Effects of Ethamivan in Patients with Chronic Respiratory Disease

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ABSTRACT

Nineteen patients suffering from chronic respiratory disease were evaluated before, during and after ethamivan administration by serial measurement of arterial pH, pCO_2, plasma ethamivan levels and alveolar ventilation. Ethamivan was administered intravenously as a single injection of 50 mg. in five patients; as an injection of 25 mg./kg. in five patients; as an intravenous injection of (a) 50 mg. over 15 minutes and (b) 150 mg. over 15 minutes in five patients; and finally as an oral dose of 300 to 500 mg. in five patients.

Plasma levels of ethamivan became unmeasurable within 15 minutes of receiving the largest dose. Alveolar ventilation increased only in patients receiving the highest intravenous dose, and no significant changes in blood gases were elicited in any patient.

SOMMAIRE

Chez 19 malades souffrant d'affections respiratoires chroniques, on a étudié la fonction pulmonaire avant, pendant et après administration d'éthamivan, au moyen de mesures en série du pH artériel, du pCO_2, des concentrations plasmatiques d'éthamivan et de la ventilation alvéolaire. L'Ethamivan a été donné par voie intraveineuse, en une seule injection de 50 mg. chez cinq malades, en injection de 25 mg./kg. chez cinq malades, en injection I.V. de (a) 50 mg. sur un laps de temps de 15 minutes et (b) de 150 mg. en 15 minutes chez cinq malades et, enfin, par voie orale, aux doses de 300 à 500 mg. chez cinq malades.

Les concentrations sanguines d'éthamivan ne purent plus être mesurées 15 minutes après le début des plus fortes doses. La ventilation alvéolaire n'a augmenté que chez les malades qui avaient reçu les plus fortes doses intraveineuses. Le médicament n'a pas modifié sensiblement les pressions gazeuses du sang chez aucun malade.

A MAJOR complication of severe obstructive lung disease is underventilation of the alveolar spaces, with retention of carbon dioxide (CO_2) and the development of respiratory acidosis. In such patients augmentation of total ventilation is not necessarily associated with clearance of carbon dioxide from the body. An increased energy cost of breathing may be such that the oxygen consumption and CO_2 production of the respiratory apparatus may aggravate rather than improve the degree of acidosis when hyperventilation is induced.[1]

Nikethamide,[2] aminophylline,[3] salicylates,[4] progesterone[5] and carbonic anhydrase inhibitors[6] have all been shown to increase ventilation, and more recently ethamivan (vanillic diethylamide) has been demonstrated to augment the alveolar ventilation of normal individuals without appreciable systemic side effect.[7]

The use of such an agent in patients suffering from severe barbiturate intoxication would seem rational[8] but there have been conflicting reports as to its efficacy in the treatment of individuals suffering from mechanical impairment of the respiratory apparatus. Miller *et al.*,[9] Said and Banerjee,[10] and Aronovitch *et al.*[11] have suggested, on the basis of clinical studies, that this is a useful adjunct to the treatment of acute respiratory acidosis, while Rodman *et al.*,[3] Golub *et al.*[12] and Cherniack and Young[18] have indicated that changes in blood gases following the administration of ethamivan to emphysematous patients are insignificant.

Since we had available to us a technique for measuring plasma levels of ethamivan, the present study was undertaken to correlate such plasma levels to measurements of respiratory function.

METHODS

Studies were conducted on 19 hospital patients whose clinical diagnosis and respiratory function tests are summarized in Table I. One patient was studied twice, the ethamivan being administered by a different route on each occasion.

All measurements were made with the patient in the supine position, resting comfortably on a stretcher. Arterial blood collections were made from a Cournand needle or polyethylene catheter inserted into the femoral artery. Ethamivan was administered *via* an infusion of 5% glucose in distilled water established in a peripheral arm vein; venous blood was collected from the contralateral vein.

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TABLE I.—PULMONARY FUNCTION

Patient No.	Age	Sex	Arterial pCO_2 (mm.Hg)	Total lung capacity*	Maximum breathing capacity*	Diffusing capacity** (c.c. per min. per mm. Hg)	Forced expiratory volume 1/2 sec.***	Functional residual capacity (l.)	Residual volume (l.)	Diagnosis
1	39	M	57	58	59	14.9	75%	2.07	1.39	Respiratory muscle paresis due to old poliomyelitis
2	47	F	43	108	67	9.7	36%	2.73	1.83	Asthma, bronchitis
3	63	M	49	111	18	7.0	15%	4.08	3.50	Emphysema
4	67	M	51	72	96	11.1	66%	2.42	1.78	Infectious polyneuritis
5	77	M	42	135	40	7.8	23%	5.15	5.04	Chronic bronchitis, emphysema
6	53	M	64	113	33	6.5	21%	3.97	3.08	Chronic bronchitis, emphysema
7	59	M	55	100	23	6.9	26%	3.50	3.16	Chronic asthmatic bronchitis, emphysema, cor pulmonale
8	63	M	42	93	54	9.5	32%	3.27	1.76	Chronic asthmatic bronchitis, pleural effusion
9	70	M	75	79	14	2.4	14%	3.52	2.42	Anemia, emphysema, cor pulmonale
10	52	M	48	111	56	14.0	22%	5.05	4.06	Asthmatic bronchitis
11	66	M	50	139	12	6.2	10%	6.94	6.20	Chronic bronchitis, emphysema
12	41	M	64	121	8	4.7	10%	5.46	4.74	Chronic bronchitis, emphysema
13	59	M	95	136	19	5.0	12%	7.09	5.92	Emphysema, cor pulmonale, CO_2 narcosis
14	75	M	39	107	25	4.4	23%	3.46	2.71	Chronic asthmatic bronchitis
15	63	M	52	104	12	2.3	12%	3.47	2.36	Asthma, emphysema, cor pulmonale
16	56	M	66	136	19	5.0	12%	5.88	5.23	Emphysema, cor pulmonale
17	70	M	54	100	16	6.0	20%	3.98	3.57	Emphysema, cor pulmonale
18	55	M	39	126	105	5.9	49%	5.19	1.84	Pulmonary fibrosis, emphysema
19	60	M	53	88	37	4.6	25%	3.67	3.13	Severe diffuse bronchiectasis

*% of predicted.
**Steady state method (Bates, D. V. *et al.*: *J. Physiol.*, 129: 237, 1955).
***Expressed as % of forced vital capacity.

Room air was breathed through a Rudolph two-way breathing valve with a Bennett seal mouthpiece, and expired air was collected in Douglas bags.

Four groups of five patients received the drug by different dosages and routes as follows: (a) orally, in a dose of 300 to 500 mg., (b) by a single intravenous injection of 50 mg. over a period of one minute, (c) by a single intravenous injection of 2.5 mg./kg. over a period of one minute, and (d) by an intravenous infusion of 50 mg. and 150 mg. either by a continuously observed intravenous drip or by a Harvard infusion pump (Model 600-900). This infusion was given over two periods, each of 15 minutes' duration. Control injections and infusions of normal saline were given in identical fashion to a number of patients receiving the drug intravenously.

Samples of arterial and venous blood were withdrawn at intervals before and after any type of administration for analysis of blood gas content and ethamivan concentration. Carbon dioxide and oxygen tensions were determined with an Epsco Blood Parameter Analyser Model 101, and pH on the Astrup apparatus. Expired air was analyzed for oxygen with a Beckman Model E-2 Oxygen Analyzer, and for carbon dioxide with a Godart Capnograph. Whole blood buffer base was estimated from the Singer-Hastings nomogram[13] and alveolar ventilation and physiological dead space were calculated from conventional equations. After centrifugation and separation of red cells, plasma was mixed with 1.5 volumes of chloroform and the ethamivan concentration, which gives a characteristic peak at 280 mμ., was measured in the chloroform extract using a Beckman D.U. spectrophotometer. Its presence in the extract was confirmed by ascending paper chromatography using Folin-Ciocalteau reagent and 10% sodium carbonate for colour development.

The control measurements represent means of three replicates taken prior to the injection of ethamivan. A standard two-way analysis of variance was carried out to obtain the laboratory variation in these measurements and is represented in the tables as 95% confidence limits. Mean values following the injection of ethamivan which lie outside the 95% confidence limits of the control values were then analyzed by a paired t-test comparing the individual values at various times with the control values obtained from the same patient. Values differing significantly are indicated in the appropriate tables.

RESULTS

An example of the data chart obtained from a single study is shown in Table II. Control measurements prior to injection were regularly made from two samples of blood and three samples of expired air.

TABLE II.—ETHAMIVAN CASE REPORT No. 17 (b)

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Name..... Hosp. No..... Sex..... Age..... Weight..... Date.....

Hematocrit 49% Ethamivan dosage 2.5 mg./kg. = 136 mg. Barometric Pressure 699 mm. Hg

Diagnosis Chronic bronchitis, emphysema Room Temperature 25 C.

	Arterial blood						*Expired gas*							
Time	*pO_2 mm. Hg*	*pCO_2 mm. Hg*	*pH*	*O_2 Sat. %*	*Ethamivan concentration µg./ml.*	*Buffer base*	*CO_2 production (l)*	*O_2 uptake (l)*	*Found vol. (l)*	*R.R.*	*A-a gradient mm. Hg*	*Alveolar ventilation (l)*	*R.Q.*	*Remarks*
10:34	38	68	7.39	70	0.0	62	.165	.217	5.78	16	18	2.19	.75	
10:36							.178	.237	6.59	16		2.26	.75	
10:38	35	69	7.40	70	0.0	63	.172	.211	6.00	15.5	25	2.25	.80	
10:40	Ethamivan injected over one minute													
10:42	47	61	7.44	84	3.2	62	.377	.377	13.97	24	33	5.44	1.0	Marked pruritus, muscle twitching (extreme)
10:43	50	61	7.44	86	2.7	62								Burning pain in arm on side of injection
10:44	50	62	7.45	86	2.8	65								
10:46	50	65	7.42	85	2.7	63	.325	.325	11.71	23	26	4.47	1.0	
10:51	46	65	7.43	83	2.7	64	.200	.241	7.21	17	20	2.75	.82	
10:56	43	64	7.43	79	2.1	64								
11:11	41	67	7.41	76	1.5	63								Dysphoria

The effects of 300-500 mg. of ethamivan taken orally by five patients are shown in Table III. The control figures represent the mean values of all determinations carried out in the five patients before drug administration.

alveolar ventilation may be related to a concomitant increased production of carbon dioxide. The level of ethamivan in the plasma which was less than half the original concentration at the end of three minutes is shown graphically in Fig. 1.

TABLE III.—THE EFFECT OF ORAL ETHAMIVAN IN FIVE PATIENTS

Dose		*Control*	*5 minutes*	*10 minutes*	*15 minutes*	*30 minutes*	*60 minutes*	*120 minutes*	*180 minutes*
Oral	pO_2 (mm. Hg)	60.5 ± 5.4	59	60	62	61	63	63	63
300-500 mg.	pCO_2 (mm. Hg)	53.5 ± 4.7	54	54	54	54	53	53	53
	pH	7.37 ± .035	7.37	7.37	7.37	7.37	7.36	7.36	7.37
	CO_2 production (l./min.)	.167 ± .055		.194			.176		
	O_2 uptake (l./min.)	.202 ± .035		.224			.200		
	Respiratory rate/min.	16 ± 3.3		16			16		
	Alveolar vent. (l./min.)	2.99 ± 1.18		3.47			3.07		
	Plasma ethamivan conc. (µg./100 ml.)		0	0	0	0	0	0	0

± = 95% confidence limits for the whole series.

There were no statistically significant changes in any of the measurements either of blood gases or expired air. The suggestive increase in alveolar ventilation may have been balanced by the increased oxygen consumption and CO_2 production, explaining the lack of change in arterial blood gases. No ethamivan was detected by spectrophotometric analysis of plasma in three hours following ingestion; however, paper chromatography did demonstrate levels of less than 1 µg./ml. in the 15- and 30-minute samples.

In Table IV the effect of a single intravenous injection of 50 mg. of ethamivan in five patients is summarized. Once again there are no alterations in the mean values for either blood gases or expired air, and minor non-significant increases in

As shown in Table IV, a single intravenous injection of ethamivan in a dosage of 2.5 mg./kg. was followed by a significant ($p < 0.05$) increase in respiratory rate and by a suggestive but not statistically significant rise in alveolar ventilation. In 10 minutes alveolar ventilation had returned to control levels. Correspondingly, the oxygen tension rose suggestively at the end of five minutes but by 10 minutes was not significantly elevated and had returned to the control levels in 30 minutes. The pH did not change, but carbon-dioxide production and oxygen consumption apparently did increase because of the greater muscular respiratory effort. This dose of ethamivan therefore did produce a significant increase in respiratory rate, and oxygen uptake along with oxygen tension rose; however,

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TABLE IV.—THE EFFECTS OF ETHAMIVAN ON RESPIRATION AND BLOOD GASES WHEN GIVEN AS A SINGLE INJECTION TO FIVE PATIENTS

Dose		Control	After injection: 1 minute	2 minutes	3 minutes	5 minutes	10 minutes	15 minutes	30 minutes
50 mg.	pO_2 (mm. Hg)	60 ± 5.4*	62	61	60	60	60	59	59
	pCO_2 (mm. Hg)	52 ± 4.7	50	50	51	53	53	54	54
	pH	7.405 ± .035	7.43	7.42	7.42	7.42	7.44	7.42	7.41
	CO_2 production (l./min.)	.197 ± .055	.233		.203	.199			
	O_2 uptake (l./min.)	.229 ± .035	.220		.222	.224			
	Respiratory rate/min.	19 ± 3.3	26		25	26			
	Alveolar vent. (l./min.)	3.34 ± 1.18	4.08		3.49	3.22			
	Plasma ethamivan conc. (μg./ml.)	Mean	3.3	2.3	2.0	1.3	1.3	1.2	1.3
		S.D.	2.0	1.2	1.3	.8	1.1	1.1	1.1
2.5 mg./kg.	pO_2 (mm. Hg)	50.0 ± 5.4	54	55	55	57	54	53	51
	pCO_2 (mm. Hg)	63.5 ± 4.7	61	62	64	64	67	66	67
	pH	7.40 ± .035	7.43	7.43	7.41	7.39	7.40	7.40	7.41
	CO_2 production (l./min.)	.201 ± .055			.308		.212		
	O_2 uptake (l./min.)	.222 ± .035			.315		.247		
	Respiratory rate/min.	22 ± 3.3			27**		22		
	Alveolar vent. (l./min.)	3.01 ± 1.18			6.26		2.93		
	Plasma ethamivan conc. (μg./ml.)	Mean	13.9	6.7	4.5	3.4	3.2	2.5	1.3
		S.D.	9.0	3.3	2.0	2.6	2.1	1.1	1.1

*Indicates 95% confidence limits.
**$P < 0.02$.

the concomitant increase in carbon-dioxide production was such that there was a negligible change in pCO_2.

The mean levels of plasma ethamivan following this dose are shown in Fig. 1. The peak mean value of 11.3 μg./ml. is present in the one-minute sample and thereafter falls rapidly to 4 μg./ml. at three minutes, at which time an effect is still observed on blood gases and respiratory rate. After 10 minutes the mean plasma level was 2.6 μg./ml.

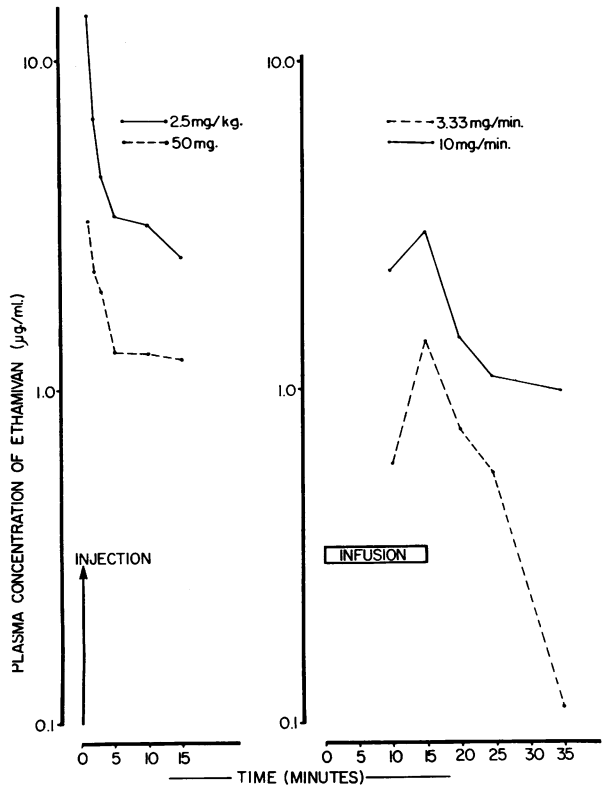

Fig. 1.—Plasma level following intravenous ethamivan.

and blood gas and expired-air measurements were unaltered from those obtained prior to injection.

Studies conducted on the effects of ethamivan continuously infused at two different rates for 15 minutes are tabulated in Table V. Each patient was given the infusion of 3.3 mg./min. for 15 minutes as well as the 10 mg./min. dose over an additional 15-minute period. The lower dose was always given first, followed by a 30-minute interval before the second infusion.

The dose of 3.33 mg./min. had no measurable effect. The infusion of ethamivan at 10 mg./min. did, however, result in a more rapid rate of breathing associated with increased metabolism and a suggestive augmentation of alveolar ventilation. These changes were evanescent, and determinations made one-half hour following the infusion corresponded to the base-line measurements.

Ethamivan plasma levels achieved during continuous infusion are graphically depicted in Fig. 1, where it can be seen that at both rates the concentration continues to rise during the period of infusion. Upon discontinuation the plasma level drops very rapidly, with a half-life which varies directly with the concentration of drug.

Respiratory effects are apparent at concentrations of greater than 4 μg./ml., a level at which the half-life of the drug is approximately three minutes. Sustained respiratory stimulation, therefore, can apparently only be achieved by continuous infusion.

The side effects noted by these patients are listed in Table VI. The most frequent complaints were elicited from the five patients receiving the large dose by single intravenous injection, and there is a distinct correlation between plasma ethamivan level and the frequency of these complaints. Four of these patients complained of nausea, and in one of them vomiting occurred. Four out of the five had muscle twitching; in one this was generalized

TABLE V.—THE EFFECTS OF ETHAMIVAN ON RESPIRATION AND BLOOD GASES WHEN GIVEN AS INTRAVENOUS INFUSION OVER 15 MINUTES IN FIVE PATIENTS

Dose		Control	After cessation of infusion: 10 minutes	15 minutes	20 minutes	30 minutes	45 minutes
10 mg. per min.	pO_2 (mm. Hg)	51.5 ± 5.4*	52	53	53	52	54
	pCO_2 (mm. Hg)	54 ± 4.7	53	53	53	54	53
	pH	7.42 ± .035	7.43	7.44	7.44	7.42	7.44
	CO_2 production (l./min.)	.203 ± .055	.217	.235		.207	
	O_2 uptake (l./min.)	.254 ± .035	.269	.290		.226	
	Respiratory rate/min.	20 ± 3.3	23	25**		22	
	Alveolar vent. (l./min.)	3.58 ± 1.18	3.91	4.17		3.47	
	Plasma ethamivan conc. (μg./ml.)	Mean	2.3	3.0	1.3	1.1	.98
		S.D.	1.4	2.0	1.2	1.6	1.0
3.33 mg. per min.	pO_2 (mm. Hg)	51.5 ± 5.4	52	51	51	51	52
	pCO_2 (mm. Hg)	54 ± 4.7	53	53	53	53	53
	pH	7.40 ± .035	7.43	7.42	7.42	7.42	7.43
	CO_2 production (l./min.)	.222 ± .055	.217	.224		.201	
	O_2 uptake (l./min.)	.268 ± .035	.226	.264		.247	
	Respiratory rate/min.	20 ± 3.3	21	21		20	
	Alveolar vent. (l./min.)	4.05 ± 1.18	3.96	3.84		3.50	
	Plasma ethamivan conc. (μg./ml.)	Mean	0.6	1.4	0.8	0.6	0.1
		S.D.	.18	1.1	.17	.12	.02

*Indicates 95% confidence limits.
**$P < 0.05$.

and persisted for almost five minutes. No side effects were noted following oral administration of the drug, and the side effects associated with continuous infusion were minimal.

TABLE VI.—SIDE EFFECTS RESULTING FROM ETHAMIVAN ADMINISTRATION

	Intravenous injection 2.5 mg./kg.	50 mg.	Intravenous infusion 50 mg./15 min.	150 mg./15 min.	Oral 300-500 mg.
Pruritus	1	1	1	1	0
Muscle twitch	4	1	0	0	0
Vertigo	2	0	0	0	0
Pain in arm on side of injection	3	2	4	2	0
Nausea	4	0	0	0	0
Vomiting	1	0	0	0	0
Dysphoria	4	1	0	0	0
Fatigue	4	1	0	0	0
Coloured lights	1	0	0	0	0
Buzzing in ears	1	0	0	0	0
Dizziness	2	2	0	0	0
Chest pain	0	0	1	0	0
Dyspnea	2	0	0	0	0
Weakness	0	1	0	0	0
Increased temperature	0	0	0	1	0
Total	29	9	6	4	0

DISCUSSION

The failure of ethamivan to produce a significant effect at the smaller dose levels used in this study is not surprising in view of the low plasma levels and the very rapid clearance of the drug from the plasma. The higher dosage levels of ethamivan did result in an increase in alveolar ventilation, although this was achieved with an increasing frequency of side effects.

In this series there were no changes in pCO_2 despite a suggestive increase of alveolar ventilation when high doses were used. The ventilatory response elicited in normal individuals by infusing ethamivan at a rate of 9 mg./min. has been shown by Anderton *et al.*[14] to be dependent upon the level of arterial CO_2 tension. They found that the hyperventilation induced by ethamivan at lower levels of pCO_2 was abolished when the arterial pCO_2 rose over 39 mm. Hg. Ethamivan, when infused at a time when the blood CO_2 level was artificially elevated by the breathing of CO_2, did not stimulate respiration any more than did an infusion of saline, and it may indeed have had a depressive effect.[14] In our emphysematous patients, most of whom had elevated levels of CO_2, a similar rate of infusion (10 mg./min.) appeared to stimulate rather than to depress ventilation. The carbon-dioxide production induced by the overbreathing, however, also rose and there was therefore no change in the arterial pCO_2.

The mechanical problem of moving air into the alveolar spaces may be aggravated by an increase in airway resistance which has been demonstrated to occur in conjunction with an elevated CO_2 tension.[15] This bronchoconstriction is apparently triggered either by the increased carbon dioxide or by an associated change in hydrogen-ion concentration.

Another explanation for the lack of change in CO_2 is that individuals with chronic compensated respiratory acidosis may not be able to rapidly alter the plasma tension of CO_2 because of an internal homeostasis between levels of CO_2 in the plasma and large carbon-dioxide reservoirs in other body compartments.[16]

Certainly, as pointed out by Miller *et al.*,[9] there is no need to stimulate the respiration of people with compensated acidosis characterized by modest increases in pCO_2 and normal levels of arterial pH. Such individuals function more efficiently at higher levels of carbon-dioxide tension because of more efficient CO_2 excretion in this range and are dyspneic if the level of pCO_2 is suddenly and artificially dropped. If decompensation has occurred and there is a lower than normal pH, respiratory centre stimulation might be indicated. We, however, failed to demonstrate a significant change in any of the indices of blood gas assessment at rates of ethamivan infusion which were increasing respiratory rate in such decompensated patients.

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The plasma ethamivan levels attained by continuous infusion at 10 mg. per minute were those previously demonstrated to occur with a higher single intravenous injection which was in turn associated with a very high incidence of undesirable side effects. These symptoms were similar in nature to those reported following the use of nikethamide,[2] suggesting that the compound in effective respiratory-centre dosage is a general cerebral stimulant. We have observed comatose hypercapneic patients (pCO_2 greater than 120 mm. Hg), undergoing continuous assistance with a mechanical ventilator, who were aroused by the injection of ethamivan without significant concomitant change in either pH or carbon dioxide tension—suggesting that the effect is one of direct cerebral stimulation.[17] In the already conscious patient the frequency of side effects restricts the dose that can be given as a single injection, and the only practical method of administration would appear to be by titration of a continuous infusion of the drug.

SUMMARY

In clinical problems of respiratory depression occurring in patients with normal pulmonary mechanics, e.g. following anesthesia and in barbiturate intoxication, the use of a stimulant such as ethamivan would seem rational. On the other hand, individuals with severe mechanical impairment of the lungs or thoracic cage are usually incapable of being effectively stimulated in such a way as to meaningfully alter the blood gases. Therapeutic attention should be primarily focused on measures to alleviate the obstruction or to improve the mechanically inefficient respiratory apparatus.

REFERENCES

1. RILEY, R. L.: *Ann. Intern. Med.*, **41**: 172, 1954.
2. DULFANO, M. J. AND SEGAL, M. S.: *J. A. M. A.*, **185**: 69, 1963.
3. RODMAN, T. *et al.*: *New Eng. J. Med.*, **267**: 1279, 1962.
4. TENNEY, S. M. AND MILLER, R. M.: *Amer. J. Med.*, **19**: 498, 1955.
5. TYLER, J. M.: *J. Clin. Invest.*, **39**: 34, 1960.
6. NAIMARK, A., BRODOVSKY, D. M. AND CHERNIACK, R. M.: *Amer. J. Med.*, **28**: 368, 1960.
7. MILLER, M. J., HAND, B. M. AND CRELLIN, J. A.: *Clin. Pharmacol. Ther.*, **2**: 689, 1961.
8. WHEELDON, P. J. AND PERRY, A. W.: *Canad. Med. Ass. J.*, **89**: 20, 1963.
9. MILLER, W. F. *et al.*: *J. A. M. A.*, **180**: 905, 1962.
10. SAID, S. I. AND BANERJEE, C. M.: *Amer. J. Med.*, **33**: 845, 1962.
11. ARONOVITCH, M. *et al.*: *Canad. Med. Ass. J.*, **85**: 875, 1961.
12. GOLUB, J. R. *et al.*: *Clin. Res.*, **11**: 234, 1963 (abstract).
13. SINGER, R. B. AND HASTINGS, A. B.: *Medicine (Balt.)*, **27**: 223, 1948.
14. ANDERTON, J. L. *et al.*: *Brit. J. Pharmacol.*, **19**: 142, 1962.
15. PARKER, J. C., PETERS, R. M. AND BARNETT, T. B.: *J. Clin. Invest.*, **42**: 1362, 1963.
16. SCHAEFER, K. E.: *Ann. N.Y. Acad. Sci.*, **109**: 772, 1963.
17. SPROULE, B. J., MILLER, J. M. AND TULLOCH, A. M.: Unpublished data.
18. CHERNIACK, R. M. AND YOUNG, G.: *Ann. Intern. Med.*, **60**: 631, 1964.