*Brit. J. Pharmacol.* (1964), **22**, 228–237.

# THE RESPIRATORY ACTION OF DICHLORPHENAMIDE

BY

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(*Received June* 27, 1963)

The action of dichlorphenamide has been investigated in three healthy subjects. After this drug had been given, ventilation increased while alveolar and oxygenated mixed venous $P_{CO_2}$ both fell. The magnitude of these changes was closely related to the degree of acidosis produced by the drug. The effect of intravenous acetazolamide, tested in one subject, was qualitatively similar to that of orally administered dichlorphenamide. Respiratory responses to carbon dioxide were studied in two of the subjects and in one of them the metabolic acidosis was sufficient to account for the short-term effect of dichlorphenamide on respiration.

Dichlorphenamide, a powerful inhibitor of carbonic anhydrase, was found by Thompson, Richardson & Wingo (1958) and by Naimark, Brodovsky & Cherniack (1960) to increase the minute volume (ventilation), reduce arterial carbon dioxide tension($P_{CO_2}$), increase arterial oxygen saturation and improve symptoms in patients with chronic respiratory insufficiency. Subsequently several reports (Simpson, 1961 ; Harris, 1961 ; Wahl & McCarthy, 1961 ; McNicol & Pride, 1961 ; Ahuja, 1961 ; Thompson, 1961 ; Christensen, 1962) have testified to the drug's ability to improve gas tensions in arterial blood, though symptoms tend usually to be replaced by side-effects.

The mechanism by which the respiratory effects of dichlorphenamide are produced is still controversial. In view of the widespread clinical use of this drug we have investigated its mode of action in healthy volunteers.

## METHODS

Gas mixtures were supplied to the subjects, and their ventilation measured, by the method of Cunningham, Cormack, O'Riordan, Jukes & Lloyd (1957). Alveolar gas was collected by the end-tidal sampling method of Anderton & Harris (1963). Oxygenated mixed venous $P_{CO_2}$ was estimated by a rebreathing method (see below). Capillary blood obtained by finger-prick, the hand having been vasodilated by immersion in warm water, was used for estimation of *p*H, $P_{CO_2}$ and standard bicarbonate concentration by the micromethod of Anderson, Engel, Jørgensen & Astrup (1960). All gas analyses were made by the Haldane method.

### *Determination of oxygenated mixed venous* $P_{CO_2}$

The rebreathing method of Campbell & Howell (1959) is now widely used to estimate oxygenated mixed venous $P_{CO_2}$. In its conventional form it allows this determination to be made only during the breathing of room air. We have modified the method so that oxygenated mixed venous $P_{CO_2}$ can be estimated during the inspiration of any desired carbon dioxide mixture.

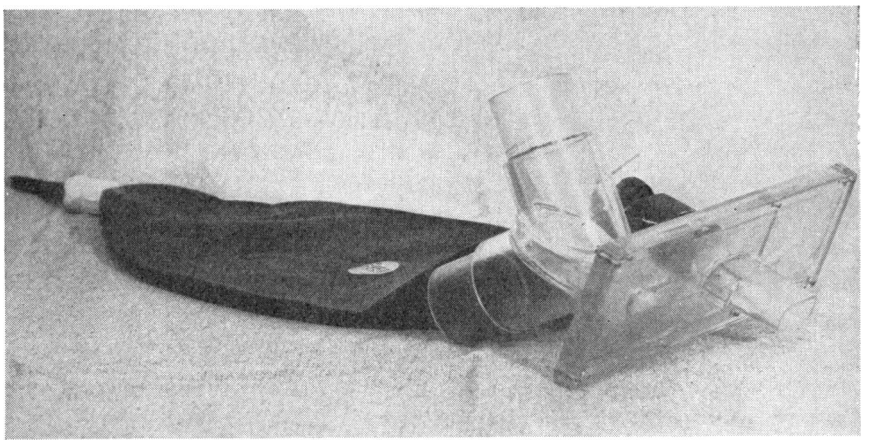

Fig. 1. Respiratory valve and rebreathing bag mounted on slide opposite mouthpiece.

The apparatus consists of a modified "Cormack" valve (Cunningham, Johnson & Lloyd, 1956) mounted side-by-side with a rebreathing bag on a Perspex sheet; this slides over a second sheet which carries the mouthpiece in its centre. Stops are arranged so that either the bag or the valve can be rapidly aligned with the mouthpiece (Fig. 1).

The procedure used must be one which disturbs the natural pattern of breathing as little as possible: we adopted the following. The first mixture of carbon dioxide in 50% oxygen

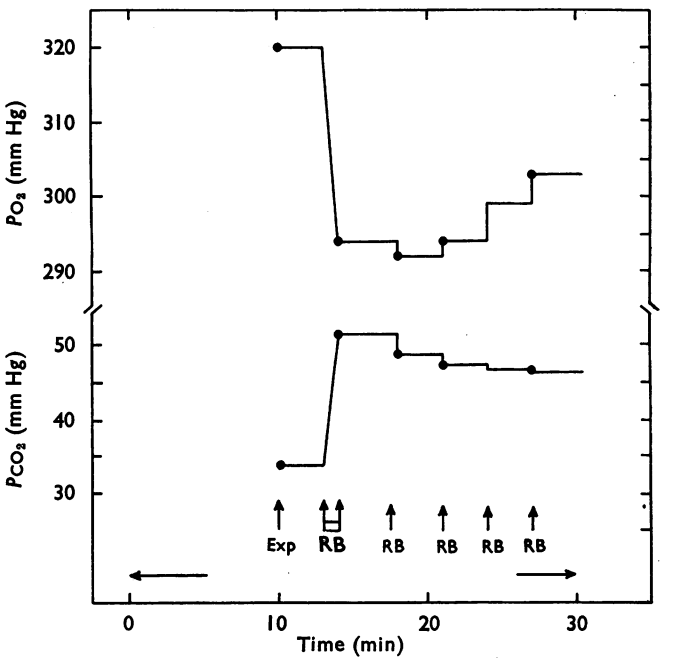


Fig. 2. $P_{O_2}$ and $P_{CO_2}$ within the rebreathing bag, measured at intervals during a period starting with a single expiration (Exp) and with rebreathing (RB) as described in text. During the 30 min period shown on the abscissa (horizontal arrows) the gas mixture inhaled by the subject, except when connected to the rebreathing bag, had a $P_{O_2}$ of 350 mm Hg and a $P_{CO_2}$ of 20 mm Hg.

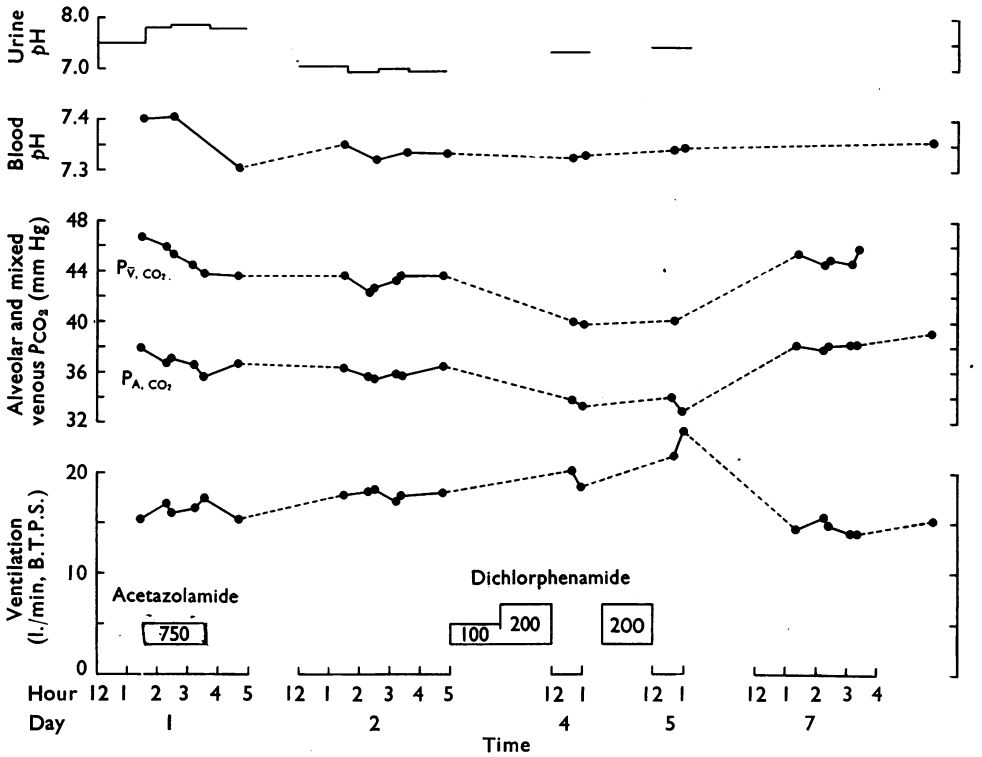


Fig. 3. From above downwards, urinary *p*H, finger-prick blood *p*H, oxygenated mixed venous ($\bar{V}$) and alveolar (A) $P_{CO_2}$, and ventilation, in subject J.A.R.S. All observations were made during inhalation of the same gas mixture ($P_{O_2}$ 350 mm Hg, $P_{CO_2}$ 20 mm Hg). Acetazolamide (750 mg intravenously) and dichlorphenamide (100 and 200 mg, orally) were given during the periods indicated.

in a given experimental session was breathed for 3 min ; the subject was then asked to inspire deeply, the empty bag moved across and he expired into it. He rebreathed quietly from the bag for 1 min and was switched back to the valve at the end of an expiration. At 2 min intervals thereafter he took three deep breaths from the bag, starting with an inspiration and ending with an expiration ; this manœuvre took about 15 sec to complete. He was then immediately returned to the valve. Fig. 2 shows that after four such three-breath manœuvres the $P_{CO_2}$ in the bag was virtually constant. The $P_{O_2}$ was not constant, but was climbing well above the level needed to saturate the mixed venous blood. Three min after the fourth three-breath period the collection of alveolar gas was started and was continued for 5 min while ventilation was measured. At the end of the 5th min, three more breaths were taken from the bag, and a sample obtained from the bag for analysis. The next inspired mixture of carbon dioxide in 50% oxygen was then started and the whole procedure repeated, except that the initial period of rebreathing lasting 1 min was omitted.

This procedure differs in several respects from the standard one and it may be particularly asked whether the bag $P_{CO_2}$, although virtually constant after four three-breath manœuvres, may be not equal to the oxygenated mixed venous $P_{CO_2}$ but somewhat lower than this. We have done experiments which indicate that if there is a discrepancy, this is unlikely to be greater than about 1 mm Hg, but rigorous proof of this is hard to devise. However, our oxygenated mixed venous $P_{CO_2}$ averaged 6 to 8 mm Hg higher than the alveolar $P_{CO_2}$ and previous work (Anderton, Cowie, Harris & Sleet, 1962) has shown good correlation between

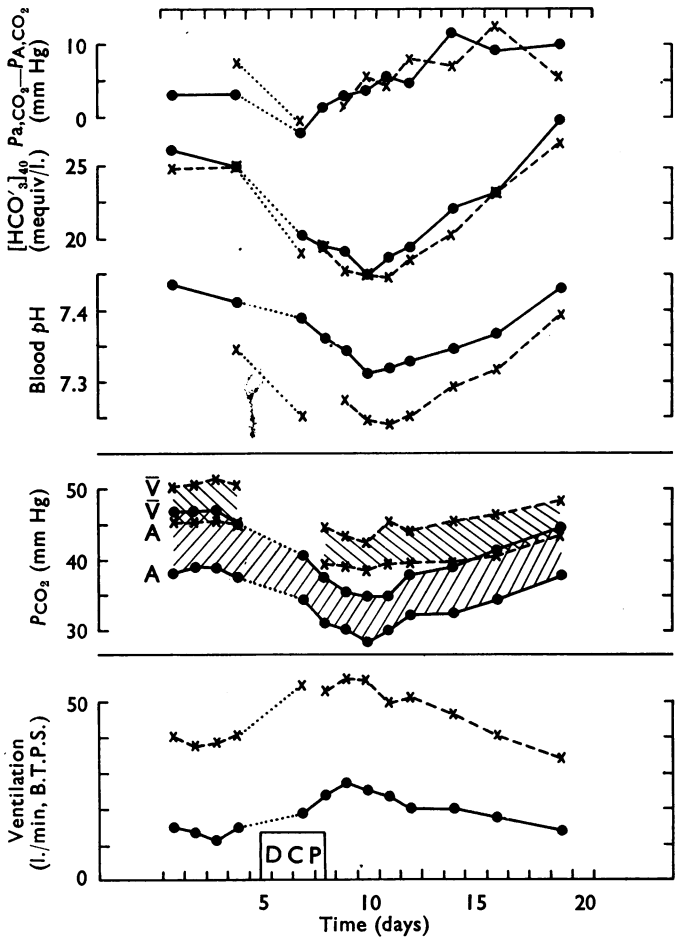


Fig. 4. From above downwards, $P_{CO_2}$-difference between finger-prick blood (symbolized $P_{a,CO_2}$ for brevity) and alveolar gas ($P_{A,CO_2}$), standard plasma bicarbonate concentration ($[HCO'_3]_{40}$ finger-prick blood *p*H, alveolar (A) and mixed venous ($\bar{V}$) $P_{CO_2}$, and ventilation, in subject K.B.S. ●——● during inhalation of low carbon dioxide mixture; × – – – × during inhalation of high carbon dioxide mixture. Dichlorphenamide (DCP, 600 mg) was given orally over the period indicated.

alveolar and arterial $P_{CO_2}$ in our hands. Since the oxygenated mixed venous/arterial $P_{CO_2}$ difference was found by Campbell & Howell (1959) to average 6 mm Hg, our own oxygenated mixed venous $P_{CO_2}$ does not appear to have been seriously underestimated.

*Procedure*

The subjects were studied in the early afternoon, after having fasted for at least 3 hr, and were semirecumbent on a comfortable bed. During ventilatory measurements they were required to read and were not allowed to fall asleep. Subject J. A. R. S. breathed the same gas mixture during each experiment ($P_{O_2}$ 350 mm Hg, $P_{CO_2}$ 20 mm Hg) and measurements were made of the ventilatory responses to an intravenous infusion of 750 mg of acetazolamide and to the oral administration of 500 mg of dichlorphenamide taken over 72 hr. Subjects K. B. S. and B. M. were studied by a different procedure. On each experimental day they first breathed a gas mixture of the composition of that used for J. A. R. S. for 20 min, during

the last 5 min of which ventilation was measured and samples of alveolar gas and capillary blood were taken. At 20 min a sample of rebreathed gas was taken for oxygenated mixed venous $P_{CO_2}$ estimation. The composition of the inspired gas was then changed ($P_{O_2}$ 350 mm Hg, $P_{CO_2}$ 35 mm Hg) and the procedure repeated. On the first five experimental days K. B. S. was given an inspired $P_{CO_2}$ of 40 mm Hg during the second period; after the drug was given his ventilation became so high at this inspired $P_{CO_2}$ that it was reduced to 35 mm Hg on subsequent days.

## RESULTS

Intravenous infusion of 750 mg of acetazolamide (Fig. 3) was followed by a rise in urinary *p*H and a fall in blood *p*H. Ventilation rose a little and alveolar and oxygenated mixed venous $P_{CO_2}$ both fell; these changes were more marked on the day after the infusion. After dichlorphenamide the rise in ventilation and the fall in alveolar and oxygenated mixed venous $P_{CO_2}$ became more pronounced, although the blood *p*H did not fall after the first day. Ventilation and $P_{CO_2}$ had

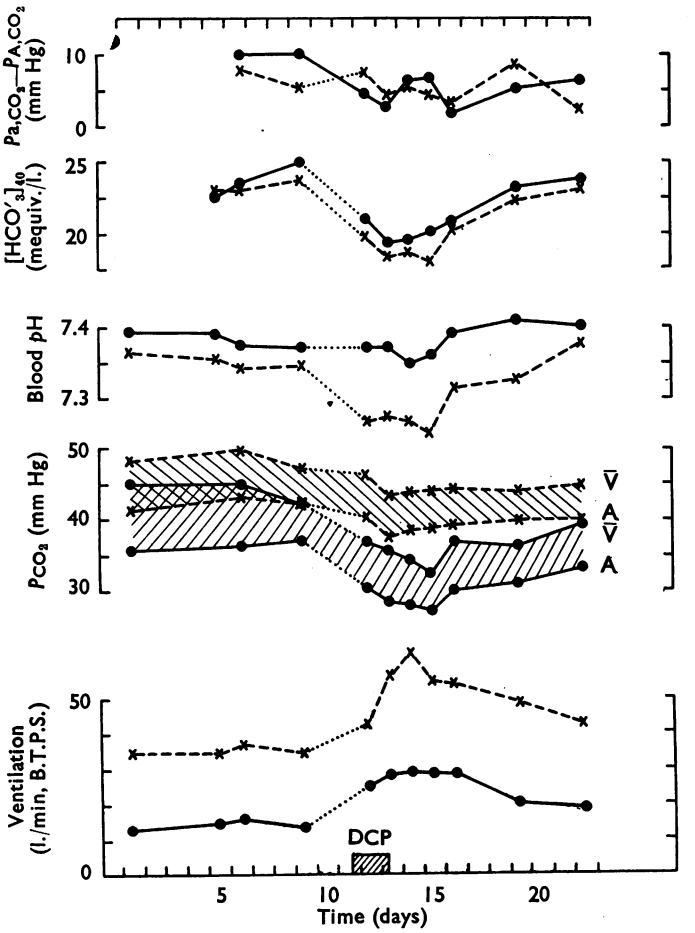


Fig. 5. Results with subject B.M., in an experiment similar to that illustrated in Fig. 4, except that 400 mg of dichlorphenamide was given.

returned to control values on the 3rd day after dichlorphenamide ; blood *p*H had not regained its control value, but only one determination of this was made (on the 17th day after the drug) and may have been erroneous.

Figs. 4 and 5 show the results in K.B.S. and B.M. Oral dichlorphenamide produced in both a metabolic acidosis (indicated by a fall of from 5.0 to 7.5 mequiv/1. in standard bicarbonate concentration), a marked rise in ventilation, a fall in alveolar $P_{CO_2}$ at each inspired $P_{CO_2}$ and a similar fall in oxygenated mixed venous $P_{CO_2}$. In K.B.S., but not in B.M., there was a tendency for the $P_{CO_2}$ difference between capillary blood and alveolar gas to increase after the drug had been given. On each day a pair of values for both ventilation and alveolar $P_{CO_2}$ was obtained, and from these the $P_{CO_2}$/response line could be drawn. Fig. 6 shows, for each subject, the

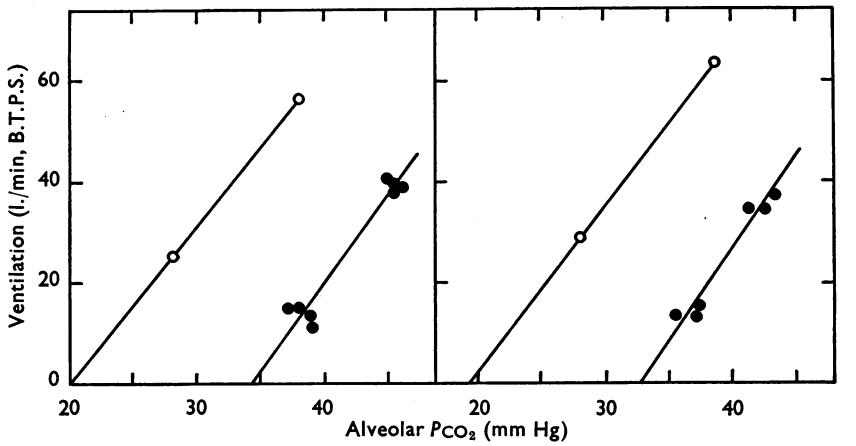


Fig. 6. Ventilation/$P_{CO_2}$ lines, before dichlorphenamide (●) and during acidosis produced by the drug (○), for subjects K.B.S. (left) and B.M. (right). The intercept on the $P_{CO_2}$ axis is parameter "B" in Fig. 7.

control $P_{CO_2}$/response line and that obtained at the height of the metabolic acidosis. In each there is a parallel shift to the left. The intercept on the $P_{CO_2}$ axis was calculated from each day's data and in Fig. 7 this value is plotted against the corresponding plasma bicarbonate concentration.

## DISCUSSION

### *The cause of the increase in ventilation after dichlorphenamide*

The metabolic acidosis caused by carbonic anhydrase inhibitors is one obvious means by which these drugs can stimulate respiration. Naimark *et al.* (1960) suggested that another factor was involved, namely an increase in $P_{CO_2}$ in the tissues (and therefore in the respiratory centre), resulting from impaired carbon dioxide transport and occurring at the same time as a fall in arterial $P_{CO_2}$. Cain & Otis (1961), who gave relatively large doses of acetazolamide to dogs, showed that a rise in mixed venous and tissue $P_{CO_2}$ may accompany a fall in arterial $P_{CO_2}$ and attributed this to inhibition of carbonic anhydrase in the red cells. Strömme & Fog (1962) showed that therapeutic doses of acetazolamide may impair carbon dioxide output

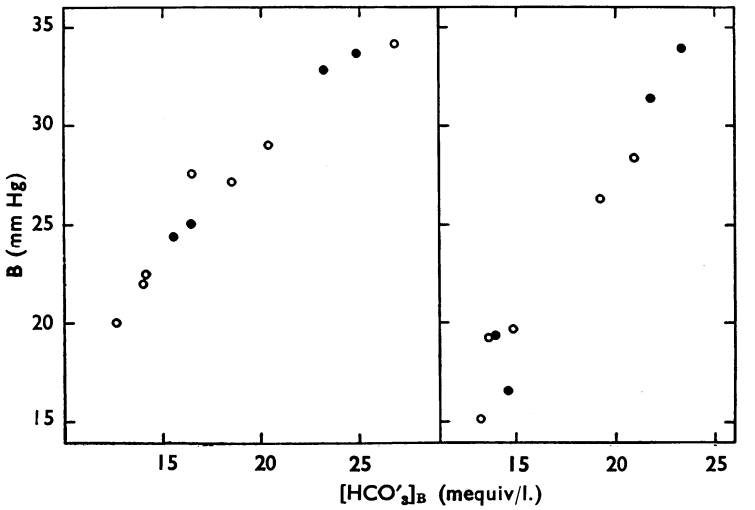


Fig. 7. Relationship of ventilation/$P_{CO_2}$ intercept (B) to plasma bicarbonate concentration ($[HCO'_3]_B$), at the same $P_{CO_2}$, before and during ingestion of dichlorphenamide (●) and after ingestion (○). for subjects K.B.S. (left) and B.M. (right). For both left-hand and right-hand plots, r=0.98

during voluntary hyperventilation in man ; presumably this also is due to an action on the red cells. The infusion of acetazolamide in J.A.R.S. was given to see whether the acute rise in mixed venous $P_{CO_2}$ demonstrated by Cain & Otis (1961) could be reproduced after a therapeutic dose in man. Neither the infusion nor the subsequent oral administration of dichlorphenamide produced this effect. A similar fall in both alveolar and oxygenated mixed venous $P_{CO_2}$ occurred in K.B.S. and B.M. after dichlorphenamide.

The interpretation of this finding must take account of the meaning of oxygenated mixed venous $P_{CO_2}$ as estimated by the rebreathing method. This value is the $P_{CO_2}$ of fully oxygenated blood with the same carbon dioxide content as mixed venous blood, and is normally a few mm Hg higher than the true mixed venous $P_{CO_2}$, the discrepancy depending on the oxygen saturation of mixed venous blood and the carbon dioxide-dissociation curves of reduced and oxygenated blood. It is therefore necessary to inquire whether dichlorphenamide, by altering carbon dioxide-dissociation, might have concealed a rise in mixed venous $P_{CO_2}$ if this did occur. No information is available on the effect of carbonic anhydrase inhibition on the carbon dioxide-dissociation curve. Presumably it has no effect provided that adequate time is allowed for equilibration of blood at different values of $P_{CO_2}$. The time available for gas exchange across the wall of the pulmonary capillary is, however, of the order of 1 sec and this might well be too short for appreciable dissociation of bicarbonate to occur as mixed venous blood becomes oxygenated. Despite partial compensation by increased breakdown of carbamino groupings, the mixed venous $P_{CO_2}$ would therefore be overestimated less during the action of dichlorphenamide than under normal conditions ; our rebreathing method might thus have concealed a rise in mixed venous $P_{CO_2}$.

Were this so, one might expect to find other evidence of disturbed carbon dioxide transport in the form of a $P_{CO_2}$ gradient beween arterial blood and alveolar gas (Shepard, Donoso, Killick, Cherniack, Johns & Riley, 1954; Cranston, Sanderson & Stapleton, 1955; Berliner & Orloff, 1956). In one of our subjects such a gradient was observed (K.B.S., Fig. 4) but in the other there was no definite change after dichlorphenamide. This question must therefore remain open in the absence of information about the effect of dichlorphenamide upon carbon dioxide-dissociation. However, our finding of similar changes in oxygenated mixed venous and alveolar $P_{CO_2}$ is most simply explained by a rise in ventilation without alteration of carbon dioxide production or cardiac output.

The ventilatory response to carbon dioxide after the administration of dichlorphenamide appears to resemble the change seen in metabolic acidosis due to ammonium chloride, in which the parallel shift of the ventilation/$P_{CO_2}$ line to the left is characteristic (Nielsen, 1936; Cunningham, Shaw, Lahiri & Lloyd, 1961).

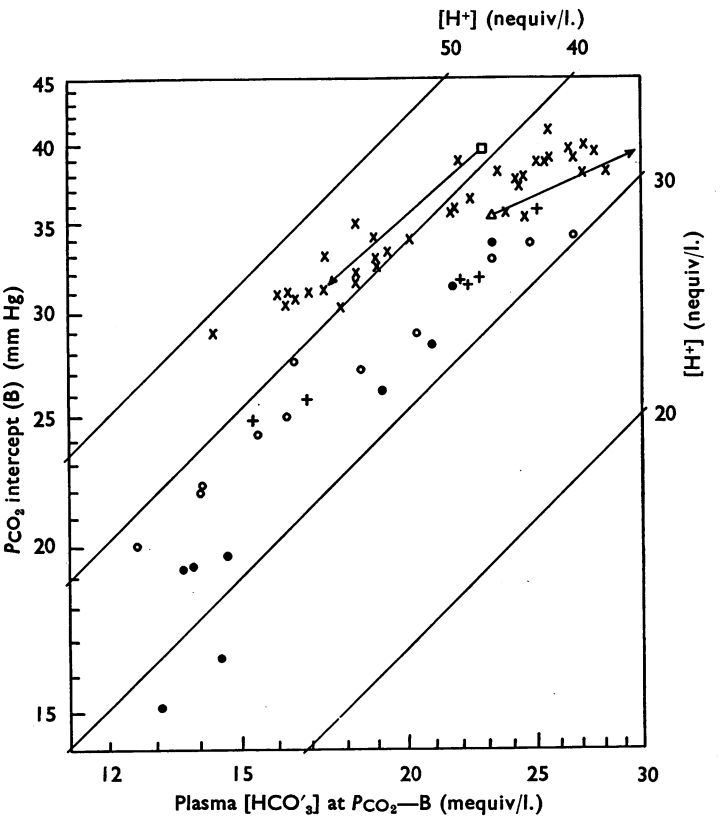


Fig. 8. Relationship between alveolar $P_{CO_2}$, plasma [$HCO'_3$] and plasma [$H^+$] at zero ventilation (that is, at the $P_{CO_2}$ intercept of the ventilation/alveolar $P_{CO_2}$ line). + Subject 68 of Cunningham *et al.* (1961) (ammonium chloride); × subjects 19, 65, 66 and 67 of Cunningham *et al.* (1961) (ammonium chloride); △——→ subjects of Katsaros *et al.* (1960) (sodium bicarbonate); □——→ subjects of Lerche *et al.* (1960) (acetazolamide); ○ K.B.S. (dichlorphenamide); and ● B.M. (dichlorphenamide).

Lerche, Katsaros, Lerche & Loeschcke (1960) have demonstrated a similar effect after acetazolamide.

Cunningham *et al.* (1961) described a linear relation between B (the $P_{CO_2}$ intercept of the ventilation/$P_{CO_2}$ line) and the plasma [$HCO'_3$] at this $P_{CO_2}$. A qualitatively similar picture was seen in our subjects during dichlorphenamide administration (Fig 7). There is, however, a quantitative difference which is best revealed by replotting the values on log co-ordinates (Fig. 8). In this plot, lines of equal [$H^+$] are parallel. Fig. 8 shows that for subject K.B.S. the slope of the log B/log [$HCO'_3$] line was similar to that of one subject studied by Cunningham *et al.* (1961) during ammonium chloride acidosis and to that of the group studied by Lerche *et al.* (1960) during the administration of acetazolamide. Four subjects of Cunningham *et al.* (1961) given ammonium chloride, and the subjects of Katsaros, Loeschke, Lerche, Schönthal & Hahn (1960) who were given sodium bicarbonate, yielded a log B/log $[HCO'_3]_B$ slope rather less than that of K.B.S. In all these subjects, however, the slope was less than that of the iso-[$H^+$] lines and in them the metabolic acid-base change was alone sufficient to account for the respiratory stimulation.

In subject B.M., however, the slope of the log B/log $[HCO'_3]_B$ relationship was not only greater than in the other groups but was, if anything, greater than that of the iso-[$H^+$] lines. Moreover his ventilation/$P_{CO_2}$ slope was not unduly great. It is therefore possible that in B.M. there was an additional stimulus to breathing during the administration of dichlorphenamide. The present experiments do not indicate what this stimulus was.

*Side-effects*

This study was primarily concerned with the pharmacological effects of dichlorphenamide, but it is appropriate to mention the sensations described by the subjects. J.A.R.S. noted breathlessness on exertion but no other effects. K.B.S. and B.M., on the other hand, had quite unpleasant side-effects. They became apathetic, had feelings of unreality and were short-tempered. Concentration was poor and B.M. eventually could make no sense of the simplest prose. While these symptoms were present B.M. was not fit to drive a car ; a suitably cautious attempt to do so convinced him that he was quite unsafe. K.B.S. noted telescoping of his appreciation of time, and gas analysis took longer to perform. Most of these effects contrast with those of acidosis induced by ammonium chloride (Haldane, 1921) and are presumably due to other actions of the drug.

We are grateful to Professor R. H. Girdwood for providing facilities for this work and to Merck, Sharp & Dohme for supplying tablets of dichlorphenamide. The capillary blood determinations were made by Dr J. L. Anderton. One of us (E. A. H.) held a Medical Research Council Grant for Scientific Assistance.

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