J. Exp. Biol. (1962), 39, 107–117
With 8 *text-figures*
Printed in Great Britain

ON THE REGULATION OF RESPIRATION IN REPTILES

II. THE EFFECT OF HYPOXIA WITH AND WITHOUT MODERATE HYPERCAPNIA ON THE RESPIRATION AND METABOLISM OF LIZARDS

BY BODIL NIELSEN

From the Zoophysiological Laboratory A, University of Copenhagen, Denmark

(*Received* 13 *October* 1961)

The chemical regulation of respiration in various species of reptiles has earlier been studied by Siefert (1896), Babak (1914*a*, *b*), v. Saalfeld (1934), Vos (1936), Boelaert (1941, 1942), Randal, Stullken & Hiestand (1944), and B. Nielsen (1961). A survey of the literature was given by Vos (1936).

The present work deals with the effect of different concentrations of O_2 in the inspiratory air on respiration of lizards. Further, the combined action of CO_2 and different O_2 percentages has been studied. Two species of lizard, *Lacerta viridis* and *Tarentola mauretanica* served as experimental animals.

METHODS AND PROCEDURE

Methods and procedure were described by B. Nielsen (1961). The fasting experimental animal was placed in a container consisting of a body-chamber and a 'helmet'. A tight-fitting rubber diaphragm was placed around its neck. When screwed together the body chamber and the helmet were separated from one another by the air-tight diaphragm, the head of the animal protruding into the helmet. Different air mixtures could be forced through the helmet from a spirometer containing 0·5 or 1·2 l. of the air mixture. The volume of air which passed out of the spirometer during each experiment was recorded on a drum. When air mixtures different from room air were used, the particular air mixture was bubbled through the water in the spirometer for about 30 min., so that an equilibrium between water and air in the spirometer could be established before the experiment.

The flow through the helmet, from which the animal breathed, could be varied, and was regulated so that the difference in CO_2 percentage between the air reaching the helmet (influx air) and leaving the helmet (exit air) was about 0·6 %. Leaving the helmet the exit air passed a sampling device where a small part of it was withdrawn into two 10 ml. glass syringes.

Volume changes in the body-chamber caused by the animal's breathing were transmitted to a small Krogh-type spirometer, 3·5 or 7 ml., which registered on a revolving drum. The animal container was placed in a thermoregulated water-bath so that the experiment could be performed at a constant temperature. The body temperature of the animal could be measured by means of a thermocouple, one junction of which, mounted in a small plastic tube, was inserted 1 cm. into the cloaca. Each experiment

lasted 15 min., but readings and sampling were not started until an initial period of 20–60 min. had elapsed, during which the body temperature became constant and the respiration regular.

Samples of the influx air, taken immediately before and after the experiment, and samples of the exit air (taken as described above during the experiment) were analysed for O_2 and CO_2 content in the Scholander 0·5 ml. analysing apparatus (Scholander, 1947). If the results of a pair of analyses differed by more than 0·05 % in O_2 or CO_2 the experiment was discarded.

From the collected data oxygen uptake, CO_2 elimination, R.Q. and O_2 uptake/100 g./hr. (at STPD) could be determined. On the spirometer record the number of respirations during the experiment was counted and the depth of the respirations measured. From this an average respiratory frequency and average respiratory depth for the experimental period were estimated. The product of these factors gave the average pulmonary ventilation per min. during the experiment. (The values were converted to BTPS.)

Seven specimens of *Lacerta viridis* (green lizard) and three of *Tarentola mauretanica* (gecko) were used. Experiments were performed at 20° C. (room temperature) and at 30° C. The air mixtures which were used contained about 20·9, 17·5, 14·5, 12·5, 9·5 and 5·5 % O_2. In the experiments with CO_2 about 2·4 % CO_2 was added to a similar oxygen series. This percentage has earlier (B. Nielsen, 1961) been shown to have the greatest effect on pulmonary ventilation.

In the following the changes in O_2 uptake, respiratory ventilation, respiratory pattern, etc., will be related to the O_2 and CO_2 contents in the exit air. The composition of the exit air must be a close approximation to the average composition of the air in the helmet from which the animal inspires. The actual expiratory air and alveolar air is not known and cannot be computed from the values obtained in these experiments (for the discussion of this problem see B. Nielsen, 1961).

RESULTS

Fig. 1 shows that the O_2 uptake decreases when the O_2 content in the inspired air is diminished to values below 10 %. The same effect of low O_2 was seen when in a series of experiments 2·4 % CO_2 was added to the O_2 mixtures (Fig. 2).

The pulmonary ventilation changes but slightly with changes in O_2 concentration in the inspired air, except at the lowest O_2 concentrations, where it decreases. This is seen in Fig. 3 ,where pulmonary ventilation per 100 g. is plotted in relation to O_2 % in the exit air. When CO_2 (2·4 %) was added to the O_2 mixtures the same effect of low O_2 was found, i.e. a decrease in ventilation at the lowest O_2 %; but the level of ventilation was 30–40 % higher, due to the CO_2 (see B. Nielsen, 1961).

Fig. 4 shows the changes in the ratio

$$\frac{[\text{pulm. ventilation/hr. (BTPS)}]}{[O_2 \text{ uptake/hr. (STPD)}]}$$

in relation to the O_2 % in the inspired air. Results with and without CO_2 addition are presented. The ratio increases with decreasing O_2 %, only little when no CO_2 is added, but much more when CO_2 is administered together with the O_2 mixtures.

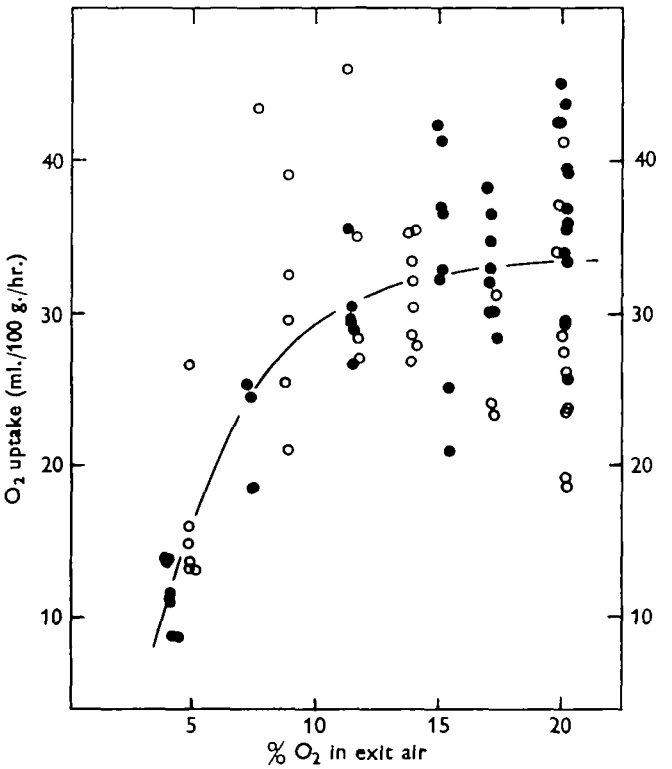

Fig. 1. Oxygen uptake in ml. per 100 g./hr. in relation to the O_2 percentage in the exit air (inspired air). CO_2 percentage 0·4–0·9 %, temp. 20° C. (Results from six animals: ●, weight about 30 g.; ○, weight about 25 g.)

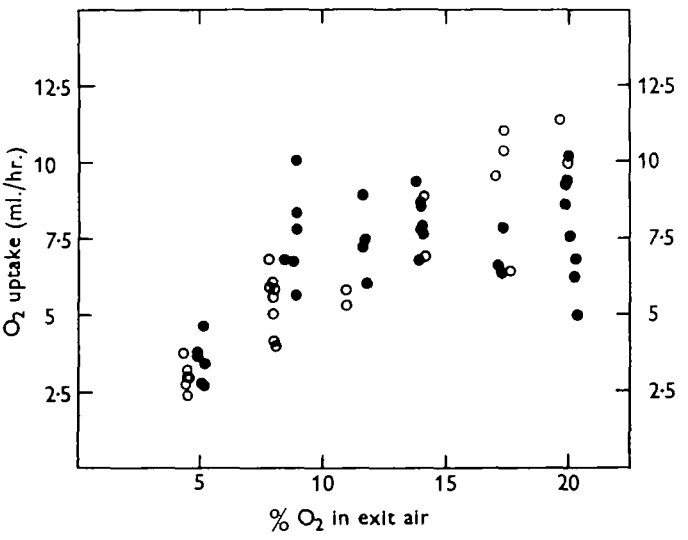

Fig. 2. O_2 uptake in ml./hr. in relation to the O_2 percentage in the exit air (two animals, weight about 25 g.). ●, CO_2 in the exit air about 0·6 %; ○, CO_2 in the exit air about 2·8 %, temp. 20° C.

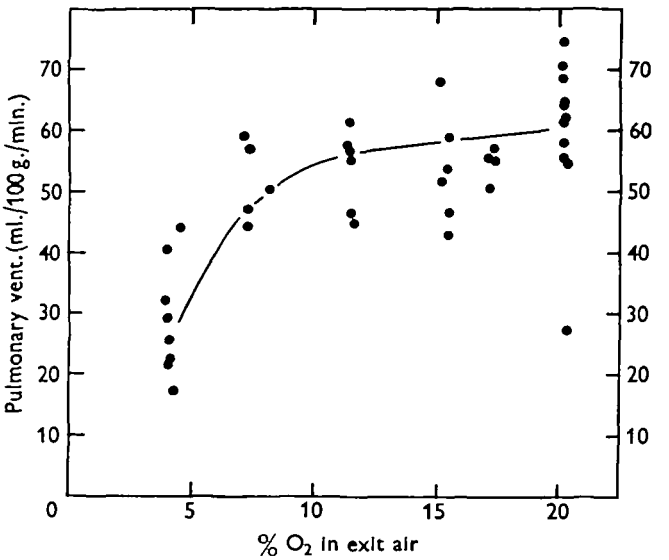

Fig. 3. Pulmonary ventilation/100 g./min. in relation to the O_2 percentage in the exit air. CO_2 about 0·6 %, temp. 20° C. (three animals, weight 30 g.).

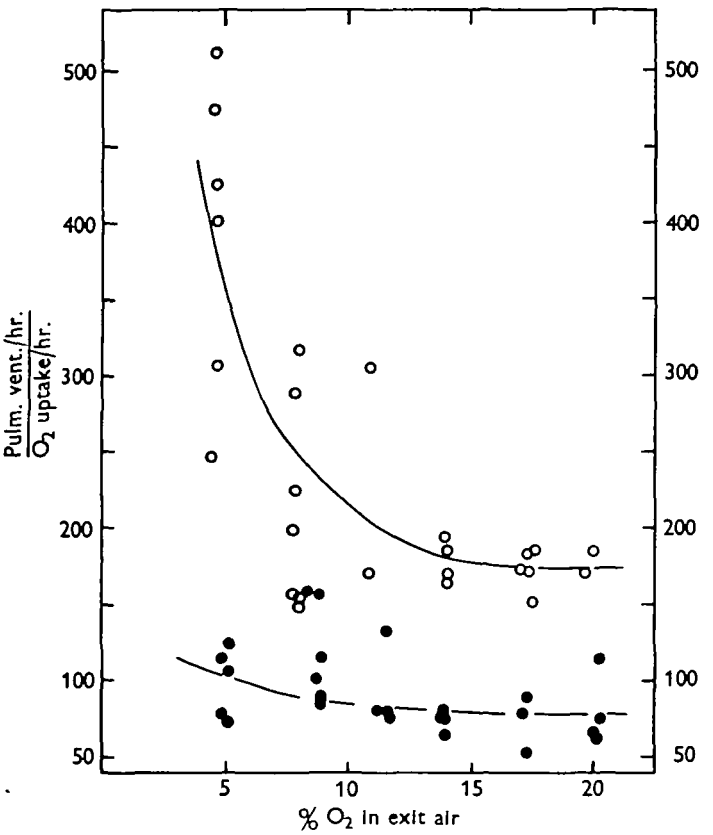

Fig. 4. [Pulm. ventilation/hr. (BTPS)]/[O_2 uptake/hr. (STPD)] in relation to O_2 percentage in the exit air. ●, CO_2 in the exit air ranging from 0·40 to 0·65 %. ○, CO_2 in the exit air ranging from 2·70 to 3·15 %, (two animals, weight 25 g., temp. 20° C.).

The effect of low O_2 on the respiratory frequency is shown in Fig. 5*a*. The frequency is found to be almost independent of changes in O_2 percentages between 20 and 10% in the inspired air. Below 10% O_2 the frequency decreases to about half the normal value. Addition of 2·4% CO_2 to the O_2 mixtures did not change this response, the average frequency being almost the same (Fig. 6). At a body temperature of 30° C. the frequency was higher than at 20° C. but decreased at all values of O_2 below 20% O_2 both with and without CO_2 addition. Hypoxia increased the respiratory depth (Fig. 5*b*). When the body temperature was about 20° C., the increase in depth occurred at O_2% below 8–10 in the exit air (inspired air). The same was found when the CO_2% in the exit air was increased to about 2·8%. The two curves relating respiratory depth and inspired O_2% were similar, but in the series with CO_2 added the depth was

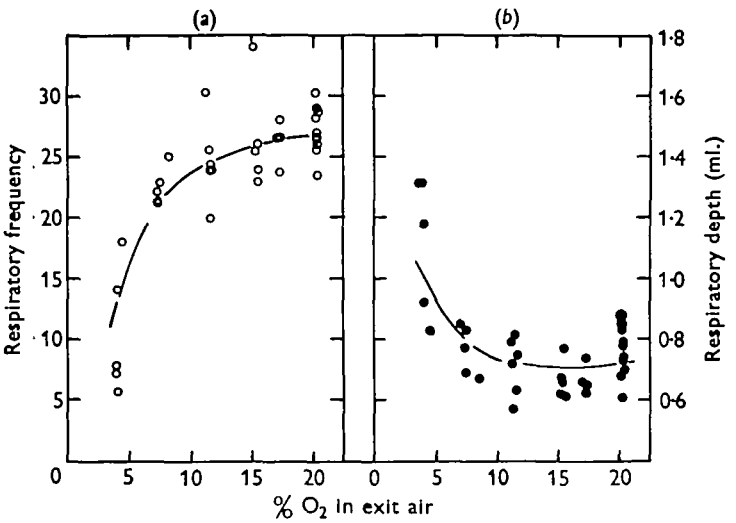

Fig. 5. (*a*) o, Respiratory frequency in relation to O_2 percentage in the exit air. (*b*) •, Respiratory depth in ml. in relation to O_2 percentage in the exit air. CO_2 0·5–0·7%, temp. 20° C. (three animals, weight *ca.* 30 g.).

doubled at all values of O_2% (Fig. 7). The combined effect of O_2 lack and CO_2 (2·4% CO_2) was studied in six animals (four *Lacerta* and two *Tarentola*). The changes in respiratory frequency, depth and ventilation with changing O_2% were fundamentally the same in the geckoes and in the green lizards. Raising the body temperature to 30° C. increased the slope of the curves relating depth and inspired O_2 both with and without addition of CO_2. With 20% O_2 in the inspired air the depth at 30° C. was a little lower than at 20° C., but the depth increased at all values of O_2% below that of room air so that the 30° C. curve crosses over the 20° C. curve when the inspired O_2% is lowered to about 12–15%. 5% O_2 was not tolerated well at this temperature. The animals became quite limp and took several hours to re-establish normal respiration (Fig. 7).

Fig. 8 contains a plot of all related values of respiratory frequency and O_2 uptake/100 g./hr. The results from ten different animals are included. The experiments were carried out between 1957 and 1960 and experiments with R.Q. values below 0·6 or

above 1·1 are omitted to ensure normal O_2 uptakes and 'steady state'. The variations in O_2 uptake/100 g. were produced by warming or cooling the animals (body temperatures between 35 and 10° C.) and by administration of different air mixtures. It appears that frequency varies linearly with O_2 uptake over a wide range of O_2 uptake.

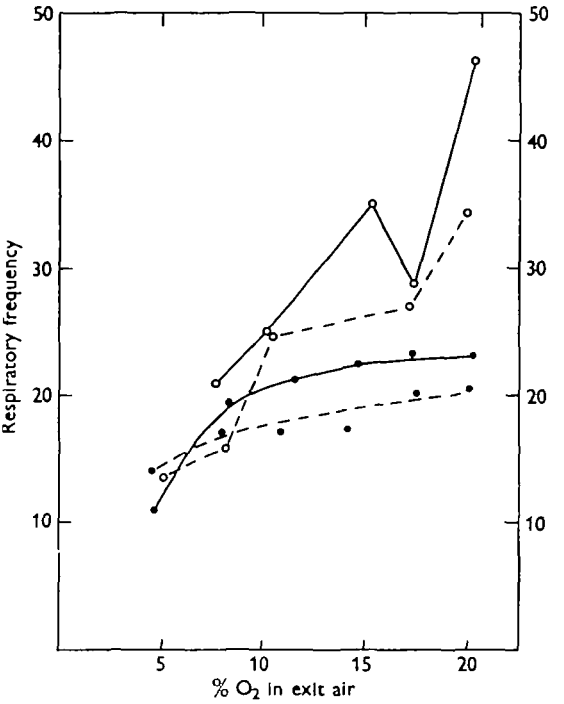

Fig. 6. Respiratory frequency in relation to O_2 percentage in the inspired air (exit air). Average values under four experimental conditions: ——●, 20° C. CO_2 content *ca.* 0·6 % 7 animals (1–7); - - - -●, 20° C. CO_2 content *ca.* 2·8 % 2 animals (1, 3); ——○, 30° C. CO_2 content *ca.* 0·6 % 4 animals (4–7); - - - -○, 30° C. CO_2 content *ca.* 3·0 % 3 animals (4–6).

DISCUSSION

The present study shows that the respiratory pattern in lizards is influenced by hypoxia: the respiratory depth increases and the frequency decreases.

The variations in pulmonary ventilation in response to the changes in inspired O_2 % (Fig. 3) found in the present experiments are strikingly small. But the close relationship between ventilation and O_2 uptake (B. Nielsen, 1961) must be considered. As Fig. 1 shows, the animal reacts to the lowered O_2 % by diminishing its O_2 uptake, the ventilation consequently decreasing. However, the ratio

$$\frac{\text{(ventilation/hr.)}}{\text{(O_2 uptake/hr.)}}$$

increases a little when the O_2 % in the inspired air decreases below 10 (Fig. 4). With CO_2 added to the inspired air a much more pronounced increase in the ratio occurs at increasing degrees of hypoxia, i.e. at low pressures the sensitivity of the respiratory apparatus towards CO_2 must have been increased, as in man (M. Nielsen & Smith, 1951)

where the combined action of increased CO_2 tension and lowered O_2 tension acts as a very strong ventilatory stimulus.

The respiratory depth increases when the inspired O_2% is lowered. This agrees

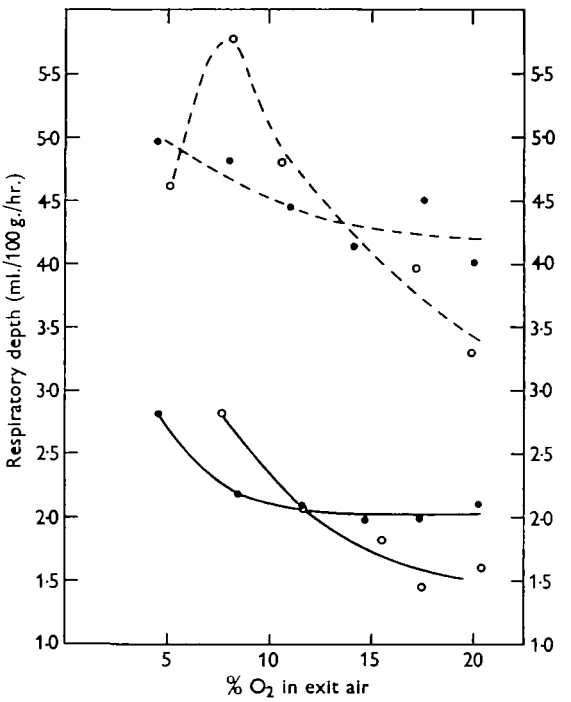

Fig. 7. As fig. 6 (see legend) but showing the variations in average respiratory depth in ml./100 g./hr. under the four conditions.

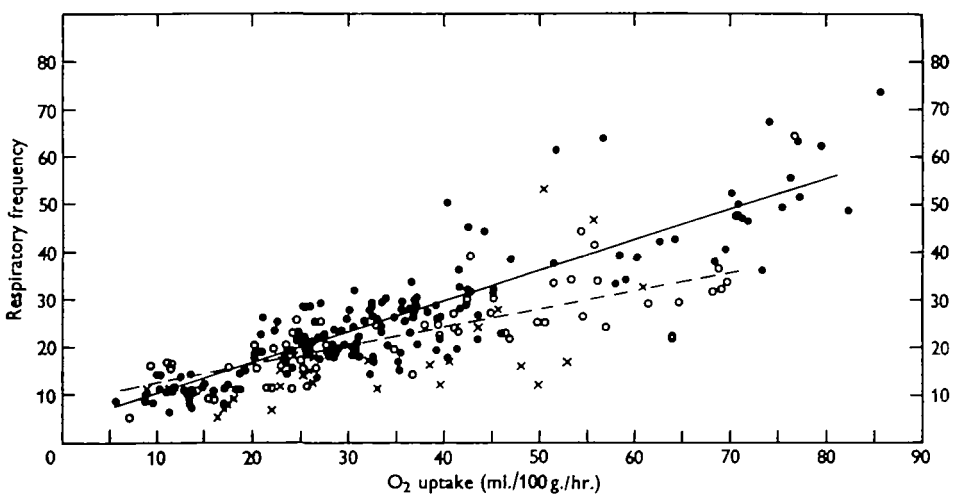

Fig. 8. The relationship between respiratory frequency and O_2 uptake in ml./100 g./hr. ●, CO_2 content in the exit air 0·4–1·1 %. ○, CO_2 content in exit air about 2·8 %. The variations in O_2 uptake were produced by warming or cooling the animals or by administrating air mixtures of different O_2 content. (Results from ten animals combined.)

with the results of most of the older investigations. But in most of the older work pure N_2 or H_2 was used to study the effect of hypoxia (Siefert, 1896; Babak, 1914*a*, *b*; v. Saalfeld, 1934; Vos, 1936; Boelaert, 1941). Few conclusions concerning the regulation of respiration can be drawn from such experiments. In the present experiments an increase in respiratory depth of about 50% was found at 5% O_2 (Figs. 5*b*, 7). But this compensation for the low O_2 content in the inspired air is counteracted by a decrease in frequency, so the *pulmonary* ventilation is not increased, and even decreases at the lowest O_2%, as mentioned above. Whether the increase in depth increases the alveolar ventilation (although the total ventilation is not increased) is questionable. The dead space of the lungs is not known but must be rather large in a simple sac-like lung like that of *Lacerta* (Milani, 1894). How a greater inflation of this lung will influence the ratio of the dead space to alveolar space cannot be predicted, but the rate of diffusion of O_2 through the lung tissue is perhaps accelerated as the walls of the more expanded alveoli must be thinner.

The increase in respiratory depth with increasing hypoxia, in the experiments with moderate hypercapnia, was found to be similar to that found in the experiments with hypoxia alone (see Fig. 7), the two curves being parallel. But an increase of 50% is more significant when the level of respiratory depth as is the experiments with hypercapnia is higher and consequently closer to the maximal inspiratory capacity. At 30° C. the slope of the curve relating O_2% to depth is greater than at 20° C. both with and without CO_2 added, and the depth is increased at all values of O_2% below that of room air, suggesting a greater sensitivity to O_2 lack at 30° C. However, no definite conclusions can be drawn from the results presented in Fig. 7 as the results are from different groups of animals and no group has been exposed to all four experimental conditions.

The effect of low O_2% in the inspired air on respiratory frequency was always a decrease in frequency. Inspired CO_2 also causes a decrease in frequency, probably due to an inhibition via chemoreceptors in the lungs (cf. B. Nielsen, 1961). But in the present experiments with low O_2 the decrease occurred gradually, and no 'inhibition' of the respiration in the form of 'Cheyne–Stokes' respiration like that found with high CO_2 was seen. Consequently, neither inspired CO_2 nor O_2 lack seems to accelerate respiration. The only way to increase the respiratory frequency in these lizards has been to increase their metabolism (O_2 uptake), for example, by warming the animals. In a plot of frequency in relation to O_2 uptake per 100 g./hr. (Fig. 8) it is seen that over a range of O_2 uptake from 5 ml./hr. to about 70 ml./hr. the frequency varies linearly with O_2 uptake. It is, therefore, possible that some metabolic breakdown products regulate frequency. This may be CO_2 from endogenous sources or other factors that influence the acid-base balance of the blood (cf. experiments with sudden shift from CO_2 breathing to room air breathing in which the frequency immediately increased to values well above the normal before gradually returning to the room air level, B. Nielsen, 1961). Experiments with CO_2 added to the inspired air do not fall on the curve relating frequency and O_2 uptake, but some of the experiments where the CO_2% of the inspired air was kept constant at about 3% form a line with a less steep slope. This is thought to be due to the 'inhibition' of respiratory frequency caused by CO_2 when inhaled (B. Nielsen, 1961). In this case neural factors change the relationship between frequency and metabolic rate.

Under low O_2% a decrease in O_2 uptake was found (Fig. 1). This may be due to a failing O_2 diffusion in the lungs or to a reduced function of other links in the O_2 transporting systems. Also tissue respiration might be inhibited under low O_2%. Altland & Parker (1955) found that O_2 uptake decreased in low O_2% in turtles but, in contrast to the findings of the present study, found abnormal R.Q. values, the CO_2 output being normal. In some warm-blooded animals also it has been reported that O_2 lack can reduce metabolism. Irving (1939), Scholander (1940) and Scholander, Irving & Grinnell (1942) found from calculations that during a quiet dive metabolism in seals and whales must have been reduced below the basal level. Hill (1958) reports that in new-born kittens and adult guinea-pigs at environmental temperatures below the neutral temperature, low O_2 (10%) causes the O_2 uptake to decrease to half the initial value. Andersen (1959), working on experimental dives in the duck, found a decrease in O_2 uptake and showed that anaerobic processes do not compensate for the reduced O_2 uptake. Eliassen (1960) stresses that the reduced metabolism found by the above mentioned authors is due to unnaturally prolonged dives and consequently is not a normal physiological response towards low O_2. Whether natural or not, the diminished metabolism enables the animals to survive a longer period of asphyxia. The above-mentioned cases are perhaps parallels to the O_2-dependent type of metabolism very often found in lower animals. Below a 'critical tension' the O_2 uptake varies linearly with the oxygen pressure. In some cases, e.g. in many fish, the activity (metabolic rate) is controlled by O_2, being reduced in O_2-poor environment. In other animals (worms, snails, etc.) the oxidative processes on the cellular level are limited by the available O_2. Fig. 1 may imply that, at least in *Lacerta*, the respiratory regulatory mechanisms do not work sufficiently below 15–18% O_2. Below this O_2% the animals became quiescent, and struggling is very rare (the available O_2 is insufficient for a working metabolism). Below 10% O_2 (the critical tension) the O_2 pressure is too low to keep up the normal resting metabolism at the temperature concerned and the O_2 uptake must decrease and varies with O_2 pressure.

The main results of this study are that the absolute values of pulmonary ventilation are not increased by hypoxia or by hypoxia combined with moderate hypercapnia. The narrow working range of the respiratory regulating mechanisms is expressed by a reduction in O_2 uptake under unfavourable conditions. However, as the ventilation ratio (ventilation/hr.)/ (O_2 uptake/hr.) increases with increasing hypoxia both without, and especially in combination with, hypercapnia, there is some regulation of ventilation which compensates for the lack of O_2. Respiratory depth seems to follow the changes in O_2 and CO_2 content in the lungs, being most sensitive to changes in inspired CO_2% (B. Nielsen, 1961), but the corresponding changes in pCO_2 (and pO_2) in the blood are unknown. The variations in respiratory frequency seem to be closely related to changes in metabolic rate.

In higher vertebrates and man O_2 lack acts upon the respiratory centre via the chemoreceptors in the aortic and carotid bodies. Such chemoreceptors must be looked for in *Lacerta* also. Smyte (1939) has shown that in the frog (*Rana esculenta*) the so-called carotid gland is an area sensitive to oxygen lack, as the hypernoea produced by administration of air mixtures poor in O_2 was almost absent when the carotid glands had been denervated. Bhatia & Dayal (1933) describe in the wall lizard *Hemidactylus*

flaviviridis a structure—the carotid gland—at the bifurcation between the carotids interna and externa. This may be present in *Lacerta* also and may have a function like that in the frog and like the carotid and aortic bodies in man.

SUMMARY

The effects of various degrees of hypoxia and the combined effects of hypoxia and moderate hypercapnia (2·8% CO_2 in the inspired air) have been studied in seven specimens of *Lacerta viridis* (green lizard) and three of *Tarentola mauretanica* (gecko).

1. It was found that hypoxia reduces the O_2 uptake.

2. Pulmonary ventilation was little changed by hypoxia until the O_2% of the inspired air was reduced below 10, when it decreased.

3. A small increase in the ratio (pulmonary ventilation/hr.)/(O_2 uptake/hr.) with decreasing O_2% was found. When CO_2 was added to the O_2 mixtures the increase was much greater, indicating an increased sensitivity of the respiratory apparatus towards CO_2 during hypoxia.

4. The effect of hypoxia on respiratory frequency was always to cause a decrease. Addition of 2·8% CO_2 to the inspired air had little effect. At a body temperature of 20° C. the decrease occurred at O_2% in the inspired air below 10, whereas at 30° C. the frequency was higher, but decreased as soon as the O_2% was below 20 (room air).

5. It was found that the respiratory frequency in *Lacerta* varies linearly with the metabolic rate (O_2 uptake).

6. The respiratory depth increases with decreasing O_2% in the inspired air, at 20° C. below 10% O_2 and at 30° C. below 20% O_2. Addition of 2·8% CO_2 in both cases doubled the respiratory depth at all degrees of hypoxia.

REFERENCES

ALTLAND, P. D. & PARKER, M. (1955). Effects of hypoxia on the box turtle. *Amer. J. Physiol.* **180**, 421–7.

ANDERSEN, H. T. (1959). Depression of metabolism in the duck during experimental diving. *Acta physiol. scand.* **46**, 234–9.

BABAK, E. (1914*a*). Über die Atembewegungen und ihre Regulation bei den Eidechsen. *Pflüg. Arch. ges. Physiol.* **156**, 531–71.

BABAK, E. (1914*b*). Über die Atembewegungen und ihre Regulation bei den Panzerechsen. *Pflüg. Arch. ges. Physiol.* **156**, 572–601.

BHATIA, M. L. & DAYAL, J. (1933). On the arterial system of the lizard *Hemidactylus flaviviridis* Rüppel (the wall lizard). *Anat. Anz.* **76**, 417–37.

BOELAERT, R. (1941). Sur la physiologie de la respiration de lacertiens. *Arch. int. Physiol.* **51**, 379–436.

BOELAERT, R. (1942). Sur la physiologie de la respiration de l'alligator mississippiensis. *Arch. int. Physiol.* **52**, 57–72.

ELIASSEN, E. (1960). Cardiovascular responses to submersion asphyxia in avian divers. *Årbok. f. Univ. i Bergen. Mat.-Naturv.* Serie 1960, no. 2.

HILL, J. R. (1958). The relation between O_2 consumption, hypoxia and environmental temperature. *J. Physiol.* **143**, 64*P*.

IRVING, L. (1939). Respiration in diving mammals. *Physiol. Rev.* **19**, 112–34.

MILANI, A. (1894). Beiträge zur Kenntnis der Reptilienlunge. I. *Zool. Jb.* (Abt. 2), **7**, 545–92.

NIELSEN, BODIL (1961). On the regulation of the respiration in reptiles. I. The effect of temperature and CO_2 on the respiration of lizards (*Lacerta*). *J. Exp. Biol.* **38**, 301–14.

NIELSEN, M. & SMITH, H. (1951). Studies on the regulation of respiration in acute hypoxia. *Acta physiol. scand.* **24**, Fasc. 4, 293–313.

RANDALL, W. C., STULLKEN, D. E. & HIESTAND, W. A. (1944). Respiration of reptiles as influenced by the composition of the inspired air. *Copeia*, pp. 136–44.

v. SAALFELD, E. (1934). Die Mechanik der Atmung bei *Uromastix*. *Pflüg. Arch ges. Physiol.* **233**, 431–48.

SCHOLANDER, P. F. (1940). Experimental investigations on the respiration in diving mammals and birds. *Hvalrddets Skr.* no. 22. Det norske Vidensk. Akad. Oslo.

SCHOLANDER, P. F. (1947). Analyser for accurate estimation of respiratory gases in one-half cubic centimeter samples. *J. Biol. Chem.* **167**, 235–50.

SCHOLANDER, P. F., IRVING, L. & GRINNELL, S. W. (1942). On the temperature and metabolism of the seal during diving. *J. Cell Comp. Physiol.* **19**, 67–78.

SIEFERT, E. (1896). Ueber die Athmung der Reptilien und Vögel. *Pflüg. Arch. ges. Physiol.* **64**, 321–506.

SMYTE, D. H. (1939). The central and reflex control of respiration in the frog. *J. Physiol.* **95**, 305–27.

VOS, H. J. (1936). Over Ademhaling en Reukzin bij Reptilien en Amphibiën. Proefschrift, Groningen.