Westinghouse lamp (wavelength spectrum 180–210 nm) on 2 consecutive days and the others acted as controls. Plasma 25-(OH)D was measured by a competitive protein-binding assay.

The mean (+SEM) total bilirubin in the Wistar rats was less than 6 µmol/l and in the CBD-ligated rats 160 ± 16 µmol/l. The mean pretreatment plasma 25-(OH)D concentrations were 5-5 ± 2-5 nmol/l in the Wistar rats (n = 11), 6-8 ± 1-8 nmol/l in the Gunn rats (n = 11) and 4-7 ± 3-3 nmol/l in the CBD-ligated rats (n = 7) and rose 1 day after the two treatments to respectively 26-5 ± 3-2, 21-0 ± 2-5 and 29-3 ± 12-0 nmol/l. Similar plasma 25-(OH)D values were seen on the following day. Small falls in plasma 25-(OH)D concentrations were seen in three groups of control rats (each n = 7) not treated with ultraviolet light.

We conclude that hyperbilirubinemia does not inhibit cutaneous vitamin D3 synthesis or hepatic 25-hydroxylation. Ultraviolet light therapy justifies further assessment as a treatment for vitamin D deficiency in jaundiced patients able to 25-hydroxylate.

94. THE SURFACE CHARGE OF CRYSTALS, AND ITS EFFECT ON INFLAMMATION


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Crystal-induced inflammation is initiated by interaction between the crystal surface and proteins or cell membranes; membrane breakdown also occurs, and hydrogen bonding and electrostatic forces are suggested mechanisms. Techniques to measure crystal surface charge have therefore been developed. Heating, grinding or sonicating of crystals before use are usually performed; the effects of these manipulations on crystal charge and inflammation have also been studied.

Urate, pyrophosphate, hydroxyapatite and brushite crystals were prepared and examined by microscopy and infrared spectrophotometry. Inflammatory responses were measured in the rat foot pad. Surface charge has been examined in media of various ionic strengths by crystal electrophoresis, with light microscopy or by laser-Doppler spectroscopy, and results expressed as mobility (μS⁻¹ V⁻¹ cm).

Crystals have a net electrophoretic mobility of sign and magnitude similar to that of erythrocytes (urate 1.15 ± 0.01, pyrophosphate 0.71 ± 0.01, hydroxyapatite 0.75 ± 0.01, brushite 0.72 ± 0.007, erythrocytes 1.07 ± 0.02). Mobility increases in low ionic strength solutions, suggesting it is a fixed property of the crystal surface charge. Urate crystals had the highest charge and caused the most inflammation.

Heat and sonication both reduced surface charge without altering the morphology or spectrophotometry of crystals. Grinding altered morphology and charge, but not spectrophotometry. Reduction in electrophoretic mobility was accompanied by reduced inflammatory responses (untreated urate: mobility 1.15, foot pad swelling at 24 h 0.38 mm; heated urate: mobility 0.79, foot pad swelling 0.27; ground urate: mobility 0.66, foot pad swelling 0.23).

These results show that crystals have a net negative charge, and suggest that their inflammatory potential may relate to this. The great susceptibility of this charge to widely used laboratory techniques questions the validity of most previous work. Alternative possible mechanisms of crystal-induced inflammation are suggested.

95. PROSTACYCLIN-LIKE ACTIVITY IN THE FEMALE RAT THORACIC AORTA AND THE INFERIOR VENA CAVA AFTER TREATMENT WITH ETHINYL OESTRADIOL AND NORETHISTERONE

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The increase in the incidence of thromboembolic disease after oral contraceptives and the occurrence of vascular lesions in women and experimental animals after treatment with contraceptive steroids is well established. The mechanism underlying these vascular changes is, however, not clear. We hence set out to investigate whether these changes were due to diminished prostacyclin (PGI2) secretion.

Four groups of rats, each of 10 female Sprague-Dawley albinos, were injected subcutaneously daily as follows: group I, ethinylestradiol (EE2, 0.01 mg in 0.1 ml of corn oil/100 g); group II, norethisterone (0.25 mg/100 g); group III, both compounds (EE2 + norethisterone); group IV, 0.1 ml of pure corn oil (control).

After 30 days of injections the thoracic aorta and the inferior vena cava were dissected out and assayed for PGI2-like activity. PGI2-like activity generated by aortic rings of EE2 (group I) treated animals (mean 55.5 ± SEM 7.1 ng/50 mg of tissue per ml; P < 0.05) was significantly greater than that in controls (46.6 ± 4.1 ng). Norethisterone (group II) alone did not produce a significant change in PGI2-like activity in the aortic rings (43.5 ± 3.9 ng). EE2 in combination with norethisterone (group III) produced a significant increase in aortic PGI2, when compared with controls (61.1 ± 5.9 ng; P < 0.01); when compared with animals treated with EE2 alone this group showed a further slight increase, which was not significant.

PGI2-like activity generated by the inferior vena cava (IVC) rings was 30-fold lower than that observed in aortic rings. Neither EE2 nor norethisterone injections, either alone or in combination, caused a significant alteration in PGI2 activity of the IVC (EE2: 1.36 ± 0.17 ng; norethisterone: 1.49 ± 0.27 ng; EE2 + norethisterone: 1.21 ± 0.27 ng; control: 1.40 ± 0.24 ng).

The pathophysiological significance of the increase in PGI2 production in the aorta after injections with EE2 alone and in combination with norethisterone is not clear. It is possible that this effect reflects a compensatory reaction to other EE2-induced intimal changes such as plaque formation.

In conclusion, the primary effect of EE2 on PGI2 production is one of enhancement rather than a reduction; an alternative mechanism underlying the pathogenesis of EE2 must hence be sought.

96. STABILITY OF PROSTACYCLIN IN HUMAN PLASMA

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Prostacyclin is a potent inhibitor of platelet aggregation. Its half-life in aqueous solution, pH 7-4 at 37°C, is less than 3 min. We have investigated the stability of prostacyclin in human and rabbit plasma.

Venous blood from either human volunteers or rabbits was collected in plastic tubes containing 3-8% sodium citrate (9 vol. of blood to 1 vol. of citrate). Platelet-rich plasma (PRP) was obtained by centrifuging the blood at 200 g for 10 min. Platelet-poor plasma (PPP) was obtained by centrifuging PRP at 400 g for 20 min at 20°C. Aliquots (0.5 ml) of PRP were placed in a Bryston aggregometer, stirred at 1100 r.p.m. at 37°C for 2 min and irreversible aggregation was induced by ADP (2.5–10 µmol/l). Authentic prostacyclin, 0.625–5 ng (a gift of Wellcome Research Laboratories), was added to PRP, stirred for 1 min at 37°C and aggregation then induced. The percentage inhibition of aggregation by the prostacyclin was then calculated.

When the contact time of prostacyclin with human PRP was prolonged for 30 min at 37°C, the anti-aggregatory activity was
not lost. In contrast, the activity was even greater than that obtained by a similar dose added 1 min before ADP challenge to PRP that had been kept at 37°C for 30 min. Thus the PRP may contain a substance that prevents prostacyclin inactivation or, alternatively, the biochemical effect that underlies the mechanism of the anti-aggregatory activity is persistent in the platelets. When prostacyclin was incubated in either human PRP or PPP for 30 min at 37°C and its activity tested there was no loss of activity. However, a similar dose of prostacyclin when incubated alone at 37°C for 30 min completely lost its anti-aggregatory activity. In similar experiments with rabbit PRP or PPP greater than 90% of prostacyclin activity was lost.

The results of these experiments clearly indicate that human plasma contains a stabilizing substance that prevents the inactivation of prostacyclin. The presence of a stabilizing factor in human plasma strengthens the role of prostacyclin as a circulating hormone. The nature of this factor is now under investigation.

97. CORONARY HEART DISEASE RISK FACTORS AND SOCIO-ECONOMIC STATUS IN A LONDON POPULATION

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British mortality statistics now indicate that death from coronary heart disease (CHD) is more common in men and women of lower socio-economic class than those with higher status. Since CHD risk-factor differences may partly explain this mortality gradient, the 1005 (males: 502; females: 503) participants in a survey of London local government workers (Fuller et al., 1978, British Heart Journal, 40, 170–176) were categorized in terms of social class and family income and comparisons made of plasma lipid and lipoprotein concentrations and nutritional factors.

There was no significant trend of total cholesterol, total triglyceride or high-density lipoprotein (HDL) cholesterol with social class in either sex. However, for men there was a significant (P < 0.005) downward trend of mean total triglyceride concentration with increasing family income. Men in the highest income group had the highest mean HDL cholesterol concentration and had higher mean body weight. In both sexes, nutrient intake of total calories, carbohydrate and fat were inversely related to family income and those in the lower income group tended to smoke more cigarettes. These lipid and nutritional relationships with family income may partly explain the present inverse association between CHD mortality and socio-economic status.

98. A SIMPLE NUMERICAL ANALYSIS OF WHOLE-BODY PLETHYSMOGRAPH DATA

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Manual methods of analysing plethysmographic data are unsatisfactory because they are tedious, give highly variable results and, if done 'blind', the results are not available for some time after the measurement. For these reasons, automated analyses are attractive since they offer the prospect of speed and less variability without bias. The method described by Lord & Brooks (1977, Thorax, 32, 60–66) proved more variable than the manual method. Chowienczyk, Rees, Payne & Clarke (1979, Clinical Science, 58, 259) described a more detailed method which effectively measured airway resistance averaged throughout the respiratory cycle. Although better coefficients of variation were obtained, the measurement is not strictly comparable with the quasi-static measurement described by Du Bois.

We describe a further method using numerical analysis. During measurements on a subject, three analogue signals for box volume ($V_o$), mouth flow ($V_m$) and mouth pressure ($P_m$) from a constant-volume pressure-compensated plethysmograph are digitized and stored on a magnetic disc. Digital processing of the data is carried out immediately after their acquisition. The three sets of data are differentiated with respect to time and peak values (positive and negative) from the $dP_m/dt$ and $dV_m/dt$ data are chosen, these peak values occurring at zero flow or pressure. Division of selected peak values of $dP_m/dt$ by values of $dV_m/dt$ corresponding in time yields the two slopes $dP_m/dV_m$ and $dV_m/dV_o$. Resistance and thoracic gas volume are computed from the means of these slopes. The peak values from $dP_m/dt$ and $dV_m/dt$ data are chosen by taking absolute values above a threshold value. This value is set in the programme as a factor of the maximum value of the data. The threshold is adjusted if an insufficient number of peaks are selected.

The identification of peaks of the derivatives of $V_o$ and $P_m$ has several advantages: (1) shutter closure is automatically recognized since peaks of $dP_m/dt$ occur only with the shutter closed; (2) artifacts are easily recognized and may be eliminated by pre-setting the peak selection criteria; (3) measurements of airway resistance are made after closure to zero flow since peak $dV_m/dt$ occurs at zero flow; (4) DC shift of signals is effectively eliminated; (5) measurements may be made at the onset or at the end of inspiration since the peaks of $dP_m/dt$ are of opposite sign at these two points of zero flow. Data processing takes approximately 90 s.

Both manual and automated analysis were made on the same data for comparison in six normal subjects. Manual analysis was made on 12 consecutive photographic records made from an oscilloscope display. The automated method calculated approximately 100 values of Raw and 70 values of Fig in the 90 s of each measurement. Data on sensitivity, within-session variability and day-to-day variability of both methods are presented.

99. HOW MANY BLOWS REALLY MAKE AN FEV1.0, FVC OR PEFR?

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In 1846 Hutchinson invented the spirometer, named the vital capacity and advised three measurements to determine the true value, 'for the first is frequently not correct' and with more 'the number of cubic inches will be found to fall short of that true value'. The literature since accepts the implied assumptions that subjects may not achieve the true value but can never exceed it providing sufficient data from the subject for reliable detection of any but the grossest intra-individual quantitative changes.

We have collected PFR, FEV1.0 and FVC in sets of 10 or 20 values at 1 min intervals from 30 normal, 49 asthmatic (22 atopic) and 26 bronchitic subjects. Analysis shows that the derivatives are compatible with the values in the sets being normally distributed so the true value is best represented by the arithmetic mean of all valid attempts. One-third of all subjects showed skewness in one or more indices but these were equally divided between the positive and negative directions. There is no sign of the dominant negative skewness resulting if the true value was indeed a maximum which could be approached or equaled but never exceeded.

There is no sign that repetition worsens performance. Seventy-two subjects showed no regression in any index and those of the remainder who deteriorated were balanced by equal numbers in all categories who improved.

There is a significant tendency for the highest and lowest values to occur earlier in any series. Probability theory suggests this is a statistical phenomenon.