weight. This gives unique dimensions for models (a) and (b), but for the Y-shapes several varieties of arm lengths and interarm angles must be considered.

$P(\theta)^{-1}$ was calculated from the formula (Guidushek & Holtzer, 1958):

$$P(\theta) = \frac{1}{N^2} \sum_n \sum_m^N (\sin hr_{nm}/hr_{nm})$$

where r_{nm} is the absolute value of the vector between the n th and m th scattering points. This was evaluated on a digital computer, and the results were plotted as $P(\theta)^{-1}$ against $\sin^2(\theta/2)$ for comparison with the experimental points.

Our conclusion was that, although the fit was not total, the experimental data corresponded best to a Y-shape molecule of equal arm lengths of four supercoils (185.6 nm), cross-sectional arm radii of 15.05 nm and an angle of 120° between each arm.

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as a measure of the number of cells present. The method depends on the enhancement of the fluorescence of ethidium bromide (2,7-diamino-10-ethyl-9-phenylphenanthridinium bromide) that occurs when the dye binds to double-stranded nucleic acids (Le Pecq *et al.*, 1964; Le Pecq & Paoletti, 1966, 1967). We measure the fluorescence intensity before and after treatment with deoxyribonuclease; this makes the method specific for DNA.

The procedure is more economical in terms of the starting material (i.e. the granulocytes) used than methods (Burton, 1956; Giles & Myers, 1965) that employ the diphenylamine reaction.

The values obtained for the DNA content of human granulocytes by the present method is 6–7 pg/cell. This agrees with the values reported by previous workers, who have used less sensitive methods and larger numbers of cells (Mandel *et al.*, 1950; Metais *et al.*, 1951; Davidson *et al.*, 1951).

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The Measurement of the Deoxyribonucleic Acid Content of Human Granulocytes

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We are currently studying some aspects of protein synthesis in human granulocytes (Andrews *et al.*, 1971). These cells have a strong tendency to form clumps when they are resuspended for biochemical studies, having been previously separated from heparinized whole blood by dextran sedimentation and differential centrifugation through an albumin solution. This makes their enumeration with either a haemocytometer or the Coulter counter unreliable, so that a cell count is a poor parameter against which to express the results of relatively more precise biochemical investigations.

We have developed a method of measuring the DNA content of as few as 1×10^6 human granulocytes with a coefficient of variation of about $\pm 3\%$. This enables the amount of DNA present to be used

Metabolism of Prostaglandin E_1 by Human Plasma

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Prostaglandins have been found in human blood in certain pathological conditions (see, e.g., Williams *et al.*, 1968) and during labour (Karim, 1968), but there has been a lack of evidence for their presence in normal human blood. We have, however, now demonstrated the presence of prostaglandins in normal human blood (Greaves & McDonald-Gibson, 1972). During this study an enzymic prostaglandin E_1 -metabolizing system in human plasma became apparent.

[5,6- ^3H]Prostaglandin E_1 was added to fresh blood (final concn. 10 ng/ml). Plasma was separated and prostaglandins and metabolites were extracted and

subjected to t.l.c. The radioactivity of appropriate areas of the chromatoplate was determined by liquid-scintillation counting. About 70% of radioactivity in the plasma was recovered as a metabolite less polar than prostaglandin E₁.

In subsequent experiments metabolism was determined by incubating 100–300 ng of prostaglandin E₁, containing tracer [5,6-³H]prostaglandin E₁, with heparinized plasma at 37°C for 15–30 s. The reaction was stopped with ice-cold ethanol, and the prostaglandins were extracted, separated and their radioactivities counted as above. With these higher concentrations of prostaglandin E₁ it was usual to observe 25–50% conversion into the less-polar metabolite under the conditions described. The rate of conversion was linear with time up to 30 s and increased with increasing substrate concentration.

There was little activity at 0°C and inactivation occurred at 100°C. The optimum pH was between 7 and 8. Storage of plasma at 4°C for up to 13 days resulted in increased activity. On further storage a decline in activity occurred. Activity was increased by EDTA (2 mM) and, to a smaller extent, by fluoride (2 mM). Iodoacetate (2 mM) caused a small inhibition, but *p*-chloromercuribenzoate (2 mM), 2,2'-bipyridyl (2 mM) and cyanide (2 mM) were without effect. Aspirin (<1 mM) produced a significant inhibition of activity, but the other anti-inflammatory agents indomethacin and prednisolone had no effect. Preliminary kinetic studies suggested some characteristics of a regulated enzyme. The activity of the enzyme in different plasmas was variable. Erythrocytes appeared to give some protection against the metabolism of prostaglandin E₁ by plasma.

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The Turnover of Glycerol and Free Fatty Acid in Blood of Genetically Obese Mice

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As part of an investigation of triglyceride metabolism in genetically obese mice, we have measured the turnover of glycerol and free fatty acid in blood.

Female obese mice aged 3–5 months, homozygous for the *ob* gene (see Abraham & Beloff-Chain, 1971) and lean controls, were anaesthetized with Avertin,

during measurements of turnover by continuous intravenous infusion of U-¹⁴C-labelled glycerol or free fatty acid (see, e.g., Armstrong *et al.*, 1961; Winkler *et al.*, 1970). Glycerol was determined by the method of Pinter *et al.* (1967), and free fatty acid by the method of Lauwerys (1969). The ¹⁴C-labelled metabolites were isolated by t.l.c. for liquid-scintillation counting.

The concentration of glycerol in blood of well-fed mice, sampled after ether anaesthesia, was 0.16 ± 0.03 (3) $\mu\text{mol/ml}$ of whole blood in lean mice (mean $\pm$ S.E.M.), and 0.36 ± 0.06 (3) $\mu\text{mol/ml}$ in *ob/ob* mice. The corresponding values in 24 h-starved mice were 0.40 ± 0.09 (3) and 0.44 ± 0.08 (3) $\mu\text{mol/ml}$. The rate of turnover of glycerol in blood (plus extravascular space) was 0.48 ± 0.08 (6) $\mu\text{mol/min}$ per mouse in well-fed lean mice and 1.13 ± 0.12 (7) $\mu\text{mol/min}$ per mouse in *ob/ob* mice.

The blood glycerol tolerance curve, after intravenous 0.3 M-glycerol, was not abnormal in *ob/ob* mice. If [U-¹⁴C]glycerol was employed in these experiments, the incorporation of ¹⁴C into liver lipids and glycogen (per g of tissue) and blood glucose, after 5 or 10 min, was not abnormal in *ob/ob* mice.

Thus the increased turnover and high blood concentrations of circulating glycerol, in obese mice, are largely a result of increased extrahepatic 'lipolysis'.

The turnover rate of circulating free fatty acid, measured by infusion of [U-¹⁴C]palmitate, was 2.5–6.0 $\mu\text{mol/min}$ per mouse in lean mice and 4–9 $\mu\text{mol/min}$ per mouse in *ob/ob* mice. Blood free fatty acid concentrations were high (0.6–1.1 $\mu\text{equiv./ml}$ of serum) in these experiments, perhaps as a result of 'stress'. Following a single intravenous trace dose of [U-¹⁴C]palmitate, the disappearance of radioactivity from blood was faster in *ob/ob* mice.

These results suggest that the process of tissue triglyceride breakdown is not impaired in obese mice (see also Abraham *et al.*, 1971). Rather, the turnover of glycerol and free fatty acid in blood is increased, in association with their greater liver and adipose-tissue mass, and blood volume.

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