acid turnover in diabetics treated by carbohydrate- and calorie-restriction, three non-diabetic obese patients on similar diets showed increased free fatty acid turnover (B. Lewis, M. Mancini, C. Lowy, M. Mattock & I. Mervart, unpublished work).

4. Other endocrine disorders more rarely cause abnormalities in plasma triglyceride metabolism. Oestrogens, alone or in oral contraceptive mixtures, raise triglyceride concentrations (Wynn, Doar & Mills, 1966). In adrenocortical hyperfunction, plasma triglyceride is often increased. Paradoxically, withdrawal of cortisol treatment in hypopituitary patients dependent on it may cause striking hypertriglyceridaemia (Summers, Hipkin & Davis, 1967; B. Tulloch, B. Lewis, I. Mervart & T. R. Fraser, unpublished work).

Growth-hormone excess, in acromegaly, has relatively little effect on plasma triglyceride concentration or clearance; free fatty acid turnover is rapid (B. Tulloch, B. Lewis, I. Mervart & T. Fraser, unpublished work).

In hyperthyroidism high plasma free fatty acid concentrations are well known. Despite increased turnover of free fatty acids, plasma triglyceride concentrations are low; the fat-tolerance test suggests rapid triglyceride clearance from the circulation (B. Tulloch, B. Lewis, I. Mervart & T. Fraser, unpublished work). Treatment of hyper- and hypothyroidism rapidly alters free fatty acid and triglyceride concentrations and turnover towards normal.

Abrams, M. E., Jarrett, R. J., Keen, H., Boyns, D. R. & Crossland, N. (1969). *Br. med. J.* 1, 599.

- Baker, N. (1968). *J. Lipid Res.* **9**, 1.
- Basso, L. V. & Havel, R. J. (1970). *J. clin. Invest.* **49**, 537.
- Boberg, J., Carlson, L. A. & Hallberg, D. (1969). *J. Atheroscler. Res.* **9**, 159.
- Carlson, L. A. (1960). *Acta Soc. Med. upsal.* **65**, 85.
- Carlson, L. A., Boberg, J. & Högstädt, B. (1965). In *Handbook of Physiology, Section 5: Adipose Tissue*, p. 625. Ed. by Renold, A. E. & Cahill, G. E. Washington, D.C. American Physiological Society.
- Farquhar, J. W., Frank, A., Gross, R. C. & Reaven, G. M. (1966). *J. clin. Invest.* **45**, 1648.
- Gidez, L. I., Roheim, P. S. & Eder, H. A. (1962). *Fedn Proc. Fedn Am. Soc. exp. Biol.* **21**, 289.
- Glueck, C. J., Levy, R. I. & Fredrickson, D. S. (1969). *Diabetes*, **18**, 739.
- Jones, D. P. & Arky, R. A. (1965). *Metabolism*, **14**, 1287.
- Lewis, B., Mancini, M., Mattock, M. & Niteckis, N. (1970). *Clin. Sci.* **38**, 33P.
- Lewis, B., Wittman, W., Krut, L. H., Hansen, J. D. L. & Brock, J. F. (1966). *Clin. Sci.* **30**, 371.
- Nestel, P. J. & Whyte, H. M. (1968). *Metabolism*, **17**, 1122.
- Salaman, M. R. & Robinson, D. S. (1966). *Biochem. J.* **99**, 640.
- Sterky, G., Larsson, Y. & Persson, B. (1963). *Acta paediat., Stockh.*, **52**, 11.
- Summers, V. K., Hipkin, L. J. & Davis, J. C. (1967). *Metabolism*, **16**, 1106.
- Wynn, V., Doar, J. W. H. & Mills, G. L. (1966). *Lancet*, **ii**, 720.

COLLOQUIUM ON 'BIOCHEMISTRY OF URINARY CALCULUS FORMATION'

Physical Chemistry of Calcium Stone Formation

By A. HODGKINSON and B. E. C. NORDIN. (*Medical Research Council Mineral Metabolism Unit, The General Infirmary, Leeds LS1 3EX, U.K.*)

The simplest explanation of calcium stone disease is that it is due to precipitation of calcium oxalate and/or calcium phosphate from an oversaturated solution. The degree of saturation of urine with respect to the stone-forming salts depends on the products of the relevant ion concentrations (or, more correctly, ion activities). Until recently it was not possible to measure activity products accurately because of the difficulty in measuring the concentrations of calcium, magnesium, phosphate and oxalate ions, as distinct from their total concentrations, but methods are now available for the measurement of Ca^{2+} by using tetramethyl murexide (Raaflaub, 1962), and liquid or solid-state electrodes (Ross, 1967; Schultz, Petersen, Mask & Buck, 1968). A few limited methods are also available for measuring Mg^{2+} concentrations (Walser, 1960; Heaton, 1967) but at present it is not possible to measure concentrations of phosphate and oxalate ions and these must be calculated from a knowledge of urine pH, ionic strength and the stability constants of the relevant complexes (Robertson, Peacock & Nordin, 1968; Pak, 1969; Finlayson & Miller, 1969).

Using these techniques Robertson *et al.* (1968) distinguished two states of supersaturation, (1) 'metastable supersaturation' in which urine may exist for long periods without precipitation occurring and (2) 'oversaturation' in which spontaneous homogeneous nucleation can occur. Most urine samples, whether from controls or stone-formers, were in a state of metastable supersaturation with respect to calcium oxalate and calcium phosphate

but the degree of supersaturation tended to be higher in the patients, owing to mainly higher concentrations of Ca^{2+} . Similar results and conclusions were reached by Pak (1969), who confined his studies to calcium phosphate stones.

Although these observations account for the growth of calculi, they do not explain the initiation of lithiasis, since nucleation requires either oversaturation or the presence of a suitable nidus (heterogeneous nucleation). Robertson *et al.* (1968) and Pak (1969) confined their studies to 24h urine collections, whereas it is clear that oversaturation need be only temporary for nucleation to occur. We have therefore examined the hour-to-hour variations in the activity products and have found that temporary oversaturation with respect to calcium oxalate or calcium phosphate occurs in both controls and stone-formers but is generally greater in patients than in controls. These periods of oversaturation generally occur after meals and are due mainly to an increased Ca^{2+} concentration, combined with a lower H^+ concentration and/or an increased oxalate ion concentration. The activity products at any given time of the day or night are generally higher in the patients than in the controls, suggesting that any crystals formed during periods of oversaturation will grow more rapidly (or dissolve more slowly) in the patients than in the controls.

These results indicate that homogeneous nucleation of either calcium oxalate or calcium phosphate may occur at some time of the day or night. The calcium crystalluria in recurrent stone-formers (Robertson, Peacock & Nordin, 1969b) and the chemical composition of small urinary-tract calculi (Hodgkinson, Peacock & Nicholson, 1969) suggested that crystallization of calcium oxalate was the first step in the formation of most calcium stones. Subsequent studies with the electron-probe microanalyser have confirmed that most small calculi consist predominantly of calcium oxalate but the