EFFECTS OF INHALATION OF 100 PER CENT AND 14 PER CENT OXYGEN UPON RESPIRATION OF UNANESTHETIZED DOGS BEFORE AND AFTER CHEMORECEPTOR DENERVATION

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Since the discovery of the aortic and carotid chemoreceptors, a large number of investigations have been performed upon these structures (for bibliography, see (13)). However there is still disagreement upon two points of fundamental importance. The first of these is the question of the existence of tonic activity. Some investigators (11, 8, 9) maintain that chemoreceptor reflexes are of great importance in the control of respiration under all conditions (normal as well as abnormal) while others (4) believe that these structures function chiefly as an emergency mechanism, of extreme importance during anoxemia, asphyxia, acidosis, and marked hypercapnia, but relatively unimportant in the control of normal breathing. The second point has to do with direct stimulation of the medullary centers by anoxia. Although experiments upon anesthetized animals indicate that anoxia usually stimulates the medullary centers only reflexly through the chemoreceptors, it has been reported (12) that anoxemia may stimulate the medullary centers directly if the anesthesia is sufficiently light. Since anesthesia may either intensify or depress chemoreceptor activity depending upon the nature and concentration of the anesthetic we decided to investigate these problems on trained unanesthetized dogs.

METHODS. Mongrel female dogs of varying size and breed were trained to lie quietly upon a table while breathing through a moulded plaster mask reinforced with rubber and fitted with inspiratory and expiratory valves. All gases, including room air, were inhaled from identical Douglas bags attached to a 3-way stopcock on the inspiratory side. Minute volume of respiration was measured by passing the expired air through a gas meter and respiratory rate was measured by a pneumograph and tambour. The periods of inhalation of each gas were usually 6 minutes; when samples of arterial blood were drawn, these were col-

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2 Ellen Mickle Fellow of the University of Toronto.

3 Chloralose (100 mgm./kilo intravenously) in the dogs used in these experiments depressed respiratory minute volume (average reduction 36 per cent) and arterial pO₂ (average reduction 25 mm. Hg) and raised arterial pCO₂ (average increase 5.2 mm. Hg). At the same time, the degree of anoxia and the amount of NaCN necessary to produce stimulation of respiration were lessened. Chloralose anesthesia therefore exaggerates the effects of chemoreceptor reflexes. Experience of other investigators (1) (6) (12) with different anesthetics has shown that the influence of anesthesia on chemoreceptor reflexes is too complicated to permit generalizations from experiments with one type and grade of anesthesia on one species of animal.

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lected under oil from the femoral artery at the end of the 6 minute periods, heparinized, covered with melted paraffin and kept in ice until analyzed. Estimations of plasma pH were made with a closed glass electrode at 38°C. and CO₂ content and whole blood O₂ content and capacity were determined by the Van Slyke manometric method. Experiments performed during the summer months were carried out in an air-conditioned room at 70°F. A complete experiment consisted of a series of observations (inhalation of room air, 100 per cent O₂, room air, 14 per cent O₂, room air) until the responses were consistent. The same series of observations was repeated after denervation of the carotid and aortic bodies.

Carotid body denervations were done under ether anesthesia; the internal carotid and occipital arteries and all attached nerve tissue were divided between ligatures and the external carotids were stripped from the origin of the internal carotid to the origin of the lingual artery. Aortic body denervations were performed by Dr. Norman Freeman in the following manner: The dog was anesthetized by intratracheal insufflation of ether; through an incision in the right third intercostal space, all branches of the right vagus and recurrent laryngeal nerves to the heart and aorta were severed below the level of the origin of the latter nerve. All branches from the stellate ganglion to the right vagus were cut and one inch of this vagus was excised below the origin of the recurrent laryngeal. Great care was taken not to injure the right recurrent laryngeal nerve itself. Through a similar incision on the left side all branches of the left vagus inside the thorax were severed including the left recurrent laryngeal at its origin from the vagus, but the stripped left vagus trunk was left intact. This operation preserves one recurrent laryngeal nerve, one abdominal vagus trunk and a few of the fibers from the pressure receptors situated in the brachiocephalic artery, but interrupts all fibers from the aortic bodies. Complete chemoreceptor denervation was attested by the lack of respiratory stimulation from intravenous injections of NaCN or inhalation of low oxygen mixtures which previously had caused marked hyperpnea; incomplete aortic pressure receptor denervation was indicated by a return of blood pressures to the normal range within 2–4 weeks, after a temporary hypertension.

Results. A. Tonic activity of chemoreceptors during quiet breathing of room air. This was investigated by substituting 100 per cent O₂ for room air while respiratory rate and minute volume were measured. We chose this procedure because the arterial blood of an animal breathing room air always shows some oxygen unsaturation, and if this is sufficient to set up tonic chemoreceptor impulses capable of stimulating respiration, inhalation of 100 per cent O₂ should result in a definite depression of respiration. The average results of 194 observations on seven trained normal dogs are shown in figure 1. The scatterings of the findings in each dog are shown in table 1. In each dog there was usually an immediate decrease in minute volume which was maximal at the end of the first minute; the magnitude of this depression varied from 11 to 31 per cent. Minute volume had returned to a normal level by the end of 3 minutes in 3 dogs and by the end of 6 minutes in another, but in 3 dogs respiration was still 10
TABLE 1  
Effect of inhalation of 100% O₂ upon minute volume of respiration

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<tr>
<th></th>
<th>TOTAL OBSERVATIONS</th>
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*DENERVATED DOG*

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![Graphs showing the effect of 100% oxygen on respiration](http://ajplegacy.physiology.org/)

Fig. 1. Average effect of 100 per cent oxygen on respiration of the unanesthetized dog. Upper charts represent average percentage change in minute volume of respiration of normal unanesthetized dogs during inhalation of room air (broken lines), 100 per cent O₂ (solid lines) and then room air again; observations are recorded at minute intervals. The numbers in squares indicate the number of experiments performed upon each dog. The numbers just above the zero percent line represent the number of experiments in which respiratory minute volume increased, and those just below the line represent the number of experiments in which respiration decreased during inhalation of 100 per cent O₂ (N.C. = no change). Arterial oxygen tensions (pO₂) and arterial CO₂ tension (pCO₂) were determined upon femoral artery blood withdrawn at the ends of the control period and of the O₂ inhalation.

Lower charts show similar data upon dogs 1 to 4 after denervation of carotid and aortic bodies.
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to 14 per cent below the control level at the end of the 6th minute of O₂ inhalation; in these, return to normal occurred promptly upon inhalation of room air. In 5 of 6 dogs in whom CO₂ tensions of arterial blood were measured, pCO₂ had increased 1.5 to 9 mm. (average 3.8 mm.) at the end of the O₂ inhalation.

The same procedures were repeated after chemoreceptor denervation in 4 of the 7 dogs (fig. 1 and table 1). (One dog died before operation, one died following carotid denervation and a third has not been operated upon as yet.) The immediate decrease in minute volume that occurred consistently when O₂ was inhaled by the intact dogs was lacking completely after the denervation.

Comment. Since all these animals usually showed an immediate depression of pulmonary ventilation when they were made to breathe 100 per cent O₂, and since chemoreceptor denervation entirely abolished this effect, it follows that some of their chemoreceptor units must have been tonically activated by the oxygen unsaturation normally existing in their arterial blood during quiet breathing of room air at sea level. The interpretation of this finding, however, should be made with due regard for the following facts:

1. Denervation of the carotid and aortic bodies did not lead to a lower resting volume of pulmonary ventilation in any of these animals (table 2), or to any significant change in arterial gas content or pH except in dog 4, which we believe had some atelectasis. Hence it would appear that tonic chemoreceptor activity was not essential for the maintenance of normal respiratory activity in these animals breathing room air. The same conclusion is suggested by the tendency of breathing to recover during the inhalation of O₂ in 4 out of 7 intact dogs, and by the qualitative variations in the responses of the same animal from day to day. This is particularly evident in dogs 1 and 2 (table 1).

2. Inhalation of 100 per cent O₂ at sea level should cause a rise in arterial pO₂ from a normal of 70 to 90 mm. Hg to something above 600 mm. Hg, which is of course far beyond the physiological range. When these dogs were made to breathe mixtures low in O₂ we obtained evidence, confirmatory of a previous report (4), indicating that a change of 30 mm. Hg in arterial pO₂ (i.e., from 80 to

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50 and back to 80 mm. Hg) causes practically no change in respiratory minute volume in the dog, but a further change of 10 mm. Hg (i.e., from 80 to 40 mm. Hg) causes definite hyperpnea and the reverse change (from 40 to 80 mm.) leads to abrupt depression of breathing. At the critical level, therefore, small changes in arterial pO₂ cause more marked alterations in chemoreceptor activity than do much larger changes in pO₂ at a normal or supernormal level. This supports the suggestion (13) that while some chemoreceptors are sensitive enough to be activated by the small degree of arterial unsaturation normally present, most of them come into action only at a definitely subnormal pO₂.

3. Even though some chemoreceptors show tonic activity in dogs at sea level, they do not appear to do so in man. It has been reported (15) that O₂ inhalation causes respiratory stimulation in healthy young adults, but the conclusion was based on the average obtained during a 15 minute period and no mention is made of the immediate effect. We investigated this point in 11 young adults. Of 19 experiments, the immediate effect of inhaling 100 per cent O₂ instead of room air was an increase in respiratory minute volume in 13, a decrease in 4, and no change in 2. The average of the 19 observations gave a 6 per cent increase. These findings suggest that tonically active, oxygen-sensitive chemoreceptors are the exception rather than the rule in normal man.

While the existence of chemoreceptors tonically activated by the degree of oxygen unsaturation normally present in unanesthetized dogs is definitely confirmed, no evidence was obtained bearing upon the question of similar activation by changes in CO₂, pH, or temperature (8, 14).

B. Direct effects of anoxia on the respiratory center. The same dogs were given 14 per cent O₂ to inhale for a 6 minute period using the same experimental technique described in A. The average results, before and after denervation, are shown in figure 2. In each of the 7 normal dogs, 14 per cent O₂ increased respiratory minute volume, the range being 17 to 29 per cent; in the 4 denervated dogs the initial effect was depression of respiration by 22 to 29 per cent. These results are similar to those reported in unanesthetized (2) and in anesthetized dogs (7). The absence of stimulation in the denervated dogs cannot be attributed to a failure of arterial pO₂ to fall, for the average arterial pO₂ (calculated according to Dill’s data (5)) during inhalation of 14 per cent O₂ was 48 mm. Hg before denervation, 33 mm. Hg afterward. The first effect of anoxia upon respiration of these dogs was unquestionably depression because rate, depth of breathing, and respiratory minute volume were decreased in each of the 4 dogs and in every one of the 25 observations made with 14 per cent O₂ after the denervation. The depression began within the first minute and lasted about four minutes, after which the minute volume began to increase, though it was still 12 to 20 per cent below normal at the end of the six minute inhalation period. This apparent recovery was associated with restlessness and in several instances convulsive movements were observed, but it is noteworthy that the increase in breathing was due entirely to acceleration, often of the rapid, shallow type; depth was never increased and was usually decreased.

Comment. These findings are presented as additional evidence that the char-
acteristic respiratory response to anoxemia (prompt increase in depth of breathing causing a diminution in the ΔpO₂ between inspired air and arterial blood) is due to chemoreceptor reflexes. This conclusion is not vitiated by the fact that, in the denervated dog exposed to atmospheres low in oxygen, the primary respiratory depression may be followed by an increase in respiratory minute volume toward or above normal (2) (12). While we have not prolonged the low O₂ inhalations in our experiments beyond 6 minutes, we did notice, following the depressed period of 4 minutes, an unmistakable tendency for the minute volume to rise despite continued anoxia; this was due entirely to an increase in rate, depth being unaffected or decreased. Goldschmidt, Brewer, Daven-

Fig. 2. Average effect of 14 per cent oxygen on respiration of the unanesthetized dog. Similar to figure 1 except that solid lines represent inhalation of 14 per cent O₂ in 86 per cent N₂.

port and Chambers (10) have analyzed the effects of prolonged anoxia in the surviving 3 of our 4 denervated dogs, and have found that minute volume eventually returned to normal and then usually rose above normal.

Several explanations might be offered for this delayed response other than regeneration of nerve fibers: 1. Chemoreceptors elsewhere (e.g., in the coccygeal body) possessing much lower sensitivity to anoxia than those in the carotid and aortic bodies might be responsible; this is unlikely because this particular response appears to be depressed easily by anesthesia (12) while the carotid and aortic chemoreceptors are characterized by a high resistance to most forms of anesthesia (4).

2. As the oxygen saturation falls to lower levels upon prolonged exposure to
low oxygen, acid metabolites may accumulate in the respiratory center and so
stimulate respiration. While there is no direct evidence favoring this explana-
tion, it is in harmony with the views of those physiologists (9) who believe that
respiration is controlled predominantly by the hydrogen ion concentration within
the cells of the respiratory center. Since anoxia of this degree probably would
result in accumulation of greater amounts of acid than under ordinary condi-
tions, it might be pertinent to inquire why acidity in this instance increases
only the rate of breathing, and why a latent period of 4 minutes must elapse
before stimulant concentrations are reached. If acidity in general (rather than
CO₂ specifically) is the characteristic stimulus to the respiratory center, it should
act promptly and should affect both rate and depth, for excess CO₂ is known to
increase both rate and depth very promptly whether given by inhalation or by
direct injection into the respiratory center (3). Since in the case of anoxia the
acid metabolites are formed presumably within the cells of the center, the ques-
tion of relative rates of diffusion of acid and CO₂ should be irrelevant. It may
be argued that anoxia by a dual mechanism is depressing while stimulating, and
consequently the acid-mechanism is working at a disadvantage. However
anoxia does not correspondingly alter the response of the center to CO₂ inhala-
tion. Dumke, Chiodi and Schmidt (7) found in denervated dogs average
increases in respiratory minute volume of 52 per cent during inhalation of 3.5
per cent CO₂ in O₂, and of 46.4 per cent on inhalation of the same CO₂ concen-
tration in 10 to 12 per cent O₂; in all cases, both rate and depth were increased and
the hyperpnea appeared promptly. If CO₂ is effective only by virtue of its acid
properties, it would be rather remarkable that the response to it is not markedly
reduced by a degree of anoxia that drastically alters the response to a direct
increase in intracellular acidity.

3. A third explanation is suggested by the observations that this polypnea
is abolished by decerebration (2) or by a slight increase in the depth of anes-
thesia (12), which also suggests a supra-tentorial origin. However, preliminary
experiments upon decerebrated cats and dogs have occasionally shown acceler-
ation of rate after an initial depression of depth and minute volume when 14
per cent O₂ was breathed after carotid denervation and vagotomy. Conse-
quently the phenomenon is not necessarily dependent upon the higher centers.

CONCLUSIONS

In 7 unanesthetized dogs, inhalation of 100 per cent oxygen for 6 minutes led
to a transient diminution in respiratory minute volume varying from −11 to
−31 per cent. After denervation of the carotid and aortic bodies, oxygen
inhalation produced no change or an increase in minute volume of respiration.
Consequently some chemoreceptors in the dog must be continually activated by
the usual degree of oxygen unsaturation of arterial blood at sea level. However
experiments upon normal men failed to reveal evidence of similar tonic activity.

Inhalation of 14 per cent O₂ in unanesthetized dogs increased respiratory
minute volume 17 to 29 per cent; after chemoreceptor denervation, initial depres-
sion of minute volume (22–29 per cent) was observed. However, unlike the
sequence in anesthetized dogs, in which respiratory depression is usually progressive until death, in the unanesthetized denervated dogs depression of depth and rate of breathing was succeeded by acceleration of rate. Therefore known chemoreceptor reflexes cannot be responsible for all the increase in rate during prolonged anoxia. The possible causes of this delayed response are discussed.

REFERENCES

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