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EFFECT OF ETHAMIVAN (VANILLIC ACID DIETHYLAMIDE) ON THE RESPIRATORY RESPONSE OF HEALTHY YOUNG MEN TO CARBON DIOXIDE, IN THE ABSENCE OF HYPOXIA

BY

J. L. ANDERTON, J. F. COWIE, E. A. HARRIS AND R. A. SLEET

From the Department of Therapeutics, University of Edinburgh

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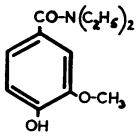
The effect of ethamivan (vanillic acid diethylamide) on the ventilatory response to carbon dioxide has been investigated in healthy young adult males. Intravenous infusion of the drug at a rate of 9 mg/min causes respiratory stimulation depending in degree upon the prevailing alveolar CO_2 tension. When the latter is low stimulation by the drug is most marked and may largely support respiration below the CO_2 threshold. As alveolar P_{CO_2} increases, the effect of the drug disappears. These findings are discussed.

Ethamivan (vanillic acid diethylamide, "Vandid") is a drug with analeptic properties which was synthesized in 1952 by Kratzl & Kvasnicka. It has the following structural formula:



Its pharmacological properties were studied by Auinger, Kaindl, Salzmann & Weissel (1952) and Ginzel (1952). Several publications refer to its use in various clinical situations. As yet there is no report of its action in controlled experiments in healthy human subjects. We present here the results of experiments in which the effect of the drug on the ventilatory response to carbon dioxide excess has been studied in healthy young adult males. In these experiments the metabolic acid-base balance was normal and the hypoxic stimulus to breathing was minimized by enriching the inspired gas with oxygen. Body temperature was not measured.

METHODS

Gas mixtures were supplied to the subjects by the method of Cunningham, Cormack, O'Riordan, Jukes & Lloyd (1957) through the intake of a low-resistance valve (Cunningham, Johnson & Lloyd, 1956). Expired gas volumes were measured with a calibrated dry gas meter and registered, with a record of individual breaths and 30-sec time-marks, on a kymograph. End-tidal alveolar gas was sampled continuously and passed through a flow-bridge CO_2 analyser (Grove-White & Sander, 1948) which gave an absolute accuracy of ± 0.5 mm Hg in the steady state. Changes of CO_2 tension were probably detected with much

greater accuracy than this. Changing concentrations of CO_2 were registered by the analyser with a delay of 1 min and accurately followed up to a maximum rate of change in CO_2 tension of 4 mm Hg per min. A full description of the apparatus and its performance will be published elsewhere.

The subjects were male volunteers without cardiac or respiratory disease, aged 19 to 27 years, most of whom were medical students or members of the laboratory staff. They attended the laboratory at 1.30 p.m., fasting for at least 3 hr, and rested in a semi-sitting position on a comfortable bed for 0.5 to 1 hr before the experiments began. During control periods isotonic saline, and during drug periods ethamivan 0.6 g per 100 ml. of saline, were infused through a polyethylene catheter into a superficial arm vein. Every effort was made to conceal details of the procedure from the subjects, who were encouraged to read but not permitted to fall asleep.

Experiments were of 3 kinds:

(1) Measurements of ventilation at different rates of infusion of ethamivan, alveolar P_{CO_2} either (*a*) being allowed to fall as ventilation increased or (*b*) being kept constant by adding more CO_2 to the inspired gas.

(2) CO_2-response experiments in 5 subjects. In these, several gas mixtures with a P_{O_2} of 350 mm Hg and a P_{CO_2} between 15 and 50 mm Hg were given in turn, each mixture for at least 15 min and in every case until no persistent rise or fall of either ventilation or alveolar P_{CO_2} was observed for 5 consecutive min. During a further period of 3 to 5 min a sample of end-tidal gas was collected for analysis by the Haldane method. Up to 4 mixtures were given in this way while isotonic saline was infused. The mouthpiece was then removed and ethamivan in saline was delivered at a rate of 9 mg/min from a constant infusion pump. After 30 min the mouthpiece was replaced and up to 6 gas mixtures given as described above, while the infusion of ethamivan continued at the same rate. In almost

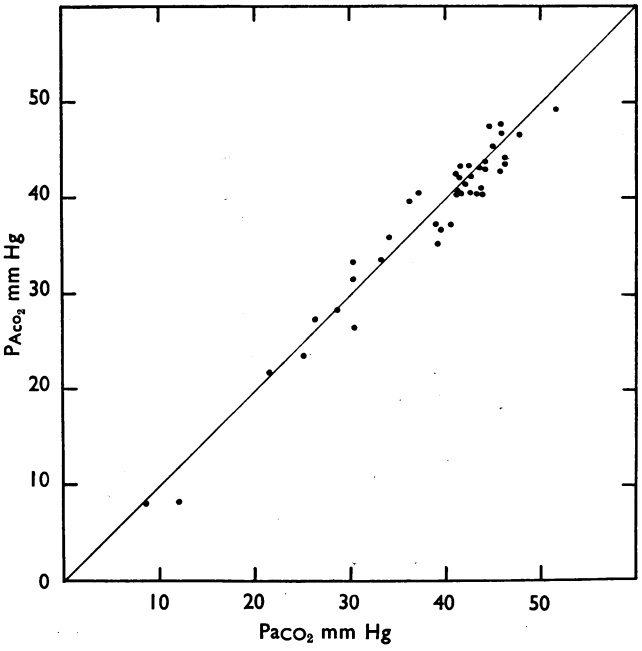


Fig. 1. Relationship between the P_{CO_2} of arterial blood (P_{aCO_2}) (*p*H interpolation method) and that of end-tidal gas (P_{ACO_2}) collected at the same time. Tidal volume was 500 ml. or more in each case.

every instance the alveolar P_{CO_2} for the CO_2-response curve was that of the sample of end-tidal gas collected during the steady state, and the flow-bridge analyser thus served mainly as an indicator of the steady state. Occasionally the sample was missed or coincided with a further change in flow-bridge readings, and the appropriate steady-state readings on the flow-bridge analyser were then used instead.

(3) Control experiments in 3 subjects, similar to (2) except that no ethamivan was given during the second half of the experiment.

The validity of end-tidal P_{CO_2} as a measure of the CO_2 stimulus rests upon its identity with the P_{CO_2} of arterial blood. In this laboratory parallel determinations have agreed well over a wide range of P_{CO_2} values (Fig. 1). In adults without gross lung disease, and provided the tidal volume is above 500 ml., end-tidal P_{CO_2} is an adequate measure of arterial P_{CO_2}.

In 3 subjects (D.D., N.McD. and A.T.) alveolar ventilation was calculated by the Bohr equation from total ventilation and determinations of the P_{CO_2} of alveolar, expired and inspired gas.

RESULTS

Fig. 2 shows the effect of ethamivan on ventilation and alveolar P_{CO_2} when inspired P_{CO_2} is constant. The start of the infusion usually caused pain in the

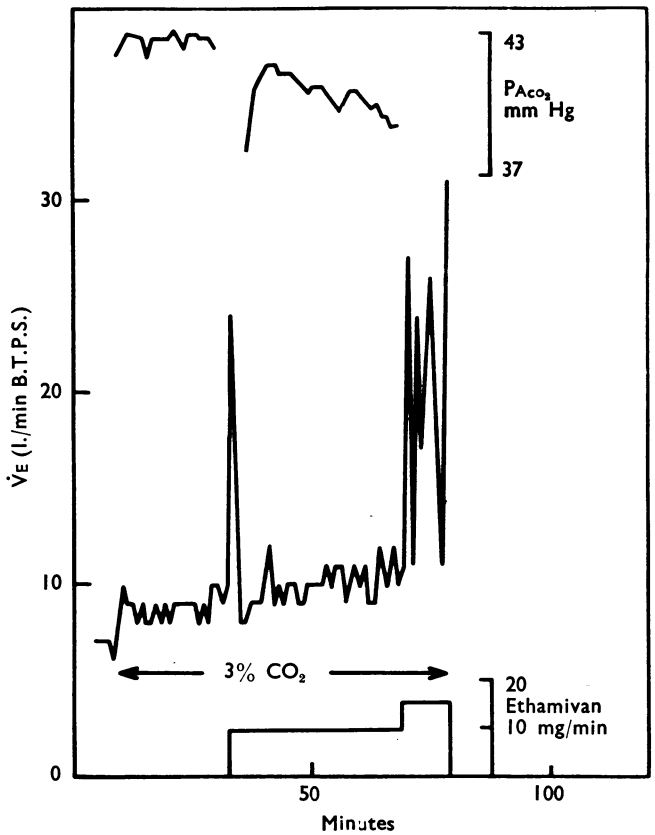


Fig. 2. Response of ventilation and alveolar P_{CO_2} to infusion of ethamivan. The first peak of ventilation is an artefact due to pain in the arm at the beginning of the infusion. The hyperventilation at the end of the record is also artificial and is due to the occurrence of severe side-effects on the bigger dose of ethamivan.

arm lasting a minute or two and there was often an associated ventilatory artefact. Thereafter ventilation slowly rose while alveolar P_{CO_2} fell, thus reducing the CO_2 stimulus and limiting the rise in ventilation. Doses of ethamivan up to 12 mg/min were usually well tolerated, although mild flushing, itching, nausea and faintness were occasionally experienced and usually persisted throughout an infusion lasting 2.5 hr. Above 12 mg/min these symptoms were nearly always severe and a crushing pain in the chest also occurred in the subject of Fig. 2. In these circumstances ventilatory measurements are of no value. For long experiments we have therefore chosen a dose of 9 mg/min which has never caused unpleasant side-effects. Neither have we observed initial apnoea or bradycardia which have been described after relatively large single intravenous injections in rabbits (Ginzel, 1952) and dogs (G. R. Sharp, personal communication).

Fig. 3 illustrates an experiment in which alveolar P_{CO_2} was prevented from falling by increasing inspired P_{CO_2} during infusion of the drug. In this experiment arterial blood samples were also taken, and Fig. 3 shows that arterial P_{CO_2} varied by not more than 1 mm Hg until the last sample. As expected, there was a bigger increase in ventilation than when P_{CO_2} was allowed to fall. Ventilation reached an approximately steady state about 30 min after the infusion started and in magnitude was

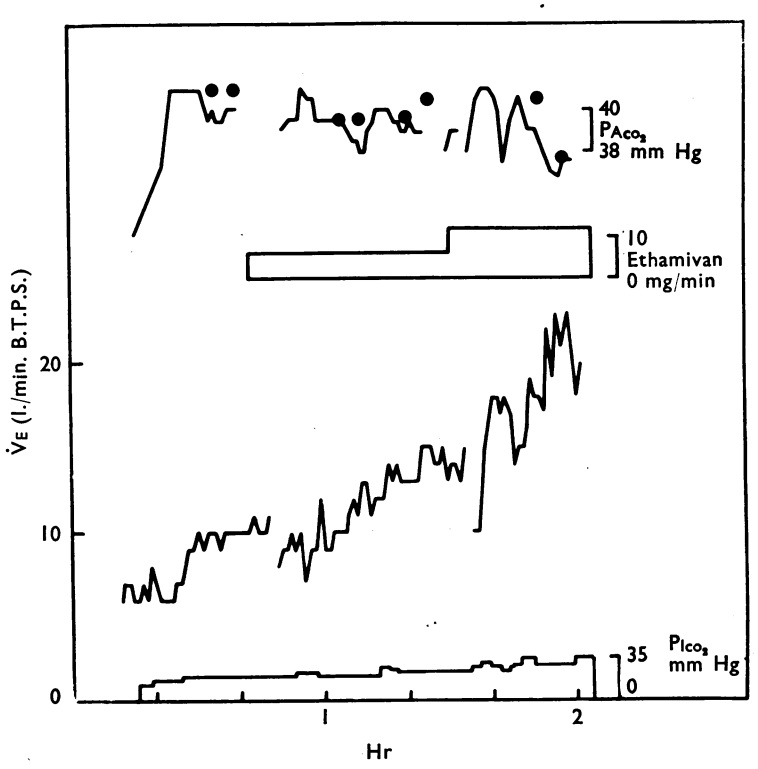


Fig. 3. Response to ethamivan at constant arterial P_{CO_2}. Upper record: P_{CO_2} of end-tidal gas (flow-bridge analyser) with arterial blood P_{CO_2} estimations (solid circles). Lower record: ventilation.

roughly proportional to the dose. Doses up to 12 mg/min can double ventilation, but the response may be less than this. It should be noted that the P_{CO_2} at which the experiment of Fig. 3 was carried out was rather low.

The results of the experiments in which different CO_2 mixtures were breathed are shown in Fig. 4. The effect of the drug was somewhat variable, but there are

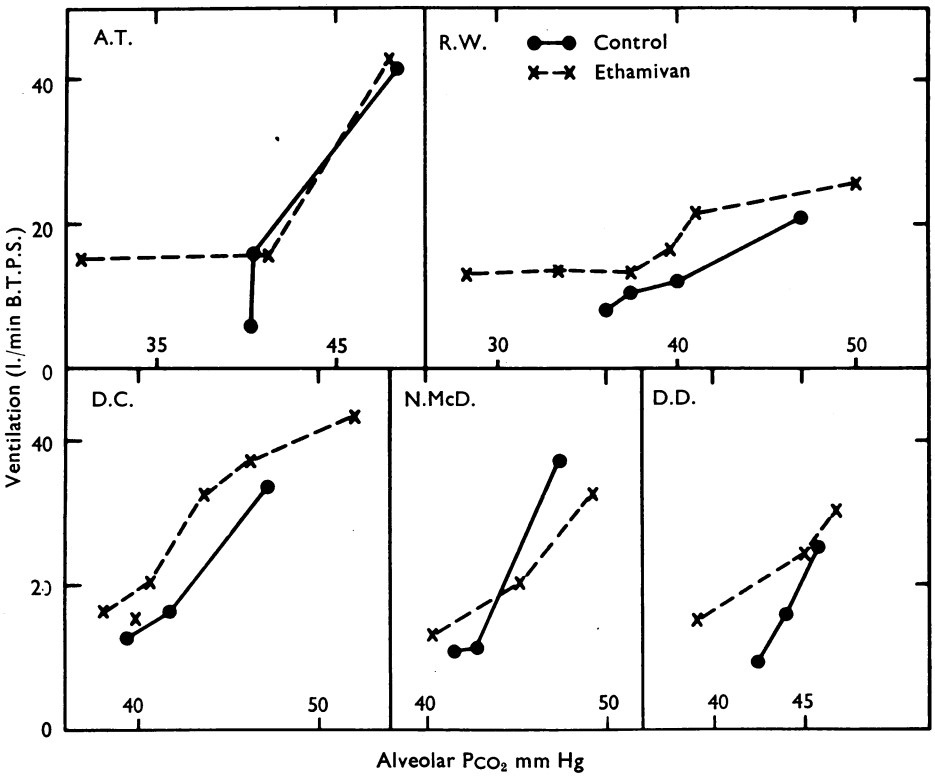


Fig. 4. Individual ventilatory responses to increases in alveolar P_{CO_2} during the infusion of saline and ethamivan respectively. Each circle and cross represents the mean of a 5-min steady state.

features common to all 5 experiments. Stimulation by the drug, shown by a shift of the points above and to the left of the saline points, was most evident at low values of P_{CO_2}. At the highest P_{CO_2} levels reached, however, no stimulation by the drug was found in any of the 5 subjects.

In any one experiment only a limited number of steady states can be studied. To obtain a more complete picture the 5 experiments have been combined in Fig. 5. For this purpose it is not enough to replot all data on the same co-ordinates, because different subjects have control CO_2-response lines with different intercepts on the P_{CO_2} axis (the parameter B of Lloyd, Jukes & Cunningham, 1958, or "CO_2 threshold") and different slopes of the $\dot{V}/P_{CO_2}$ line (CO_2 "sensitivity"). Differences

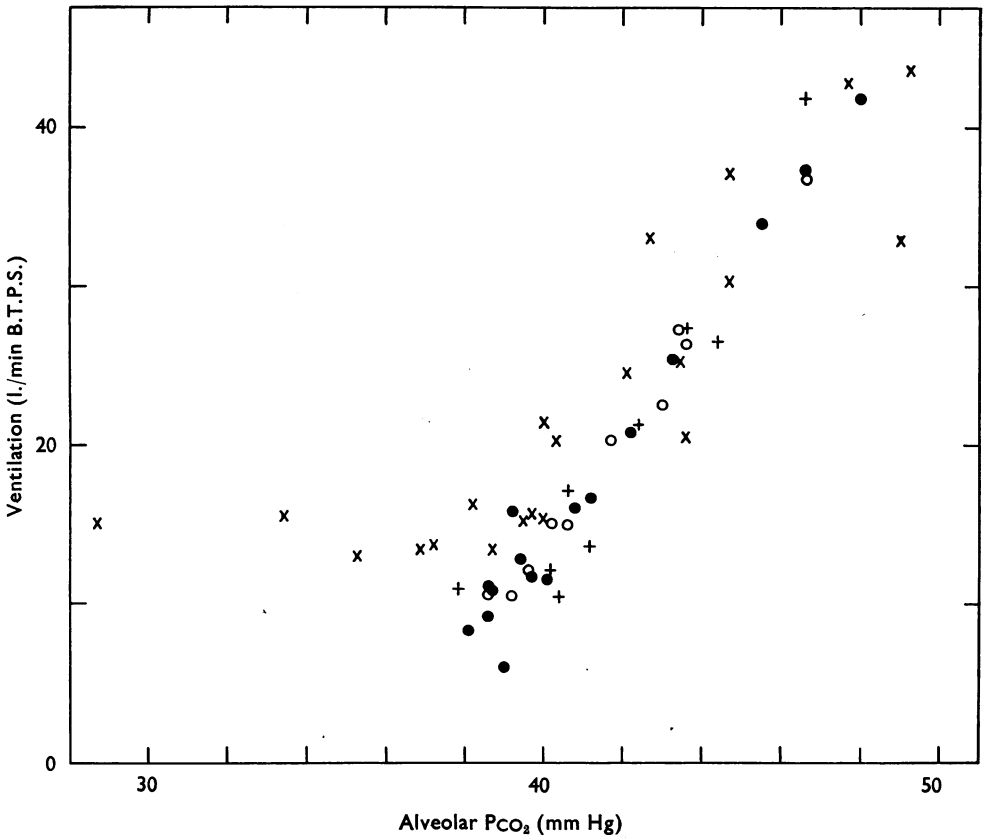


Fig. 5. The data of Fig. 4 have been replotted (● saline and X ethamivan) after altering the individual P_{CO_2} axes as described in the text. Data from the control subjects have been added after similar adjustment (O, control 1st period, and +, control 2nd period).

in body size, if not corrected, emerge as apparent differences in CO_2 sensitivity. To overcome these difficulties, the best-fitting straight line was calculated by the method of least squares for the " saline " points of each drug experiment and the parameter B and the slope of this line noted. The mean " saline " intercept and slope for the 5 subjects were calculated and the nearest convenient figures (B = 36 mm Hg, slope = 3.5 l./min/mm Hg) were taken. The P_{CO_2} axis was then altered for each subject in such a way that the individual " saline " regression lines all had this intercept and slope with respect to the new axis. New values for P_{CO_2} were then read off for both " saline " and ethamivan points, using the corrected P_{CO_2} axis. The data from the three control experiments, in which no drug was given, were dealt with in the same way, the points for the first period being substituted for " saline " points and those for the second period for the ethamivan points. Finally, all experiments were replotted on the same co-ordinates, using the original values for ventilation and the corrected values for P_{CO_2}.

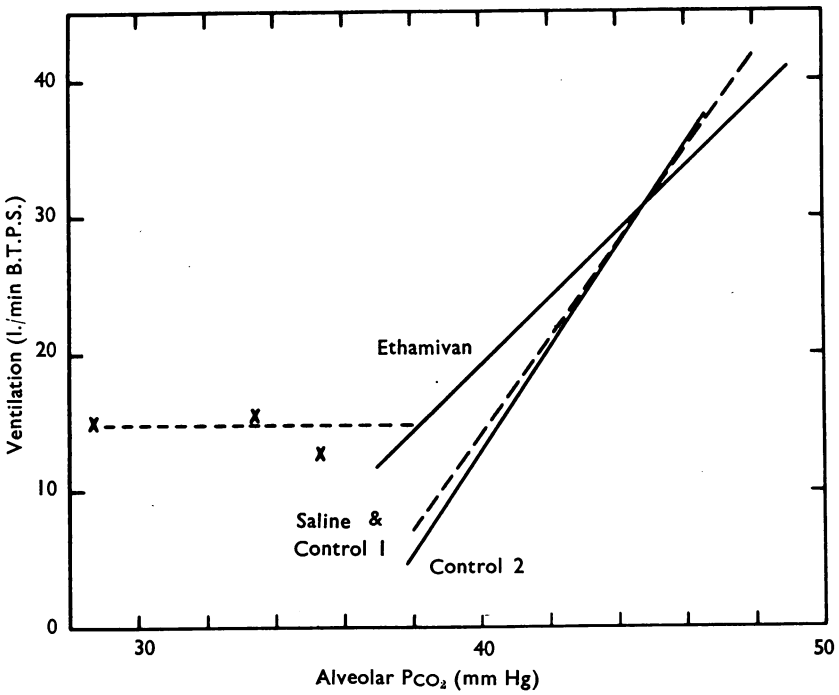


Fig. 6. Regression lines for the four groups of data shown in Fig. 5. The regression line for the ethamivan data was calculated after exclusion of the three points shown (X).

The result is shown in Fig. 5. Regression equations for the pooled results in the four groups are as follows. Regression lines are shown in Fig. 6.

Saline

$$\dot{V}_E = 3.46\ P_{CO_2} - 124.43$$
$$r = 0.982$$
$$B\ (P_{CO_2}\ \text{intercept}) = 35.95\ \text{mm Hg}$$

Ethamivan. (a) *Excluding 3 points with* $P_{CO_2} < 36$ *mm Hg*

$$\dot{V}_E = 2.42\ P_{CO_2} - 77.48$$
$$r = 0.912$$
$$B = 32.08\ \text{mm Hg}$$

(b) *Excluding 7 points with* $P_{CO_2} < 39$ *mm Hg*

$$\dot{V}_E = 2.51\ P_{CO_2} - 81.75$$
$$r = 0.879$$
$$B = 32.56$$

Control, 1st period

$$\dot{V}_E = 3.44\ P_{CO_2} - 123.60$$
$$r = 0.993$$
$$B = 35.93\ \text{mm Hg}$$

Control, 2nd period

$$\dot{V}E = 3.73\ P_{CO_2} - 136.27$$
$$r = 0.965$$
$$B = 36.51 \text{ mm Hg}$$

Tests of significance of differences in slope were carried out as follows:

(1) Ethamivan data with 3 points excluded and saline data.

(2) Ethamivan data with 7 points excluded and saline data.

(3) Ethamivan data with 3 points excluded and combined saline and control 1 data.

(4) Ethamivan data with 3 points excluded and combined saline, control 1 and control 2 data.

TABLE 1

END-TIDAL CO_2 TENSION, TOTAL VENTILATION, ALVEOLAR VENTILATION AND RESPIRATORY FREQUENCY IN SUBJECTS GIVEN INFUSIONS OF ETHAMIVAN

Subject	Saline (S) or ethamivan (E)	End-tidal P_{CO_2} mm Hg	$\dot{V}E$ l./min, B.T.P.S.	$\dot{V}A$ l./min, B.T.P.S.	f Breaths/ min
D. D.	S	42·5	9·2	5·8	12·8
		44·1	16·1	9·7	15·2
		45·8	25·5	17·5	16·2
	E	38·9	15·5	9·8	24·0
		45·0	24·6	17·2	32·0
		46·8	30·4	18·5	30·4
N. McD.	S	41·6	11·2	7·5	17·8
		42·7	11·6	8·2	15·4
		47·5	37·4	29·9	23·6
	E	40·3	13·3	9·3	17·8
		45·3	20·6	15·6	18·8
		49·3	32·9	26·2	21·0
A. T.	S	40·2	6·0	4·4	9·0
		40·4	15·9	12·9	12·0
		48·4	41·8	35·8	17·6
	E	30·8	15·0	12·3	14·0
		41·1	15·4	11·5	12·4
		48·1	42·8	34·2	20·2
D. C.	S	39·4	12·9	—	22·0
		41·8	16·7	—	20·6
		47·3	34·0	—	26·8
	E	38·0	16·3	—	42·4
		40·6	20·3	—	38·3
		43·7	33·0	—	43·7
		46·2	37·2	—	45·8
		52·1	43·7	—	30·0
		39·9	15·7	—	30·6
R. W.	S	36·0	8·3	—	17·0
		37·5	10·8	—	17·7
		40·1	11·7	—	16·3
		47·0	20·9	—	16·6
	E	28·2	12·9	—	26·0
		33·4	13·7	—	23·4
		37·5	13·4	—	19·0
		39·6	15·3	—	16·1
		41·0	21·5	—	20·0
		50·1	25·4	—	18·0

(5) Ethamivan data with 3 points excluded and control 2 data.

(6) Control 1 data and control 2 data.

None of these differences was significant at the 5% level; the most significant were differences (3) ($P=0.14$) and (4) ($P=0.11$).

Values for alveolar ventilation for subjects D.D., N.McD. and A.T. are given in Table 1. These follow total ventilation almost proportionately. The same three subjects had normal standard bicarbonate concentrations in samples of arterial blood; in the remaining subjects standard bicarbonate was not measured.

DISCUSSION

The data for the ethamivan periods can be considered as composed of two phases. Inspection of Fig. 5 shows that three ethamivan points lie to the left of the saline and control 1 CO_2 threshold, and suggests that ventilation in this region is independent of alveolar P_{CO_2}. When these three points are excluded, the slope of the regression line through the remaining 18 points is less than that for the saline data, but this difference is not statistically significant. Testing of the difference in r between the saline, control 1 and control 2 data shows that these form a homogeneous population, but even when these data are combined the difference between their slope and that of the ethamivan data is still not significant at the 5% level. When the seven ethamivan points below 39 mm Hg are excluded, the slope of the remaining 14 points is almost unchanged. We conclude that at low values of P_{CO_2} the ventilation during infusion of ethamivan remains constant with rising P_{CO_2} until the normal $\dot{V}/P_{CO_2}$ line is approached or actually reached. At levels of P_{CO_2} higher than this we have been unable to show that ventilation is significantly higher than during the control periods.

In these experiments we tried to exclude a hypoxic stimulus, so far as possible, by using inspired gas mixtures with a P_{O_2} of 350 mm Hg, and we estimate that alveolar P_{O_2} was of the order of 250 to 300 mm Hg although we did not measure it. The work of Lloyd *et al.* (1958) suggests that there is little change in slope of the $\dot{V}/P_{CO_2}$ line with rising P_{O_2} at levels of alveolar P_{O_2} above 150 mm Hg. On the other hand, pure oxygen may increase ventilation and was therefore avoided in the present study. It may fairly be assumed that the hypoxic drive was constant in all our experiments and of a magnitude quite insufficient to account for the ventilation of 12 to 16 l./min observed below a P_{CO_2} of 39 mm Hg.

Body temperature was not measured in these studies and a drug-induced rise in temperature could conceivably be responsible for some of the stimulation observed during the infusion of ethamivan. However, in this case one would expect stimulation to continue into the higher range of P_{CO_2} and this did not occur. We therefore conclude that at low values of P_{CO_2} (below 39 mm Hg on our arbitrary scale) ventilation is mainly supported by ethamivan during infusion of this drug.

Our failure to show significant stimulation by the drug at P_{CO_2} levels above 39 mm Hg may be due to the scatter of the ethamivan points which is evident in Fig. 5. The combined discomforts of ethamivan and hypercapnia make proper relaxation

difficult, and we ascribe much of the scatter to this cause. It is reasonably certain that the results in the higher range of P_{CO_2} are not artificial. That the drug does not lose its effect as infusion continues is indicated by three observations: (1) Side-effects such as faintness and itching frequently continue unchanged until the infusion is stopped; (2) no falling off in ventilation has been observed in experiments on hypoxia at low P_{CO_2} (to be published); and (3) apparent depression of respiration by the drug at high P_{CO_2} and high P_{O_2} has been converted to stimulation at the same P_{CO_2} when P_{O_2} was reduced (to be published).

It is, perhaps, of minor importance whether the drug component of ventilation disappears abruptly at around 39 mm Hg or whether it is progressively suppressed as P_{CO_2} increases above this level, as the regression lines suggest. Hesser (1949) found that impulses from the carotid bodies in anaesthetized dogs could be blocked by acute hypercapnia; although the frequency of impulses in the sinus nerve was unchanged or even increased, the carotid body component of ventilation ("chemoreceptor drive") was reduced while the "central drive" was increased. It was shown by Ginzel (1952) that in dogs ethamivan acts on both the medullary centre and the carotid body chemoreceptors, but he used considerably bigger relative doses of the drug than we have found to be tolerated in conscious human beings. If, in the doses we have used, ethamivan acts mainly on the carotid bodies, raising P_{CO_2} acutely might block the carotid body afferents as in Hesser's experiments. We would, however, contrast the effect of ethamivan in man with that of hypoxia, which is known to act mainly through the chemoreceptors. The first phase of the action of ethamivan, in which P_{CO_2} is driven below the threshold, is similar to that of acute hypoxia (Nielsen & Smith, 1951). Above the threshold, however, hypoxia results in an increased sensitivity to CO_2 whereas with ethamivan the sensitivity is either unchanged or reduced. It seems unlikely that CO_2 is itself responsible for the disappearance of the stimulation due to the drug; we have observed that ethamivan may stimulate in the presence of severe but compensated hypercapnia due to chronic pulmonary disease. It may be, therefore, that the effect of increasing P_{CO_2} is brought about by the acidosis it produces.

Two ethamivan points in Fig. 5 (P_{CO_2} 43.6 and 49.0 mm Hg) lie well below the control points and suggest that there might be depression of breathing by the drug. We have noted this effect in subsequent experiments to which reference has already been made. Although our results, so far, do not allow a definite statement regarding depression, this is a matter of some importance. It has been widely held among anaesthetists (Dundee, 1956) that analeptic drugs may be inactive or may even depress breathing in severe barbiturate poisoning, whereas they usually stimulate to some extent when poisoning is less severe. This view is partly based on some rather inconclusive work by Mousel & Essex (1941) and deserves further study. Patients in deep barbiturate coma, given (as they nearly always are) pure oxygen to breathe, present the conditions—hyperoxia and acute respiratory acidosis—which we have found to be associated with inactivity of, or possibly depression by, ethamivan.

An increase in total ventilation may not be accompanied by an increased alveolar ventilation if the respiratory frequency increases greatly. Table 1 shows, however,

that changes in total ventilation were accompanied by roughly proportional changes in alveolar ventilation in the 3 subjects in whom it was determined. Respiratory frequency behaved very variably, though in general the frequency was greater, for a given alveolar P_{CO_2} or total ventilation, when ethamivan was infused than when it was not. Some of the increase in total ventilation, therefore, was " wasted " in the respiratory dead space.

In our experiments we have used the biggest dose consistent with reasonable comfort of the subject. Our data on the effect of doses smaller than this are few, but suggest a stimulating action proportional to dose. If given clinically to patients who are less than fully conscious bigger doses might possibly be used without undesirable effects and with greater respiratory stimulation. We do not, however, wish to imply that our results in healthy subjects are directly relevant to the clinical use of this drug. Patients with respiratory depression, hypoxia, chronic hypercapnia, metabolic acid-base disturbance and mechanical ventilatory limitation present separate problems which we are investigating.

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