H^+ and pCO_2 as chemical factors in respiratory and cerebral circulatory control[1]

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LAMBERTSEN, C. J., S. J. G. SEMPLE, M. G. SYMTH, AND R. GELFAND. *H^+ and pCO_2 as chemical factors in respiratory and cerebral circulatory control.* J. Appl. Physiol. 16(3): 473–484. 1961.—The relationships of changes in respiration and brain blood flow index to alterations in arterial and internal jugular venous blood pCO_2, $[HCO_3^-]$, and pH were studied in normal men. Observations during control of alveolar pCO_2, first at 44 and then at 50 mm Hg, represented the effects of CO_2 breathing. Intravenous infusion of $NaHCO_3$ solution (ca. 2.4 mEq/kg) while maintaining alveolar pCO_2 at 50 mm Hg revealed the responses to a lowering of blood $[H^+]$ without concurrent change in arterial or internal jugular venous pCO_2. Brain blood flow index varied directly with alteration in blood pCO_2 and was unaffected by changes in blood pH not produced by pCO_2 change. Respiratory measurements indicated a prominent relationship between respiration and blood hydrogen ion concentration, the reversal of the acidemia normally associated with CO_2 administration removing approximately 45% of respiratory stimulation induced by hypercapnia. The remaining 55% of the increased ventilation caused by CO_2 breathing was not directly related to changes in arterial or internal jugular venous blood pH or $[HCO_3^-]$. The residual respiratory effect of CO_2 administration was correlated, not only with alteration of pCO_2, but with calculated changes in the pH of cerebrospinal fluid. Thus, the total respiratory stimulation produced by CO_2 breathing, and its diminution by bicarbonate infusion, can be quantitatively described either in terms of a single stimulus index, hydrogen ion concentration, or in terms of two factors, pH and pCO_2. Choice between single and multiple acid-base factors as indices of chemical stimuli in respiratory control remains arbitrary. However, the discussion re-emphasizes that, while respiratory changes do occur when blood pH is altered without change of blood or central pCO_2, comparable stimulant effects of molecular CO_2 cannot be demonstrated without somewhere producing concurrent modification of pH.

IN MEN BREATHING AIR at sea-level, the precise homeostatic regulation of the acid-base and oxygen environment of the brain must depend largely upon the selective reactivity of respiratory neurons to minute chemical changes in their own local environment. This concept of response to local environment, often expressed (1, 2) and undoubtedly sound, appears also to apply to a secondary mechanism for central nervous system homeostasis, i.e., chemical regulation of the tone of brain vascular smooth muscle cells (3–5). In neither instance does this rational concept yet lend itself to definitive study, since in neither instance have there been established the mechanisms by which cellular excitation is affected by local chemical changes.

The exceptional potency of increased inspired CO_2 as a respiratory stimulant and relaxant of brain vessels has led to extensive studies aimed at determining mechanisms whereby intrinsically produced CO_2 might influence the activity of respiratory neurons and affect cerebral vascular tone. On the basis of these studies various investigators have expressed convictions concerning the individual or collective roles of such specific chemical factors as bicarbonate ion, hydrogen ion, and CO_2 itself. The history of these important investigations is presented in several recent reviews which point to the continued uncertainty regarding the qualitative nature and even the site of action of chemical factors in normal respiratory and brain circulatory control (1, 2, 6, 7). Certainly the possibilities for quantitative appraisal of the independent influences of proposed stimuli have not yet been thoroughly explored.

The present study was carried out in an attempt to make a quantitative assessment in man of the relationships of changes in ventilation and in cerebral arteriovenous oxygen difference to the alterations in blood acid-base variables produced by inhalation of carbon dioxide. Levels of hypercapnia in the region of the normal homeostatic adjustment of pCO_2 were chosen. The primary technical objective of the study to be reported was the experimental separation of the normally interdependent blood parameters pCO_2 and pH in both arterial and internal jugular venous blood. This was accomplished by intravenous infusion of sodium bicarbonate solution during artificial regulation of alveolar (arterial) pCO_2 at an essentially constant, selected level

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TABLE 1. *Ages and body measurements of subjects*

Subj. No.	Age, yr.	Ht., in.	Wt., lb.	B.S.A., m^2
1	22	68.5	157	1.84
2	20	71.3	153	1.88
3	21	69.5	156	1.88
4	22	71.5	166	1.96
5	20	68.9	129	1.70
Mean	21	69.9	152	1.85

above normal (8, 9, 10). By this means an effort was made to determine responses of unnarcotized subjects to CO_2-induced acidemia and the nearly exact reversal of acidemia during persistent hypercapnia.

METHODS

The experiments were conducted at the prevailing atmospheric pressure and room temperature (Table 2).

General Procedure

Each experiment consisted of four main parts, hereinafter called *phases I, II, III,* and *IV.* In *phase I,* measurements were obtained when the subject's alveolar pCO_2 had been artificially adjusted to approximately 44 mm Hg. Immediately thereafter, alveolar pCO_2 was adjusted to approximately 50 mm Hg and measurements repeated (*phase II*) to obtain indices of respiratory and circulatory responses to simultaneous increase in blood pCO_2 and cH.[2] A solution of sodium bicarbonate was then infused intravenously to abolish the CO_2-induced acidemia, and while again maintaining alveolar pCO_2 at 50 mm Hg, measurements were repeated (*phase III*). In all but one subject a delayed duplication of sampling was obtained after the sodium bicarbonate infusion (*phase IV*).

Subjects

Table 1 shows the ages and body measurements of the five normal men used as subjects for this study. All these subjects were familiar with the sensations of carbon dioxide inhalation and intravenous infusion, but were not informed regarding the order of experimental procedures or the exact agent to be infused on a particular day. The subjects reported to the laboratory in the morning in a fasted state and rested in a supine position for approximately 20 minutes following completion of vascular punctures and other preparations. The subjects were blindfolded throughout each phase of the experiments.

[2] For convenience the term cH will be used in figures and discussion to represent pH with reversal of the positive or negative sign. Thus, an increase in acidity will be considered an increase in cH rather than being cited as a fall in pH. Over the small range of values in the present study ΔcH may be considered linearly related with $\Delta[H^+]$, which may be substituted for ΔcH upon introduction of the appropriate constant.

Artificial Control of Alveolar pCO_2

The method employed for regulation of end-tidal pCO_2 at supranatural levels has been described in detail elsewhere (8). Pertinent to this study is that respiratory measurements and blood sampling were carried out only after the subject had been held stable at the desired pCO_2 for a minimum of 7 minutes. Gases employed or pCO_2 control throughout all phases of the study consisted of water-pumped, compressed air, and mixtures of CO_2 and 21 % O_2 in N_2. A mouthpiece and nose clip were employed in preference to a mask to minimize lag time in alveolar pCO_2 control. Since ventilation in all phases was greater than normal, it was expected that influences of change in pO_2 upon chemoreceptor activity would be negligible.

Infusion of Sodium Bicarbonate Solution

Prior to beginning measurements, an indwelling needle was inserted into an antecubital vein and an intravenous infusion of 5% glucose in water was established at a rate of approximately 0.5 ml/min. The infusion continued during the first two phases of the study and provided a means for administering the solution of sodium bicarbonate without disturbing the subject.

A dose of 2.4 mEq sodium bicarbonate/kg body wt was injected as a .89 molar solution into the tubing of the infusion apparatus, beginning promptly after completing the prebicarbonate measurements (*phases I* and *II*). The injection of bicarbonate was accomplished in an average time of 24 min (range 15–33 min). The dosages of bicarbonate were purposely matched to those employed by Singer et al. (11) to permit subsequent intercomparisons of effects. Subsequent to the infusion measurements were repeated, sometimes with a delay for urination to assure subject comfort.

Respiratory Measurements

Expired gas was directed by means of low dead-space, low resistance-breathing valves and 1-inch i.d. smooth-bore tubing through a high capacity (142 liters/min), rotary, wet-test gasometer. From measurements of gas volume expired and the number of respirations over a 5-min sampling period, average rate, depth, and minute volume of respiration were determined.

Blood Sampling and Analysis

Nineteen-gauge femoral arterial and internal jugular venous blood sampling needles were inserted after infiltrating the respective puncture sites with 3 ml of 2% and 1% procaine hydrochloride solution. These indwelling needles and the 1-mm bore plastic tubing connecting them to manifolds of Luerlok stopcocks (12) permitted blood to be sampled without the awareness of the subject. A pair of specially designed, electrically powered, heparinized, magnetically stirred syringes

connected to the manifolds permitted a single individual to carry out simultaneous, constant-rate sampling of arterial and internal jugular venous blood over the entire 5-min period of respiratory measurements (13). All blood measurements except hemoglobin O_2 capacity were performed upon anaerobically transferred aliquots of these integrated samples. The arterial blood sampled for O_2 capacity was obtained in a separate, heparinized syringe after flushing the syringe dead space with 2 ml of blood prior to actual sampling. Blood samples for gas-content measurements were stored in ice-water slush prior to analysis; samples for pH determinations were not iced, but warmed to 37 C for immediate measurement.

All blood measurements were performed in duplicate. Blood CO_2 and O_2 contents were performed on 1-ml samples by the Van Slyke-Neill manometric method (14). Hemoglobin O_2 capacity was determined by manometric measurement (15), after rotating a 6-ml blood sample at room temperature for 1 hour in a 100-cc tonometer equipped to minimize evaporation of water.

Blood pH measurements were completed anaerobically within 15 min of sampling, using a McInnes-Belcher electrode system equilibrated to 37 C in a thermostatically controlled, shielded air bath, together with a Cambridge model R pH meter. Buffer checks were performed immediately before and after each blood pH measurement, using commercial buffers calibrated against National Bureau of Standards buffers. Actual temperature of blood pH measurement was checked to within ±0.1 C by means of a calibrated thermistor thermometer, the thermistor being mounted within the lumen of the tubular glass electrode. The subject's deep body temperature was determined to within ±0.1 C during each phase of the experiment, using for the purpose an indwelling rectal thermistor and the Wheatstone bridge-galvanometer circuit of the above-mentioned thermistor thermometer. Final pH and pCO_2 values at the subject's body temperature were obtained by the procedures outlined by Severinghaus et al. (16). In 26 consecutive pairs of pH measurements on samples of arterial and venous blood obtained in these experiments, the average difference and the standard deviation of the individual differences were .003 ± .002 pH units. Since the present study is based upon comparison of differences in pH and pCO_2, error in duplicate pH measurements is more critical than is absolute error.

Calculations of percentage hemoglobin saturation and pO_2 were carried out in the normal manner (15). For estimation of blood pCO_2 and plasma bicarbonate a pK′ of 6.105 and a Bunsen solubility coefficient (15) of 0.501 were used. The values for pK′ and CO_2 solubility in cerebrospinal fluid were those presented in a separate study (17).

Circulatory Measurements

Cerebral blood flow index is defined for the purposes of this study as the ratio of the arterial-internal jugular venous O_2 difference in *phases II, III,* or *IV* to the basic control arteriovenous O_2 difference of *phase I* (44 mm pCO_2). As such, it differs but slightly in numerical value from previous usage in which the A-V O_2 during air breathing at rest provided the denominator of the ratio (3, 18).

Mean arterial blood pressure was measured with an air-damped mercury manometer with zero pressure referred to a point 10 cm anterior to the skin of the back. Pulse rate was counted during the period of respiratory measurements.

RESULTS

Mean measured and derived values obtained in the several phases of these experiments[3] are presented in Table 2, and the significance of observed changes in Table 3.[4]

Fig. 1*A* and *B* show the relationships of respiratory minute volume to arterial and internal jugular venous pCO_2 and pH during *a*) control of end-tidal pCO_2 at 44 mm Hg (*phase I*), *b*) systemic "respiratory" acidosis produced by elevating end-tidal pCO_2 to 50 mm Hg (*phase II*), and *c*) reversal of the CO_2-induced acidosis by infusion of sodium bicarbonate intravenously while maintaining pCO_2 at 50 mm Hg (*phase III*). The data of *phase IV*, obtained on only four subjects, is similar to that of *phase III*, but is insufficient for statistical appraisal and will not be presented graphically. Relationships of blood pCO_2 and pH to tidal volume and to respiratory rate are essentially the same as illustrated for respiratory minute volume in Fig. 1*A* and *B*. For ease of comparison, the figures are so scaled that the CO_2-induced change in pH is equivalent in size to the corresponding change in pCO_2. The straight lines connecting the experimentally determined points of *phases I* (44 mm pCO_2) and *II* (50 mm pCO_2) represent only a convenience and an approximation over the short range to the actual CO_2-sensitivity curves for respiratory minute volume (19) and cerebral blood flow index (3, 18). In the lower regions of the normal CO_2-response curves and with the small change in pCO_2 selected for this study, the illustrative use of straight connecting lines introduces no appreciable error.

Blood Acid-Base Parameters

The figures reveal that changes in arterial blood pCO_2 and pH produced by elevating alveolar pCO_2 from 44

[3] Mean values presented in these tables on 5 subjects differ in minor respects from values reported in abstract form for 4 of these subjects (9).

[4] Additional tabular data have been deposited as Document number 6637 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D.C. A copy may be secured by citing the Document number and by remitting $1.25 for photoprints, or $1.25 for 35-mm microfilm. Advance payment is required. Make checks or money orders payable to: Chief, Photoduplication Service, Library of Congress.

TABLE 2. *Effects on respiration and composition of arterial and internal jugular venous blood of elevated CO_2 tension before and after sodium bicarbonate infusion*

Measurement	Phase I 44 mm Hg Alv. pCO_2 Prebicarbonate	Phase II 50 mm Hg Alv. pCO_2 Prebicarbonate	Phase III 50 mm Hg Alv. pCO_2 Postbicarbonate	Phase IV 50 mm Hg Alv. pCO_2 Postbicarbonate
Respiratory				
Min vol, liters/min/m², BTPS	6.25±0.94	14.82±1.96	9.81±1.97	11.81±2.46
Tidal vol, liters, BTPS	0.627±0.031	1.309±0.132	0.893±0.050	0.913±0.020
Rate, per min	18.5±2.6	21.9±3.8	19.8±3.2	24.0±4.8
End-tidal pCO_2, mm Hg	44.4±0.5	50.7±0.3	50.6±0.5	50.1±0.8
Arterial blood				
pCO_2, mm Hg	44.4±0.2	50.2±0.7	50.8±1.1	49.5±1.3
pH, units	7.385±0.002	7.344±0.007	7.396±0.009	7.397±0.009
CO_2 content, vol %	52.2±0.5	53.9±0.5	61.2±0.8	59.5±0.6
$[HCO_3^-]$, mM/liter	26.7±0.1	27.4±0.2	31.4±0.4	30.7±0.3
pO_2, mm Hg	120±9	127±7	127±8	126±9
Hb sat., %	97.9±0.6	97.9±0.4	99.4±1.0	98.1±0.3
O_2 Content, vol %	19.2±0.7	19.5±0.7	19.3±0.6	19.5±0.8
O_2 Capacity, vol %	19.2±0.8	19.5±0.7	19.0±0.7	19.4±0.8
Buffer slope	28.02	28.24	27.77	28.21
Internal jugular venous blood				
pCO_2, mm Hg	54.0±0.3	59.2±0.4	58.7±0.8	59.4±1.0
pH, units	7.339±0.002	7.296±0.003	7.352±0.006	7.343±0.004
CO_2 content, vol %	58.1±0.7	58.0±0.6	64.9±0.8	64.2±0.6
$[HCO_3]$, mM/liter	29.2±0.2	28.9±0.2	32.7±0.4	32.3±0.3
pO_2, mm Hg	38.1±1.1	46.8±1.3	46.7±2.4	43.5±2.5
Hb sat., %	67.9±1.7	76.4±1.2	78.2±2.1	74.7±2.4
O_2 content, vol %	13.2±0.7	15.0±0.6	15.0±0.8	14.7±0.8
General				
A-V O_2 diff., vol %	6.0±0.3	4.5±0.3	4.2±0.3	4.9±0.4
Cerebral blood flow index	1.00	1.35	1.43	1.27
MABP, mm Hg	103±5	110±5	106±4	111±4
Pulse rate, per min	73±4	81±5	80±4	86±5
Body temp, C	36.8	36.8	37.0	37.1
Barometric pressure, mm Hg	764.7	764.5	764.0	763.9

Values are means ± SEM.

to 50 mm Hg are closely paralleled by the pCO_2 and pH changes of internal jugular venous blood. This parallelism persisted when sodium bicarbonate solution was infused. It is thus also evident that control of end-tidal pCO_2, through fixation of arterial CO_2 tension, provided a high degree of artificial control over central levels of pCO_2. This method of indirect, experimental stabilization of central pCO_2 would, of course, be interfered with by any change in brain blood flow or metabolism. Nevertheless, in this study, it was possible to control internal jugular pCO_2 so that the average values observed before and after bicarbonate infusion differed by only 0.5 mm Hg.

When alveolar pCO_2 was elevated, the expected rise in arterial $[HCO_3^-]$ occurred, but brain venous $[HCO_3^-]$ fell slightly. Thus, in CO_2-induced hyperventilation as in bicarbonate administration, respiratory changes were negatively correlated with alterations of internal jugular venous $[HCO_3^-]$.

Respiration

The data and Fig. 1*A* and *B* indicate that the respiratory stimulation produced by a 6-mm Hg elevation of blood pCO_2 was considerably reduced, though not completely abolished, when bicarbonate infusion lowered blood cH. That the bicarbonate-induced reduction of rate, depth, and minute volume of respiration was unrelated to change in the pCO_2 of either arterial or internal jugular venous blood is evident, since pre-bicarbonate injection levels of blood pCO_2 were closely sustained during and after the infusion of base. It is likewise evident that the residual respiratory stimulation following bicarbonate infusion was unrelated to acidemia, since sufficient bicarbonate was administered to more than reverse the original elevation of blood cH by carbon dioxide. It should be noted that the figures illustrate changes in respiratory minute volume rather than alveolar ventilation, since in normal subjects the former, like work of breathing (20), is more representative of total respiratory drive than is alveolar ventilation.

Circulation

Changes in cerebral blood flow index produced by hypercapnia and by bicarbonate infusion are shown in Fig. 2*A* and *B*. Since the changes in pCO_2 and pH produced in these experiments are known not to be associated with significant alterations of cerebral O_2 consumption or CO_2 production (5), the observed changes in cerebral blood flow index provide quantita-

tive expressions of alteration of cerebral blood flow. Though of course only relative, the observed changes should be, quantitatively, at least as precise as values provided by the more complex nitrous oxide method for absolute measurement of cerebral blood flow (12). The figures show that the cerebral blood flow index was elevated approximately 35% by changing end-tidal pCO_2 from 44 to 50 mm Hg, and that reversal of the CO_2-induced acidemia did not lower cerebral blood flow below the level associated with 50 mm Hg end-tidal pCO_2 prior to bicarbonate infusion. Thus, gross increase in blood bicarbonate concentration and pH exerted no significant effect upon CBF index when blood pCO_2 was not allowed to change (Table 3).

The small average changes in pulse rate and mean arterial blood pressure associated with the induced alterations in blood pCO_2, $[HCO_3^-]$, and pH were not statistically significant.

TABLE 3. *Statistical significance of average changes in measured values between various study conditions*

Difference In:	Comparison of Experimental Phases			
	I-II	II-III	I-III	II-III′
RMV	<.01	<.05, >.01	<.1, >.05	<.05, >.01
TV	<.01	<.1, >.05	<.05, >.01	<.05, >.01
RR	<.1 >.05	<.3, >.2	<.6, >.5	<.3, >.2
CBF index	<.01	<.6, >.5	<.01	
Art. pCO_2	<.01	<.6, >.5	<.01	
Art. pH	<.01	<.01	<.3, >.2	
Art. $[HCO_3^-]$	<.01	<.01	<.01	
Ven. pCO_2	<.01	<.7, >.6	<.01	
Ven. pH	<.01	<.01	<.1, >.05	
Ven. $[HCO_3^-]$	<.01	<.01	<.01	

Probabilities indicate whether observed difference in means occurred by chance.

DISCUSSION

Much of the difficulty encountered in interpreting the extensive studies concerned with respiratory and brain circulatory control has been related to limited adaptability of data to quantitative appraisal. In the discussion which follows, emphasis will therefore be placed primarily upon quantitative aspects of the results obtained in the present experiments. Initially, it will be convenient for the relationships of acid-base factors to respiratory and brain circulatory control to be considered separately.

Respiratory Control

Arterial and internal jugular venous blood. Of the conventionally considered chemical respiratory "stimuli" in arterial or central venous blood, only cH fell when sodium bicarbonate was infused and pCO_2 held constant across the brain (*phase III*). Assessment of the relationship of fall in blood cH to the concomitant lowering of respiratory rate, depth, and minute volume is somewhat complicated by the use of a dose of bicarbonate slightly in excess of that required for exact reversal of the acidemia produced by raising alveolar pCO_2 from 44 to 50 mm Hg pCO_2. Fig. 3 illustrates the approximate respiratory effect of more exact reversal of the CO_2-induced acidemia, using the values for cH and pCO_2 obtained upon internal jugular venous blood. The dashed line connecting points II and III indicates the pathway for the relationship of respiration to venous cH as increasing doses of sodium bicarbonate are administered and central pCO_2 is held essentially constant (in this case at about 59 mm Hg). This proposed pathway should pertain if infusion of base does not alter the slope of the respiratory response to CO_2-induced changes in cH. That this condition actually applies was verified by the results of a second series of experiments which will be reported separately in full (10) and by the recent study of Loeschcke et al. (20a). It further appears that administration of HCl in dogs (21) or production of acidosis with NH_4Cl, $CaCl_2$, or acetazoleamide in man (22) fails to alter the slope of the pCO_2-response curve.

Fig. 3 shows that reversal of acidemia (II to III′), while allowing hypercapnia to persist, removed approximately 45% of the respiratory stimulation originally produced by elevating alveolar pCO_2 from 44 to 50 mm Hg. The remaining 55% of the total increase in ventilation brought about by raising pCO_2 must have been due to changes somehow connected with elevation of pCO_2, but unrelated to alterations of the pH or $[HCO_3^-]$ of arterial or internal jugular venous blood (9, 10). Similar proportionate association of respiration with changes of blood cH and pCO_2 in normal men can be derived (about 45%) from the equation of Gray (23) and from the data (about 43%) of Loeschcke et al. (24).

The change in pulmonary ventilation associated with change in blood cH observed in these and certain other studies in normal man (22–24) is large in comparison with the results of many experiments in animals and man (1, 2). In numerous instances, alteration of respiration during metabolic acidosis or acid infusion has been slight, even in the face of gross change in arterial cH (1, 2) (25). Usually in such experiments, the normal readjustments of blood pCO_2 to change in alveolar ventilation have occurred as blood cH was altered. The use of anesthetic agents in animal studies should further limit the respiratory response to acid infusion. Fig. 4 shows the magnitude of the association of changes in ventilation with change in arterial cH found in three different situations. It is evident from the figure that the degree of respiratory stimulation per unit change in blood cH, observed during pCO_2 control in the unnarcotized normal subjects of the present study, is over 500% greater than that described by Domizi et al. (26) as a powerful effect of HCl infusion on the basis of a study also involving controlled alveolar pCO_2, but using anesthetized dogs. It is noteworthy that the respiratory

response to an increase in cH produced by HCl in the study by Domizi et al. (26) can, as in man, be shown to average about 45% of the response to an equivalent acidemia induced by CO_2 administration. In the normal subjects, change in ventilation per unit increase in cH is further seen to be more than ten times greater than that found by Kety et al. (25) to accompany the severe metabolic acidosis and hypocapnia of diabetes. The finding in the present experiments, averaging approximately 1.7 liters/min/m^2 for each .01 unit change in blood cH, is in close quantitative agreement with the observations made by Gray (23) and Loeschcke et al. (24), using different methods for appraising the respiratory response to acidosis, and may be considered to indicate the actual magnitude of the association of ventilation to blood cH in normal individuals breathing CO_2.

Extravascular factors. While the change in ventilation accompanying alterations of blood cH is large in normal man as compared with the narcotized dog or the patient in metabolic acidosis, it remains impossible to associate more than about 45% of CO_2-induced hyperventilation with alterations in the level of acidity in either arterial or internal jugular venous blood. This effect is not related to change in pCO_2, hence it is not related to changes in intracellular cH. The remaining 55% of the total ventilatory response to hypercapnia is distinctly unassociated with change in the cH of circulating blood, but does somehow relate to change in blood pCO_2, or, secondarily, to the cH or pCO_2 of extravascular fluids (1, 2, 9). The present experiments do not provide the means for identifying either the nature or the actual location of this latter effect of CO_2 administration. Change in dissolved CO_2, as indicated by arterial pCO_2 in Gray's multiple factor equation (23, 27), could represent a stimulus distinct from change in cH and could exert its effect centrally, at or within peripheral chemoreceptors, or at both locations (1, 2). On the other hand, recent studies by Loeschcke et al. (28) and Mitchel et al. (29) indicate that a respiratory response can be elicited to changes in the cH of fluid perfusing the fourth ventricle, even when the pCO_2 of the fluid remains stable. These responses in cats (28) and dogs (29), while not gross in degree, suggest that at least some cells concerned with respiratory control are so located anatomically that they can be influenced in activity by the composition of adjacent cerebrospinal fluid.

Prediction of effects of CO_2 breathing and sodium bicarbonate infusion upon cerebrospinal fluid. It was not practical to sample cerebrospinal fluid (CSF) from the subjects of the present study. However, two features of cerebrospinal fluid and the blood-cerebrospinal fluid barrier make it possible to employ the Henderson-Hasselbalch equation to calculate for these experiments the probable changes in its acid-base composition during hypercapnia and the subsequent intravenous infusion of sodium bicarbonate at controlled, elevated pCO_2. First, it is known from studies in animals and man that intravenous injection of sodium bicarbonate does not significantly increase the $[HCO_3^-]$ of CSF (1, 2, 30–32). Second, considering the known free physical diffusibility of molecular carbon dioxide (33), even into cerebrospinal fluid (2), it is reasonable to expect that alteration of cerebrospinal fluid cH in acute, stable-state experiments would be determined almost exclusively by induced changes in central pCO_2 (2, 10, 31). To calculate the cH of cerebrospinal fluid in the present study, the pCO_2 of CSF was considered to be essentially the same as that of internal jugular venous blood [in dogs the pCO_2 of CSF appears to be approximately 6 mm Hg higher that that of arterial blood (31), and in a study of this particular question in man, no difference could be found between the pCO_2 of cisternal fluid and internal jugular venous blood (32)]. In addition to selection of reasonable values for pCO_2, and the assumption that $[HCO_3^-]$ of cerebrospinal fluid was not appreciably altered throughout the several phases of the study, calculation of cH in cerebrospinal fluid required appropriate values for pK′ and solubility of CO_2 (17). Actually, estimation of the changes induced in the cH of cerebrospinal fluid by administration of CO_2 or $NaHCO_3$ does not require knowledge of the exact value for $[HCO_3^-]$ in CSF. For the purposes of this study, the $[HCO_3^-]$ in cerebrospinal fluid was considered to remain at 26.0 mEq/liter, close to that in the arterial blood during air breathing at sea level (18). This may be slightly in excess of the normal value for $[HCO_3^-]$ in man (32).

Fig. 5*A* and *B* illustrate the relationships between calculated values for the cH of CSF and the observed values for respiration, and the pCO_2 and cH of venous blood in the three phases of the present study. In these stable states, the respiratory stimulation produced by raising alveolar CO_2 tension should be accompanied by increases of both pCO_2 and cH both in the blood and the cerebrospinal fluid. However, when bicarbonate is infused to render the blood alkaline while the pCO_2 of the blood and brain is held steady at the selected, elevated level, the cerebrospinal fluid should remain in the state of acidosis induced by CO_2 administration.

Composite factors in blood and fluids of central nervous system. The present data can be employed to examine combined influences upon respiration of acid-base factors in blood and a central acidosis which persists during hypercapnia, unaffected by intravascular infusion of sodium bicarbonate. The composite, stimulant-inhibitory influences upon respiration of simultaneous administration of CO_2 and $NaHCO_3$ may be described quantitatively in several different ways, as follows:

A. Independent effects of cH and CO_2.

$$\text{total } \Delta RMV_{CO_2} = K_1 \Delta cH_{blood} + K_2 \Delta pCO_2 \qquad (1)$$

where K_1 represents the observed respiratory reactivity to change in blood $[H^+]$ in terms of liters/min/.01 unit change in cH, i.e. (= .45 total $\Delta RMV_{CO2}/\Delta cH_{blood}$). K_2 is the residual respiratory response to the increased pCO_2 in liters per min per millimeter Hg pCO_2 (= .55 total $\Delta RMV_{CO2}/\Delta pCO_2$), and ΔpCO_2 represents

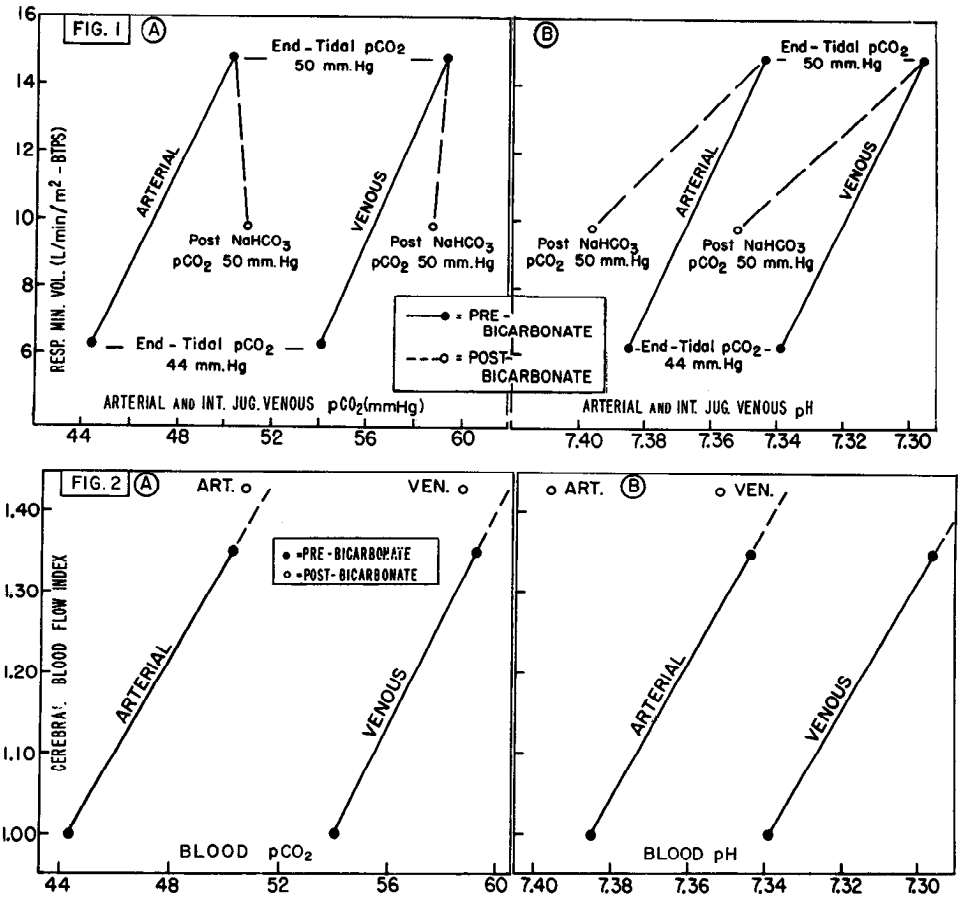


FIG. 1. *A* and *B:* relationships of respiratory minute volume to pCO_2 and pH of arterial and internal jugular venous blood before and after i.v. infusion of sodium bicarbonate solution (mean values in 5 normal men). *Solid lines* connecting *closed circles* represent influence of change from control of end-tidal pCO_2 at about 44 to control of pCO_2 at about 50 mm Hg. *Dashed lines* to *open circles* approximate influence of bicarbonate infusion while maintaining end-tidal pCO_2 close to 50 mm Hg. Infusion of bicarbonate solution resulted in prominent decrease in respiratory minute volume although no significant change occurred in arterial or venous pCO_2. Reversal of CO_2-induced acidemia with bicarbonate did not completely abolish hypercapneic respiratory stimulation.

FIG. 2. *A* and *B:* influence of change in arterial and internal jugular venous pCO_2 and pH on cerebral blood flow index. *Solid lines* connecting *closed circles* represent composite influence of change in pCO_2 and pH on elevation of end-tidal pCO_2 from 44 to 50 mm Hg. *Dashed lines* indicate an approximate extrapolation of observed effects of hypercarbia. Following complete reversal of CO_2-induced acidemia by infusion of sodium bicarbonate, values for cerebral blood flow index at 50 mm Hg remained elevated. Change in pCO_2 unrelated to effects on blood pH therefore appears responsible for prominent influence of CO on cerebral vascular resistance.

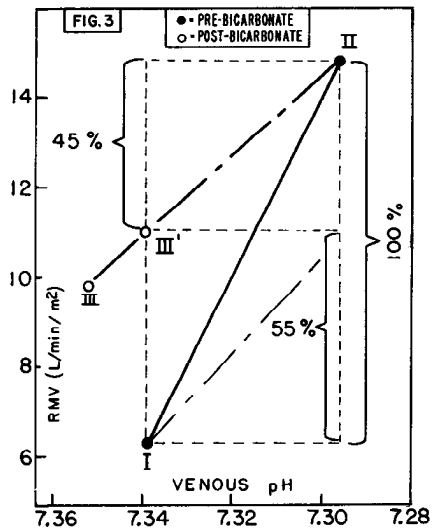
change in pCO_2 at an undesignated location. The location, which is unknown, may be arterial, mean capillary or central venous blood, cerebrospinal or other central extracellular fluid, or intraneuronal (19, 27). This expression for ΔRMV is thus the equivalent to that derived by Gray to relate the "ventilation ratio" and acid-base factors in arterial blood (23) and relates to that of Lloyd et al. (34). It suggests independent actions of different potential stimuli but, aside from identification of a positive contribution of change in blood cH to respiratory stimulation, offers no suggestion regarding the nature or location of an effect of ΔpCO_2. Nevertheless, like the original "multiple factor equation" (23),[5] *equation 1* is closely descriptive of a large number of respiratory acid-base disturbances. Because sensitivity to CO_2 is extremely variable, the constants for this equation should, of course, be determined for each subject or subject group (19). In this study, arbitrarily using values of cH and pCO_2 obtained for brain venous blood, K_1 = 1.66 liters/min/m^2/ΔcH and K_2 = 1.68 liters/min/m^2/mm Hg ΔpCO_2.

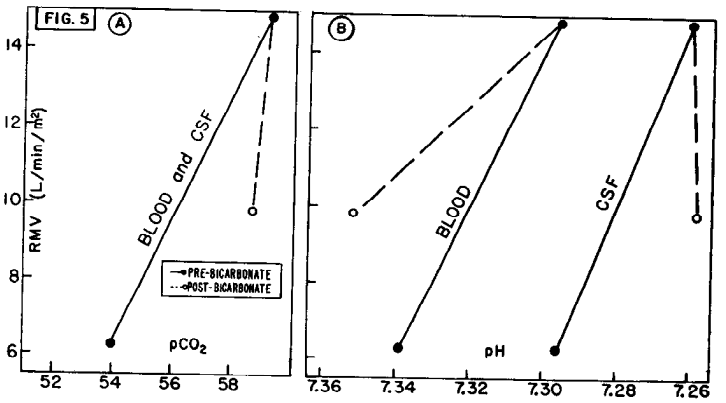
B. Actions of change in hydrogen ion concentration at multiple locations. The data of the present study can equally well be described as

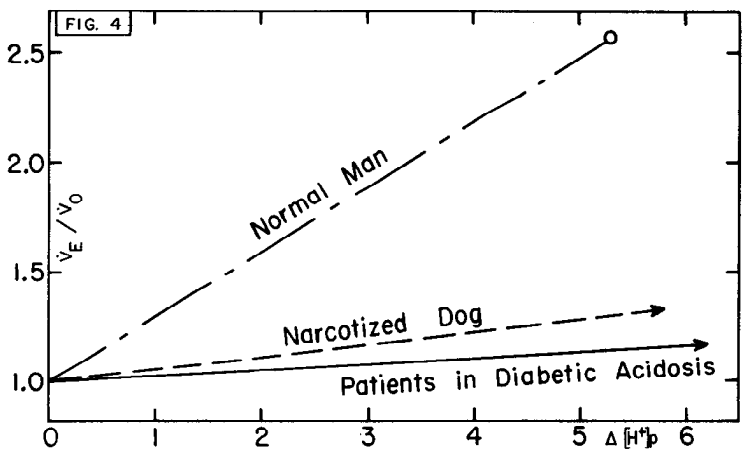
$$\text{total } \Delta RMV_{CO_2} = A\Delta cH_{blood} + B\Delta cH_{CSF} \qquad (2)$$

A indicates the slope of the respiratory change per unit of variation in blood $[H^+]$, and equals .45 total $\Delta RMV_{CO2}/\Delta cH_{blood}$. Similarly, B indicates the residual respiratory reaction to CO_2 tension elevation, this time expressed as .55 total $\Delta RMV_{CO2}/\Delta cH_{CSF}$. ΔcH_{blood} represents change of hydrogen ion concentration in arterial or central blood and ΔcH_{CSF} represents change in $[H^+]$ of cerebrospinal or other extravascular fluids. Here no specific stimulant effect of molecular CO_2 is invoked. Rather, it is suggested that the respiratory stimulation not related to change in blood $[H^+]$ may still be related quantitatively to change in $[H^+]$, but in this instance by way of independent actions at anatomically distinct sites. Again, whether the effect is exerted upon medullary or other respiratory centers, or even upon peripheral chemoreceptors, is uncertain. The influence of change in blood cH remains the 1.66

[5] VR_{H,pCO_2} = 0.22 H + 0.262 pCO_2 − 18, where VR is the ratio of an altered ventilation to ventilation during air breathing at rest, H is the absolute hydrogen ion concentration of arterial blood in terms of a unit which, when multiplied by 10^{-9}, is converted to mEq/liter, and pCO_2 is the absolute arterial CO_2 tension in mm Hg (23).







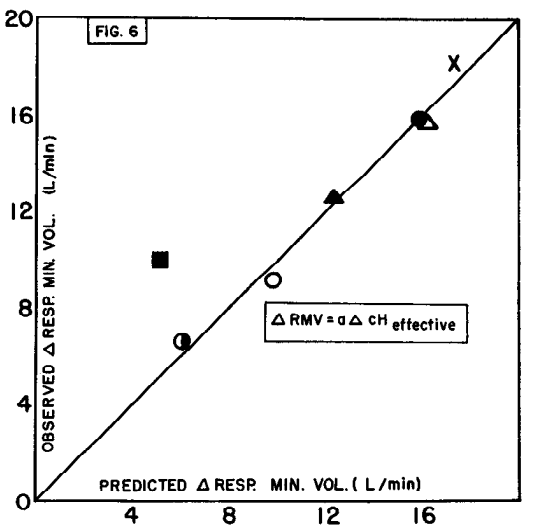


FIG. 3. Proportion of total respiratory stimulation by CO_2 which can be related to change in pH of blood (mean values for respiration and internal jugular venous blood from Table 2). *Solid line* connecting *phases I-II* represents total (100%) response to combined increase in CO_2 tension and cH, *II* to *III'* indicates that, without change in pCO_2, the interpolated respiratory change on exact reversal of CO_2-induced acidemia removes 45% of the ventilatory response to CO_2. Remaining 55% of respiratory response to hypercapnia is unrelated to change in blood cH.

FIG. 4. Change in ventilation ratio in response to elevation of blood H^+. $\dot{V}o$ = control ventilation per minute; $\dot{V}E$ = ventilation per minute during acidosis. *A:* data indicating sensitivity of normal man is from present study in which change in interval jugular venous pH occurs without change in pCO_2. Values for $\dot{V}o$ of 3.28 liters/min and resting $[H^+]$ of 40.1 obtained as supplementary measurements (10). Note that ventilatory response to $\Delta[H^+]p$ is over 500% greater for normal man than for narcotized dogs, and more than 10 times greater than in uncompensated diabetic acidosis. *B:* results obtained in narcotized dogs by Domizi et al. (26) in response to change in $[H^+]$ induced by HCl infusion with arterial blood pCO_2 held at 40 mm Hg. *C:* average relationship of respiration to change in arterial or internal jugular venous $[H^+]$ observed by Kety et al. (25) in *patients JS, MM, TH, CB* and *CC* before and after treatment for diabetic acidosis. In these patients acidosis and increased ventilation were accompanied by hypocapnia.

FIG. 5. *A* and *B:* comparison of observed effects of sodium bicarbonate infusion on internal jugular venous pCO_2 and pH with predicted effects on pCO_2 and pH of cerebrospinal fluid. Values for pH in cerebrospinal fluid were calculated with the Henderson-Hasselbalch equation, using values reported elsewhere for the pK′ of cerebrospinal fluid (17), an assumed stable cerebrospinal fluid $[HCO_3]$ equal to an observed mean arterial blood $[HCO_3]$ of 26.0 mEq/liter and cerebrospinal fluid pCO_2 levels considered equal to those observed in internal jugular venous blood. While *A* indicates that bicarbonate infusion lowered ventilation at an essentially constant level of pCO_2 in blood, cerebrospinal fluid, and brain, *B* suggests that reduction in ventilation accompanying decreased acidemia occurred in spite of sustained acidosis of cerebrospinal fluid and, presumably, intracellular fluids of respiratory neurons or even glomus cells of peripheral chemoreceptors.

FIG. 6. Comparison of measured changes in respiratory minute volume with changes predicted by calculation from observed alterations of pH in blood and assumed changes in cerebrospinal fluid. *Diagonal line* represents absolute agreement between observed and predicted values. Data pertinent to prediction of change in RMV from measurements of cH and pCO_2 are shown in Table 4. *Symbols* indicate findings in various forms of acid-base disturbance. These include: ●, CO_2 breathing; ○, bicarbonate infusion at 50 mm Hg alveolar pCO_2; or ◑, combined hypercapnia and alkalosis (this study); ■, diabetic acidosis (25); and experimental acidosis due to administration of Δ, NH_4Cl; ▲, $CaCl_2$; or ×, acetazoleamide (22, 24). In this figure all changes used as examples are positive except for bicarbonate infusion at 50 mm Hg alveolar pCO_2.

liters/min/m²/ΔcH shown in *equation 1*. B is 2.42 liters/min/m²/ΔcH_{CSF}.

C. Composite effect of change in [H⁺] of blood and CSF. *Equation 2*, in which respiration is correlated with a single type of chemical stimulus, can be further simplified as

$$\text{total } \Delta RMV_{CO_2} = a\Delta cH_E \qquad (3)$$

In this instance, *a* is the slope of the over all ventilatory response to CO_2 breathing, in terms of liters per min per square meter per .01 unit change in cH_E. ΔcH_E represents change in an "effective extracellular cH," or the composite influence of H^+ changes in blood and cerebrospinal fluid or other extracellular fluid of the central nervous system, resulting in an alteration of $[H^+]$ which correlates with changes in activity of the respiratory neurons. Thus, in the present study,

$$\text{effective } \Delta cH = .45\ \Delta cH_{blood} + .55\ \Delta cH_{CSF}, \qquad (4)$$
$$a = 4.07 \text{ liters/min/m}^2/.01\ \Delta cH,$$

and

$$\text{total } \Delta RMV_{CO_2} = 4.07\ (.45\ \Delta cH_{blood} + .55\ \Delta cH_{CSF}) \qquad (5)$$

Predictions of respiratory change in acute acid-base imbalance. In *equations 1* and *2* the constants describing the degree of respiratory response to change in blood cH and in pCO_2 or CSF cH were obtained by employing the values, summarized in Table 4, for elevation of pCO_2 in the present study (change from *phase I* to *II*). To compare the effects of CO_2 and sodium bicarbonate administration with various other forms of acute acid-base derangement, constants for $\Delta RMV/.01\ \Delta cH_E$ in *equation 3* were derived for CO_2 administration, using the subjects of this study and that of Loeschcke et al. (24), in which the effects of CO_2 breathing upon respiratory minute volume and the pCO_2 and pH of arterial blood were measured as controls prior to administration of NH_4Cl, $CaCl_2$ or acetazoleamide (22, 24). The unreported values for pH were kindly provided by these authors. The constants for CO_2 administration, together with the data from which they were derived and the observed changes during acid-base alteration, appear in Table 4, along with information pertaining to the previously mentioned group of patients in diabetic acidosis studied by Kety et al. (25).

Using the data summarized in Table 4, the experimentally observed effects of the several acid-base disturbances are compared with the values of ΔRMV predicted from change in cH on the basis of the descriptive *equations 3* and *4*. (Table 4, Fig. 6). No predicted values appear in the table for CO_2 breathing, since the data for simple hypercapnia were the basis for determining the constants in the predictive equation. Fig. 6 shows that the concept of change in cH as a single index of the chemical respiratory stimulus in regulation of acid-base homeostasis provides striking agreement between observed and predicted values for change in ventilation in the normal subjects available for test. Even the hyperventilation of severe diabetic acidosis, often cited as an example of the inadequacy of change in $[H^+]$ as an index of the respiratory stimulus, is now seen to be not grossly different from that calculated from changes in blood and "CSF" cH. Actually, if the expected slight reduction of $[HCO^-_3]$ in CSF occurred in these patients, the discrepancy in Fig. 6 would be in part erroneous. Similar close agreement between calculated and observed values can be shown for respiratory rates and tidal volumes in these conditions.

Relation to "multiple factor" and "reaction" theories. The preceding analysis relates, in normally oxygenated individuals, both to the reaction theories of Winterstein (1, 2) and the multiple factor theory of Gray (23). As mentioned elsewhere (1, 2), the reaction theory has successively emphasized the absolute influence upon respiration of change in hydrogen ion concentration in arterial blood, within the respiratory neurons and, ultimately, at both central and chemoreflex receptors. Whereas the support for the reaction theory has thus far depended largely upon qualitative studies, the present analysis indicates a quantitative correlation in man between change in ventilation and change in hydrogen ion concentration.

The present studies resemble the multiple factor theory in that each provides a quantitative description of a variety of acid-base disturbances, each attributes essentially the same value to a respiratory effect of change in blood cH, and each associates the remaining respiratory drive to an action of CO_2 not related to its effect upon blood cH. Thus, the major differences between the present interpretations and the multiple factor theory include use in the latter of pCO_2 as a factor separate from the extravascular changes in cH which must be induced by change in pCO_2, and emphasis upon arterial blood values for all indices of respiratory stimuli.

Limitations. Because of the extreme permeability of cells and membranes to molecular CO_2 (33), an acute elevation of the physical pressure of CO_2 in arterial blood will result in an increase of cH both in intracellular and extracellular fluids of the central nervous system. It is for this well-known reason that unequivocal separate appraisal of $[CO_2]$ and cH as potential respiratory stimuli has not yet been accomplished, even when techniques of direct injection into respiratory centers have been used (35). In describing the respiratory response to CO_2 administration, it therefore remains necessary to select, on purely arbitrary grounds, either change in CO_2 tension, or extravascular change in cH, as an index of the respiratory stimulus not related to change in cH of the blood itself. With these limitations clearly in mind, the chief objection to a multiple factor concept and to the continued designation of arterial pCO_2 as a distinct factor in respiratory control is that change in pCO_2 (or $[CO_2]$) has never been shown to affect respiration even qualitatively without the pCO_2 change being somewhere accompanied by a corresponding alteration of pH. Thus, respiratory effects

TABLE 4. *Mean values employed for comparison of observed and calculated Δ RMV in Fig. 6*

Conditions and References	No. of Subj.	Change in pCO_2		Change in cH				Response to CO_2, liters/min/Δ .01 $cH_{eff.}$	Change in RMV		Symbol in Fig. 6
		Arterial	Venous	Arterial	Venous	CSF	Effective		Observed	Predicted	
A (this study)											
Increase in end-tidal pCO_2 from 44 to 50 mm Hg (I–II)	5	+5.8	+5.2	+.041	+.043	+.036	+.039	4.07	+15.86		●
Bicarbonate infusion at 50 mm Hg (II–III)		−0.6	+0.5	+.052	+.056	−.002	+.024		+9.27	+9.77	○
Combined hypercapnia and alkalosis (I–III)		+6.4	+4.7	−.011	−.013	+.038	+.015		+6.59	+6.11	◑
B (22, 24)											
Inhalation of 4% CO_2 in 35% O_2 in N_2	7	+2.3		+.018		+.019	+.019	3.85	+6.93		
Inhalation of 6% CO_2 in 35% O_2 in N_2		+7.0		+.053		+.058	+.056		+22.70		
Ingestion of NH_4Cl, $\Delta\dot{V}_0$		0		+.093		0	+.042		+15.70	+16.17	△
Ingestion of $CaCl_2$, $\Delta\dot{V}_0$		0		+.070		0	+.032		+12.50	+12.32	▲
Ingestion of acetazoleamide, $\Delta\dot{V}_0$		0		+.101		0	+.045		+18.20	+17.33	×
C (25, this study)											
Diabetic acidosis	5	−25.4	−14.4	+.270	+.250	−.183	+.012	4.07	+10.00	+4.88	■

Predicted values for Δ RMV were calculated using the expression Δ RMV = $a\Delta cH_{effective}$, where $\Delta cH_{effective}$ = .45 ΔcH_{blood} + .55 ΔcH_{CSF}. In *conditions A* and *C*, data for change in internal jugular venous blood were available and, on an arbitrary basis, were used in place of values for Δ cH and Δ pCO_2 in arterial blood. In *condition B*, only arterial blood data were available. No predicted Δ RMV is shown for simple CO_2 breathing since observations of Δ RMV during CO_2 inhalation were used to determine "sensitivity" constants in respiratory control equations; for this reason observed and predicted values would be identical. In test of data for experimental acidosis (22, 24), technique used for graphic equalization of arterial pCO_2 in 2 experimental situations was considered as accomplishing the same fixation of central pCO_2 as was achieved for the present study by artificial regulation of end-tidal pCO_2 (8). Prediction of change in RMV during diabetic acidosis (25) was based on *patients JS, MM, TH, CB, and CC* and the use of average response to CO_2 breathing of 4.07/.01 effective Δ cH in the 5 subjects of *conditions A* in this table.

designated as change in threshold to pCO_2 (36) may in fact be related to change in stimulus level. Since change in pH of the blood (23, 26) or of the cerebrospinal fluid (28) does affect respiration without change in pCO_2, it must be realized that, in selecting a stimulus index for respiration, the choice to be made remains between *1*) changes in a single chemical stimulus index which can be quantitatively correlated with changes in ventilation (Fig. 6) and *2*) changes in two different factors, one of which has not yet been demonstrated to have any independent respiratory stimulant properties. Whereas *2*) appears unreasonable, there are situations such as simple CO_2 breathing when, for practical purposes, it may be convenient and adequate to employ changes of pCO_2 as the sole index of the respiratory stimulus (19).

The descriptive expressions relating the single factor, cH, and respiration in the present study also have limitations. Even if it were presumed that CO_2 breathing increases ventilation only via acidification of blood and other fluids, it remains uncertain how or even where the clearly demonstrable respiratory effects of changes in blood cH are effected. Central neurons are not directly exposed to circulating blood. Certainly *equations 2* and *3*, though expressing the quantitative adequacy of a single-stimulus index in the respiratory response to CO_2 breathing, do not distinguish among *a*) the uniform influence of a single, composite cH change upon central respiratory neurons, *b*) actions of cH at different sites upon cells having perhaps different sensitivities to change in cH, *c*) integration of concurrent, but separate, influences of blood and cerebrospinal fluid acid-base reaction upon nerve cells exposed both to blood and to cerebrospinal fluid, and *d*) possible direct or chemoreflex influences of change in blood cH upon the threshold of central respiratory neurons to changes in the cH of their own local environment.

There are further limitations which, while apparent, deserve mention here. In spite of the clear correlation of respiration with change in measureable factors such as cH, the mechanism by which any stimulant factor affects the activity either of central respiratory neurons or peripheral chemoreflex receptors is essentially unknown. Second, the quantitative relationships observed in the present study appear to pertain to conditions of acute respiratory stimulation and its removal; they do not necessarily relate to depression of breathing below the normal, eupneic level (11, 20a, 37), or to some chronic alterations of acid-base balance (38, 39). Finally, there must be added the obvious fact that, in contrast to the responses in CO_2 breathing, respiratory regulation in eupnea appears to involve fluctuations in reactivity of respiratory neurons to chemical stimuli, with inverse correlation between the magnitude of ventilation and the levels of cH or pCO_2 in blood and central fluids.

Brain Circulation

Fig. 2 shows the increase in CBF index observed in the present study, with elevation of alveolar pCO_2 from 44 to 50 mm Hg. However, when arterial pCO_2 was

maintained at 50 mm Hg and the CO_2-induced acidemia abolished by infusion of sodium bicarbonate, the CBF index remained as high as when pCO_2 and cH were elevated together. Thus, in contrast to respiratory drive, the tone of cerebral vessels appears to be related almost quantitatively to change in pCO_2 and to be unaffected by change in blood cH unless the latter is accompanied by alteration of CO_2 tension. These findings in the region of normal acid-base balance may differ only in degree from those of severe metabolic acidosis (25), in which gross change in the acidity of blood is associated with a detectable diminution of cerebral vascular resistance, even though blood pCO_2 is lower than normal.

The apparent relationship of cerebral blood flow index to change in the pCO_2 and essential independence of change in the cH of blood perfusing the brain still does not assure a direct role for molecular CO_2 in control of brain circulation. As in the case of part of the respiratory response to CO_2 breathing, it is not yet possible to effect experimental separation of the roles of CO_2 and H^+. Therefore, although the effects of increased CO_2 tension in blood upon the tone of brain vessels may be brought about by some primary action of the CO_2 molecule (such as inert gas narcosis), the ready passage of CO_2 into cells (33) may produce the relaxation of vascular smooth muscle cells indirectly, as through the changes this induced in intracellular cH.

Interactions of Respiratory and Cerebral Circulatory Control

Changes in the CO_2 tension of arterial blood are determined by, and at the same time indirectly contribute to, the existing ventilatory state. CO_2 also provides the major link between the mechanisms of respiratory control and those concerned with the regulation of overall brain circulation. Under normal circumstances, breathing air, the linkage of respiratory and brain circulatory control via arterial pCO_2 should tend to minimize fluctuations in the cH, pCO_2, and pO_2 of respiratory neurons and other components of brain tissue (5, 19). It is for such reasons that the background regulation of the brain circulation and, hence, of the internal environment of the brain can be considered in large measure to be passive consequences of an active response by respiratory neurons to acid-base changes in their own local environment (40, 41). Even in a system of interrelated respiratory and brain circulatory control, the different effects upon respiration and cerebral blood flow index resulting from change in cH and pCO_2 are not surprising. While CO_2 is prominently involved in the regulation of each function, there is no reason to expect that the ultimate biophysical mechanisms by which change in pCO_2 produces relaxation and contraction of smooth muscle cells in cerebral vessels are the same as the mechanisms which increase and decrease the rate of electrical discharge by the neurons of the respiratory centers. The results of the present study offer no direct information bearing upon this question. However, they are quantitatively compatible with the possibilities that $[H^+]$ may be intimately involved within each system (perhaps extracellularly for respiratory neurons and intracellularly for smooth muscle cells), and that molecular CO_2 has no direct role in either.

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