single evening dose of prednisolone and (ii) 28 days after this change.

The domiciliary collection of urine (Hillier & Knapp, 1974) was used as this avoids many of the disturbances of physiological rhythms associated with hospital admission. The subjects were asked to record the clock-time of micturition and to measure the volume of urine passed. An aliquot was retained and later analysed for sodium, potassium, calcium, creatinine and osmolality. The subjects also recorded their body temperature, weight, and assessed their joint stiffness. Six out of ten patients showed nocturia 4–6 days after the change to a single evening dose. Analysis of the urinary data (Fort & Mills, 1970) showed changes in the timing of peak electrolyte excretion rate and urine flow rate when prednisolone was taken as a single dose in the evening.

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The effect of diazepam on the threshold of the ventilatory response to CO_2

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The ventilatory response to CO_2 ($\dot{V},P_{CO_2}$ response) is measured as the linear relation between ventilation and P_{CO_2}. The slope of the line describes the sensitivity to CO_2 and the intercept on the CO_2 axis represents the threshold of the response. Recent investigations into the onset of electromyogram activity in respiratory muscles during breath-holding (Sempik, 1978) have indicated that it may be governed by the threshold of the $\dot{V},P_{CO_2}$ response and to test this hypothesis some means of changing the threshold would be useful.

There is some discrepancy in the literature as to the depressant effect of diazepam on respiration assessed by a shift of the $\dot{V},P_{CO_2}$ response. No significant respiratory depression was found by Cohen, Finn & Steen (1969) using intravenous doses up to 0.266 mg/kg. However, respiratory depression was reported by Catchlove & Kafer (1971) using intravenous doses of 0.14 mg/kg and by Utting & Pleuvry (1975) using oral doses of 5 mg.

In view of this disagreement it was decided to examine the effects of single oral doses of diazepam (5 and 10 mg) by measuring the $\dot{V},P_{CO_2}$ response of ten healthy subjects immediately before, and 1 h after the drug, using the CO_2-rebreathing method of Read (1967). Table 1 shows the results obtained. In a paired *t*-test, doses of 5 mg caused an insignificant fall in

Table 1 The mean slopes and thresholds of the $\dot{V},P_{CO_2}$ responses obtained before and after 5 or 10 mg diazepam

	Slope (l min^{-1} torr^{-1})		*Threshold (torr)*	
	mean ± s.e. means	*differences ± s.e. means*	*mean ± s.e. means*	*differences ± s.e. means*
Control	4.49 ± 0.62		45.4 ± 1.44	
		−0.82 ± 0.26		−0.1 ± 0.78
Diazepam (5 mg) $n = 7$*	3.67 ± 0.51	(*P* < 0.025)	45.3 ± 1.26	(n.s.)
Control	3.35 ± 0.48		42.7 ± 2.17	
		−0.01 ± 0.26		+2.76 ± 0.76
Diazepam (10 mg) $n = 10$	3.34 ± 0.53	(n.s.)	45.4 ± 2.36	(*P* < 0.01)

* 3 subjects were excluded from the 5 mg study because of non-linearity of the $\dot{V},P_{CO_2}$ responses.

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% ($n = 12$).