RESPIRATORY RESPONSES TO ADRENOCORTICOTROPIN AND DEXAMETHASONE IN UNANESTHETIZED GOATS

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ABSTRACT

RESPIRATORY RESPONSES TO ADRENOCORTICOTROPIN AND DEXAMETHASONE IN UNANESTHETIZED GOATS

By

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Adrenocortical influences on chemically regulated respiratory excitability were measured subsequent to intramuscular administration of adrenocorticotropin (80 I.U.) and dexamethasone (2.5 mg) to adult female goats with hormone treatment schedules of 12 and 36 hours. Hormone injections were given at 12 and 3 hours before carbon dioxide (CO_2) inhalation in the 12 hour regimes, while the 36 hour treatments involved similar hormone injections at 36, 24, 12 and 3 hours before the inspiratory studies. Minute ventilation, respiratory frequency, fraction of expired oxygen, fraction of expired CO_2, rectal temperature, heart rate, mean arterial blood pressure, arterial pH, arterial bicarbonate concentration and arterial CO_2 tension were monitored during the administration of inspiratory gases consisting of room air, and gas mixtures of $2\frac{1}{2}\%$ CO_2 plus 21% O_2 in nitrogen and 5% CO_2 plus 21% O_2 in nitrogen. These measurements allowed the calculation of tidal volume (V_T), respiratory dead space, alveolar ventilation ($\dot{V}_A$) and oxygen consumption. Estimations of cisternal cerebrospinal fluid (CSF) bicarbonate

concentration, CSF CO_2 tension and CSF pH were made from corresponding arterial values by employing comparisons of arterial and CSF measurements existent in the literature.

No significant changes occurred in respiratory frequency, blood pressure, heart rate, rectal temperature, arterial bicarbonate concentration or CSF bicarbonate concentration in any of the groups following the development of hypercapnic respiratory drive.

Oxygen consumption was not significantly affected by any of the hormone treatments. Additionally, mean rectal temperatures of the treated groups were consistently less than those of control.

Mean arterial blood pressure during both dexamethasone treatments was 17 mm Hg greater than those values of either control or of the two adrenocorticotropin (ACTH) treatments. This effect was presumably associated with high-level glucocorticoid potentiation of vasoconstrictor activity. Further, the bicarbonate concentration in both the blood and CSF compartments was significantly elevated in the 36 hour ACTH regime over that found in any other group, probably resulting from the development of metabolic alkalosis due to the enhanced mineralocorticoid activity induced by this treatment.

Tidal volume, $\dot{V}_A$, arterial hydrogen ion (H^+) concentration and CSF H^+ concentration all were elevated upon increasing inspired CO_2 content. The increased respiratory activity during induced hypercapnia was related to the

elevated CSF H^+ concentration which presumably stimulated the central chemoreceptors.

Correlations between $\dot{V}_A$ and blood or CSF CO_2 tension (P_{CO_2}) indicated that all treatment groups except the 36 hour ACTH regime exhibited greater respiratory excitability than control animals, since the $\dot{V}_A$ of the hormonally treated animals was greater than that of control animals at all measured CSF or arterial P_{CO_2}. Evaluation of $\dot{V}_A$ in terms of arterial pH indicated that all hormone treatments other than the 12 hour dexamethasone group exhibited greater respiratory excitability than control animals throughout the measured range of arterial pH. Comparison of $\dot{V}_A$ as a function of CSF H^+ concentration showed that all hormone treatments consistently increased respiratory excitability above control by depressing the ventilatory response set-point for CSF H^+ concentration by approximately 3.0 nM H^+/kg H_2O. The ventilatory response sensitivity was essentially unaltered since the magnitude of change in $\dot{V}_A$ for unit change in H^+ concentration was similar in all treatment regimes to that of control.

Since both the ACTH and dexamethasone treatments induced nearly identical increases in respiratory activity at specific levels of CSF pH, the effective mediators of these response changes were presumably glucocorticoids. The ability of ACTH to alter ventilation was thus associated with this hormone's action to enhance adrenocortical hormone release. The similarity of the 12 and 36 hour responses

suggests that the glucocorticoid effects on respiratory mechanisms occur within 12 hours after initiation of hormone treatment. It is possible that the site of glucocorticoid action is directly on the medullary respiratory control center to decrease its set-point for respiratory response to CSF H^+ concentration. The glucocorticoid effect might also be to enhance chemoreceptor activity at either central or peripheral sites. Finally, glucocorticoids may act to alter the strength of an input to the respiratory control center from some source other than that of the chemoreceptors.

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INTRODUCTION

The first reports concerning the effects of stages of the menstrual cycle and pregnancy on ventilation appeared in the literature nearly 60 years ago. Subsequent investigations suggested that several steroid hormones may be responsible for increasing respiratory excitability. These studies, conducted with untrained human subjects under poorly controlled controlled conditions, used the partial pressure of either alveolar or arterial carbon dioxide as an index of the level of respiratory activation. The quantitative effect of any hormone on respiration has yet to be determined. The site of hormonal influence and the mechanism of action have likewise not yet been elucidated. The present study was designed to attempt a more extensive and precise evaluation of adrenocortical influence on respiratory excitability.

LITERATURE REVIEW

There is evidence that certain gonadal (Hasselbalch and Gammeltoft, 1915; Loeschcke and Sommer, 1944; Heerhaber et al., 1948; Goodland et al., 1953; Huang and Lyons, 1966; Lyons and Huang, 1968) and adrenal steroids (Koepchen, 1953) increase respiratory activity. Koepchen et al. (1954) suggested that ACTH may have a similar stimulatory effect upon respiration.

In order to adequately evaluate mechanisms of hormonal action on respiration, it is necessary to present a brief review of respiratory control. Consideration must also be given to the multiple physiological effects of those hormonal agents employed in the course of this research.

Respiratory Control

Gross localization of the respiratory control center was first performed by Julian Legallois in 1811 (Lambertson, 1968). By progressively ablating increasingly primitive areas of rabbit brain, he isolated the site of the control center to within the medulla. He found that the medulla alone was capable of generating spontaneous rhythmical respiration. This function of the medulla has since been

well substantiated (Hoff and Breckenridge, 1948; Brodie and Borison, 1957; Wang et al., 1957).

Lumsden (1923 and 1924) was the first investigator to describe the neural interactions by which the medullary center induces and regulates rhythmic breathing. He showed that extramedullary influences from the vagus and pontile pneumotaxic centers make possible the medullary rhythm. Lumsden found that when vagal and pontile-medullary connections were severed, respiratory cyclic activity stopped in the state of an inspiratory cramp.

Lumsden's findings led to further analysis of the genesis of rhythmicity within the medulla. By observing the effects of stereotaxic stimulation of the internal medulla, several investigators (Pitts et al., 1939; Brookhart, 1940; Ngai and Wang, 1957; Cohen and Wang, 1958) were able to differentiate inspiratory and expiratory groups of neurons. Pitts et al. (1939) found a spatial separation of these groups, with inspiratory neurons generally lying dorsal and caudal to the expiratory cell groups. On the other hand, Brookhart (1940) reported the groups to be diffuse and intermingled with one another, rather than existing as separate and discrete neurological centers.

With the discovery of these intramedullary cell groups, it became evident that understanding of respiratory control was dependent on knowledge of the basic interactions of the two cell groups which constitute the respiratory control center. Upon direct stimulation of the inspiratory

cell group (Haber et al., 1957), the inspiratory neurons in the control center were activated via cellular recruitment as evidenced by increased inspiration amplitude and the associated increase in impulse frequency occurring in the phrenic nerve. Any input causing an increased activity level in either the inspiratory or expiratory cell groups also brings about a simultaneous inhibitory influence upon the opposed group (Salmoiraghi and Burns, 1960). The inspiratory group is believed to display spontaneous self re-excitation in initiating the inspiratory phase of the respiratory cycle (Burns, 1963).

Cohen and Wang (1959) showed that two types of "phase spanning" neurons (inspiratory-expiratory and expiratory-inspiratory neurons) in the medulla and lower pontile region provide a modulating influence upon the medullary control center. The inspiratory-expiratory neurons start firing during inspiration, reaching a peak activity level at the end of inspiration, and then stop firing during expiration. It was believed that these neurons inhibit inspiration. Conversely, neurons of the expiratory-inspiratory type are active from the middle of expiration until mid-inspiration, presumably acting to inhibit expiration.

There is evidence for further modulation of the medullary respiratory control center by certain areas of the central nervous system. According to Lumsden (1923 and 1924), the pontile pneumotaxic center supplies constant

inhibition to the inspiratory cell group. Fink et al. (1962) postulated an excitatory influence on respiration originating from an area between the superior and mid-collicular level in cats because decerebration rostral to the superior colliculus increased minute ventilation and respiratory frequency more than did mid-collicular decerebration. A major afferent to the respiratory control center is from central chemoreceptors located either on the surface of the medulla (Mitchell et al., 1963) or at an internal medullary site (Pappenheimer et al., 1965; Fencl et al., 1966).

The regulatory activity of the respiratory control center is also modified by inputs from the periphery. In 1889, Head found that decreasing lung volume provided a stimulus for inspiration that was mediated through the vagus. This was a reversed Hering-Breuer response, where lung deflation apparently decreased the inhibitory input from the pulmonary stretch receptors. Chemoreceptive tissue in the carotid and aortic bodies has also been identified (Comroe and Schmidt, 1938; Comroe, 1939) as having reflex influences upon respiration.

The most comprehensively studied peripheral chemoreceptor, the carotid body, has a large blood flow, allowing it to rapidly and reliably respond to blood chemistry alterations in the arterial supply which perfuses it (Daly et al., 1954). A significant arterio-venous oxygen difference

across the carotid bodies of the cat subsequent to partial common carotid occulusion suggested that the receptors have appreciable metabolic rates (Daly et al., 1954). Under normal conditions, tonic afferent activity can be monitored in the sinus nerve, which originates from the carotid body (Eyzaguirre and Lewin, 1961). Perfusing carotid or aortic bodies with fluids containing high CO_2 tensions or low partial pressures of arterial oxygen (Comroe and Schmidt, 1938; Comroe, 1939) results in a stimulation of respiration. Decreasing the pH of similar perfusates was shown by Schmidt et al. (1939) to enhance respiration. Decreasing blood flow through the carotid bodies induced respiratory stimulation, where rapid metabolic utilization of oxygen and formation of CO_2 and other acid metabolites in the slowly moving blood were postulated to be the ultimate source of stimulation to the carotid receptors (Daly et al., 1954). Hornbein and Roos (1963) reported that afferent carotid discharge frequency was a function of arterial pH rather than of arterial CO_2 tension (P_{aCO_2}).

The work of Gemmill and Reeves (1933) suggested that although peripheral chemoreceptors are important in monitoring changes of the partial pressure of oxygen, they are not necessary for a respiratory response to hypercapnia. More recent investigations by Dutton (1967) indicated that oscillations of the CO_2 tension of blood perfusing the

carotid bodies results in greater increases in ventilation than do comparable steady state elevations of P_{aCO_2}. This work suggests that P_{aCO_2} oscillations resulting from cyclic changes in the alveolar P_{CO_2} may be sensed in the carotid bodies and provide a fine adjustment of respiration.

As early as 1926, Gesell and Hertzman observed that the cisternal cerebrospinal fluid (CSF) pH was different from that of arterial samples. The inhalation of CO_2 rich gases results in increasing the hydrogen ion concentration in both arterial blood and in cerebrospinal fluid. Robin and Bromberg (1959) hypothesized a more rapidly achieved equilibrium of CO_2 between blood and CSF than equilibriums with either hydrogen or bicarbonate ions. Since the partial pressure of CO_2 in internal jugular blood is essentially in equilibrium with CSF P_{CO_2}, Lambertson et al. (1961) were of the opinion that the parameters of CSF hydrogen ion concentration and internal jugular venous P_{CO_2} are equally valid indicators of ventilatory drive to central respiratory chemoreceptors in the steady state.

Several groups of investigators (Leusen, 1954; Mitchell et al., 1963; Pappenheimer et al., 1965) have indicated that cerebral ventricular perfusion of bicarbonate buffer solutions containing high hydrogen ion concentrations or low bicarbonate concentrations acted as a stimulus to respiration. A depression of respiration was observed when CSF perfusates contained low hydrogen ion concentrations.

These effects were mediated centrally since respiration was modified before changes were detected in arterial blood chemistry (Leusen, 1954). However, there remain conflicting viewpoints concerning the exact location of the central chemoreceptor site.

Direct application of acetylcholine, nicotine or acidic solutions (Mitchell et al., 1963) upon the ventrolateral surface of the medulla caused a prompt and marked stimulation of respiration. These data support the view that the chemoreceptor site is on the medullary surface, and therefore anatomically separated from the respiratory control center.

Other experimental techniques have provided results which are inconsistent with the hypothesis of superficial medullary chemoreceptors. Leusen (1954) used boric acid/sodium borate buffers in solutions perfusing the brain ventricles, which allowed him to alter CSF hydrogen ion concentration independently of CO_2 or of bicarbonate. He observed no ensueing changes in respiration following intraventricular perfusions containing high boric acid concentrations. It was found that dynamic respiratory responses to administration or withdrawal of inspired CO_2 were more rapid than CSF changes, but appeared to lag behind variations of arterial pH, Pa_{CO_2} or alveolar P_{CO_2} (Fuleihan et al., 1963; Lambertson et al., 1965).

The fact that respiratory responses to inspired CO_2 occur at a time intermediate between detectable CSF and

$P_{a_{CO_2}}$ changes suggests that the chemoreceptor location is such that it is simultaneously affected by chemical changes in both blood and CSF. Lambertson et al. (1961) found that the total respiratory response to inhaled CO_2 was decreased by 45% when the respiratory acidosis was compensated with bicarbonate infusion. They concluded that 45% of the respiratory response was induced by arterial changes following CO_2 inhalation, leaving 55% of the response due to CSF changes. In that there is no appreciable blood-CSF gradient for CO_2 (Lambertson et al., 1961), Pappenheimer and his associates (1965) concerned themselves with bicarbonate and hydrogen ion gradients between CSF and blood. When the CSF was perfused with solutions of varying bicarbonate concentrations, they found that alveolar ventilation in goats was a single function of the hydrogen ion concentration at a point approximately $\frac{3}{4}$ the way down a bicarbonate concentration gradient between the medullary-CSF surface and an idealized interface at the capillary-glial boundary. This point along the gradient was designated as the location of the central chemoreceptor, in that ventilation was a single function of the calculated hydrogen ion (H^+) concentration at this site. According to this hypothesis, the ultimate regulatory mechanism adjusting ventilation to various acid-base disturbances is a bicarbonate pump which maintains simultaneous gradients of hydrogen and bicarbonate HCO_3^- ions between blood and CSF (Pappenheimer, 1966).

Fencl et al. (1966) showed that in the steady state, alveolar ventilation of goats is directly related to CSF hydrogen ion concentration, regardless of chronic metabolic acid-base disturbances. In these studies, no net ionic fluxes between the CSF and blood compartments were observed. Assuming a bicarbonate pump at the glial-capillary boundry adjusted ionic movement, it was concluded that the ionic concentrations in the CSF were essentially identical with those of brain interstitial fluid. The work of Fencl's group thus suggested that under steady state conditions, measurements of CSF H^+ concentration is a valid reflection of hydrogen ion concentration at a brain interstitial chemoreceptor site.

Characterization and Effects of Adrenocorticotropin

Hypophysectomy results in marked atrophy of the adrenal cortex (Smith, 1930) because the pituitary releases a hormone, adrenocorticotropin (ACTH), capable of maintaining adrenocortical development. Adrenocorticotropin is a randomly coiled globular protein consisting of thirty-nine amino acid residues with a total molecular weight of about 4,500. The complete amino acid sequence of this protein has been determined for several mammals including the sheep, pig and cow (Li et al., 1958; Harris, 1960). There is minimal interspecies variation in the amino acid sequence. The COOH-terminal is phenylalanine and N-terminal is serine, which

is essential for characteristic action of the hormone (Sheperd et al., 1956). Most variation among species occurs at positions between residues 25 and 33 of the polypeptide chain (Li et al., 1958).

Adrenocorticotropin is synthesized and stored in the adenohypophysis. This area of the pituitary is highly vascular, receiving its blood supply from the hypothalamic-hypophyseal portal system (Wislocke, 1937). The portal system provides the means by which the hypothalamus controls ACTH secretion (Harris, 1948). Cells in the median eminence of the hypothalamus are rich in small polypeptides which activate synthesis and release of ACTH (Vernikos-Danellis, 1965), and thus cause adrenal cortical activation (Matsuda et al., 1964). According to Schally et al. (1958), the corticotropin releasing factor (CRF) peptide is similar in amino acid composition and sequence to that of vasopressin. This releasing factor is produced by cells located in the hypothalamic median eminence and secreted into the hypophyseal portal system, with CRF acting as the hormonal messenger to target cells within the adenohypophysis (Guillemin, 1964).

Hypophyseal release of ACTH is thought to be controlled by at least two negative feedback mechanisms. Several investigators (McCann et al., 1958; Smelik and Sawyer, 1962; Chowers et al., 1963; Davidson and Feldman, 1963) have shown that adrenocortical hormones injected or

implanted into the rat's hypothalamus bring about a decreased level of adrenal cortical function, and cause pronounced adrenocortical atrophy during chronic administration. In 1964, Beaven et al. found that intravenous injections of 10 mg of dexamethasone, a synthetic glucocorticoid, completely suppresses adrenocortical secretion in sheep for twelve hours. This inhibition is of central origin since the adrenals were still responsive to exogenous ACTH. Further, Brodish and Long (1962) reported that plasma CRF decreased after cortisol treatment of hypophysectomized rats. These results support the concept that adrenal steroids provide negative feedback by inhibiting the hypothalamic release of CRF. When cortisol was injected directly into the pituitary (Smelik and Sawyer, 1962; Chowers et al., 1963), no decrease in adrenocortical activity ensued. It was concluded that cortisol does not directly inhibit the pituitary. However, data in support of steroid feedback on the pituitary was presented by Russel et al. (1969), who noted that dexamethasone injected into the adenohypophysis was able to prevent ACTH release in response to elevated CRF release.

The findings of Kitay et al. (1959) suggested that ACTH has the capability of inhibiting its own release, although was uncertain whether this action occurs at the hypothalamus or hypophysis. Brodish and Long (1962) observed that the CRF concentration in the peripheral blood of rats increased after hypophysectomy and adrenalectomy,

probably resulting from loss of inhibition of ACTH upon the hypothalamic synthesis or release of CRF.

Other extra-adrenal effects of ACTH have been recognized. Adrenocorticotropin is known to possess some melanocyte-stimulating hormone (MSH) activity which is attributable to similarities between MSH and the N-terminal ACTH amino acid sequences (Li et al., 1960). Hollenberg et al. (1961) hypothesized that the acknowledged lipolytic activity of ACTH on adipose tissue is brought about by activation of a lipase other than lipoprotein lipase to induce enzymatic hydrolysis of triglycerides. Adrenocorticotropin is also known to have an anabolic effect upon protein which is dependent on the maintenance of permissive levels of glucocorticoids (Engel and Feredicks, 1959).

Torda and Wolff (1952) studied the effects of ACTH on the electrical activity of the brain. They made use of the electroencephalogram and the sensitivity to a convulsion-inducing drug as indices of brain excitability in adrenalectomized and hypophysectomized rats. Brain activity increased at from one to three minutes after ACTH injections, while chronic ACTH treatment which was of three to four days duration resulted in decreasing electrical activity of the brain.

The most documented effect of ACTH is its action upon the adrenal cortex. Removing the ACTH influence by hypophysectomy results in atrophy of the fasciculata and reticularis cell layers of the adrenal cortex (Deane and

and Greep, 1946). Conversely, ACTH injections result in hypertrophy of these same layers (O'Donnell et al., 1951). The ACTH induced adrenal hypertrophy is in part due to incorporation of amino acids into adrenal proteins (Farese, 1965). Stachenko and Giroud (1959) reported that ACTH added to slices of bovine fasciculata and reticularis cell cultures causes an increase of the corticosteroid concentration in the medium.

The glomerulosa layer of the adrenal cortex, whose histological integrity is essentially independent of ACTH, is responsible for the synthesis of aldosterone. The effect of ACTH on aldosterone release is not as clear as ACTH effects on other portions of the adrenal cortex. Data of Bartter et al. (1959) suggest that ACTH does stimulate aldosterone release in man, whereas Axelrad (1954) found no increase of plasma aldosterone after ACTH treatment. Stachenko and Giroud (1959) showed that ACTH added to glomerulosa cell cultures does not significantly change the production rate of either glucocorticoids or aldosterone. The effects of ACTH on the zona glomerulosa are thought to be greatly overshadowed by the more specific effects of the renin-angiotensin system to stimulate aldosterone release (Tobian, 1960).

The primary effect of ACTH on the adrenals is to stimulate the synthesis and release of adrenocortical hormones which are high in glucocorticoid activity. Recent investigations (Saffran and Rowell, 1969; Jaanus et al.,

1970) have utilized *in vitro* adrenal gland perfusion. By continuously monitoring corticosterone output it was found that, after a five to ten minute lag period, corticosterone output varies directly with the ACTH concentration and the duration of its perfusion. The response to a constant ACTH infusion decreases with time, suggesting an adaptation phenomenon. The perfusion of isolated adrenal cells, of which 90% are of the reticularis or fasciculata type, provides another method to assay the effect of ACTH on glucocorticoid release (Kitabchi and Sharma, 1971). Results from this perfusion assay indicated that both ACTH and 3', 5'-cyclic adenosine monophosphate nucleotides stimulate corticosterone production to an equal extent, implying that the response to ACTH may be mediated through this nucleotide.

Glucocorticoid Actions

The large volume of information that has accumulated concerning glucocorticoid effects has been extensively reviewed (Moon, 1961; Eisenstein, 1967). Circulating glucocorticoids alter carbohydrate metabolism by stimulating hepatic glycogen synthesis (Glenn, 1961). The hyperglycemia following glucocorticoid administration indicates that these steroidal hormones may act to decrease peripheral glucose utilization (Jeanrenaud and Renold, 1960). There is evidence suggesting that glucocorticoids exhibit insulin antagonistic properties on fat metabolism, causing an inhibition of hepatic lipogenesis while enhancing lipolysis

in adipose tissue (Brady et al., 1951; Fain, 1964). Glucocorticoids apparently act to increase whole body protein catabolism as evidenced by elevated urinary nitrogen excretion (Long et al., 1940), while inducing increased hepatic amino acid accumulation for protein synthesis (Weber et al., 1965). Cortisone has been reported to antagonize the vitamin D dependent uptake of calcium from the small intestine (Harrison and Harrison, 1960). Large, non-physiological doses of glucocorticoids have also been demonstrated to alter immunological and inflammatory reactions, and to inhibit wound healing (Bjørnboe et al., 1951; Holden and Adams, 1957; Weissmann and Thomas, 1964).

Natural glucocorticoids exhibit some mineralocorticoid activity, and it has been shown that significant mineralocorticoid effects result from exposure to substantial levels of glucocorticoids. Administration of aldosterone and deoxycorticosterone acetate causes decreased urinary sodium and increased urinary potassium excretion (Ross et al., 1959; Ferrebee et al., 1941; Ganong and Mulrow, 1958). Conn's (1955) description of primary aldosteronism includes the development of metabolic alkalosis and decreased renal tubular reabsorption of water. Bartter and Fourman (1962) reported that after injection of ACTH in humans, potassium and hydrogen ion excretion increased independently of changes in sodium excretion.

Massive treatment doses of cortisol have been reported to increase cardiac output and decrease peripheral resistance without significantly altering arterial blood pressure (Sambhi, 1965). The findings of Zweifach (1960) indicate that glucocorticoids function to potentiate cardiovascular responses to catacholamine vasoconstrictors. On the other hand, Small et al. (1959) were not able to demonstrate the existence of such potentiation in the pulmonary arterioles of Starling heart-lung preparations.

Adrenocortial steroids provide negative feedback upon the hypothalamic CRF releasing cells (Beaven et al., 1964; Russel et al., 1969), suggesting that specific receptor sites for glucocorticoids exist in the central nervous system. The brain is known to take up labeled blood cortisol, which disappears more rapidly from blood plasma than from the brain, suggesting that the steroid is accumulated in the brain (Peterson and Chaikoff, 1963; Walker et al., 1971). Touchstone et al. (1966) demonstrated that whole human brain homogenate contains a cortisol concentration of 400 μg/100 gm compared to 15 μg/100 ml in blood. McEwen et al. (1969) postulated that the rat brain has two separate mechanisms for the uptake of labeled corticosterone: (1) a generalized concentrating mechanism in the whole brain; and (2) a limited active uptake in specific areas of the septum and hippocampus.

Electro-shock seizure threshold has been used as a measure of brain excitability. Woodbury and Sayers (1950) demonstrated that cortisone treatment caused a decrease in the shock threshold, indicating an increase in whole brain electrical activity. On the other hand, analysis of electro-encephalograms after cortisone or ACTH administration to medical students showed no change in electrical activity (Friedman and Engel, 1956).

It is possible to monitor electrical activity at various isolated points within specific brain structures by the use of multiple or single unit recording electrodes. Feldman et al. (1961) made unit recordings of cat brain stem potentials evoked by sciatic nerve stimulation. Increases in amplitude of the evoked responses in hypothalamic and midbrain reticular sites were noticed within five to fifteen minutes after cortisol injection. Similar potential changes occurred in these same areas two hours after ACTH treatment. Feldman et al. concluded that the induced response was directly due to the steroid hormone, and that the increased potential amplitude indicated an alteration of membrane potential to lower the threshold for synaptic transmission. Intravenous injection of cortisol also acts to alter the spontaneous rate of firing of hypothalamic and midbrain reticular units in cats (Slusher et al., 1966). After injecting cortisone into the third cerebral ventrical, rapid increases in discharge frequency occurred in units of the lateral hypothalamus and of the hippocampus. However this

procedure did not indicate noticeable changes in midbrain reticular unit activity following such cortisone injections (Feldman, 1966).

The possibility exists that glucocorticoids may only indirectly effect brain activity by functioning to alter the availability of certain chemical transmitter substances. Steiner et al. (1969) have demonstrated that acetylcholine and dexamethasone have opposite effects upon the activity of mesencephalic units in the rat. Maas and Mednieks (1970) showed that hydrocortisone, added to cultures of rat cerebral cortex, produces increased norepinepherine uptake.

Adrenocortical Activation Following Respiratory Stress

Certain relationships between respiration and adrenocortical function have been formulated. One route of investigation has been to elucidate the influence of changes in respiratory drive and alterations of arterial acid-base chemistry upon adrenal release of 17-hydroxycorticosteroids. It has been reported that moderate hypoxia (100 mm Hg inspired oxygen) results in increased peripheral plasma 17-hydroxycorticosteroid concentration in man (Hale et al., 1957). However, Biddulph et al. (1959) reported no such steroid increase in peripheral plasma 17-hydroxycorticosteroid concentration in anesthetized dogs during hypoxia (5% oxygen in inspired gas). Analysis of lumbo-adrenal vein plasma has demonstrated that hyperoxia produced by breathing

100% oxygen (Marotta et al., 1965) and hypoxia resulting from 10% inspired oxygen (Hirai et al., 1963; Marotta et al., 1963; Marotta et al., 1965) both result in significant increases in the release of 17-hydroxycorticosteroids in dogs. Anichkov et al. (1960) found that the adrenocortical response to hypoxia disappeared after either hypophysectomy or sinus nerve section. They suggested that the adrenocortical response to hypoxia is regulated by hypoxic carotid chemoreceptor stimulation which provides input into the hypothalamic-adrenophypophyseal system for ACTH release.

Richards and Stein (1957) demonstrated that hypercapnia also induces adrenocortical activation for 17-hydroxycorticosteroid release. They found that inspired gas mixtures containing over 10% CO_2 results in maximal activation of 17-hydroxycorticosteroid output into the adrenal venous blood of dogs. They concluded that the response was mediated centrally since hypophysectomized dogs did not exhibit increased adrenal steroid output even after 20% carbon dioxide administration, while the adrenal cortex was still capable of responding to exogenous ACTH.

In 1957, Richards infused hydrochloric acid into anesthetized dogs and noted a drop in arterial pH and $P_{a_{CO_2}}$, and a marked elevation of the 17-hydroxycorticosteroid concentration in the lumbo-adrenal vein. When sodium bicarbonate was infused, arterial pH and $P_{a_{CO_2}}$ increased, but 17-hydroxycorticosteroid release also increased. Lau and Marotta (1970) reported that adrenocortical activation in

dogs was primarily due to oxygen lack and secondarily due to blood acid-base changes. They also demonstrated that intact carotid and aortic body innervation was necessary for such adrenocortical activation.

Hormonal Effects on Respiratory Excitability

In 1915, Hasselbalch and Gammeltoft reported that the alveolar partial pressure of carbon dioxide ($P_{A_{CO_2}}$) decreased in women during pregnancy, and promptly returned to normal non-pregnant values at the end of gestation. They also showed that the $P_{A_{CO_2}}$ of women was depressed during the luteal phase of the menstrual cycle. Plass and Oberst (1938), and Goodland et al. (1954) have confirmed these findings and reported an increased alveolar ventilation during pregnancy. Loeschcke and Sommer (1944) studied the respiratory responses of pregnant women to inspired CO_2. Carbon dioxide response curves (minute ventilation plotted as a function of $P_{A_{CO_2}}$) were shifted to the left during pregnancy, indicating a decreased stimulus threshold for CO_2. However, the slope of the CO_2 response curve during pregnancy was not significantly different from that of the non-pregnant controls, indicating that pregnancy did not alter the respiratory sensitivity to $P_{A_{CO_2}}$. In 1958 Antonio and Lyons used the ventilatory ratio (CO_2 induced minute ventilation divided by resting minute ventilation) as an indicator of respiratory excitability, and found that this

ratio was higher in pregnant than non-pregnant women, suggesting that pregnancy does increase central respiratory responsiveness to hypercapnic drive.

These data implicate progesterone as the hormone which depresses the stimulus threshold for respiratory responsiveness to inhaled CO_2. Using both pre-pubertal and post-menopausal women, it was demonstrated that pregnanediol injections decreased the $P_{A_{CO_2}}$ for six hours after treatment (Heerhaber et al., 1948). Lyons and Antonio (1959) found that progesterone injections given to men and post-menopausal women resulted in both increasing respiratory excitability and in increasing the sensitivity of the central respiratory control center, when evaluated in terms of a carbon dioxide response curve. In 1966, Huang and Lyons reported that the decreased respiratory response threshold to CO_2 was not mediated through central chemoreceptors since progesterone treatment induced no significant alterations in CSF pH. The action of progesterone in lowering the arterial CO_2 tension ($P_{a_{CO_2}}$) is clinically useful for treatment of emphysema (Tyler, 1960) and alveolar hypoventilation syndrome of the obese (Lyons and Huang, 1968).

Other hormones besides progesterone and pregnanediol have been shown to affect respiration. Koepchen (1953) demonstrated that men treated with cortisone acetate exhibited a depression of alveolar P_{CO_2} lasting for approximately 24 hours. He was unable to detect any changes in $P_{A_{CO_2}}$

after treatment with testosterone. In 1954, Koepchen et al. reported that ACTH treatment caused a decrease in $P_{A_{CO_2}}$ which lasted for about thirty hours after intramuscular injection. In the view of his earlier work, Koepchen concluded that the ACTH effect was secondary to that directly attributable to adrenocortical activation.

MATERIALS AND METHODS

Animals

Three, three year old non-pregnant goats (2 Nubians; 1 Togenberg) whose body weights ranged from 45 to 60 kg were used during the period between July 13, 1970 and March 27, 1971. The animals had been surgically prepared with externalized carotid loops two years prior to testing. The goats were trained to stand in a restraining stanchion and to wear a respiratory mask and valve assembly while in a thermally controlled chamber. Throughout the time of the trials, the animals were housed in sheltered pens and fed a diet of hay and water *ad libitum*.

Experimental Conditions

Once inside the environmental chamber, the animal was confined in an angle iron stanchion equipped with a vinyl sling which both supported the weight of the goat's trunk and effectively restricted its movement (Figure 1). Chamber temperature was monitored by a tele-thermometer (Yellow Springs Instrument Co., model 43 TD). Temperature control was maintained using Thermistemp Temperature Controller (Yellow Springs Instrument Co., model 71) which

regulated the pumping of hot or cold water through convective heat exchangers located in front of fans within the chamber. Chamber temperature was adjusted to 25°C ± 0.5°C through the summer until November 1st, at which time growth of winter fur necessitated lowering the controlled environmental temperature to 20°C in order to remain within the animals' thermoneutral zone.[1]

A respiratory mask and valve assembly (Lloyd Valve by Warren E. Collins, Inc.) was connected to flexible respiratory hosing to allow collection of expired gas and administration of inspiratory gases consisting of room air and mixtures of $2\frac{1}{2}\%$ CO_2 plus 21% O_2 in 76.5% N_2, and 5% CO_2 plus 21% O_2 in 74% N_2. A 150 liter Douglas bag (Warren E. Collins, Inc.) was employed as an inspiratory buffer reservoir for the administration of the test gases from high pressure gas cylinders. In view of the findings of Lambertson et al. (1961) that pulmonary equilibrium was achieved 7 minutes after beginning CO_2 administration to dogs, data collections in this study were made after the goats had breathed each gas mixture for 10 minutes

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[1]Unpublished data characterizing seasonal fluctuations in thermoneutral environmental temperature zones of the goat have been previously determined in this lab by W. F. Hofman, G. D. Riegle and B. E. Watkins.

Fig. 1. Environmental chamber for data collection.

Each goat was positioned in a restraining stanchion (RS) equipped with a vinyl sling (SL) to restrict its movement. Chamber temperature was monitored using a thermister probe (TP) connected to a temperature controller (TC) which governed the pumping of water from hot or cold baths (HB/CB) through convective heat exchangers (CH) located in front of circulation fans (F). Inspiratory gas mixtures (IG) were administered through the respiratory mask and valve assembly (RMV). The expired gas was passed through a gasometer for the determination of minute ventilation ($\dot{V}_E$); a sample of this gas was analyzed for O_2 and CO_2 content. The carotid loop (CL) was catheterized (CA) to monitor heart rate (HR) and blood pressure (BP), and to obtain anaerobic blood samples (BS). Respiratory frequency was obtained using a mercury-in-rubber strain gauge (SG) around the animal's abdomen. A thermocouple inserted into the goat's lower colon was used to monitor rectal temperature (TR).

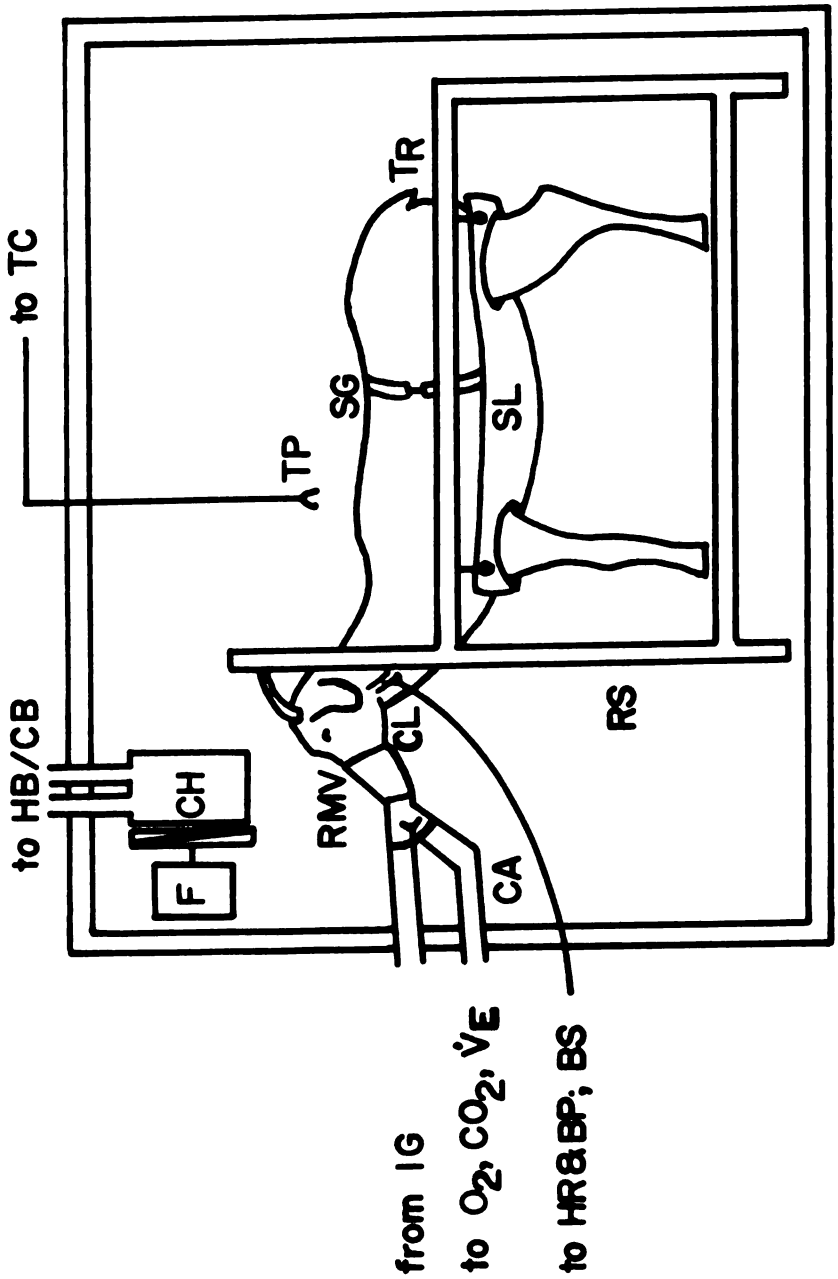

FIGURE 1
ENVIRONMENTAL CHAMBER FOR DATA COLLECTION

Experimental Design

All experiments were performed at the same time of day to avoid variability which might be due to circadian changes in plasma adrenocortical steroid levels. Ninety CO_2 response curves were performed in this study, where two CO_2 response curves were determined from one animal on any experimental day. For the generation of each CO_2 response curve, the animal was positioned in the environmental chamber at 8:30 A.M. Following a 90 minute adaptation period, the respiratory mask and valve assembly was fitted on the snout and the animal was given an additional 30 minutes to adjust to breathing room air through the mask. During the ensuing 15 minutes, four data recordings were made at 5 minute intervals, followed by the drawing of an anaerobic arterial sample (Figure 2). The animal was then given $2\frac{1}{2}\%$ CO_2 as inspired gas for 10 minutes. Four additional data recordings followed by a second arterial blood sample were then obtained. After the goat breathed 5% CO_2 for 10 minutes, the last data recordings were made, followed by the drawing of the third blood sample. Subsequent to a 90 minute recovery period, a second CO_2 response curve was generated as described above.

The experimental design included hormone treatment regimes of ACTH and dexamethasone, with therapy durations of both 12 and 36 hours for each hormone (Figure 2). All animals were allowed at least a two week recovery period

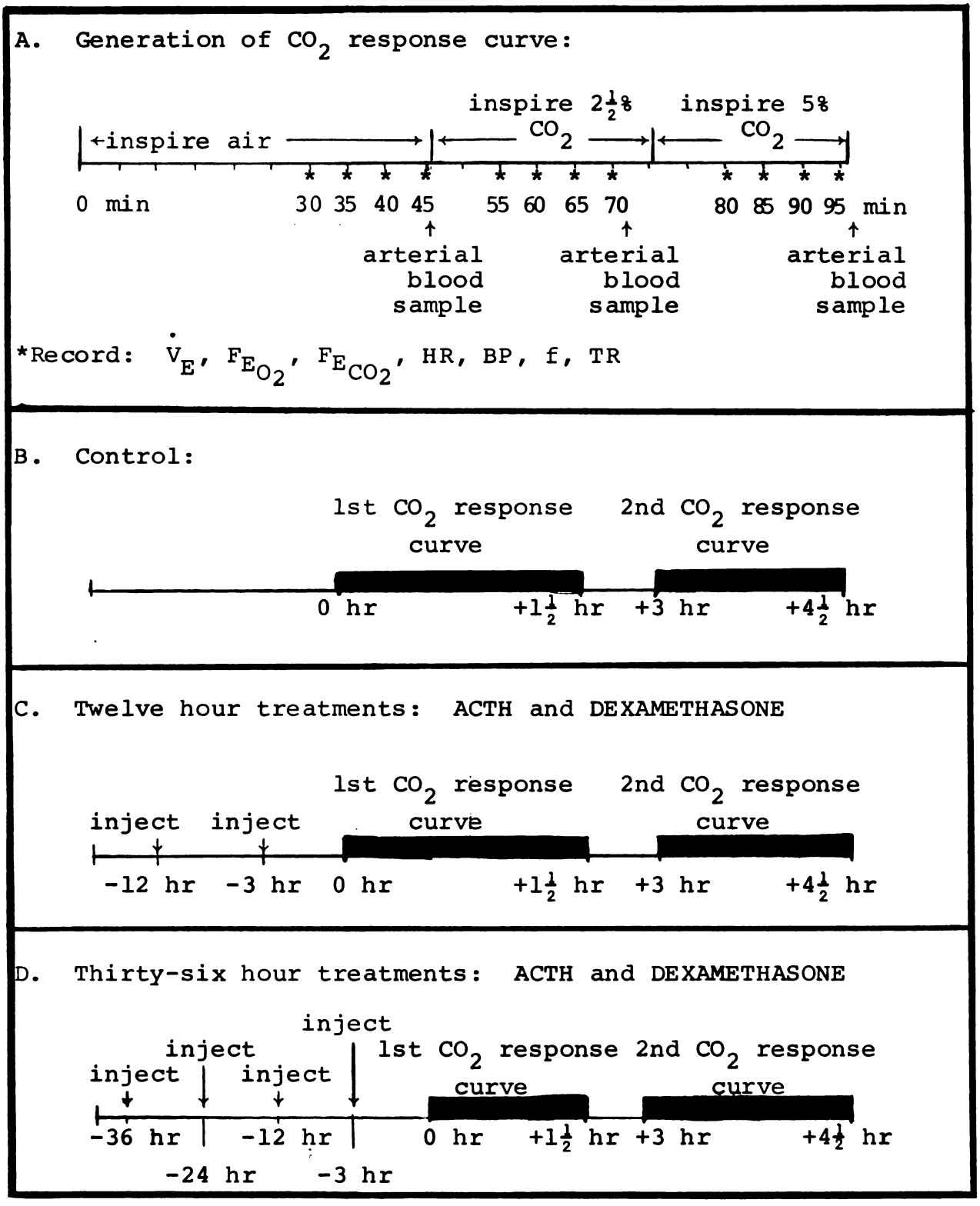

FIGURE 2

TREATMENT SCHEDULES

between the completion of one hormone treatment and the initiation of another. The 12 hour ACTH treatment group received intramuscular injections of 80 I.U. ACTH[2] at 12 and 3 hours before the respiratory studies. To evaluate possible temporal effects of the treatment, a 36 hour ACTH group receiving similar injections at 36, 24, 12, and 3 hours pre-experiment was included in this study. A second series of experiments utilized intramuscular injections of $2\frac{1}{2}$ mg of dexamethasone suspended in corn oil to determine whether the respiratory effects caused by ACTH were due directly to ACTH or to subsequent adrenal activation. Dexamethasone treatment periods were of 12 and 36 hours, with similar injection intervals as described for the ACTH regimes. Data obtained from the animals during each of the hormone treatments were compared to data generated from control animals which did not receive hormone therapy. These uninjected control animals were deemed suitable since they did not exhibit response characteristics distinguishable from those receiving corn oil injections at 12 and 3 hours before CO_2 inhalation.

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[2]The ACTH preparation was Depo-ACTH®, developed by the Upjohn Co. for respiratory corticotropin injection.

Measured Variables

Rectal temperature was continuously monitored by means of a copper-constantan thermocouple encased in a polyethylene catheter and inserted approximately 10 cm into the lower colon. The thermocouple was referenced to an ice bath and calibrated with an accuracy of 0.1°C as displayed on a strip chart recorder (Hewlett-Packard, model 7100 BM).

Respiratory frequency (f) was recorded using a variable resistance mercury-in-rubber strain gauge affixed to an elastic band which was strapped around the animal's abdomen. Respiratory movements induced changes in strain gauge resistance which led to a matching electrical bridge (model 270 plethysmograph; Parks Electronics Laboratory) and was displayed on a Mosley strip chart recorder (model 7100 BM).

The carotid catheter consisted of a 20 gauge needle connected to a multi-fit stopcock manifold (Becton-Dickenson, model 3170) by means of a length of "Intramedic" polyethylene tubing with an inside diameter of 0.034". The manifold served both as a blood sampling site and as a relay to a pressure transducer (Stathan, model P 23 AC, Hatorey Products). Signals from the transducer were amplified through a D.C. preamplifier (Grass Instrument Co., model 5 P 1 K) for calibrated read-out of mean arterial blood pressure and heart rate on a strip chart recorder (Mosley, model 7100 BM).

The expired gas volume was collected for timed intervals at atmospheric pressure and temperature, and saturated with water vapor (ATPS), by passing the gas through a respiratory gasometer (Max Plank Institute of Physiology). Minute ventilation ($\dot{V}_E$) was then computed in one of the following manners:

$$\dot{V}_E\ (BTPS) = \dot{V}_E\ (ATPS) \times \left[\frac{P_B - P_T\ (H_2O)}{P_B - P_A\ (H_2O)}\right]\left[\frac{273 + T_R}{273 + T}\right] \quad (1)$$

where:

$\dot{V}_E$ (BTPS) = minute ventilation (l/min) at body temperature and pressure, and saturated with water vapor

$\dot{V}_E$ (ATPS) = minute ventilation (l/min) at ambient temperature and pressure, and saturated with water vapor

P_B = barometric pressure (mm Hg)

$P_T\ (H_2O)$ = partial pressure of water vapor at ambient temperature (mm Hg)

$P_A\ (H_2O)$ = partial pressure of water vapor at body pressure (mm Hg)

T_R = rectal temperature (°C)

T = ambient temperature (°C).

$$\dot{V}_E\ (STPD) = \dot{V}_E\ (ATPS) \times \left[\frac{P_B - P_T\ (H_2O)}{760}\right]\left[\frac{273}{273 + T}\right] \quad (2)$$

where:

$\dot{V}_E$ (STDP) = minute ventilation (l/min) at 0°C, 760 mm Hg, and free of water vapor

$\dot{V}_E$ (ATPS) = minute ventilation (l/min) at ambient temperature and pressure, and saturated with water vapor

P_B = barometric pressure (mm Hg)

P_T (H_2O) = partial pressure of H_2O vapor at ambient temperature (mm Hg)

T = ambient temperature (°C)

The fraction of expired carbon dioxide ($F_{E_{CO_2}}$) was measured by passing a continuously flowing sample of the expired gas through a Mead CO_2 analyzer (Harvard Apparatus Co., model 2000) which had been calibrated with gases of known CO_2 composition as measured on a Haldane gas analyzer. Similarly, the fraction of expired O_2 ($F_{E_{O_2}}$) was determined by channeling the expired sample through a Beckman paramagnetic oxygen analyzer (model C 2).

At the end of each period of breating a gas mixture, an anaerobic arterial sample was drawn from the stopcock manifold into a 5 cc heparinized syringe and promptly sealed with a syringe cap filled with mercury. The blood samples were stored in an ice water bath for subsequent determination of arterial pH, $P_{a_{CO_2}}$, and bicarbonate (HCO_3^-) concentration. Arterial pH measurements were made from a microelectrode chain attached to a Radiometer pH meter (Radiometer Co., model 27). In determining $P_{a_{CO_2}}$ and bicarbonate concentrations, the samples were allowed to equilibrate with gases of known CO_2 tensions in a Radiometer Microtonometer

(Radiometer Co., model AMT-1). Values for HCO_3^- concentrations in mM/l and for $P_{a_{CO_2}}$ were determined by plotting the anaerobic and equilibrated pH's on a Siggard Anderson (1962) nomogram.

Calculated Parameters

From measurements of minute ventilation and the fractional composition of expired gas, one can calculate various respiratory quantities. Tidal volume (V_T) defines that amount of gas expired in one breath, and is calculated as:

$$V_T = \frac{\dot{V}_E}{f} \tag{3}$$

where:

V_T = tidal volume (ml BTPS)

$\dot{V}_E$ = minute ventilation (ml BTPS)

f = respiratory frequency (breaths/min)

Respiratory dead space (V_D) was determined to be 239 ± 19 ml (BTPS), which included both the goat's own physiological dead space plus that in the mask and respiratory valve. This quantity was calculated by using the Bohr dead space formula:

$$V_D = V_T \left[\frac{F_{E_{CO_2}} - P_{a_{CO_2}} / (P_B - P_{H_2O})}{F_{I_{CO_2}} - P_{a_{CO_2}} / (P_B - P_{H_2O})}\right] \tag{4}$$

where:

V_D = dead space (ml BTPS)

V_T = tidal volume (ml BTPS)

$F_{E_{CO_2}}$ = fraction of expired CO_2

$F_{I_{CO_2}}$ = fraction of inspired CO_2

$P_{a_{CO_2}}$ = partial pressure of arterial CO_2 (mm Hg)

P_B = barometric pressure (mm Hg)

P_{H_2O} = partial pressure of H_2O vapor at body temperature (mm Hg)

Alveolar ventilation ($\dot{V}_A$) is a term that describes that amount of gas actually flushing through the alveoli in one minute, and is calculated as:

$$\dot{V}_A = (V_T - V_D) \times f \qquad (5)$$

where:

$\dot{V}_A$ = alveolar ventilation (ml/min BTPS)

V_T = tidal volume (ml BTPS)

V_D = ventilatory dead space (ml BTPS)

f = respiratory frequency (breaths/min)

Determinations of oxygen consumption ($\dot{V}_{O_2}$) under standard conditions of: 0.0°C, 760 mm Hg and free of water vapor (STPD); were calculated only from the air breathing phase of each experiment because the fraction of inspired oxygen in the CO_2 rich gases was not precisely known. The derivation formula is presented as follows:

$$\dot{V}_{O_2} = \frac{\dot{V}_E}{1 - F_{I_{O_2}}} \left[F_{I_{O_2}} - (F_{I_{O_2}})(F_{E_{CO_2}}) - F_{E_{O_2}} \right] \quad (6)$$

where:

$\dot{V}_{O_2}$ = oxygen consumption (ml/min STPD)

$\dot{V}_E$ = minute ventilation (ml/min STPD)

$F_{I_{O_2}}$ = fraction of inspired oxygen

$F_{E_{CO_2}}$ = fraction of expired carbon dioxide

$F_{E_{O_2}}$ = fraction of expired oxygen

Estimations of CSF P_{CO_2} were derived from data of Pappenheimer et al. (1965) which indicated that in goats similar to those of this study, CSF P_{CO_2} was higher than $P_{a_{CO_2}}$ by nine mm Hg for arterial CO_2 tensions between 30 and 50 mm Hg. These findings provided the following conversion formula:

$$\text{CSF } P_{CO_2} = P_{a_{CO_2}} + 9.0 \text{ mm Hg} \quad (7)$$

The values of arterial bicarbonate concentrations in mM/l were multiplied by a conversion factor of 1.11 (Cantarow and Schepartz, 1967) in order to express arterial bicarbonate as mM/kg H_2O. Cerebrospinal fluid bicarbonate concentration was then calculated by employing the relation between arterial and CSF bicarbonate in mM/kg H_2O, which was formulated for unanesthetized goats by Fencl et al. (1966). The correlation is as follows:

$$\text{CSF } HCO_3^- = 11.3 + 0.352 \times \text{arterial } HCO_3^- \quad (8)$$

where:

HCO_3^- = bicarbonate concentration in mM/kg H_2O

Cerebrospinal fluid pH could then be calculated from the Henderson-Hasselbalch equation. The CSF HCO_3^- concentration in mM/l, as used in the Henderson-Hasselbalch equation, was found to be not measurably different from that bicarbonate concentration expressed as mM/kg H_2O (Cantarow and Schepartz, 1967; Potts and Parry, 1964). Assuming a CSF pK of 6.126 and the soluability coefficient of 0.0314 for the CSF P_{CO_2} (Fencl et al., 1966), the equation appears as:

$$\text{CSF pH} = 6.126 + \log \frac{\text{CSF } HCO_3^-}{(0.0314)(\text{CSF } P_{CO_2})} \qquad (9)$$

where:

CSF P_{CO_2} = partial pressure of CSF CO_2 (mm Hg)

CSF HCO_3^- = CSF bicarbonate concentration (mM/l)

Statistical Analysis

Eighteen CO_2 response curves were obtained for each treatment group. The mean and standard error of the mean for each measured or calculated quantity was determined. Analysis of variance between groups was determined utilizing Student's "t" test, critical to a 5% significance level. All statistical computations were performed on an Olivetti-Underwood Programma 101 computer.

RESULTS

The mean values obtained for the measured variables of any control animal were not significantly different from those of any other control animal for all inspired gas mixtures. This uniformity suggested using comparisons among test groups rather than among individual animals.

Response Changes Due to CO_2 Inhalation

Mean arterial blood pressures, heart rates and rectal temperatures are shown in Table 1. The mean arterial blood pressure of control and both ACTH treated groups was unaffected by breathing CO_2, while both dexamethasone treatment groups showed a higher mean blood pressure when breathing 5% CO_2 than when breathing air. Heart rate and rectal temperature of control and all hormone treatment groups were unaffected by various inspired CO_2 contents.

Table 2 contains mean values of respiratory frequency, V_T and $\dot{V}_A$ for the various hormone treatments at the different inspiratory gas mixtures. Tidal volume and $\dot{V}_A$ were significantly greater in all groups while inhaling $2\frac{1}{2}$% CO_2 than when breathing room air, and were also greater at the 5% CO_2 level than when the animals breathed either room air or $2\frac{1}{2}$% CO_2. Respiratory frequency was generally not affected by CO_2 inhalation.

Tables 3 and 4 contain data concerning arterial and CSF P_{CO_2}, pH and bicarbonate concentrations for each hormone treatment while breathing various levels of CO_2. All treatment groups exhibited significantly greater arterial and CSF P_{CO_2} values at the 5% CO_2 level than during air breathing, while the control and the 12 hour ACTH group showed significantly greater P_{aCO_2} and CSF P_{CO_2} levels while breathing $2\frac{1}{2}$% CO_2 than when breathing room air. For all treatments, both arterial and CSF pH values while breathing 5% CO_2 were significantly lower than when breathing either room air or the $2\frac{1}{2}$% CO_2 gas mixture. Arterial and CSF bicarbonate concentrations were not affected by CO_2 inhalation.

Response Differences Between Hormone Treatment Groups

Blood Pressure, Heart Rate, Rectal Temperature and Oxygen Consumption

The data in Table 1 suggest that dexamethasone appears to affect blood pressure in that the mean arterial blood pressures of both dexamethasone therapies were significantly elevated above those of control and of both ACTH treatments at all inspired CO_2 levels. None of the hormone treatments affected the animals' heart rates. There was a significant depression of rectal temperature below control in the 36 hour treatment regimens of both ACTH and dexamethasone for every inspired gas mixture (Table 1), while

oxygen consumption (Table 5) was not significantly affected by any of the hormone treatments.

Respiratory Frequency, Tidal Volume and Alveolar Ventilation

While breathing 5% CO_2, respiratory frequency, V_T and $\dot{V}_A$ for all hormone treatment groups were consistently elevated above the corresponding control values, though this difference was not significant in every case (Table 2). Although tidal volume in all treatment groups was elevated above control during the 2½% CO_2 inspiration, this difference was only significant for the 12 and 36 hour ACTH treatments. Alveolar ventilation in the 12 hour ACTH group was significantly greater than control while breathing 2½% CO_2. As the animals breathed room air, the hormone treatments did not significantly affect respiratory frequency, V_T or $\dot{V}_A$. The hormone injections similarly had no affect on respiratory frequency while breathing 2½% CO_2.

Arterial P_{CO_2}, pH and Bicarbonate Concentration

The mean control $P_{a_{CO_2}}$ levels were consistently greater than those of all hormone treatments other than the 36 hour ACTH regime during each level of inspired CO_2 (Table 3). Control $P_{a_{CO_2}}$ values were significantly greater than those of the 36 hour dexamethasone treatment group at all inspiratory gas levels, and greater than the 12 hour dexamethasone group's $P_{a_{CO_2}}$ values when the animals breathed 2½% CO_2 and 5% CO_2. The mean control values for $P_{a_{CO_2}}$ were

greater than those of the 12 hour ACTH group while breathing room air or 2½% CO_2. The tabular data also indicate that the mean values of the 36 hour ACTH group were in every case greater than control and all other treatment groups while breathing any gas mixture. Though not significantly different from control, the 36 hour ACTH treatment resulted in P_{aCO_2} levels which were significantly greater than those of both dexamethasone groups at essentially every test gas, and also greater than the 12 hour ACTH P_{aCO_2} values when the goats breathed 2½% CO_2. The data further demonstrate that the 36 hour dexamethasone treatment's P_{aCO_2} levels were significantly lower than in the 12 hour ACTH group during the inspiration of room air and 5% CO_2.

The arterial bicarbonate concentration (Table 3) of the 36 hour ACTH treatment was significantly elevated above control and all other treatment groups for each inspiratory gas mixture. Also, the 12 hour ACTH group exhibited a bicarbonate level significantly greater than control while breathing air.

The arterial pH data (Table 3) show that mean control pH values were consistently lower than those of all hormone treatments for every test gas. Control pH values were significantly lower than those of either of the ACTH treatments at each inspiratory mixture, and lower than the 36 hour dexamethasone treatment's arterial pH values while breathing room air and 2½% CO_2. Though not statistically different from control during any inspired gas, the arterial

pH of the 12 hour dexamethasone group was lower than that of the 36 hour ACTH treatment while breathing all test gases. The 12 hour dexamethasone group resulted in arterial pH values significantly lower than those of either the 12 hour ACTH or 36 hour dexamethasone treatments while breathing air and $2\frac{1}{2}\%$ CO_2. Table 3 also shows that the arterial pH levels of the 12 hour ACTH group were depressed significantly below those of the 36 hour ACTH group when the animals inhaled room air and 5% CO_2.

Cerebrospinal Fluid P_{CO_2}, pH and Bicarbonate

Hormone-induced variations in estimated CSF P_{CO_2} (Table 4) were necessarily similar to those measured changes occurring in P_{aCO_2}. At every inspired CO_2 level, the mean control values of CSF P_{CO_2} were greater than the CSF P_{CO_2} levels resulting from all hormone treatments other than the 36 hour ACTH group. Control CSF P_{CO_2} was significantly greater than that of the 36 hour dexamethasone treatment at all inspired CO_2 levels, and was greater than that of both the 12 hour ACTH and 12 hour dexamethasone therapies while breathing $2\frac{1}{2}\%$ CO_2. The standard errors of the 36 hour ACTH CSF P_{CO_2} values were so large that though mean CSF P_{CO_2} levels were greater than those of control or any other treatment group for every inspiration gas, the differences were not generally significant.

Because of the procedure for estimating CSF HCO_3^-, differences in estimated values of CSF bicarbonate concentration among all treatment groups (Table 4) were similar to those variations of arterial HCO_3^- across treatments. The most noticeable difference between therapies is that the 36 hour ACTH group exhibited CSF bicarbonate levels greater than those of all other groups at every level of inspiratory CO_2. During air breathing, the 12 hour ACTH group's CSF HCO_3^- level was also significantly greater than control.

The calculated CSF pH of the control group was significantly lower than that of all hormone treated groups for essentially every test gas (Table 4). Aside from this, the only CSF pH difference among the treatments was that the 12 hour dexamethasone pH was significantly lower than that of the 36 hour dexamethasone group while breathing room air and $2\frac{1}{2}\%$ CO_2.

Relationships Between Alveolar Ventilation and Blood and CSF Chemistry

Figure 3, plotted from the data in Tables 2 and 3, illustrates CO_2 response curves which were obtained both for control animals and for those receiving hormone treatments. The curves are generated for each test group by plotting $\dot{V}_A$ on the ordinate as a function of the corresponding $P_{a_{CO_2}}$ at various levels of hypercapnic drive to respiration. Each curve is drawn through three points representing

values obtained during the inspiration of room air and gas mixtures of 2½% CO_2 plus 21% O_2 in 76½% N_2, and 5% CO_2 plus 21% O_2 in 74% N_2. The 36 hour dexamethasone group's responses were significantly shifted to the left of control at all three inspiration gases, while the 12 hour dexamethasone treatment resulted in a significant leftward shift of the response curve during both levels of induced hypercapnic drive to respiration. The curve corresponding to the 12 hour ACTH treatment was also significantly shifted to the left of the control response at the room air and 2½% CO_2 points. The 36 hour ACTH treatment resulted in a response curve which tended to lie to the right of control, but this shift was not significant at any point.

Figure 4, depicting data from Tables 2 and 3, graphically represents the relationships between mean $\dot{V}_A$ and arterial pH values obtained for all test groups while breathing the three gas mixtures. The response curves of both ACTH groups and of the 36 hour dexamethasone treatment were significantly shifted to the left from the curve generated by the control group at each level of inspiratory CO_2. On the other hand, the response curve described by the 12 hour dexamethasone treatment did not result in any significant deviation from that of control for any of the test gas inspirations.

In Figure 5, alveolar ventilation is plotted as a function of CSF H^+ concentration (data from Tables 2 and 4). All hormone treatments tended to exhibit response curves

which were shifted to the left from that of control. The 36 hour dexamethasone treatment resulted in a response which was significantly to the left of control for all three gas mixtures. Values for both the 12 hour dexamethasone and 12 hour ACTH treatments are shifted to the left from corresponding controls at the room air and $2\frac{1}{2}\%$ CO_2 inspiration points. The 36 hour ACTH treatment was also significantly different from control at the room air and 5% CO_2 inspiration points.

Table 1. Effects of hormone treatments and inspiratory CO_2 on arterial blood pressure,[1] heart rate,[2] and rectal temperature[3]

Physiological Measurement & Hormone Treatment Regime	No. of Trials	Inspired Gas		
		Room Air	$2\frac{1}{2}$% CO_2	5% CO_2
Blood Pressure				
Control	18	147 ± 3	150 ± 1	150 ± 1
12 hr ACTH	18	145 ± 1	149 ± 1	148 ± 1
36 hr ACTH	18	140 ± 4	147 ± 2	150 ± 6
12 hr Dex.	18	162 ± 2 A B C	166 ± 3 A B C	171 ± 2 a A B C
36 hr Dex.	18	154 ± 3 B C D	161 ± 2 A B C	167 ± 1 a b A B C
Heart Rate				
Control	18	104 ± 13	111 ± 11	113 ± 15
12 hr ACTH	18	104 ± 8	111 ± 6	115 ± 7
36 hr ACTH	18	103 ± 15	108 ± 13	120 ± 8
12 hr Dex.	18	109 ± 7	118 ± 6	128 ± 8
36 hr Dex.	18	104 ± 10	109 ± 8	116 ± 9
Rectal Temp.				
Control	18	40.4 ± 0.2	40.3 ± 0.2	40.3 ± 0.2
12 hr ACTH	18	40.1 ± 0.0	40.2 ± 0.1	40.2 ± 0.1
36 hr ACTH	18	39.6 ± 0.3 A	39.6 ± 0.2 A B	39.5 ± 0.2 A B
12 hr Dex.	18	39.9 ± 0.1 A	39.9 ± 0.1 B	39.9 ± 0.2
36 hr Dex.	18	39.3 ± 0.2 A B D	39.3 ± 0.3 A B	39.4 ± 0.2 A B

[1]mm Hg/ mean values ± SEM. [2]Beats/min; mean values ± SEM. [3]°C; mean values ± SEM.

[a]Significantly different from room air period during same hormone treatment ($P<0.05$).
[b]Significantly different from $2\frac{1}{2}$% CO_2 period during same hormone treatment ($P<0.05$).

[A]Significantly different from control at corresponding inspired gas mixture (P 0.05).
[B]Significantly different from 12 hr ACTH at corresponding inspired gas mixture ($P<0.05$).
[C]Significantly different from 36 hr ACTH at corresponding inspired gas mixture ($P<0.05$).
[D]Significantly different from 12 hr Dex. at corresponding inspired gas mixture ($P<0.05$).

Table 2. Effects of hormone treatments and inspiratory CO_2 on respiratory frequency,[1] tidal volume,[2] and alveolar ventilation[3]

Physiological Measurement & Hormonal Treatment Regime	No. of Trials	Inspired Gas		
		Room Air	$2\frac{1}{2}$% CO_2	5% CO_2
Respiratory Frequency				
Control	18	27 ± 8	24 ± 2	28 ± 1
12 hr ACTH	18	33 ± 12	28 ± 4	32 ± 2
36 hr ACTH	18	26 ± 5	26 ± 2	32 ± 1 b A
12 hr Dex.	18	17 ± 3	21 ± 3	29 ± 4 a
36 hr Dex.	18	20 ± 3	23 ± 3	29 ± 4
Tidal Volume				
Control	18	457 ± 26	670 ± 17 a	910 ± 26 a b
12 hr ACTH	18	444 ± 33	736 ± 22 a A	963 ± 32 a b
36 hr ACTH	18	416 ± 32	657 ± 28 a B	980 ± 36 a b
12 hr Dex.	18	450 ± 20	695 ± 14 a	1,041 ± 44 a b
36 hr Dex.	18	483 ± 50	710 ± 10 a	1,068 ± 48 a b A
Alveolar Ventilation				
Control	18	4.9 ± 0.5	9.5 ± 0.6 a	18.8 ± 1.6 a b
12 hr ACTH	18	5.0 ± 0.7	11.7 ± 0.6 a A	21.6 ± 0.6 a b
36 hr ACTH	18	4.3 ± 0.5	10.1 ± 0.6 a	22.6 ± 1.4 a b A
12 hr Dex.	18	4.1 ± 0.4	9.4 ± 0.9 a	23.7 ± 2.0 a b
36 hr Dex.	18	5.1 ± 0.7	10.4 ± 1.1 a	24.5 ± 2.5 a b

[1]Breaths/min; mean values ± SEM. [2]ml BTPS; mean values ± SEM.
[3]l/min BTPS; mean values ± SEM.

[a]Significantly different from room air period during same hormone treatment ($P<0.05$).
[b]Significantly different from $2\frac{1}{2}$% CO_2 period during same hormone treatment ($P<0.05$).

[A]Significantly different from control at corresponding inspired gas mixture ($P<0.05$).
[B]Significantly different from control at corresponding inspired gas mixture ($P<0.05$).

Table 3. Effects of hormone treatments and inspiratory CO_2 on arterial P_{CO_2},[1] arterial bicarbonate,[2] and arterial pH[3]

Physiological Measurement & Hormone Treatment Regime	No. of Trials	Inspired Gas: Room Air	Inspired Gas: 2½% CO_2	Inspired Gas: 5% CO_2
Arterial P_{CO_2}				
Control	18	42.9 ± 0.7	45.9 ± 1.1 a	48.6 ± 0.8 a b
12 hr ACTH	18	41.3 ± 0.3 A	42.2 ± 0.3 a A	47.6 ± 0.7 a b
36 hr ACTH	18	45.0 ± 2.3	48.4 ± 3.0 B	53.9 ± 3.2 a
12 hr Dex.	18	41.0 ± 1.2	41.7 ± 1.4 A C	46.4 ± 0.5 a b A C
36 hr Dex.	18	38.1 ± 1.3 A B C	39.8 ± 1.8 A C	44.8 ± 0.7 a b A B C
Arterial Bicar.				
Control	18	29.4 ± 0.3	29.7 ± 0.7	29.6 ± 0.7
12 hr ACTH	18	30.5 ± 0.2 A	30.2 ± 0.7	30.5 ± 0.9
36 hr ACTH	18	36.3 ± 1.1 A B	37.1 ± 0.7 A B	37.2 ± 1.2 A B
12 hr Dex.	18	29.4 ± 0.9 C	28.9 ± 1.3 C	29.1 ± 1.0 C
36 hr Dex.	18	28.9 ± 1.0 C	28.9 ± 1.5 C	29.2 ± 1.3 C
Arterial pH				
Control	18	7.412 ± 0.006	7.394 ± 0.009	7.359 ± 0.004 a b
12 hr ACTH	18	7.443 ± 0.006 A	7.431 ± 0.010 A	7.379 ± 0.004 a b A
36 hr ACTH	18	7.475 ± 0.012 A B	7.457 ± 0.018 A	7.410 ± 0.012 a b A B
12 hr Dex.	18	7.421 ± 0.008 B C	7.406 ± 0.001 B C	7.360 ± 0.011 a b C
36 hr Dex.	18	7.449 ± 0.005 A D	7.430 ± 0.004 a A D	7.377 ± 0.018 a b

[1]mm Hg; mean values ± SEM. [2]mM/kg H_2O; mean values ± SEM. [3]Mean values ± SEM.

[a]Significantly different from room air period during same hormone treatment ($P<0.05$).
[b]Significantly different from 2-% CO_2 period during same hormone treatment ($P<0.05$).

[A]Significantly different from control at corresponding inspired gas mixture ($P<0.05$).
[B]Significantly different from 12 hr ACTH at corresponding inspired gas mixture ($P<0.05$).
[C]Significantly different from 36 hr ACTH at corresponding inspired gas mixture ($P<0.05$).
[D]Significantly different from 12 hr Dex. at corresponding inspired gas mixture ($P<0.05$).

Table 4. Effects of hormone treatments and inspiratory CO_2 on calculated CSF P_{CO_2},[1] CSF bicarbonate,[2] and CSF pH[3]

Physiological Measurement & Hormone Treatment Regime	No. of Trials	Inspired Gas: Room Air	Inspired Gas: 2½% CO_2	Inspired Gas: 5% CO_2
CSF P_{CO_2}				
Control	18	51.3 ± 0.3	54.6 ± 0.8 a	56.6 ± 0.7 a
12 hr ACTH	18	50.5 ± 0.3	51.5 ± 0.1 a A	56.4 ± 0.9 a b
36 hr ACTH	18	54.0 ± 2.3	57.4 ± 2.9	62.9 ± 3.2 a B
12 hr Dex.	18	50.0 ± 1.2	50.7 ± 1.4 A	54.5 ± 1.3 a b
36 hr Dex	18	47.1 ± 1.3 A B C	48.8 ± 1.8 A C	53.8 ± 0.6 a b A B C
CSF Bicarbonate				
Control	18	21.6 ± 0.1	21.8 ± 0.2	21.7 ± 0.2
12 hr ACTH	18	22.0 ± 0.1 A	21.9 ± 0.2	22.0 ± 0.3
36 hr ACTH	18	24.1 ± 0.4 A B	24.4 ± 0.2 A B	24.4 ± 0.4 A B
12 hr Dex.	18	21.6 ± 0.3 C	21.5 ± 0.4 C	21.5 ± 0.4 C
36 hr Dex.	18	21.5 ± 0.3 C	21.5 ± 0.5 C	21.6 ± 0.4 C
CSF pH				
Control	18	7.253 ± 0.004	7.230 ± 0.004 a	7.212 ± 0.003 a b
12 hr ACTH	18	7.269 ± 0.004 A	7.256 ± 0.006 A	7.219 ± 0.001 a b A
36 hr ACTH	18	7.278 ± 0.011 A	7.256 ± 0.018	7.219 ± 0.016 a
12 hr Dex.	18	7.263 ± 0.005 A	7.256 ± 0.004 A	7.226 ± 0.008 a b A
36 hr Dex.	18	7.287 ± 0.005 A D	7.272 ± 0.006 a A D	7.233 ± 0.006 a b A

[1]mm Hg; mean values ± SEM. [2]mM/kg H_2O; mean values ± SEM. [3]Mean values ± SEM.

[a]Significantly different from room air period during same hormone treatment ($P<0.05$).
[b]Significantly different from 2½% CO_2 period during same hormone treatment ($P<0.05$).

[A]Significantly different from control at corresponding inspired gas mixture ($P<0.05$).
[B]Significantly different from 12 hr ACTH at corresponding inspired gas mixture ($P<0.05$).
[C]Significantly different from 36 hr ACTH at corresponding inspired gas mixture ($P<0.05$).
[D]Significantly different from 12 hr Dex. at corresponding inspired gas mixture ($P<0.05$).

Table 5. Effects of hormone treatments on oxygen consumption[1]

Treatment	Number of Trials	$\dot{V}O_2$ ml/min (STPD)
Control	18	241 ± 18
12 hr ACTH	18	256 ± 25
36 hr ACTH	18	247 ± 39
12 hr Dex.	18	236 ± 20
36 hr Dex.	18	320 ± 41

[1]Mean values ± SEM.

Fig. 3. Effect of hormone treatments on the ventilatory response to induced changes of arterial P_{CO_2}.

These data represent CO_2 response curves for all test groups, which relate alveolar ventilation ($\dot{V}_A$) on the ordinate with arterial carbon dioxide tension ($P_{a_{CO_2}}$) on the abscissa. Each curve is drawn through three points corresponding to $\dot{V}_A$ and $P_{a_{CO_2}}$ values obtained while breathing room air and modified air mixtures containing $2\frac{1}{2}\%$ CO_2 and 5% CO_2. The control response is represented by circles connected with a solid line. Crosses linked by dashed lines correspond to values obtained during the ACTH treatments, with the 12 and 36 hour treatment durations labeled in the figure. The responses obtained during the 12 and 36 hour dexamethasone treatments are likewise identified by circles connected by dashed lines.

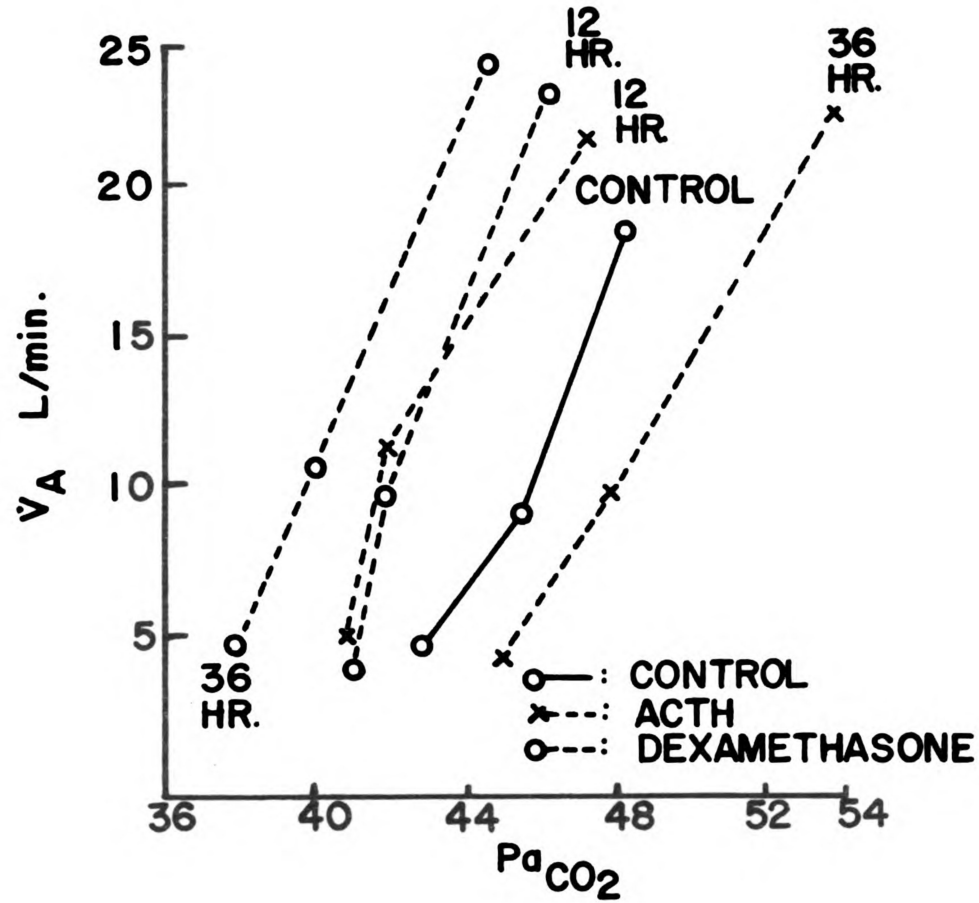

FIGURE 3
EFFECT OF HORMONE TREATMENTS ON THE VENTILATORY RESPONSE TO INDUCED CHANGES OF ARTERIAL P_{CO_2}

Fig. 4. Effect of hormone treatments on the ventilatory response to induced changes in arterial pH.

These data represent response curves for all test groups, which relate alveolar ventilation ($\dot{V}_A$) on the ordinate with arterial pH on the abscissa. Each curve is drawn through three points corresponding to $\dot{V}_A$ and arterial pH values obtained while breathing room air and modified air mixtures containing $2\frac{1}{2}\%$ CO_2 and 5% CO_2. The control response is represented by circles connected with a solid line. Crosses linked by dashed lines correspond to values obtained during the ACTH treatments, with the 12 and 36 hour treatment durations labeled in the figure. The responses obtained during the 12 and 36 hour dexamethasone treatments are likewise identified by circles connected by dashed lines.

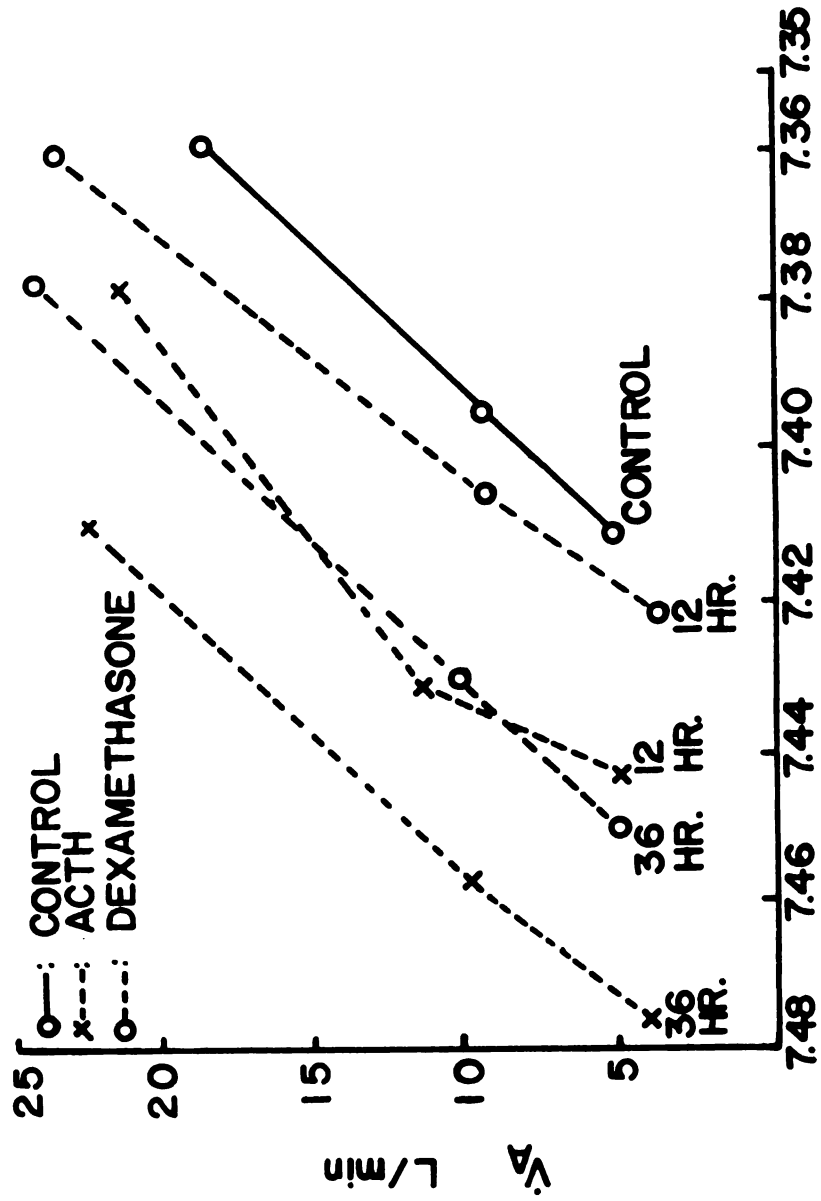

FIGURE 4
EFFECT OF HORMONE TREATMENTS ON THE VENTILATORY RESPONSE TO INDUCED CHANGES IN ARTERIAL pH

Fig. 5. Effect of hormone treatments on the ventilatory response to estimated changes of CSF H^+ concentration.

These data represent response curves for all test groups, which relate alveolar ventilation ($\dot{V}_A$) on the ordinate with a combined abscissa of CSF pH and CSF H^+ concentration. Each curve is drawn through three points corresponding to $\dot{V}_A$ and CSF pH values obtained while breathing room air and modified air mixtures containing $2\frac{1}{2}\%$ CO_2 and 5% CO_2. The control response is represented by circles connected with solid lines. Crosses linked by dashed lines correspond to values obtained during the ACTH treatments, with the 12 and 36 hour treatment durations labeled in the figure. The responses obtained during the 12 and 36 hour dexamethasone treatments are likewise identified by circles connected by dashed lines.

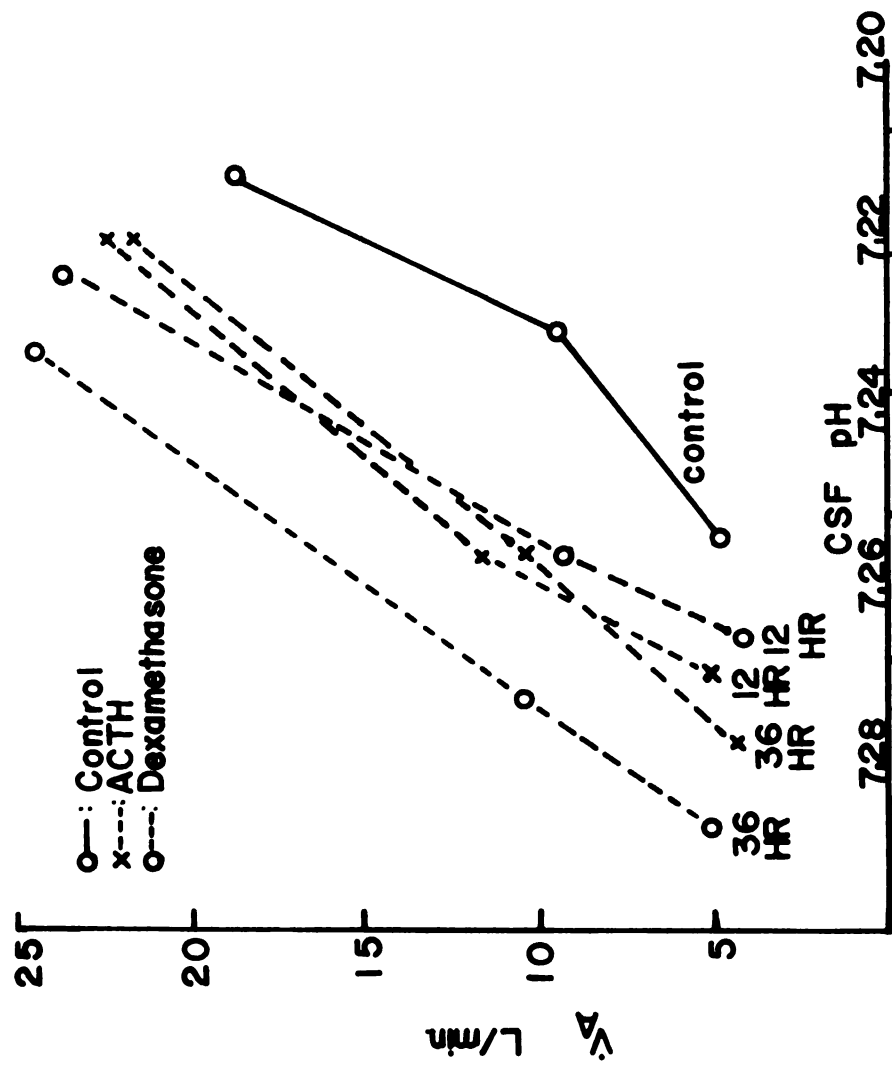

FIGURE 5
EFFECT OF HORMONE TREATMENTS ON THE VENTILATORY RESPONSE TO ESTIMATED CHANGES OF CSF $[H^+]$

DISCUSSION

Heart rate was unaffected by either the hormone treatments or by hypercapnia induced with inspired CO_2 (Table 1). The mean arterial blood pressure of the dexamethasone treatments was significantly greater than that of both control and the ACTH injected animals for all inspiratory gas mixtures (Table 1). The maintenance of blood pressure associated with ACTH injections agrees with the findings of Sambhi et al. (1963), who reported increased cardiac output, decreased peripheral resistance and unchanged mean arterial blood pressure following ACTH treatment to normal and hypotensive subjects. The increase in blood pressure during dexamethasone therapy, with no significant alteration in heart rate, suggests that the dexamethasone effect was brought about by increasing peripheral resistance or by increasing cardiac output. It is possible that dexamethasone increases peripheral resistance by exhibiting the known glucocorticoid action to potentiate the vasoconstrictive response of adrenergic drugs (Zweifach, 1960). Although reasons for the different effects of the two types of hormone treatment on blood pressure are not apparent, they may be related to specific biological actions

of natural glucocorticoids and dexamethasone or to differences in effective hormone dosages available to responsive tissue.

Oxygen consumption (Table 5) was not significantly affected by any of the hormone treatments. Control rectal temperature was consistently greater than that exhibited by either of the 36 hour treatment groups for all levels of inspired CO_2 (Table 1). These results indicate that the hormone treatments did not significantly alter whole body metabolic heat production as evidenced by the unchanging $\dot{V}_{O_2}$, and may have induced the development of slight hypothermia as indexed by a depression of rectal temperature. Since both ACTH and dexamethasone treatments resulted in lowering rectal temperature without changing $\dot{V}_{O_2}$, the effect may have been caused by glucocorticoids to activate certain thermoregulatory heat loss mechanisms without measurably altering heat production as indexed by unaltered $\dot{V}_{O_2}$. Since $\dot{V}_A$ was generally not significantly elevated above control in either of the 36 hour treatment groups at any level of inspired CO_2 (Table 2), the increased heat loss was likely mediated cutaneously rather than by means of increasing evaporative water loss. The data indicate that the hypothermic response begins at a time between 12 and 36 hours after hormone treatment initiation.

The inhalation of gas mixtures rich in CO_2 resulted in increased P_{CO_2} and decreased pH in blood and cerebrospinal fluid. It is generally accepted that, in the absence

of hypoxia, the increased respiratory activity induced by CO_2 breathing results to a much greater extent from stimulation of central respiratory chemoreceptors than from those at the peripheral level (Gemmill and Reeves, 1933; Lambertson, 1968). Alterations in the level of respiratory activation were measured in terms of changes in $\dot{V}_A$, presumably reflecting fluctuations of the efferent output from the medullary respiratory control center. The respiratory control center, which is assumed to consist of the inspiratory and expiratory neuron groups in the medulla generates electrical signals which travel along such avenues as the phrenic nerve (Lourenco et al., 1966) to regulate activity of respiratory musculature. Differences of $\dot{V}_A$ between control and hormone treated animals at specific arterial or CSF levels of pH and P_{CO_2} were assumed to reflect hormonal effects upon the activity of the respiratory control center.

Tidal volume and alveolar ventilation both increased with elevated inspiratory CO_2 content (Table 2). On the other hand, respiratory frequency was unchanged with increased levels of inspired CO_2, indicating that the increase in $\dot{V}_A$ was primarily due to elevated V_T. These findings are in agreement with data reported by Lambertson et al. (1961) and Mitchell et al. (1963). At the 5% level of inspired CO_2, all hormone treatment groups had greater mean values for f, V_T and $\dot{V}_A$ than those of the control group. Although most of the increases in f, V_T and $\dot{V}_A$ were not significantly greater than the corresponding control values, the consistency of

the elevations provides support that both hormones may be involved in the alteration of these respiratory responses.

Although breathing CO_2 had no effect on the arterial HCO_3^- levels in any of the groups tested, comparisons of bicarbonate levels between treatment groups showed that only the 36 hour ACTH treatment group had arterial bicarbonate concentrations different from the control group (Table 3). The bicarbonate concentration of the 36 hour ACTH treatment averaged 7.0 mM/kg H_2O higher than that of any other groups, indicating development of metabolic alkalosis in these animals. However, the extent of this development was variable as evidenced by the high standard error associated with these bicarbonate measurements. The 36 hour ACTH treatment regime likely induced maximal adrenocortical activation to lead to the development of a Cushingoid syndrome which includes the development of metabolic alkalosis. This treatment presumably resulted in the release of appreciable amounts of adrenocortical steroids, all of which possess some mineralocorticoid activity. It has been shown that mineralocorticoids induce an increased renal loss of potassium. Migration of hydrogen ions into intracellular sites may then have occurred to compensate for the potassium lost. The result of these electrolyte shifts would be the development of extracellular metabolic alkalosis (Nocenti, 1968). This decrease in extracellular hydrogen ion concentration would then tend to drive the bicarbonate buffer equilibrium toward the formation of increased bicarbonate.

$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^- \quad (9)$$

The mean $P_{a_{CO_2}}$ at each test gas mixture was greater in the control group than in any of the hormone treatments except the 36 hour ACTH group (Table 3). This relationship implies that the respiratory response to arterial P_{CO_2} was less in the control than in all hormone treatment groups other than the 36 hour ACTH treatment, in that the control animals exhibited a greater tolerance to arterial P_{CO_2} for each inspiratory gas mixture than did the hormonally treated groups. Similar decreases in alveolar P_{CO_2} have been reported during pregnancy and after pregnanediol treatment (Hasselbalch and Gammeltoft, 1915; Heerhaber et al., 1948; Goodland et al., 1954). The mean $P_{a_{CO_2}}$ values of the 36 hour ACTH group were higher than those of any other treatment group. It is possible that the elevated $P_{a_{CO_2}}$ levels at all gas mixtures in the 36 hour ACTH group were related to the development of metabolic alkalosis during this treatment, where the increased blood bicarbonate concentrations would cause a shift of the bicarbonate buffer equilibrium to increase the arterial tension of CO_2. The tolerance of elevated $P_{a_{CO_2}}$ by this group suggests that the arterial CO_2 tension does not in itself constitute a sufficiently strong respiratory stimulus to decrease the $P_{a_{CO_2}}$ level.

Figure 3 illustrates the relationships between $\dot{V}_A$ and arterial P_{CO_2} values which were obtained for control and hormone treated groups while breathing the three test gases.

The leftward shift of both dexamethasone treatment groups and the 12 hour ACTH group from that of control suggests that those hormone treatments acted to increase respiratory excitability. Increased respiratory excitability can be visualized as a parallel leftward displacement in a respiratory response curve (Figures 3, 4, and 5), and defined as an increased $\dot{V}_A$ above control when associated with a specific level of arterial or CSF pH or P_{CO_2}. These indications of hormone-induced increased respiratory excitability concur with similar CO_2 response curve alterations that have been reported following injections of cortisone (Koepchen, 1953), ACTH (Koepchen et al., 1954) or progesterone (Huang and Lyons, 1966), and during pregnancy (Loescheke and Sommer, 1944; Lyons and Antonio, 1959). On the other hand, the mean 36 hour ACTH response fell to the right of the control group response at each inspiratory gas, suggesting a decreased respiratory excitability in this group. However, the variability of the 36 hour ACTH responses was so great that the shift toward respiratory depression was not significant. The difference in ventilatory excitability between the 12 and 36 hour ACTH regimens was unexpected. The observation that respiratory excitability was not different from control in the 36 hour treatment group may be due to the existence of metabolic alkalosis in this group. By shifting the bicarbonate buffer equilibrium toward elevating P_{aCO_2}, this metabolic condition could act to mask any hormone-induced

alterations in respiratory excitability when $\dot{V}_A$ is gauged as a function of $P_{a_{CO_2}}$.

Arterial pH in all groups decreased with increasing inspired CO_2 (Table 3; Figure 4). The decrease in pH is directly associated with the increase in $P_{a_{CO_2}}$ which shifts the bicarbonate buffer equilibrium to favor the formation of hydrogen ions in the blood. The average values of arterial blood pH for the control group were significantly lower than those of any treatment group except the 12 hour dexamethasone therapy. The findings indicate that the respiratory excitability of these hormone treatments is greater than control for every tested level of hypercapnic drive to respiration when arterial pH is used as an indicator of respiratory drive. The response curve described by the 36 hour ACTH treatment tended to be shifted further to the left of control than were the curves of the other hormone treatments. The presence of metabolic alkalosis in the 36 hour ACTH group presumably causes a displacement of hydrogen ions intracellularly from vascular stores, thereby increasing arterial pH values above those that would be recorded if the treatment group were not in an acid-base imbalance.

As has been noted, the arterial values of pH and $P_{a_{CO_2}}$ became appreciably altered during the development of metabolic alkalosis (Table 3, Figures 3 and 4). An attempt was thus made to estimate values for CSF pH, P_{CO_2} and HCO_3^- levels from the corresponding arterial data in order to evaluate the effects of CSF hydrogen ion concentration on

the central chemoreceptor sites. To make possible the calculation of CSF pH, estimations of CSF P_{CO_2} and CSF HCO_3^- concentrations were derived from the data of Pappenheimer et al. (1965) and Fencl et al. (1966), respectively, who simultaneously compared these aspects of CSF and arterial composition in goats similar to those used in this study.

Upon increasing inspired CO_2 content, the calculated CSF P_{CO_2} was elevated as shown in Table 4, supporting the hypothesis that with increased inspired CO_2 concentrations, substantial diffusion of CO_2 occurs from the blood across the "blood-brain" barrier and into the cerebrospinal fluid. It was observed that the control group maintained a higher CSF P_{CO_2} than did all treatments other then the 36 hour ACTH group, suggesting a difference between control and hormone treated groups in the central respiratory response to CSF P_{CO_2}. It is again assumed that the difference between the 36 hour ACTH group's CSF P_{CO_2} and that of the other treatment groups is related to the metabolic acid-base imbalance occurring during the 36 hour ACTH treatment.

The calculated CSF pH values during CO_2 inhalation indicates that the CSF pH while breathing 5% CO_2 was significantly lower than corresponding pH values during room air and 2½% CO_2 breathing for all treatments. While breathing air and 2½% CO_2, the mean CSF pH of the control group was lower than the mean calculated CSF pH of all treatment groups. Although the measured mean arterial pH of the

36 hour ACTH treatment was higher than other groups at all inspiratory gas mixtures, the computed hydrogen ion concentrations in CSF during the 36 hour ACTH treatment were not significantly different from the other hormone treated groups. These data demonstrate a deviation of hydrogen ion concentration alterations between the CSF and vascular compartments associated with metabolic alkalosis. Although metabolic alkalosis acts to increase blood pH, it does not measurably alter CSF pH from that occurring in the other hormonally treated groups.

The increase in respiratory excitability as indexed by CSF pH (Figure 5) was consistent across most treatment groups, with only the room air and 2½% CO_2 points of the 36 hour dexamethasone treatment different from those of the 12 hour dexamethasone treatment group. Generally, respiratory excitability increased by a similar magnitude above control for all hormone injection groups while breathing any gas mixture. The increased respiratory excitability can be quantified with the observation that all hormone treatments decreased the effective set-point of the output from the respiratory control center by a calculated hydrogen ion concentration of about 3.0 nM/kg H_2O. These results are inconsistent with those of Huang and Lyons (1966) who reported that no alterations in CSF pH occurred in response to progesterone treatment of dosages sufficient to increase $\dot{V}_A$. The slope of the ventilatory response curve for the control group was 2.6 liters/min per nM H^+ while the slopes

of all hormone treated groups averaged 2.7 liters/min per nM H^+, which was not significantly different from the control slope. This relationship suggests that the sensitivity (change in $\dot{V}_A$ for given change in CSF H^+ concentration) of the respiratory control center in response to changes of CSF pH was not measurably altered by increased glucocorticoids.

The data suggest that arterial blood P_{CO_2} or pH are not totally reliable indicators of the effective degree of respiratory activation when the test animals experience a metabolic acid-base disturbance. The development of metabolic alkalosis from mineralocorticoid activity in the 36 hour ACTH group presumably altered the bicarbonate buffer equilibrium toward increasing CO_2 formation. This increased CO_2 would result in biasing the $P_{a_{CO_2}}$ ventilatory response curve to the right of values that would have been expressed under normal acid-base conditions (Figure 3). The intracellular migration of hydrogen ions to replace the loss of potassium ions during metabolic alkalosis would then lower the arterial pH below normal levels. This decrease in arterial pH may then be associated with a shift in the arterial pH related ventilatory response curve (Figure 4) further to the left than would have occurred under normal conditions (Nocenti, 1968). These results indicate that arterial pH or $P_{a_{CO_2}}$ cannot themselves account for the observed alterations in respiratory activity during the development of an abnormal metabolic acid-base status.

Fencl et al. (1966) demonstrated that the respiratory response to inhaled CO_2 in unanesthetized goats was a single function of cisternal CSF hydrogen ion concentration, regardless of metabolic acid-base status. In the present study, all the hormone treatments were associated with a shift of the respiratory excitability, producing an increase in ventilation above control group values for all levels of CSF pH. On the other hand, analysis of the respiratory response to arterial pH or P_{aCO_2} changes did not produce a similar relationship capable of explaining ventilatory alterations on the basis of changes in these arterial quantities.

The data suggest that cisternal CSF hydrogen ion concentration estimations appear to be the preferred index of respiratory excitability in that the relationship between $\dot{V}_A$ and estimated CSF pH was alone able to reflect response changes of consistent direction and magnitude for all glucocorticoid elevating treatments. The alteration in respiratory excitability, as indexed by CSF pH, was similar after both 12 and 36 hours of ACTH stimulation, illustrating response consistency even during the development of metabolic alkalosis.

The similarity of the responses of the 12 and 36 hour ACTH and dexamethasone groups to CSF pH indicates that the effectiveness of ACTH was secondary to its recognized action of activating the adrenal cortex. The comparability of the 12 and 36 hour data from both treatments implies that

the alteration in respiratory excitability occurred within the first twelve hours after initiation of hormone treatment.

The glucocorticoid effect on respiration may be brought about by directly increasing the activity of the medullary respiratory control center to increase respiratory excitability by approximately 3.0 nM H^+/kg H_2O. The glucocorticoids may instead function on the central or peripheral chemoreceptors to increase their level of afferent neural input to the respiratory control center, thereby altering respiratory excitability. It is also possible that glucocorticoids alter the strength of an input to the respiratory control center from some source other than that of the chemoreceptors.

It may be that glucocorticoids do not in themselves directly increase respiratory excitability. These adrenocortical hormones may in fact function by increasing the availability of certain nervous tissue chemical mediators or transmitter substances. As feedback inhibitors of corticotropin releasing factors, glucocorticoids are recognized to effect the synthesis and/or release of central neuro-transmitters (Vernikos-Danellis, 1965). It is conceivable that the glucocorticoid effect on respiratory excitability could involve similar mechanisms. Glucocorticoid effect on adenosine triphosphate available for neuro-transmitter substance synthesis, or hormone effects on the secretion or uptake of such neuro-transmitter

substances as norepinepherine (Maas and Mednieks, 1971) could ultimately be responsible for increasing the level of respiratory activation.

SUMMARY

This investigation was designed to assess the influence of ACTH and glucocorticoids on respiratory activity. Adrenocorticotropin and dexamethasone treatments were separately administered in 12 and 36 hour therapy durations to resting, unanesthetized adult female goats. Hormonal effects on the alteration of respiratory responses and blood chemistry were measured during various levels of hypercapnic respiratory drive, while concomitant changes in cisternal CSF chemistry were calculated from the arterial data.

Oxygen consumption was unaffected by hormone treatments, while the rectal temperatures of the 36 hour ACTH and 36 hour dexamethasone therapies were both lower than control. The results suggest that glucocorticoid treatment of more than 12 hours resulted in the development of a hypothermic effect, presumably by activating a thermoregulatory heat loss mechanism because metabolic heat production as indexed by $\dot{V}_{O_2}$ was unchanged.

Another observed effect of the hormone therapies used in these studies was that the 12 and 36 hour dexamethasone treatments resulted in an elevation in arterial blood pressure with no alteration in heart rate. This increased blood pressure presumably reflects an increase

in either peripheral resistance or cardiac output during dexamethasone administration.

Analysis of the arterial data suggested that the 36 hour ACTH treatment induced the development of metabolic alkalosis which was presumably due to mineralocorticoid activity of the natural hormones of the adrenal cortex. The electrolyte shifts associated with this metabolic state caused significant increases in arterial bicarbonate concentration, CSF bicarbonate concentration, $P_{a_{CO_2}}$ and arterial pH above corresponding values in the other treatment groups. On the other hand, metabolic alkalosis did not significantly alter the calculated CSF pH from values observed in the other hormone treatment groups.

Mean values of V_T, f and $\dot{V}_A$ for all hormone treated animals inspiring 5% CO_2 were higher than the corresponding control means. The arterial P_{CO_2} data indicated that all treatments except the 36 hour ACTH group (which experienced metabolic alkalosis) exhibited greater respiratory excitability than control since the $\dot{V}_A$ during these hormone therapies was greater than that of control at all $P_{a_{CO_2}}$ levels measured. Ventilatory excitability gauged as a function of arterial pH was likewise inconsistent in that all treatment groups other than the 12 hour dexamethasone regime displayed significant increases in respiratory excitability above control at all levels of arterial pH. When $\dot{V}_A$ was evaluated in terms of calculated CSF hydrogen ion concentration, it was found that all treatment groups exhibited an

increased respiratory excitability consistently greater than control by 3.0 nM/kg H_2O. Since the degree of $\dot{V}_A$ elevation for a unit change in CSF H^+ concentration was similar for all hormone treatment groups and for the control animals, no change in respiratory sensitivity was evident. The data suggest that CSF H^+ concentration is a more reliable index of respiratory excitability than arterial pH, $P_{a_{CO_2}}$ or CSF P_{CO_2}.

Since both the ACTH and dexamethasone treatments induce similar alterations in respiratory responses when compared in terms of CSF H^+ concentration, glucocorticoid hormones probably mediate the observed respiratory activation. The ability of ACTH to alter ventilation appears to be a function of its capacity to induce adrenocortical hormone release. The response uniformity between the 12 and 36 hour treatments suggests that maximal effects on the respiratory mechanism occur within 12 hours after the initiation of hormone treatment. The glucocorticoid hormones most likely induce increased respiratory excitability in one of the following manners: (1) by acting directly on the respiratory control center to increase respiratory excitability, (2) by functioning at the central or peripheral chemoreceptor level to increase receptor activity, or (3) by affecting an input to the respiratory control center from some source other than that of the chemoreceptors.

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