Hypoxia and the carotid body

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Since the publication of the classical studies of Heymans *et al* (1930) it has been accepted that the carotid body is a chemoreceptor which monitors the oxygen tension of systemic arterial blood, and it has been extensively investigated by physiologists (Biscoe, 1971). Because chronic hypoxia is a major hazard of life at high altitude and of cardiorespiratory disease at sea level, it might be thought that much is known about the pathology of the carotid body in such situations. This, however, is not the case. It was not until 1969 that Arias-Stella reported enlargement of the carotid bodies in high-altitude dwellers in the Peruvian Andes and 1970 that Heath *et al* described such enlargement in sea-level patients with emphysema. Thus until the last decade, the only condition of the carotid body which the general pathologist was aware of was the rare tumour known as the chemodectoma. Since the pioneering observations of Arias-Stella, pathological studies by Heath and Edwards (1971) of the carotid body have been made on human subjects and animals born and living at high altitude, and also on animals living at subatmospheric pressure in hypobaric chambers. These chambers are very convenient for working with small animals in the laboratory without the inconvenience and expense of long journeys to countries with mountain ranges over 3600 metres high. However, a distinction must be made between the examination of animal tissues derived from experiments using hypobaric chambers and the examination of tissues from human subjects and animals born and living for many years at high terrestrial altitude. Hypobaric chambers can accurately reproduce the low atmospheric pressure of high altitude but not the climatic and other environmental features of life in a high mountainous region.

This paper briefly reviews the structure and function of the normal carotid body, and then deals with the morphological changes associated with acute and chronic alveolar hypoxia in human subjects and animals.

Structure of the normal carotid body

Although the chemoreceptor function of the

carotid body has been known for decades, the identity of the receptors and mechanism by which they are stimulated remains unknown. Previous light and electron microscope studies (reviewed by Biscoe, 1971) have shown that the carotid body is composed of clusters of small cells surrounded by nerves and an anastomosing system of small blood vessels (fig 1). Two types of parenchymal cell can be distinguished: a type I, chief or glomic cell; and a type II, sheath or sustentacular cell (fig 2). The type I cells, which are arranged in compact microscopic nests (*zellballen*), have a rounded contour and contain a single vesicular nucleus with an open chromatin pattern. There are two varieties of type I cell: a light cell with clear vacuolated cytoplasm and a dark cell with dense-staining eosinophilic cytoplasm. The type II cell has an elongated nucleus with dense chromatin pattern. The cell is fusiform in shape with processes which envelop the type I cells. The distinction between type I and type II cells can be made in histological sections stained with haematoxylin and eosin, but better definition is obtained

Fig 1 Normal rat. The carotid body is seen in the centre of the picture lying to the left of the carotid artery (C) and above the superior cervical ganglion (S). Haematoxylin and Van Gieson $\times 60$.

Fig 2 Carotid body of normal rat. The type I cells (I) have rounded vesicular nuclei and are arranged in small clumps ('zellballen'). The type II cells (II) are at the periphery of the clumps of type I cells and have elongated dark-staining nuclei. Epoxy section (1 μ m thick) stained with toluidine blue $\times 1500$.

with the electron microscope (fig 3). The type I cell contains numerous membrane-bound granules consisting of a central osmiophilic core with a clear halo subjacent to the limiting membrane (fig 4). The dark variety of type I cell has larger and more angular dense-core secretory granules than the light variety. Two subclasses of type I cell have been recognized by analysing the diameters of dense-core vesicles, but the significance of distinguishing 'small vesicle cells' from 'large vesicle cells' has yet to be determined (Hellström, 1975). The vesicles contain biogenic amines of which dopamine is the most abundant in the carotid bodies of rabbits, cats and humans. On the basis of their cytochemical characteristics Pearse (1969) has included the type I cells of the carotid body in the APUD series and suggests that they produce a polypeptide hormone,

'glomin', of unknown function which has not yet been identified. Nerves end on type I cells but their function is disputed. It used to be thought that the type I cells were receptor cells and that nerves ending on such cells were afferent. This hypothesis was not seriously questioned until electron microscopy revealed that nerves ending on type I cells contain synaptic vesicles suggesting that such nerves were efferent rather than afferent. The hypotheses regarding the identity of the chemoreceptor and relative merits of the type I cell, type II cell and nerve endings as candidates for this role are discussed in the publications of Biscoe (1971), McDonald and Mitchell (1975) and Osborne and Butler (1975).

Carotid body in acute hypoxia

Mitchell *et al* (1972) found that free nerve endings regenerated from the central part of a cut cat sinus nerve have chemoreceptor properties which are similar to those of the carotid body as a whole, suggesting that type I cells are not primarily responsible for the chemoreceptor function of the carotid body. It has been postulated that catecholamines in the type I cells may function as transmitters in an efferent neural pathway which inhibits chemoreceptor discharge. It is thus an important question as to whether the catecholamines in the type I cells are released by the physiological stimulus, hypoxia.

Blümcke *et al* (1967) carried out electron microscope and fluorescence studies on the carotid bodies of rats exposed to acute and severe oxygen deficiency. They found that extreme hypoxia equivalent to a reduction of the oxygen concentration in the air to 2.5 per cent led to a decrease in the number of dense-core vesicles of the type I cells. In extreme hypoxia, the vesicles moved to the periphery of the cell until their bounding membranes were in contact with the cytoplasmic membrane. The entire contents of the vesicles were discharged into the intercellular space, and after discharge the former membrane of the dense-core vesicle remained as part of the cytoplasmic membrane of the type I cell. The almost total discharge of catecholamines from the type I cells after 20 minutes of extreme hypoxia was confirmed by fluorescence microscopy. They also noticed swelling of the mitochondria, disintegration of the chromatin particles of the nucleus and an increase in the number of nuclear pores which may suggest that they were observing severe pathological changes rather than a physiological response. Chen *et al* (1969) did not find any change in the number or density of dense-core vesicles in hamsters exposed to hypoxia. Al-Lami and Murray (1968), however, found in cats that the

Fig 3 Carotid body of normal rat fixed by perfusion with glutaraldehyde and hydrogen peroxide (McDonald and Mitchell, 1975). Two type I cells (I) are surrounded by the cytoplasmic processes of a type II cell (II). The type I cells contain abundant mitochondria and tiny dense-core vesicles. Two capillaries (cap) which are devoid of fenestrations are situated on the right side of the picture. Electron micrograph $\times 7500$.

Fig 4 Type I cell from carotid body of normal rat. Note the large nucleus (N) and numerous tiny cytoplasmic vesicles with a central electron-dense core surrounded by a clear halo (arrow). Electron micrograph $\times 25\,000$.

number and density of the dense-core vesicles was increased by hypoxia lasting for 45 minutes.

In electron microscope studies which report a change in the number or density of dense-core vesicles in type I cells after hypoxia, it is frequently assumed that there is a corresponding change in type I cell catecholamine content. However, the electron density of secretory vesicles may reflect the amount of complexing substances in the vesicle and not its catecholamine content. Mills and Slotkin (1975) measured the catecholamine content of the carotid bodies of cats after ventilation with hypoxic gas mixtures for 60 to 90 minutes. They showed that the content of catecholamines in the carotid body decreased at a rate directly proportional to the inspired oxygen concentration in the range 8 to 40 per cent oxygen. The decrease in content during hypoxia was mediated mainly through an efferent neural pathway in the carotid sinus nerve.

Human carotid body in chronic hypoxia

Anatomical changes in the carotid body in subjects exposed to chronic hypoxia were first described by Arias-Stella (1969) who found that the carotid bodies of high-altitude dwellers in Peru were substantially larger than those in sea-level subjects. Recently, these changes have been described in detail by Arias-Stella and Valcarcel (1976) who carried out a necropsy study comparing the carotid bodies of subjects dying in Cerro de Pasco (4300 metres) with those from people dying at sea level in Lima. Cases of cardiovascular and pulmonary disease were excluded from their study and subjects were divided into three age groups: 10 to 20 years; 21 to 40 years; and 41 to 70 years. The length, transverse diameter and depth were significantly greater in the three age groups from high altitude. Specifically, in the 41 to 70 age group, the mean dimensions of the carotid body at sea level were $4.00 \times 1.48 \times 1.12$ mm whereas at high altitude they were $6.56 \times 3.12 \times 2.18$ mm. Similarly in this age group the mean combined weight of the carotid bodies was 23.66 mg at sea level and 61.76 mg at high altitude. The mean carotid body weights varied very little within the three age groups at sea level, but a progressive increase occurred with age at high altitude. Thus, the mean combined carotid body weights in the age groups 10 to 20 years, 21 to 40 years and 41 to 70 years were respectively 28.13 mg, 36.04 mg and 61.76 mg. In general, the high-altitude carotid bodies showed a dark brown colour in contrast to the pale brown colour of the sea-level specimens. Histological studies showed that the number of lobes in the high-altitude carotid bodies (mean 6.16) was greater than that in the sea-level cases (mean 4.94). The high-

altitude carotid bodies showed a reduction in the width of the interlobar fibrous connective tissue septa accompanied by an apparent hyperplasia of chief cells which possessed highly vacuolated cytoplasm. When examined in a fluorescence microscope green-yellow naturally fluorescent granules indicative of biogenic amines were present in the sea-level carotid bodies while such fluorescence was scarce or absent in the high-altitude specimens. Another distinctive feature was that the high-altitude specimens generally showed congestion of their capillaries and veins, a characteristic not often seen in the sea-level carotid bodies.

Chronic hypoxia affects many patients with respiratory disease as well as people living at high altitude. If it is accepted that the carotid bodies of high-altitude dwellers enlarge in response to the stimulus of living in a hypoxic environment, then it would be expected that such enlargement would also occur in sea-level patients suffering from diseases like chronic bronchitis and emphysema which may give rise to chronic hypoxia. This is indeed the case. In a necropsy study of the carotid body in subjects who had lived at sea level in Liverpool, England, it was found that the average weights of the right and left carotid bodies were respectively 12.9 mg and 11.3 mg (Heath *et al*, 1970). In two patients with right ventricular hypertrophy complicating pulmonary emphysema, the combined weight of the carotid bodies was 60.9 mg and 68.8 mg, respectively. The first of these patients was known to have had a systemic arterial pO_2 of 36 mm Hg and a pCO_2 of 54 mm Hg during life. Another patient with the primary alveolar hypoventilation (Pickwickian) syndrome had enlarged carotid bodies which together weighed 45.5 mg. The increase in size of the carotid bodies in these three cases could not be attributed to hyperplasia of any one type of cell. However, in one of the emphysema cases, there were hyperplastic nodules showing a predominance of the dark variety of type I cell. Fluorescence microscopy for the study of biogenic amines in the carotid bodies of sea-level patients with respiratory diseases complicated by chronic hypoxia has not yet been carried out. However, Steele and Hinterberger (1972) measured the weight and content of norepinephrine, epinephrine, dopamine and 5-hydroxytryptamine in 76 pairs of human carotid bodies obtained at necropsy and attempted to correlate their results with the nature of the terminal illness. Dopamine was uniformly the most abundant amine and it occurred in significantly higher concentration in eight normotensive subjects dying of cerebral vascular disease. The second most abundant amine was 5-hydroxytryptamine and its concentration equalled that of dopamine in nine patients

with renal and essential systemic hypertension. The pattern of amine distribution did not differ significantly in patients dying in respiratory failure secondary to diseases such as chronic bronchitis, emphysema and fibrosing alveolitis, when compared with other disease groups. They found that the weight of the carotid body was increased in patients with abnormally high cardiac weight. This latter observation corroborated the observations of Heath *et al* (1970) and Edwards *et al* (1971a). In the former paper it was shown that there was a significant correlation between the weight of the carotid bodies and the weights of the left and right ventricles in 40 successive cases coming to necropsy. In the latter paper the type and severity of emphysema, carotid body weight and left and right ventricular weights were recorded in 44 consecutive cases referred for routine necropsy. The mean combined weight of the carotid bodies in 13 cases with no evidence of emphysema was 21.1 mg. In four of 11 cases of emphysema studied, the right ventricular weight was normal and the mean combined carotid body weight was 32.4 mg. In the seven cases of emphysema with right ventricular hypertrophy the mean combined carotid body weight was 56.2 mg. In one case in this latter group the combined carotid body weight was as high as 89.3 mg. There was no relationship between the type and severity of emphysema on the one hand and the size of the carotid bodies on the other. Rather there appeared to be a relationship between the weight of the carotid bodies and the weight of the right ventricle. Since the latter was probably associated with hypoxic vasoconstriction in the lung elevating pulmonary vascular resistance, it was assumed that the link between enlarged carotid bodies and right ventricular hypertrophy was chronic hypoxia. There was also an unexplained relation between the weight of the carotid bodies and the weight of the left ventricle in the 44 cases studied which included several patients with left ventricular hypertrophy.

Animal carotid body in chronic hypoxia

The carotid bodies of guinea pigs, rabbits and dogs which had been born and lived all their lives in Cerro de Pasco, Peru (altitude 4330 metres) were found to be enlarged when compared with those of similar animals which had been reared at sea level in Liverpool, England (Edwards *et al*, 1971b). This enlargement was attributed on histological examination to a hyperplasia of the type I cells which showed highly vacuolated cytoplasm. In the guinea pigs and rabbits the hyperplasia affected the light variety of type I cells. In the dog all the type I cells are normally of the light variety. Enlargement of the carotid

Fig 5 An enlarged carotid body from a rat exposed to chronic hypoxia in a hypobaric chamber at a simulated altitude of 4300 metres (460 mm Hg) for 98 days. The carotid artery (C) is on the right and the superior cervical ganglion (S) is at the bottom of the picture. Compare with figure 1. Haematoxylin and Van Gieson $\times 60$.

body also occurs in experimental rats exposed to simulated high altitude in a hypobaric chamber (fig 5).

We have used stereological methods to perform quantitative morphological studies of the carotid bodies of young rats to determine precisely what changes occur in the various tissue components on exposure to chronic hypoxia (Laidler and Kay, 1975a and b). Ten young rats (initial body weight 27 to 50 g) were confined in a hypobaric chamber at a pressure of 460 mm Hg simulating the altitude of Cerro de Pasco 4300 metres above sea level. All the animals died spontaneously after being in the chamber for periods ranging from 25 to 96 days. When these animals died the bifurcations of their carotid arteries were removed, fixed in formalin, and, together with the carotid bodies from 10 untreated control rats, were processed for histological examination. Each bifurcation was cut into serial transverse sections which were mounted on microscope slides and stained by the Van Gieson method so that collagen appeared red and other tissues yellow. All the serial sections were examined using an automatic sampling microscope. The image of the carotid body was displayed on a projection head which was equipped with a detachable transparent foil bearing the multipurpose test system described by

Weibel. Using the technique known as point counting we measured the area of the carotid bodies in selected serial sections and were then able to calculate the volume of the carotid body using a formula known as Simpson's rule. We also used the point-counting method to determine the proportion of the volume of each carotid body occupied by five major tissue constituents. In the normal rat four tissue constituents can be recognized in the carotid body. These are the specialized functional glomic cells, fibrous tissue, capillaries and other tissues such as large arteries and nerves. In the hypoxic carotid bodies an amorphous hyaline material was present and we also measured the volume proportion of this. In all the hypoxic rats the volume of the carotid bodies was greatly increased. There was a four-fold increase in the mean combined volume of the carotid bodies from $47.16 \times 10^6 \mu\text{m}^3$ in the control rats to $187.39 \times 10^6 \mu\text{m}^3$ in the hypoxic animals. However, this increase in carotid body volume was not accompanied by a simple proportional increase in the various tissue constituents (fig 6). There was an absolute three-fold increase in the volume of glomic cells in the hypoxic carotid bodies but the actual proportion of glomic cells was significantly decreased. The most dramatic change in the composition of the hypoxic carotid bodies was an increase in their vascularity. There was a ten-fold increase in the volume proportion of the capillaries in the hypoxic rats when compared with the controls. The proportion of fibrous connective tissue remained constant. In all the hypoxic carotid bodies an amorphous hyaline material was present which occupied between 0.2 and 8 per cent of their volume. This appeared to be within the lumen of some capillaries and within the substance of the carotid body in relation to specialized glomic cells and is probably the result of capillary platelet thrombosis followed by fibrosis (Blessing and Wolff, 1973). In the hypoxic rats, although there was no direct linear relation between the combined carotid body volume and the duration of exposure to hypoxia, there was a tendency for the carotid bodies to be larger in the rats which survived longer in the hypobaric chamber. Further analysis revealed that there was, however, a linear relation between the volume of specialized glomic tissue and the duration of exposure to chronic hypoxia (fig 7). This experimental observation is in accordance with that of Arias-Stella and Valcarcel (1976) who observed a progressive increase in carotid body weight with increasing age in high-altitude residents of Cerro de Pasco. There was no relation between the carotid body volume in our hypoxic rats and the left and right cardiac ventricular weights. We counted the number of type I cells in our control and hypoxic rats to see if this cell proliferated on exposure to

Fig 6 This diagram illustrates the difference in mean volume and volume proportions of various tissue components between the carotid bodies of 10 normal rats and 10 rats exposed to chronic hypoxia in a hypobaric chamber (460 mm Hg) for periods ranging from 25 to 96 days.

Fig 7 The volume of specialized glomic tissue in the left and right carotid bodies of 10 rats exposed to chronic hypoxia in a hypobaric chamber at a simulated altitude of 4300 metres (460 mm Hg) for periods ranging from 25 to 96 days. Figure reproduced by kind permission of the Editor of the Journal of Pathology.

chronic hypoxia. In normal rats we found that the number of type I cells in the left and right carotid bodies ranged from 15.92 to 30.77×10^3 with mean of 22.91×10^3 . In the hypoxic rats the number ranged from 15.16 to 93.65×10^3 with mean of 40.79×10^3 . In six of the hypoxic animals the number of type I cells was less than or slightly exceeded the highest figure encountered in the controls. In the other four hypoxic rats there was a substantial increase in the number of type I cells. In both groups there was a linear relationship between the number of type I cells and duration

Fig 8 The number of type I cells in the left and right carotid bodies of 10 rats exposed to chronic hypoxia in a hypobaric chamber (460 mm Hg) for periods ranging from 25 to 96 days. The lower regression line relates to six rats in which the number of type I cells did not exceed the number found in 10 untreated control animals. The upper regression line depicts four rats in which there was an abnormal increase in the number of type I cells. Figure reproduced by kind permission of the Editor of the American Journal of Pathology.

Fig 9 Carotid body of a rat exposed to chronic hypoxia in a hypobaric chamber (460 mm Hg) for 35 days. The capillaries (cap) are dilated. The type I cells (I) and their nuclei are enlarged. Compare with figure 2. Epoxy section (1 μ m thick) stained with toluidine blue $\times 1500$.

exposure to hypoxia (fig 8). The type I cells in the hypoxic rats were enlarged and had pale vacuolated cytoplasm (fig 9). Because the cytoplasmic borders of the cells are poorly delineated, it was not possible to measure their diameters. However, the mean diameter of the nuclei of type I cells of hypoxic rats (5.5 μ m) was greater than that of the controls (5.0 μ m). The largest type I cell nuclei were seen in those rats which had been subjected to hypoxia for the shortest time.

Reversibility of hypoxic enlargement of the carotid body

The pulmonary hypertension, right ventricular hypertrophy and muscularization of the small pulmonary arterial vessels which occur in animals exposed to chronic hypoxia, are reversible when such animals are removed from the hypoxic environment and allowed to breathe air at normal atmospheric pressure (Abraham *et al*, 1971). Similarly, the increase in size of the carotid bodies in response to chronic hypoxia is reversible once the hypoxic stimulus is withdrawn. The volume of the carotid body was studied in three groups of adult rats (Heath *et al*, 1973). In the first group kept for five weeks at a simulated altitude of 5500 metres (380 mm Hg), the mean carotid body volume was $47.8 \times 10^6 \mu\text{m}^3$. The volume in the second group kept hypoxic for the same period of time and then allowed to recover in room air at sea level for five weeks was $19.82 \times 10^6 \mu\text{m}^3$. In a group of untreated control rats the mean carotid body volume was $13.45 \times 10^6 \mu\text{m}^3$. A recent study has, however, shown that although to the light microscope the carotid body appears to be normal after recovery from exposure to simulated high altitude, at the ultrastructural level there is an apparent permanent distortion in the composition and cellular arrangement of the glomus (Blessing and Kaldeweide, 1975). These workers investigated the effects of chronic exposure to a simulated altitude of 7000 metres (308 mm Hg) and subsequent recovery at normal atmospheric pressure on the carotid bodies of rats. At high altitude the carotid bodies were enlarged and their lobular configuration was lost. The capillaries were dilated and contained thrombi associated with vacuolation, degeneration and disappearance of type I cells which were replaced by collagen. After recovery for 41 days at sea-level pressure the carotid body enlargement had regressed and the histological pattern looked normal. However, at the ultrastructural level, the capillaries were found to be separated by collagenous tissue containing intact type II cells but only scanty, atrophic type I cells.

Ultrastructure of the carotid body in chronic hypoxia

Ultrastructural changes in the carotid bodies of animals that had been born and lived all their lives at high altitude have been described by Edwards *et al* (1972) and Møller *et al* (1974). The former group of workers compared the carotid bodies of seven guinea pigs derived from Cerro de Pasco (altitude 4300 metres) with those obtained from five guinea pigs born and bred in Liverpool. Changes were noted in dense-core vesicles in the cytoplasm of type I cells. In the high-altitude guinea pigs the clear halo separating the central electron-dense core from the outer limiting membrane was increased in width. The osmiophilic core tended to be smaller, excentric and less dense so that in some animals only faint remnants were seen. In others, the core was absent so that clear vesicles replaced the osmiophilic bodies. In some cells large clear intracytoplasmic spaces were present which were thought to be derived from the fusion of microvacuoles originating from the dense-core secretory granules. These clear spaces were thought to be responsible for the vacuolated appearance of the cytoplasm of type I cells seen in histological sections of high altitude carotid bodies. Møller *et al* (1974) compared the ultrastructure of the carotid bodies of sea-level rabbits with that of rabbits exposed to a simulated altitude of 7000 metres for seven days, and with that of rabbits which had always lived at an altitude of 4000 to 4300 metres above sea level in Bolivia. No difference was detected in the ultrastructure between the two groups of hypoxic rabbits. In the type I cells the Golgi region appeared enlarged and the number of mitochondria and dense-core vesicles was greatly increased compared with those of sea-level rabbits. The size and electron density of the dense-core vesicles varied considerably, but, in general, the electron density of the granules was increased and the relative number of large granules was increased. Contradictory findings were reported by Blessing and Kaldeweide (1975) who studied the carotid body in rats adapted in consecutive steps to a simulated altitude of 7000 metres (308 mm Hg) over a period of 103 days, after which they remained at this altitude for 26 days. The type I cells showed swelling and vacuolation of their mitochondria. Large cytoplasmic vacuoles were present and the number of dense-core vesicles was decreased.

We have studied the carotid body in three rats which lived in a hypobaric chamber at a simulated altitude of 4300 metres (460 mm Hg) for periods ranging from 27 to 35 days. At the end of the experimental period, the carotid bodies of the three hypoxic rats and three untreated controls were

Fig 10 Carotid body from a rat exposed to chronic hypoxia in a hypobaric chamber (460 mm Hg) for 28 days. The capillary (cap) is lined by an attenuated endothelial cell which shows numerous fenestrations (arrows). A narrow interstitial zone separates the capillary from a type I cell (I) which contains mitochondria and vesicles with an eccentric electron-dense core and central clear zone (encircled). Fixed by perfusion with glutaraldehyde and hydrogen peroxide. Electron micrograph $\times 25\,000$.

fixed by perfusing the carotid arteries with a mixture of glutaraldehyde and hydrogen peroxide. The chronically hypoxic rats showed pronounced dilatation of their capillaries which were lined by attenuated endothelial cells showing an increased number of fenestrations compared with control animals (fig 10). In some areas the capillary dilatation was so marked that it caused distortion of the lobular pattern as a result of displacement of glomic cells. The type I cells appeared unchanged in the hypoxic animals.

Fig 11 Type I cell from the carotid body of a rat exposed to chronic hypoxia in a hypobaric chamber (460 mm Hg) for 35 days. Between the nucleus (N) and cell membrane (CM) are several enlarged vesicles (arrows) with the dense core situated eccentrically and adherent to the limiting membrane. Fixed by perfusion with glutaraldehyde and hydrogen peroxide. Electron micrograph $\times 50\,000$.

but there was obvious enlargement of the type I cells. The density of free ribosomes and endoplasmic reticulum was unchanged in these cells but the cytoplasm contained scattered myelin figures. The density and distribution of dense-core vesicles were similar in the control and hypoxic animals with a concentration around the periphery of the cell, particularly in relation to areas of blurring and fusion of the cell membrane with that of an adjacent cell. These specialized portions of the cell membrane are thought to represent areas of synaptic contact between adjacent type I cells or between type I cells and nerve endings. We found changes in the dense-core vesicles similar to those described by Edwards *et al* (1972) in guinea pigs derived from Cerro de Pasco. The vesicles were enlarged and there was an increase in the width of the clear halo separating the dense core from the limiting membrane (fig 11). The size of the electron-dense core was reduced and its position became eccentric so that it was adherent to limiting membrane. These changes in the dense-core vesicles within type I cells did not occur in the synaptic vesicles located in

an adjacent nerve ending, suggesting that they are a specific effect of chronic hypoxia on the type I cell.

The lack of uniformity in descriptions of the ultrastructural changes in the type I cell in chronic hypoxia may be the result of species differences and differences in experimental technique, particularly with regard to fixation. Another problem is the subjective assessment as to whether or not certain organelles, such as mitochondria and dense-core vesicles, are increased in number. Subjective assessment is notorious for interobserver variation and variation in the same observer on different occasions. It seems that this problem will only be solved when quantitative methods are applied to studies of the ultrastructure of the carotid body.

Significance of carotid body changes in chronic hypoxia

Studies in human subjects and animals living at high altitude or simulated high altitude have shown that the carotid body is enlarged in chronic hypoxia. This

enlargement is due to an increase in the volume of functional glomic tissue and also to an increase in the volume of capillary blood vessels. Various histological and ultrastructural changes have been described in the type I cells but not in the type II cells, suggesting that it is the type I cell which responds to chronic hypoxia. Interpretation of the ultrastructural changes is difficult because one group of workers (Blessing and Kaldeweide, 1975) demonstrated apparent depletion of the dense-core vesicles while another group describes apparent increased synthesis of such vesicles (Møller *et al*, 1974). A depletion of natural yellow-green fluorescence was noted by Arias-Stella and Valcarcel (1976) in the carotid bodies of high-altitude dwellers suggesting a reduced content of biogenic amines.

The enlargement of the carotid bodies which occurs at high altitude is associated with a blunted ventilatory response to hypoxia which is said to be permanent (Sørensen and Severinghaus, 1968). It is difficult to reconcile this permanence with the reversibility of enlargement of the carotid body in experimental rats in a hypobaric chamber (Heath *et al*, 1973). Young rats were kept in hypobaric conditions for two to 11 weeks by Barer *et al* (1976). Their carotid bodies enlarged and immediately on removal from the hypoxic chamber the ventilatory response to hypoxia was impaired. This impairment disappeared after three days' recovery under normoxic conditions. Arias-Stella and Valcarcel (1976) report that although at sea level there is no significant variation in hypoxic ventilatory drive in relation to age, a progressive depression of ventilation occurs with aging at high altitude. There is a close relation between increased carotid body weight and diminished chemoreceptor sensitivity with age. However, unlike man, many animal species living at high altitude have normal ventilatory responses to hypoxia (Brooks and Tenney, 1968; Mines and Sørensen, 1969; Hornbein and Sørensen, 1969; Lahiri *et al*, 1971; Lahiri, 1972; Bissgard *et al*, 1974). Attenuation of chemoreceptor function does occur in a small proportion of patients with chronic bronchitis and emphysema (Flenley *et al*, 1970).

The increased vascularity of the chronically hypoxic carotid body is a feature common to all organs in people from the Andes (Arias-Stella and Valcarcel, 1976). It may be a non-specific reaction designed to increase blood flow and thus oxygen transport to an organ with increased metabolic activity. An increase in the vascularity of brain, heart and skeletal muscle has been reported in rats adapted to high altitude (Miller and Hale, 1970). However, it may be that the capillary bed in the carotid body responds to chronic hypoxia in a way not seen in other organs. In the human subject, the

media of the glomic artery is highly elastic and the intima contain large globular cells reminiscent of type I cells (Heath and Edwards, 1971). Furthermore, arteriovenous anastomoses have been found in the carotid body of the cat (Schäfer *et al*, 1973).

Pearse (1969) has included type I cells in the APUD series and suggests that they produce a polypeptide hormone, 'glomins', of unknown function, which has not yet been identified. The structural findings in the chronically hypoxic carotid body suggest that if such a hormone is produced by type I cells, its rate of secretion may be increased during prolonged hypoxia.

Chronic hypoxia and chemodectoma

It is well known that climate can influence the development of neoplasms. Thus in countries with plentiful sunshine, people with light skins are prone to basal cell carcinoma and malignant melanoma. However, there are few reports of altitude influencing the neoplastic process. The discovery of the influence of altitude on Burkitt's lymphoma excited great interest but it now seems that high altitude may predispose to tumours by more subtle means than external factors, such as a virus or insect vector. The evidence comes from South America where Saldaña *et al* (1973) drew attention to the high incidence of chemodectomas of the head and neck in Peruvian adults born and living at high altitude in the Andes. They studied a series of 25 subjects with 26 chemodectomas. Twenty-three of the 25 patients (92 per cent) were born and had lived at altitudes between 2105 and 4350 metres. Estimates of the prevalence of chemodectomas in Peru revealed that they are 10 times more frequent at high altitude compared with sea level. Saldaña *et al* (1973) also refer to two other large published series of carotid body tumours comprising 21 patients and 40 patients derived respectively from the upland areas of Colorado (altitude 1500 to 3000 metres) and Mexico City (altitude 2240 metres). Of Saldaña's 23 high-altitude patients, 22 had carotid body chemodectomas while one patient had a malignant glomus jugulare tumour which produced osteolytic metastases in several ribs. The two sea-level patients both had carotid body tumours one of which was malignant with spinal metastases. The other sea-level patient and one of the high-altitude subjects died of widely metastasizing thyroid carcinoma. An association between thyroid carcinoma and chemodectoma has been noted before (Albores-Saavedra and Duran, 1968). The chemodectomas in the sea-level and high-altitude patients usually presented as a slowly

growing mass below the angle of mandible. The average dimensions of the high-altitude carotid body tumours were 3.9 by 2.8 by 2.6 cm. Histologically they consisted of clear and dark chief cells, the latter predominating, arranged in characteristic nests or *zellballen*. Focal obliterative arterial lesions were present within the tumour. These consisted of pronounced intimal fibrosis associated with medial destruction. Some of the occluded vessels and their accompanying collateral channels formed vascular complexes reminiscent of the dilatation lesions seen in the lungs in advanced hypertensive pulmonary vascular disease. The high-altitude carotid body chemodectomas appeared benign, since 16 cases were followed up for periods ranging from one to 14 years with no evidence of recurrence or metastasis. Three interesting features were noticed in this group of patients. First, the carotid body tumours in Peruvians living at high altitude were six times more frequent in females than in males. This observation is in keeping with the 13:1 predominance of females over males in the series of 40 carotid body tumours reported from Mexico City (altitude 2240 metres). This is in marked contrast to the situation at sea level, where carotid body tumours are evenly distributed between the sexes. The second interesting feature was that left-sided carotid body tumours were three times commoner than right-sided lesions. One patient had bilateral carotid body chemodectomas and Saldaña *et al* (1973) also refer to a 53-year-old woman from Bolivia with bilateral carotid body tumours who had lived at an altitude of 3700 metres until the age of 30 and afterwards at an altitude of 2200 metres. The third interesting fact about the Peruvian high-altitude chemodectoma patients was that half of them also had significant respiratory abnormalities such as emphysema, pulmonary fibrosis and pneumoconiosis. Saldaña *et al* considered that carotid body chemodectomas occurring in high-altitude natives represent an extreme degree of hyperplastic response of the chemoreceptor tissue to prolonged hypoxia. This concept that high-altitude chemodectomas represent hyperplasia rather than neoplasia is supported by their slow rate of growth and benign clinical course.

If chronic hypoxia were a factor in the development of carotid body tumours, it might be expected that such lesions would also occur in animals at high altitude and in patients with chronic hypoxia due to respiratory disease. This may indeed be the case. Arias-Stella and Valcarcel (1976) have recently studied the carotid bodies in cattle living at high altitude. They reported extreme chief cell hyperplasia and the presence of chemodectoma in 40 per cent of animals. Chedid and Jao (1974) described 11 tumours of the carotid body and one chemodecto-

ma of the ganglion nodosum in six members of two consecutive generations of a family. All but one of the affected members had bilateral carotid body tumours, and four of the six had associated chronic obstructive pulmonary disease with persistently low arterial pO_2 and high pCO_2 levels. The authors speculated that chemodectomas of the carotid bodies begin as hyperplasias initiated by variations in the arterial pO_2 and pCO_2 levels in genetically predisposed individuals unable to maintain normal values because of respiratory disease.

Laidler and Kay (1976) investigated this problem experimentally by studying the effects of chronic hypoxia combined with the neurotropic carcinogen N-ethyl-N-nitrosourea (ENU) on the carotid body of the rat. Since the carotid body is of neural crest embryonic derivation, we wondered if the administration of ENU would convert the hypoxic hyperplasia of the carotid body type I cells into neoplasia. One hundred and ten newborn Wistar albino rats of either sex were divided into four groups. Group 1 rats were control animals and were not exposed to chronic hypoxia or given ENU. Animals in groups 2 and 4 were given a single injection of ENU 24 hours after birth. After weaning at approximately 21 days of age, rats in group 4 were transferred to a hypobaric chamber at a pressure of 460 mm Hg (equivalent to an altitude of 4300 metres above sea level) for the remainder of their lives. Rats in group 3 were not given ENU, but after weaning were transferred to a hypobaric chamber under the same conditions as rats in group 4. No significant difference was found between the mean combined carotid body volumes of the two groups of rats (1 and 2) which were not exposed to chronic hypoxia, so that it is unlikely that treatment with ENU alone can induce carotid body enlargement. Exposure to chronic hypoxia (50 to 414 days) produced enlargement of the carotid bodies due to capillary dilatation and increase in volume of type I cells. None of the carotid bodies had the histological appearance of chemodectoma. Treatment with ENU did not increase the degree of carotid body enlargement produced by chronic hypoxia. In their study of high-altitude chemodectomas, Saldaña *et al* (1973) described 24 carotid body neoplasms. The smallest of these was increased 10-fold as compared with the mean carotid body weight of people born and living at high altitude. None of our chronically hypoxic rats showed such a degree of enlargement of their carotid bodies. Our inability to produce carotid body chemodectomas in rats exposed to the combined stimuli of chronic hypoxia and a neurotropic carcinogen supports the view that tumours arising in high-altitude natives are hyperplastic lesions rather than true neoplasms.

Carotid body in the sudden infant death syndrome

Prolonged apnoeic episodes during sleep have been described in some infants who became victims of the sudden infant death syndrome (SIDS) (Stevens, 1965; Steinschneider, 1972). Such apnoeic episodes may be associated with chronic alveolar hypoventilation, since, according to Naeye *et al* (1976), many SIDS victims have anatomical evidence of chronic alveolar hypoxia and hypoxaemia in the form of right ventricular hypertrophy, increased muscle in small pulmonary arteries, increased adrenal medullary chromaffin tissue, an abnormal proliferation of brain stem astroglial fibres and an abnormal retention of extramedullary erythropoiesis and brown fat. Naeye *et al* (1976) studied the carotid body in SIDS victims because a defect in the chemoreceptor function of the organ might have contributed to abnormalities in ventilatory control in such cases. Furthermore, the organ would be expected to be enlarged if a state of chronic alveolar hypoxia had existed before death. Seventeen male and 14 female infants (mean age 2.4 months) were classified as non-infected victims because death was sudden, unexpected and unexplained by any routine clinical or postmortem findings. Another 14 males and 11 females (mean age 3.4 months) were classified as SIDS with infection since they had tracheobronchitis, pneumonia, laryngitis or otitis media of too mild a degree to explain death. The control group consisted of 14 male and 12 female infants (mean age 3.8 months) who died as a result of accident, fire or homicide. The carotid bodies were serially sectioned and examined by a point counting method so as to determine the volume of the organ and the respective volume proportions of glomic cells, neural tissue, blood vessels and other elements. When the volume of glomic tissue was expressed as a ratio of the body weight, 63 per cent of SIDS victims had a subnormal volume and 23 per cent an enlarged volume of glomic tissue in their carotid bodies. These abnormalities were not due to a failure of nerve fibres to grow into the organ. Quantitative evidence of antecedent chronic alveolar hypoxia in the form of increased right ventricular weight, increased pulmonary arterial muscle and increased brown fat and hepatic erythropoiesis was found in both groups but more severe in the victims with enlarged glomic tissue volumes. A subnormal volume of carotid glomic tissue was less common in infected than in non-infected SIDS victims, and the infected infants had less evidence of chronic alveolar hypoxia. It is not easy to interpret the carotid body abnormalities in the sudden infant death syndrome. The finding of both increased and decreased volumes of glomic tissue in various SIDS victims suggests that diverse

mechanisms are involved in their deaths and neither confirms nor excludes the possibility that abnormalities in respiratory control play a significant role.

Carotid body and erythropoiesis

The first reports of an effect of the carotid body on erythropoiesis were published by Latner (1937; 1938) who showed that after denervation and probable destruction of both carotid and aortic bodies rabbits developed an initial macrocytic anaemia which was followed by a reticulocytosis and a return to normal after three weeks. During the initial anaemic phase, which was not considered to be due to haemorrhage during operation, the peripheral blood film showed normoblasts and megaloblasts. He postulated that lack of oxygen activates a reflex mechanism involving the carotid body and brain which influences the bone marrow. However, different results were obtained by Schafer (1945) in experiments on dogs in which the same type of denervation was done. He found that a lasting increase in haematocrit and red cell mass developed in several animals some months after operation. Recently, Tramezzani *et al* (1971) have suggested that in the cat the carotid bodies are endocrine organs concerned in the control of erythropoiesis. Their suggestion was based on the observation that in a group of six cats, removal of the carotid bodies led to a fall in the reticulocyte count and anaemia. When a carotid body extract was injected into glomectomised cats and normal cats there was a reticulocytosis. When glomectomised cats were subjected to daily bleeding by phlebotomy, they failed to increase their reticulocyte counts. Daily bleeding was said to increase the weight of the carotid body, and efferent venous blood collected from the carotid body produced an increase in ^{59}Fe incorporation in newly formed red cells when assayed in polycythaemic rats. In these experiments, the carotid body was postulated to be an endocrine gland secreting one or more erythropoietic hormones. However, these findings were not confirmed by Gillis and Mitchell (1972) who serially measured the haematocrit, reticulocyte count, plasma iron removal, red cell iron incorporation and blood volume in five glomectomised cats, one sham-operated control and two unoperated control animals. The values in glomectomised cats did not differ significantly from those in the controls and it was concluded that the carotid body does not directly regulate erythropoiesis. The authors suggested that the anaemia occurring in the cats described by Tramezzani *et al* was attributable to secondary infection resulting from an indwelling femoral vein catheter.

There is evidence that the carotid body does *not* directly regulate erythropoiesis in man. Lugliani *et al* (1971) found no evidence of anaemia in 57 patients who had undergone bilateral carotid body resection as a treatment for asthma and chronic obstructive lung disease. Furthermore, five of these patients showed a normal reticulocyte response after the removal of 1 litre of blood. An interesting observation in view of the work cited above (Schafer, 1945) and below (Grant, 1951; Paulo *et al*, 1973) was that six of the patients developed secondary polycythaemia requiring phlebotomy after removal of their carotid bodies. Winson and Heath (1973) weighed the carotid bodies at necropsy in 50 patients, and in each case related the combined carotid body weight to a representative haemoglobin level measured during life. The carotid bodies were found not to be enlarged in anaemia, and were thus unlikely to be endocrine organs concerned with the control of erythropoiesis.

The role of the carotid body in the response to an erythropoietic stimulus has been clarified by comparing the responses to hypoxia of glomectomised rabbits and sham-operated rabbits. Grant (1951) showed that when glomectomised rabbits were exposed to chronic hypoxia in a hypobaric chamber (400 mm Hg) for six hours a day for several weeks their haematocrits, red cell counts and reticulocyte counts were greatly increased while control animals exposed to similar hypoxic conditions showed no change. The systemic arterial pO_2 levels in rabbits without carotid bodies were much lower than those in normal rabbits, while the pCO_2 was higher. Grant concluded that the intense polycythaemic response of rabbits without carotid bodies exposed to chronic hypoxia was attributable to pronounced depression of the systemic arterial pO_2 level. Paulo *et al* (1973) found that the plasma erythropoietin levels in rabbits exposed to hypoxia (0.42 atmospheres for 18 hours) 48 hours after removal of the carotid bodies were significantly higher than those in sham-operated controls exposed to the same hypoxic stimulus. Plasma erythropoietin levels were also higher in glomectomised rabbits when they were exposed to hypoxia for an 18-hour period two weeks after removal of the carotid bodies. During the two-week period following ablation of the carotid bodies, there was an increase in the haematocrit and reticulocyte count accompanied by a decrease in the systemic arterial pO_2 levels.

From the work reviewed it would seem that there is no convincing evidence that the carotid body is an endocrine organ producing erythropoietin. However, the carotid body does influence erythropoietic activity through an indirect mechanism. It would appear that the response of the carotid body to changes in

pO_2 level in systemic arterial blood modifies the erythropoietic response of the animal. Intact animals subjected to hypoxia respond with an increase in rate and depth of respiration. Animals deprived of their carotid bodies show a pronounced reduction in this ventilatory response to hypoxia (Lugliani *et al*, 1971) leading to excessive systemic arterial hypoxaemia which may possibly necessitate an increased rate of erythropoietin production to compensate for the decreased delivery of oxygen to the tissues.

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