threshold of 0.1 torr, whereas 10 mg caused a significant increase of threshold of 2.76 torr ($P < 0.01$), i.e. a respiratory depression. Doses of 5 mg caused a small, significant depression of slope of the $\dot{V},P_{CO_2}$ response line of 0.82 l min^{-1} $torr^{-1}$ ($P < 0.025$) which is also indicative of respiratory depression. Doses of 10 mg did not cause a significant change of slope.

These results confirm that diazepam at commonly prescribed doses causes significant respiratory depression by raising the threshold or depressing the slope of the $\dot{V},P_{CO_2}$ response, and will provide a basis for further work on e.m.g activity in respiratory muscles.

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A simple microassay for human tissue renin

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Renin in tissue homogenates is measured by its ability to form angiotensin I from renin substrate. The rate at which angiotensin I is generated is taken as a measure of the quantity of renin present. In currently used methods the generation and estimation steps are performed in separate tubes, aliquots of the incubation mixture being removed and assayed for angiotensin I. This approach introduces an unnecessary complication. A simple two step assay for the measurement of renin is described, using a single tube and conventional inhibitors. In this method all of the angiotensin I generated is estimated, enabling the sample volume to be kept to a minimum. The assay has high sensitivity and renin can be measured adequately when the tissue sample available is very small.

Renin was mobilised from human endometrium by homogenisation in distilled water (10 µl/ml). The homogenate was freeze-thawed three times and the supernatant clarified by centrifugation and stored at −20°C until assayed. A mixture of 5 µl of sample, 5 µl buffered inhibitor (5-OH-quinoline, EDTA, dimercaprol buffered in TRIS pH 7.2) and 20 µl sheep renin substrate (11.6 µM) (Skinner, 1967) was incubated at 37°C for 2 h. After incubation the reaction was stopped by cooling the mixture to 1°C. Blanks were prepared as samples, but were incubated at 0°C for 2 h. Angiotensin I formed was measured by radioimmunoassay. The standard curve was prepared by adding unlabelled asp-ileu-angiotensin I in 5 µl TRIS albumen buffer (pH 7.4) to 20 µl of sheep renin substrate and 5 µl inhibitor. Five hundred microlitres of a specific angiotensin I antiserum, diluted 1 in 10,000 in barbital (pH 8.6) was added to each tube, together with 500 µl of monoiodinated asp-ileu-angiotensin I (10,000 counts min^{-1} ml^{-1}).

This mixture was left to equilibrate for 16 h at 4°C. Bound angiotensin I was separated from free angiotensin I with charcoal. Both the supernatant and the pellet were counted separately for 2000 counts and the results expressed as percentage bound.

Using this method angiotensin I generation was linear for 3 h and the rate of generation was directly dependent upon the quantity of enzyme present. During the incubation step less than 0.2% of the substrate was hydrolyzed. Measurement of the Michaelis constant, K_m, (0.27 µM) indicated that the substrate concentration used was ten times greater than K_m and therefore the generation of angiotensin I followed zero order kinetics. The use of the inhibitors allowed complete recovery of generated angiotensin I. The sensitivity of the radioimmunoassay enabled the detection of as little as 150 nu of human renin/ml of sample (i.e. 2.5 ng angiotensin I ml^{-1} h^{-1}). The inter-assay variability was 9.3% (in 6 separate assays) and the intra-assay variability was 10.8% ($n = 12$).

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Plasma sex hormone binding globulin and oestrogen therapy

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Oestrogens increase sex hormone binding globulin (SHBG) in human plasma and SHBG levels are a sensitive indication of oestrogenisation.

Using the method of Rudd, Duignan & London (1974), with [^{14}C]-labelled testosterone as a ligand, SHBG levels were measured in groups of women on oestrogen or oestrogen-progestogen therapy and the levels compared with those seen in comparable groups of untreated women. The levels of SHBG are expressed as μg of bound testosterone per 100 ml plasma (± 1 s.e. mean).

The first group were twenty-two pre-menopausal women on a combined oral contraceptive. Of these, eleven were taking a Pill containing 50 μg ethinyl oestradiol and eleven a Pill containing 35 μg or less ethinyl oestradiol. Plasma SHBG levels in these women were compared with those of fifty-two normal, ovulating women who had not received hormone therapy over the previous 6 months. The mean SHBG levels in women on (a) a 50 μg Pill was 2.73 ± 0.13, (b) a 35 μg or less Pill was 2.2 ± 0.12 and (c) in the untreated women was 2.31 ± 0.05 μg testosterone/100 ml. Statistical comparison between these three groups of premenopausal women showed that the levels in women on the 50 μg Pill were significantly elevated over the levels in the women taking a 35 μg or less Pill as well as over the levels seen in the untreated ovulating group ($P < 0.01$ and $P < 0.005$).

The second group consisted of nine women with hypergonadotrophic ovarian failure eight of whom were post-menopausal and all of whom had been receiving a variety of different hormone replacement regimes over the previous three months. The SHBG levels in the treated women were compared with those seen in twenty-six untreated post menopausal women who had never received any therapy. The mean SHBG level in the treated group was 2.75 ± 0.16 μg testosterone/100 ml while in the untreated group the mean level was 1.84 ± 0.05 μg testosterone/100 ml. The levels of SHBG in the treated patients were significantly elevated ($P < 0.001$) over those in the untreated group.

A further comparison showed a significant elevation of SHBG levels in the hormone-treated post menopausal women over those seen in the normal ovulating women ($P < 0.02$).

These results show that the combined oral contraceptive containing 35 μg or less of ethinyl oestradiol more closely mimics the SHBG levels in the normal menstrual cycle than those seen in women on a 50 μg combined Pill. Standard hormone replacement therapy substantially elevates the SHBG to levels higher than those seen in normal ovulating women and suggests an element of overtreatment rather than true replacement therapy in the post menopausal women. These results also suggest that oral contraceptives containing 35 μg or less of ethinyl oestradiol would more closely resemble, in terms of SHBG levels, true replacement therapy than do the preparations more commonly used for this purpose.

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