Adrenocortical Secretion in Anesthetized Dogs During Hyperoxia, Hypoxia and Positive Pressure Breathing.* (30008)

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It is well known that a variety of adverse environmental conditions can stimulate the adrenal cortex; however, many contradictory reports have appeared regarding the effects of alterations in inspiratory oxygen tensions on adrenal activity. Hale *et al*(1) noted that human peripheral plasma levels of 17-hydroxycorticosteroids (17-OHCS) were slightly depressed after breathing 100% O_2 at ground level for 4 hours. On the other hand, exposure of rats to pure oxygen at 2 to 7 atmospheres caused a reduction in adrenal ascorbic acid content(2,3). Others have shown that although adrenalectomy offers protection against the toxicity of high oxygen pressures(4,5), relatively small doses of adrenocortical extracts counteract this protective action while larger doses are beneficial (5). At low oxygen tensions, rapid increases in adrenocortical secretory rates were noted in dogs breathing 10% O_2 at ground level or exposed to simulated altitude(6,7), whereas no significant changes in adrenal ascorbic acid and peripheral 17-OHCS levels were observed in dogs made hypoxemic by breathing 5% O_2(8).

Since a majority of the investigations assessing adrenocortical activity during altered oxygen tensions were based primarily on indirect methods such as adrenal ascorbic acid and peripheral plasma 17-OHCS, the present study was designed to determine the effects of hyperoxia, hypoxia and continuous positive pressure breathing (CPPB) on 17-OHCS concentrations in adrenal venous blood of anesthetized dogs.

Materials and methods. The first series of experiments was designed to determine a) the time at which 17-OHCS production reaches a steady baseline secretory rate after lumbo-adrenal vein cannulation and b) whether the adrenal cortex is responsive to adrenocorticotropin (ACTH) at this time. Eight mongrel dogs, weighing 14.2-19.5 kg, were anesthetized with sodium pentobarbital and maintained at a surgical level of anesthesia throughout the experiment. The left lumbo-adrenal vein was cannulated and blood samples were collected according to a procedure previously described(6,7). Immediately after completing the surgery, heparinized blood samples were taken from 5 animals at one-half hour intervals for 4.5 hours. In the remaining 3 dogs, control samples were obtained 1.5 hours after cannulation and at various intervals after the infusion of 5 USP units of ACTH (Acthar, Armour Pharmaceutical Co., Kankakee, Ill.) at 2 and 3.5 hours.

In the second series of experiments 27 mongrel dogs weighing 11.0-19.5 kg were anesthetized with sodium pentobarbital. The trachea was cannulated in order to administer various gas mixtures and the animals prepared as above for the collection of adrenal venous blood. Based on data obtained in the first series of experiments, the dogs were allowed to recover from the trauma of the operation for 90 minutes prior to obtaining 3 control blood samples at 15-minute intervals. The animals were then treated as follows: a) 7 control dogs continued to breathe ambient air at ground level (750 mm Hg) throughout the 120-minute experimental period, b) 7 were made hyperoxic by breathing 100% O_2 at ground level, c) 6 respired a gas mixture containing 10% O_2 in nitrogen, and d) a type A-16 breathing regulator connected to the laboratory compressed air line was attached to the tracheal cannula of 7 dogs and permitted 14-18 mm Hg continuous positive pressure breathing (CPPB). All experiments were performed at room temperature (22 ± 2°C) and blood samples taken at 15, 30, 60, 90 and 120 minutes after administering the

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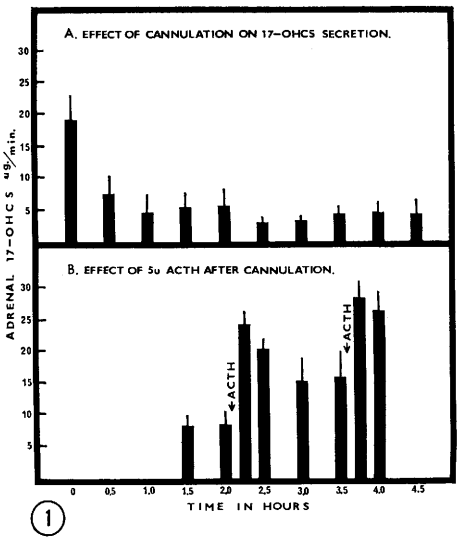

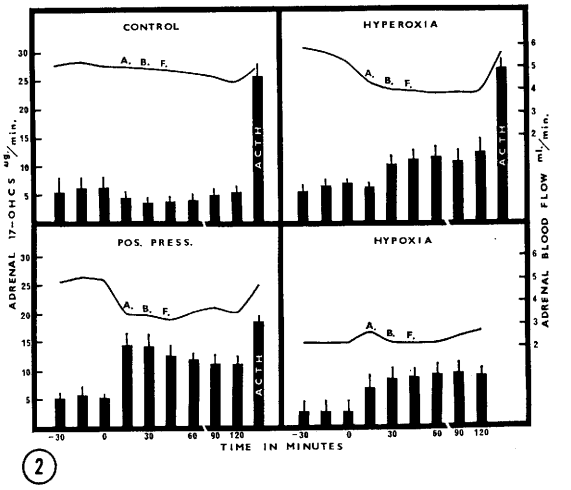

FIG. 1. A) Effect of lumbo-adrenal vein cannulation on 17-OHCS secretory rates (mean ± SE) of anesthetized dogs. B) Responsiveness of adrenal cortex to exogenous ACTH administered at 2.0 and 3.5 hr after adrenal vein surgery.

FIG. 2. Secretion of 17-OHCS and lumbo-adrenal vein blood flow (A.B.F.) in anesthetized dogs breathing ambient air (control), 100% oxygen (hyperoxia), 10% oxygen (hypoxia) and ambient air against 14-18 mm Hg (Pos. Press.). The prescribed gas mixtures were applied immediately after collecting the 0 sample and 5 units of ACTH infused at the end of experimental period in all groups except hypoxic animals.

gas mixtures. Immediately after collection of the last sample, 5 USP units of ACTH were infused intravenously over a 2-minute period in all groups except hypoxia and a blood sample was collected 15 minutes later while the animals continued to breathe the prescribed gas mixtures. Microhematocrits were determined on all blood samples to permit the calculation of plasma flow. The remaining heparinized blood was immediately centrifuged and the plasma stored at —25°C until analyzed for 17-OHCS(9). Secretory rates, based on plasma flow and 17-OHCS concentration, are expressed in μg/min and all t values calculated by the independent method (10).

Results. The mean 17-OHCS secretory rate immediately after lumbo-adrenal vein cannulation was 19.4 ± 3.6 μg/min and significantly ($P<.05$) decreased to 7.4 ± 2.9 μg/min within the next one-half hour (Fig. 1A). At one hour these rates had further declined to 4.4 ± 2.9 μg/min and subsequently remained relatively constant. Lumbo-adrenal vein blood flow varied from 4.3 ± 0.4 to 5.3 ± 0.5 ml/min and the coefficient of correlation (r) between μg 17-OHCS/ml plasma and ml plasma/min for individual dogs (first sample after surgery not included) ranged from +.25 to —.49 with a mean of —.30 ($P>.20$). The infusion of 5 units of ACTH at both 2.0 and 3.5 hours after cannulation significantly elevated 17-OHCS secretory rates (Fig. 1B). The initial administration of ACTH caused a 2.7-fold elevation ($P<.02$), whereas the second injection, given when the secretory rates were still relatively high (15.9 ± 4.1 μg/min), approximately doubled the secretion of 17-OHCS ($P<.01$). Although blood flow increased following ACTH infusion, it was not sufficient to account for the marked increases observed in 17-OHCS secretion.

In the second series of experiments, the first 3 samples were taken at 15-minute intervals beginning at 30 minutes prior to the initiation of hyperoxia, hypoxia or CPPB (1.5 hours after cannulation). The 17-OHCS secretory rates of the control, hyperoxic and CPPB groups ranged from 5.1 ± 1.0 to 6.8 ± 1.5 μg/min during the pre-period (—30 to 0 min; Fig. 2) and were not significantly lower than the mean 2.8 ± 1.6 μg/min observed in the hypoxic group at similar times.

During the experimental period (0-120 min) the secretory rates of control animals varied from 3.6 ± 0.8 to 5.1 ± 1.2 μg/min ($P > .30$) while blood flow remained relatively constant. The correlation between steroid concentration and plasma flow during this time was —.21 ($P > .20$). Upon infusion of ACTH a 5-fold increase ($P < .001$) in 17-OHCS occurred concomitantly with a 19% increase in blood flow.

During the first 15 minutes of hyperoxia a transient insignificant decrease was observed in 17-OHCS secretion which subsequently increased 69-95% ($P < .05$). From 0 to 120 minutes blood flow decreased 25% and the correlation with steroid concentration was —.16 ($P > .30$). After ACTH injection the secretory rate rose from 12.0 ± 2.5 to 26.9 ± 1.5 μg/min ($P < .001$) together with an elevation in blood flow from 3.8 ± 0.5 to 5.7 ± 0.8 ml/min ($P < .01$). Immediately upon application of CPPB, adrenal blood flow markedly decreased while adrenocortical secretion increased approximately 3-fold ($P < .001$) followed by a gradual decline from 14.5 ± 1.9 to 10.9 ± 1.7 μg/min over the 120-minute period. However, the latter value remained significantly ($P < .01$) higher than pre-pressure breathing rates and the correlation of plasma flow with concentration was —.47 ($P < .001$). ACTH infusion elevated 17-OHCS secretion and blood flow 67% and 30% respectively. Within 15 minutes after induction of hypoxia, a 2.4-fold ($P < .05$) increase in 17-OHCS secretion and a 23% elevation in blood flow were observed. Although the flow rapidly returned to pre-hypoxic levels, 17-OHCS continued to rise until attaining a maximum secretory rate at 90 minutes.

When the mean secretory rates of animals in the hyperoxic, hypoxic and CPPB groups are compared to those of control animals at similar times during the experiment, all secretory rates were significantly ($P < .05$) higher in "stressed" than control animals with the exception of the rates observed in hyperoxic and hypoxic dogs at 15 minutes.

Discussion. The data obtained one hour after cannulation reveal that the 17-OHCS secretory rates had reached low levels which were relatively stable for the succeeding 3.5 hours. These values are comparable to those of Gann *et al*(11) in which basal adrenal blood samples were collected approximately 24 hours after cannulation. Moreover, in dogs with dorsal spinal roots (T_{11} to L_3) sectioned at least 3 weeks before adrenal vein surgery, and control samples collected approximately 18 hours after cannulation(7), the 17-OHCS secretory rates are very similar to those presented herein. These findings, in conjunction with the observed constancy of the secretory rates of control animals breathing ambient air and the responsiveness of the adrenal cortex to ACTH administered after cannulation, indicate that this preparation is suitable for ascertaining the acute effects of various stresses on adrenocortical 17-OHCS secretion.

The increased 17-OHCS secretory rates noted during hypoxia in anesthetized animals are in accord with the findings observed in both conscious and anesthetized dogs exposed to simulated altitude(7). The maximal mean secretion rate during hypoxia was slightly lower than during hyperoxia or CPPB; however, the percentage increase from pre-stress rates was as large as those found in other stressed animals. Since the secretion rates were lower during hypoxia and exogenous ACTH was not administered to determine if the adrenal cortex could be further stimulated, it is possible that the biosynthesis of 17-OHCS was slightly depressed. Although it has recently been demonstrated that pituitary and blood ACTH concentrations are elevated in rats breathing 10-15% O_2(12), hypoxia *per se* may not be the direct stimulus for ACTH release since altered acid-base balance is capable of increasing 17-OHCS secretion in intact but not hypophysectomized dogs(13,14). Furthermore, Anichkov *et al* (15) have shown that the carotid sinus nerves must remain intact for hypoxemia to activate the adrenal cortex. In our experiments alterations in pH and CO_2 tensions resulting from hyperventilation accompanying hypoxia may have stimulated the hypothalamic-hypophyseal system *via* the carotid body reflex and thus released ACTH.

Although our data on hyperoxic dogs are essentially in agreement with those of others who have used oxygen pressures from 2-7

atmospheres(4,5), the experimental conditions are not comparable since our animals were breathing 100% O_2 at only one atmosphere. However, our findings are not consistent with those of Hale *et al*(1) who found that breathing 100% O_2 at one atmosphere caused a mild depression in peripheral 17-OHCS levels of conscious men. Observations from this laboratory(16) have shown that in control animals the half-life and excretion of infused cortisol are similar to those observed in animals exposed to various environmental stresses including hyperoxia. This would indicate that the discrepancy is not related to an alteration in the metabolism of cortisol. Perhaps the differences are related to respiratory depression occurring during pentobarbital anesthesia in the present investigation with a subsequent alteration in acid-base balance. This explanation is supported by the data which reveal that an increase in 17-OHCS secretion was not noted until 30 minutes after initiation of 100% O_2 breathing and that the percentage increase in secretory rate throughout the experiment was the smallest observed in "stressed" animals. It is possible that the initial depression in respiratory rate occurring during hyperoxia led to a gradual accumulation of CO_2 which stimulated the release of ACTH as previously described.

Several factors may be involved in the elevation of 17-OHCS secretion during CPPB. Since it is known that the pressor receptors are stimulated during CPPB as a result of hypotension(17) and further that carotid constriction, which simulates a drop in blood pressure, increases 17-OHCS secretory rates in acutely prepared intact animals(18), it may be the CPPB can activate the adrenal cortex *via* this mechanism. In addition, the hemoconcentration resulting from the increased hydrostatic pressure in peripheral capillary beds due to the rapid rise in venous pressure(19) may cause the release of ADH which in turn is known to enhance the secretion of ACTH(20). Finally, the altered acid-base balance which results upon application of CPPB may also contribute to the release of ACTH. This latter explanation is supported by the fact that a large increase in 17-OHCS secretion occurs immediately upon application of CPPB when there is a temporary cessation of respiration which causes a period of hypoxemia and hypercapnia. Upon continuation of elevated intra-pulmonary pressure, respiratory rates gradually returned toward control and thus the disturbed acid-base balance was partially corrected. This may account for the decline in 17-OHCS secretion noted in the latter part of the experiment.

It is interesting to note that although secretory rates were markedly elevated during the stresses employed in this investigation, the adrenal cortex was capable of further stimulation by 5 units of ACTH. In addition, responses to stress or ACTH injections were not dependent upon a proportional change in blood flow.

Summary. Experiments on acutely prepared anesthetized dogs revealed that 17-OHCS secretion after adrenal vein cannulation reached a low baseline level within one hour and remained relatively stable for the next 3.5 hours during which time the adrenal cortex was responsive to exogenous ACTH. Using this preparation, 17-OHCS secretory rates were significantly increased during hyperoxia, hypoxia and continuous positive pressure breathing. No significant positive correlations between steroid concentration and plasma flow were observed. The possible mechanism(s) for stimulation of the adrenal cortex during these stresses are discussed.

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Resistance to Experimental Tuberculosis Stimulated by Fractions from Attenuated Tubercle Bacilli. (30009)

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It was reported from this laboratory that disruption of viable BCG suspended in light mineral oil led to isolation of a product, consisting predominantly of cell walls, which could be heat-sterilized with retention of immunogenic potency for mice(1). In contrast to these findings, Youmans *et al*(2-5) isolated from viable BCG or H37Ra after mechanical disruption of the cells in buffered aqueous sucrose solution cytoplasmic particles which were immunogenic for mice. This fraction was reported to lose its protective capacity following heat sterilization.

The two types of vaccines reported above cannot be directly compared from the data reported, because different criteria were chosen in evaluation of protective potency. In the test used to evaluate the oil disruption product, mice were vaccinated intravenously and later challenged with an aerosol containing a few virulent bacilli(6). After this type of challenge, tuberculosis takes a relatively mild course; the animals are sacrificed to determine the presence or absence of visible tuberculous lesions in lungs and the number of virulent tubercle bacilli in lungs and spleens. The dose of the oil disruption product needed to produce significant protection was about 100 μg (dry weight). The assay employed by Youmans to determine the immunogenicity of the particulate fraction was based on intravenous challenge of vaccinated and control mice with massive doses of virulent tubercle bacilli. This type of tuberculosis is much more severe than that induced by pulmonary challenge, and the effectiveness of the vaccine is judged by the number of mice that survive 30 days after challenge or by the median survival times. In this test, Youmans' particulate fraction was protective only at a dose of 20 mg (moist weight) administered intraperitoneally.

In view of the apparent differences between the two vaccines, it seemed advisable to compare their immunogenicity by preparing the oil disruption product and the particulate fraction from the same starting lot of cells and testing the preparations in parallel, together with Rosenthal's viable BCG vaccine, in both of the above mentioned assays.

Materials and methods. Preparation of vaccines. a) *Particulate fraction and oil disruption product from BCG.* A strain of BCG from the Pasteur Institute, which had been

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