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## RESPIRATORY PROPERTIES OF BLOOD AND RESPONSES TO DIVING OF THE PLATYPUS, *ORNITHORHYNCHUS ANATINUS* (SHAW)\*

**Abstract-1.** The O<sub>2</sub>-Hb dissociation curve for platypus blood shows a typical sigmoid shape with a pronounced Bohr effect.

2. The O<sub>2</sub> capacity for one specimen was 23.5 vol % with a corresponding hematocrit of 50 per cent.

3. The blood displayed a considerable Haldane effect and a large difference in buffer capacity between reduced and oxygenated blood.

4. Arterial blood samples taken during experimental submersion were analyzed for O<sub>2</sub> and CO<sub>2</sub> pressure and content. CaO<sub>2</sub> falls rapidly during diving and reaches a low of 2 vol % after approximately 3 min, which was close to the endurance limit during experimental conditions. A quick restoration of arterial O<sub>2</sub> saturation characterized the recovery.

5. Platypus showed a distinct bradycardia upon submersion. The rate of decline in heart rate was more pronounced if the arterial blood pressure - increased in response to submersion.

6. The findings are discussed in relation to the normal diving habits of the platypus.

### INTRODUCTION

THE platypus, *Ornithorhynchus anatinus* (Shaw), occupies a unique position among the mammals. As a member of the order Monotremata it represents the most primitive mammal known. A reptilian ancestry is suggested by the presence of a cloaca, an episternum, the structure of the orbit and certain skull features. A labile body temperature crudely regulated around 30°C is a functional feature often taken to imply an intermediate position between reptiles and higher mammals (Semon, 1894). The platypus is a habitual diver. It is adapted to semi-aquatic life by having, broadly webbed feet, a beaver-like flattened tail and no pinna of the ear. Its remarkable bill serves as an excellent tool for procuring food from the bottom of rivers and lakes.

The distinctive phylogenetic ranking coupled with a truly amphibious nature of the platypus stimulated the authors to study some respiratory properties of the blood and cardio-respiratory responses to experimental diving.

### MATERIALS AND METHODS

The platypus is indigenous to Eastern Australia from Cape York to Victoria on both sides of the dividing range as well as in rivers and lakes of Tasmania (Le Souef & Burrell, 1926): The animal has been rigidly protected since 1905 when it faced extinction.

The two male specimens of platypus forming the basis for the present work were collected in a river near Tambour in Queensland, Australia.

For experimentation each animal was lightly tied to a board in supine position. Local infiltration with 2% xylocaine anesthetized the area about the right femoral artery which was exposed and cannulated with a polyethylene catheter (PE 20). After the incision was closed the animal was untied, turned to the prone position and lightly tied to a small board. In this way the platypus remained floating at the surface when placed in a large basin of water. Complete submersion was effected by gently forcing the board deeper into the water: The animals often reacted to submersion by struggling, but in some cases they remained quiescent throughout the submersion period. Forced dives lasting from 25 or 30 sec up to 3 min were repeated on each animal, and blood samples were secured anaerobically before, during and after each successive dive by sampling through the indwelling catheter. The arterial blood pressure was monitored continuously except when blood samples were being taken, using a Statham pressure transducer and a

Beckman-Offner system of amplifiers and recorder. A thermometer inserted into the cloaca of the animals was used to determine the body temperature before and after each experimental series.

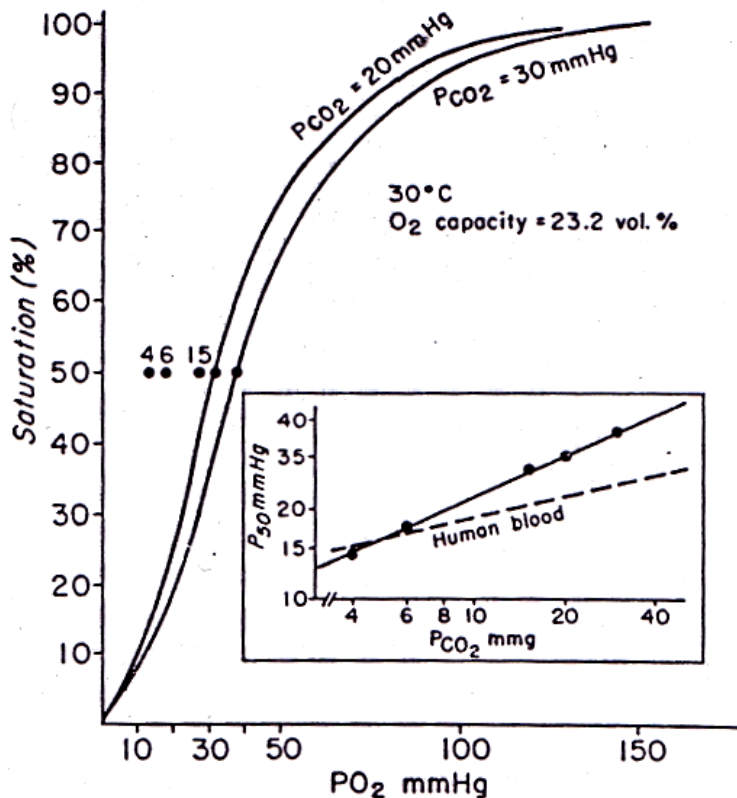
The arterial blood samples were analyzed for oxygen and carbon dioxide tension ( $p_aO_2$  and  $p_aCO_2$ ) and pH using the Beckman gas analyzer, model 160. The  $O_2$  and  $CO_2$  contents of arterial blood ( $C_aO_2$  and  $C_aCO_2$ ) were analyzed by gas chromatography. In vitro studies on respiratory properties of blood were made in a mixture of the blood from the two animals.

A detailed description of the technique used for determination of oxyhemoglobin and  $CO_2$  dissociation curves has been published earlier (Lenfant & Johansen, 1965). All measurements were made at  $30^\circ C$ .

## RESULTS

### *Oxy-hemoglobin dissociation curves*

Figure 1 depicts two  $O_2$  dissociation curves at  $pCO_2$  values of 20 and 30 mm Hg. Additional values of  $P_{50}$  at 4, 6 and 15 mm Hg  $pCO_2$  are also shown. The dissociation curves display a typical sigmoid shape closely resembling that typical for man and other mammals. The  $O_2$  capacity measured for one of the specimens was 23.5 vol% with a corresponding hematocrit of 50 per cent. Figure 1 reveals the presence of a Bohr effect far more pronounced than usual for mammals. The insert compares the Bohr effect in platypus and human blood.



**Fig. 1.**  $O_2$ -Hb dissociation curve of platypus blood. The insert demonstrates the magnitude of the Bohr effect compared to human blood.

### *$CO_2$ dissociation curves and buffering capacity*

In Fig. 2 the  $CO_2$  dissociation curves for reduced and fully oxygenated blood are shown. The curves conform in shape with typical mammalian curves. The absolute values are comparable with those for higher mammals. The effect of oxygenation on the ability of the blood to carry  $CO_2$  was, however, prominent and a considerable reduction in  $CO_2$ -carrying power of oxygenated blood was apparent (Haldane effect). The difference between oxygenated and reduced blood in  $CO_2$ -carrying ability naturally extends to the buffer capacity as expressed in Fig. 3.

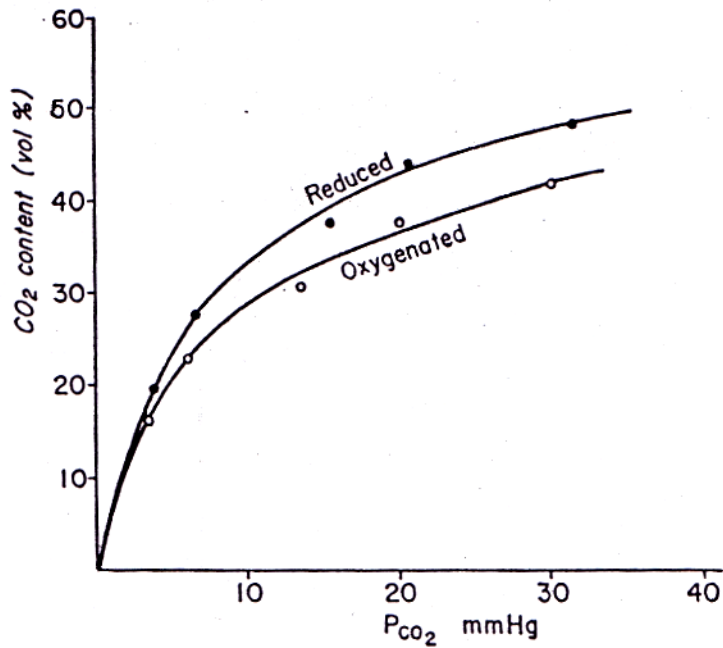


Fig. 2. CO<sub>2</sub> dissociation curves of oxygenated and reduced whole blood.

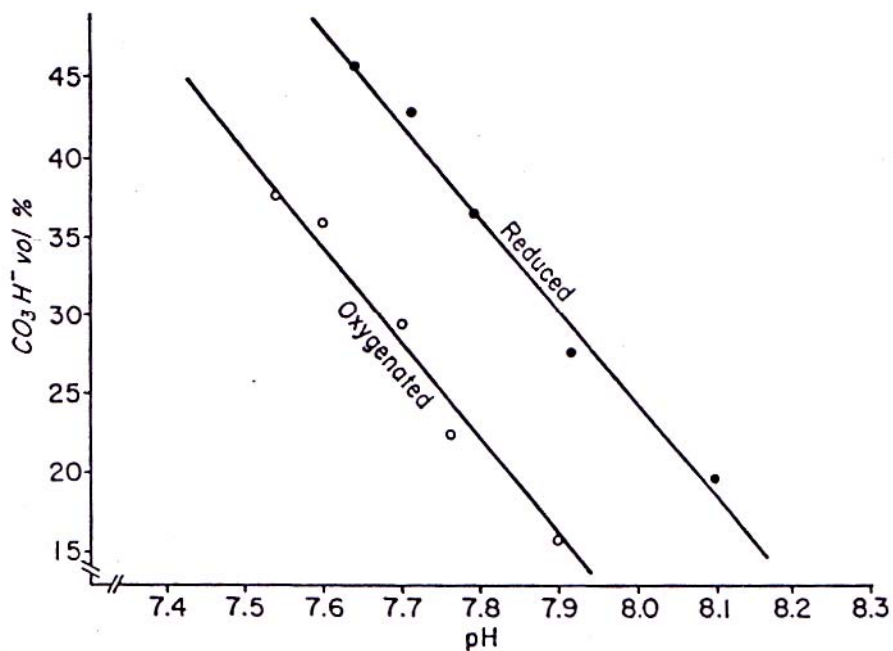


Fig. 3. Bicarbonate-pH diagram showing buffering capacity of the oxygenated and reduced whole blood.

#### Blood gas analysis

In Fig. 4 the  $C_aO_2$  and  $C_aCO_2$  are shown before and during diving and in the subsequent recovery period. The resting levels of  $C_aO_2$ , and  $C_aCO_2$  were 20 and 40 vol % respectively. The depletion of  $O_2$  from arterial blood during submersion was rapid and  $C_aO_2$  approached 2 vol % at the end of a 3 min dive. The recovery phase was characterized by a very quick restoration of full arterial  $O_2$  saturation, whereas a slightly longer time was needed to bring the  $C_aCO_2$  back to pre-dive level. Figure 5 illustrates the course of  $p_aO_2$ ,  $p_aCO_2$  and pH before, during and after successive dives. The filled circles indicate consecutive dives in one specimen and the open circles one dive in the other animal. The broken lines are drawn between the extreme values. Note the extremely low values of  $p_aO_2$  at the end of a dive. The arterial pH values suggest a slower recovery from the acidosis incurred during submersion in the later dives of a series. It is noteworthy that the lowest pH values are reached within the actual submersion and not during the recovery phase. The body temperature ranged between 29° and 31° during the experiments.

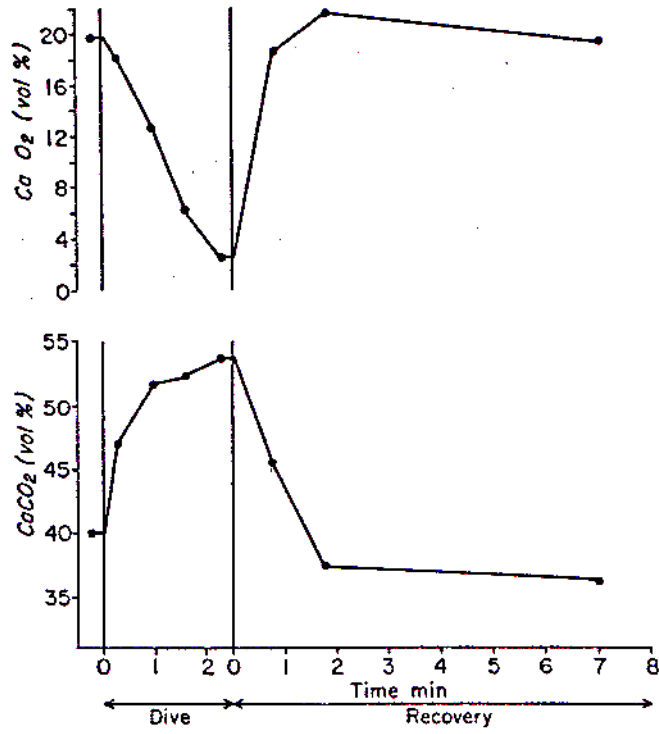


Fig. 4. Changes in arterial O<sub>2</sub> and CO<sub>2</sub> content during, before and after submersion.

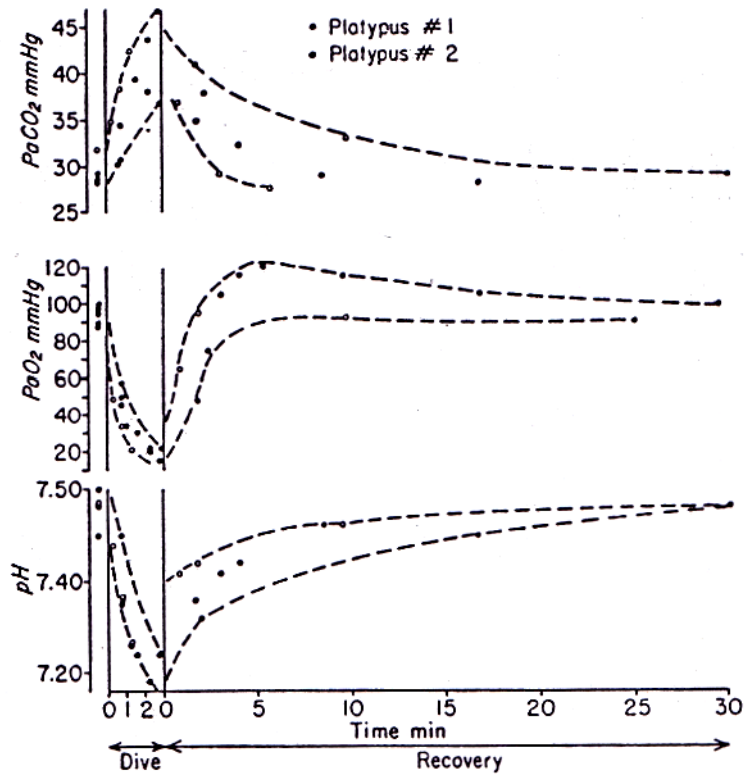


Fig. 5. Changes in arterial pO<sub>2</sub>, pCO<sub>2</sub> and pH during, before and after submersion.

### Cardiovascular responses to submersion

The bradycardia commonly observed in diving animals upon submersion was also clearly evident in the platypus. The onset of the bradycardia was variable and did not as a rule coincide with commencement of submersion. The slowing of the heart seemed to develop gradually and the heart rate reached a minimum value approximately 30-35 sec after submersion. Figure 6 shows the change in heart rate during submersion for seven different dives all lasting approximately 34-40 sec. The rate is seen to change from an average of 140 beats/min before submersion to about 20 beats/min at its lowest. The rate of decline in heart rate seemed to be influenced by a number of factors such as struggling and the release of gas from the lungs.

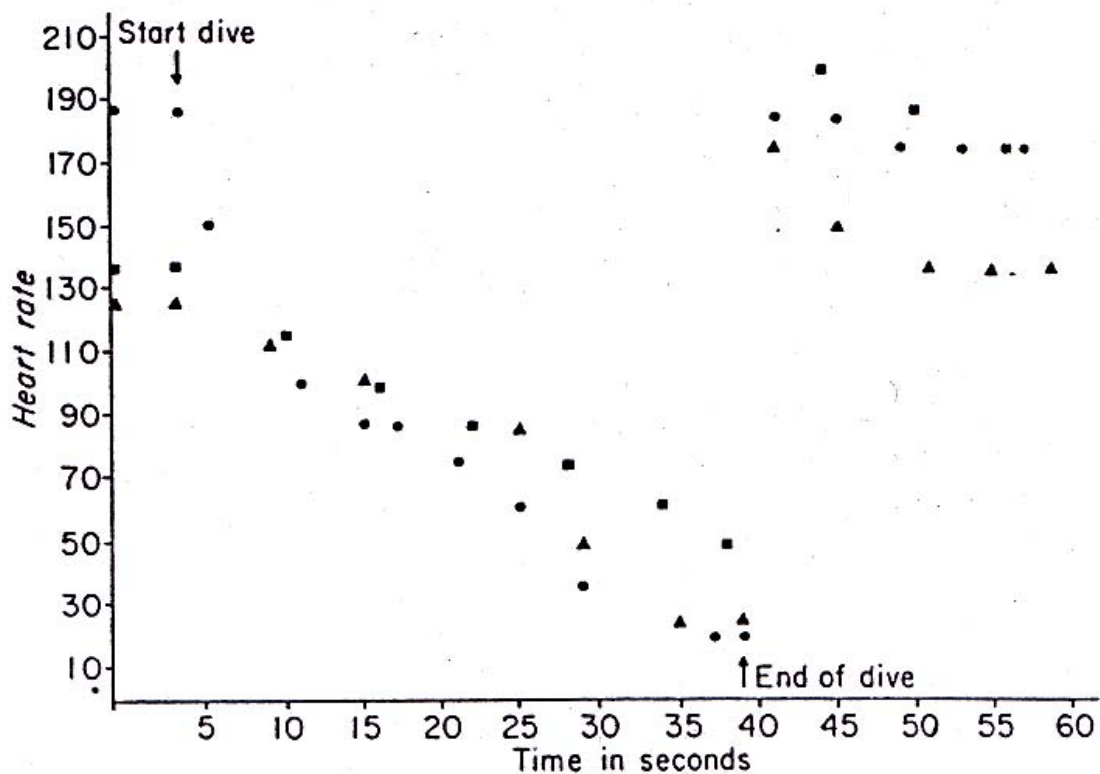
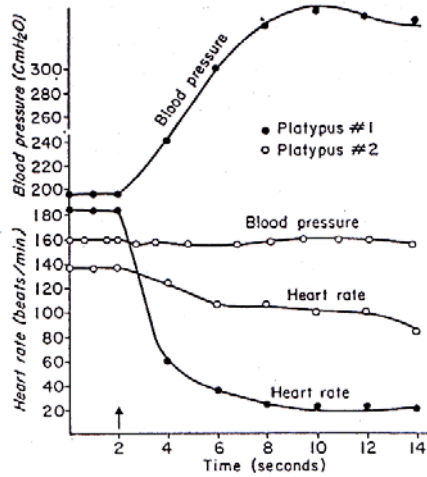


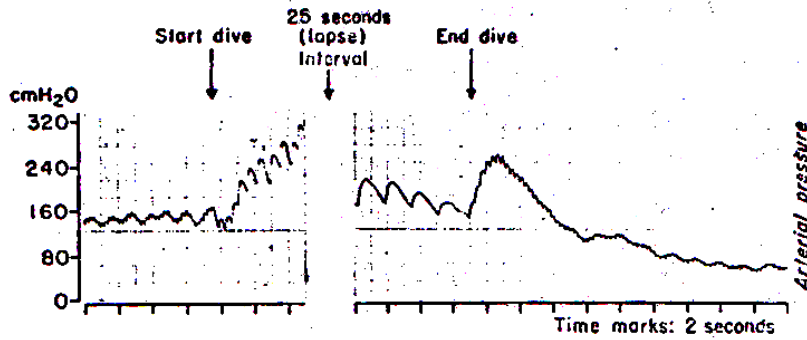
Fig. 6. Heart rate before, during and after submersion. Composite plot for seven different dives on two specimens.

The arterial blood pressure never decreased during submersion. It either followed the pre-dive level or showed various degrees of increase. In the latter instance the pressure could in some cases rise sharply and more than double its value within the first few seconds of submersion. Such an abrupt rise was usually followed by a gradual decline to the pre-dive level. Upon comparing the initial responses in heart rate and arterial pressure it became distinctly evident that in those cases where the pressure rose sharply upon submersion, there was a much more rapid decline in heart rate than if the pressure stayed unchanged or rose insignificantly. Figure 7 depicts two selected extremes of this relationship. The development of bradycardia is seen to bear an obvious relationship to the initial response in arterial pressure.

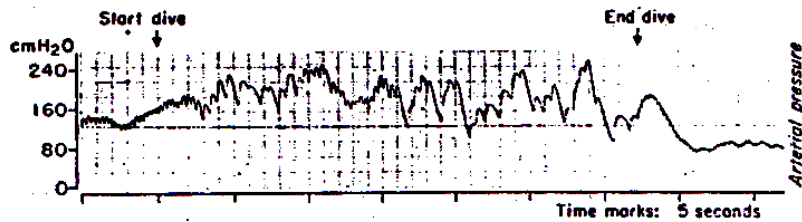


**Fig. 7.** Relationship of initial blood pressure response and the concurrent change in heart rate during diving.

At the end of a dive the response consisted uniformly of a sudden change in heart rate with a transient period of tachycardia showing rates up to 200 beats/min. The blood pressure similarly always rose transiently during the tachycardia and declined subsequently to levels far below the pre-dive values. Figures 8(a) and (b) show two representative types of responses to submersion. Figure 8(a) illustrates a case in which there is a rapid early pressure rise with a concomitant pronounced fall in heart rate. The terminal phase of the submersion and the recovery follow the general trend described above. Figure 8(b) depicts a 30 sec dive with a more gradual rise in blood pressure accompanied by a slowly developing bradycardia.



**FIG. 8(a).**



**FIG. 8(b).**

**Fig. 8.** Arterial blood pressure during experimental submersion. (a) Shows slight increase in blood pressure and a moderate fall in heart rate. (b) Shows a steep increase in blood pressure accompanied by a markedly reduced heart rate.

## DISCUSSION

The intermediate phylogenetic position of the platypus with morphological characters strongly resembling reptilian conditions and others of selective mammalian type furthers inquiry about whether physiological features might also attest to such an intermediate position.

The present discussion is presented with awareness of the limitations posed by having had only two specimens available for experimentation. Few generalizations are made and emphasis is put more on trends than actual numerical values.

### *Respiratory adaptations to diving*

The blood from both specimens gave an immediate impression of being hemoconcentrated. A hematocrit of 50 per cent with corresponding O<sub>2</sub> capacity of 23-5 vol % is higher than for most mammals and does not point to any reminiscence of a transitional stage between a therapsid reptilian ancestor and an early warm-blooded mammalian type. Reptiles show O<sub>2</sub> capacities commonly ranging between 5-10 vol % (Prosser, 1962) and lower ranking mammals among the marsupials have been listed with O<sub>2</sub> capacities in the neighborhood of 15 vol % (Burke, 1953). Among the true placental mammals there is no trend demonstrated in the relationship of O<sub>2</sub> capacity of the blood to phylogenetic ranking. Most likely all mammals that enjoy the freedom of warm-bloodedness and the accompanying high metabolism, need a fairly high O<sub>2</sub> capacity to support such operational ability. Meanwhile, there seems to be a definite correlation between the progressive phylogenetic differentiation of the erythrocytes and their concentration of hemoglobin. It is established in this respect that the mature erythrocytes are nonnucleated in all mammalian orders including the monotremes (Briggs, 1936). The high blood O<sub>2</sub> capacity in platypus should hence be looked upon as an attendant to the amphibious habits of the animal, which benefits from an increased oxygen storage capacity in the circulating blood during the intermittent suspension of breathing. Such adaptation has been amply manifested among other naturally diving mammals like seals and whales (Sudzuki, 1924; Florin & Redfield, 1931; Green & Redfield, 1933; Irving et al., 1935; Irving, 1939).

Let us next assess to what extent the O<sub>2</sub>-Hb and CO<sub>2</sub> dissociation curves for platypus blood show traces of phylogenetical conformity or secondary adaptive features to meet the diving habits of the animal. The delineation of the O<sub>2</sub>-Hb dissociation curve reveals the sigmoid shape characteristic of both reptiles and higher mammals. The Bohr effect is more pronounced in platypus than in seals, the only other diving mammal whose Bohr effect has been measured (Irving et al., 1935; Scholander, 1940). Pronounced Bohr effect constitutes a significant adaptation to the diving habits of the platypus, since it allows a complete utilization of the blood O<sub>2</sub> content while keeping the blood to tissue gradient in O<sub>2</sub> tension relatively high. If we extend the comparison to reptiles and in particular to aquatic forms like crocodiles and alligators, we find a prominent effect of CO<sub>2</sub> or acids in reducing the affinity of hemoglobin for O<sub>2</sub> (Dill & Edwards, 1931). The obvious adaptive nature of this feature seems to be retained in the platypus.. The usefulness of CO<sub>2</sub> accumulation during suspended breathing for better utilization of the available O<sub>2</sub> may perhaps be regarded as a primary adaptation in phylogenetical perspective.

When comparing the effect of reduction of hemoglobin on the increase in CO<sub>2</sub> carrying ability, the platypus blood seems again to be intermediate between reptiles and higher diving mammals so far studied. The crocodiles hold in this respect a phenomenal record among the vertebrates by having a very conspicuous Haldane effect (Dill & Edwards, 1931).

The difference in acidity between reduced and oxygenated hemoglobin is shown in the bicarbonate-pH diagram for platypus blood (Fig. 3). Aside from this difference there is a notably low buffering capacity of platypus blood compared with terrestrial mammals like dog and man. A poor buffering capacity is also a characteristic of crocodilian blood (Dill & Edwards, 1931) and, quite unexpectedly, also of seal blood (Irving et al., 1933). This is surprising on the background of the diving habits of these animals. An intermittent suspension of breathing associated with diving and the concurrent accumulation of acid metabolites would presumably call for potent buffering mechanisms. The apparent absence of these may be put in interesting relevance to the presence of an unusual tolerance and irresponsiveness to increased hydrogen ion concentration in the diving mammals (Irving, 1938a). The commonly observed pH changes in diving mammals consist in a gradual decline throughout the dive with a precipitous but transient drop in the first part of the recovery period (Scholander et al., 1942). Even the so-called air diving in fish (Leivestad et al., 1957) showed a transient drop in pH during the recovery after return to water. The mechanisms underlying the delayed release of lactic acid to the recovery period is attributed to a striking redistribution of the circulating blood during submersion resulting in a virtual close down of circulation through the bulk of the skeletal muscle mass (see later discussion). The present data on platypus do not fall into line with this general picture. The minimum level of pH was reached as the dive terminated with no further drop in the early recovery.

One may assume that the accumulation of lactic acid has been relatively modest during the short dive or entirely masked by mobilization of reserve buffer mechanisms.

The rate of decline in arterial O<sub>2</sub> content is a reliable indication of the depletion of the oxygen resources during suspended breathing. The platypus showed an O<sub>2</sub> depletion from the arterial blood much more rapid than for other diving animals studied under similar conditions. Scholander (1910) has suggested a proportionality between diving ability and O<sub>2</sub> capacity based on his work on seals. Obviously differences in baseline metabolism must influence such a relationship. Animals may also differ in their ability to economize with the O<sub>2</sub> resources during diving by temporarily defraying energy costs on anaerobiosis or by a general depression of their total energy metabolism (Scholander, 1940; Andersen, 1959).

Domestic ducks with O<sub>2</sub> capacities of about 15-18 vol % and with weights comparable to the platypus seem to reach an arterial O<sub>2</sub> saturation of approximately 10 per cent in 5-6 min (Scholander, 1940) Andersen & Hustvedt (1965), also working on ducks, predicted that the arterial O<sub>2</sub> saturation would decrease to 10 per cent during a 14 min dive. Our data demonstrate a nearly constant rate of decline in O<sub>2</sub> content throughout the dive. On the other hand, the rate of increase in arterial CO<sub>2</sub> content diminishes in the latter part of the submersion. This finding is in agreement with Scholander's observations on seals (1910).

The literature offers conflicting reports on the ability of the platypus to endure submersion. Irving (1964) has listed 10 min. Burrell's (1927) monograph on the platypus describes an incidence of 2 animals diving voluntarily for 6 min. when disturbed. The recent compendium *Mammals of the World* (Walker, 1964) grants the platypus an ability to stay submerged for 5 min. However most observers list 30-40 sec as the normal diving time for undisturbed animals in their normal environment (Allport, 1878; Semon, 1894). The present results on the rate of O<sub>2</sub> depletion from arterial blood during experimental submersion give reason to question the long diving times estimated by earlier authors. A diving time around 3 min was close to the endurance limit under experimental conditions. Meanwhile, it seems important to point out that the usefulness of habitual diving may be very inadequately measured by the endurance time to individual submersions. To the platypus diving is closely associated with his feeding habits and occasionally with temporary refuge. The platypus feeds in shallow water by diving for 30-40 sec, then returning to the surface to masticate the food where after the cycle is repeated (Burrell, 1927). The platypus spends 60-90 min each morning and evening foraging for food. During these periods half the time is spent under water. Such a pattern of behavior seems best supported physiologically by rapid recovery between short dives in close succession, whereas ability to sustain prolonged submersion is of no common consequence. The limited data presently obtained are suggestive that this is exactly the manner in which platypus is physiologically adapted for diving.

#### *Circulatory responses to submersion*

It has long been alleged that the marked cardiovascular adjustments observed during submersion of naturally diving animals are dominating factors in the exceptional endurance to asphyxia in these animals. A conspicuous reflexly onset bradycardia and a redistribution of the circulating blood, reducing flow through muscle, skin and splanchnic areas with a maintained or augmented flow to the central nervous system and myocardium, are the most essential features of the circulatory adjustments (Irving, 1938b; Johansen, 1964). The platypus proved no exception in this case. The bradycardia was gradual in development, but with a sudden release of the cardiac inhibition when the dive was terminated.

The present study was not designed to elucidate the reflex chain leading to the bradycardia. It was, however, consistently observed that in those cases where a marked and promptly onset arterial blood pressure increase accompanied the submersion, a much more rapid slowing of the heart rate occurred (Figs. 7 and 8). Development of bradycardia caused by baroreceptor stimulation from the profound vasoconstriction may be a potentiating factor in the slowing of the heart.

Downing et al. (1962), experimenting on dogs, reported that hypoxic stimulation of the carotid bodies, when respiration is controlled artificially, produces an increased sympathetic discharge to peripheral vascular beds, together with a general cardiac sympathetic withdrawal and a vagal slowing of the heart. Such a dichotomous response of the sympathetic nervous system is a characteristic feature in the diving animal. The above authors also postulated that the primary cardiac response to systemic hypoxia in mammals in general is normally overridden by reflex effects of the increased respiratory activity, which incidentally is also stimulated by the hypoxia. It seems warranted to suggest that a basic adaptation in the diving animals may consist in an ability to suppress the stimulatory effect of hypoxia and hypercapnia on respiration.

In diving animals the arterial blood pressure commonly stays unchanged or increases slightly during submersion in spite of the notable bradycardia. Since recent studies on sea lions (Elsner et al., 1964) have disclosed a marked reduction in cardiac output during submersion, the maintained blood pressure



indicates that large portions of the vascular bed have been reduced or shut down in flow. The platypus shows a phenomenal vasoconstriction upon submersion, in some instances resulting in a doubling of the arterial pressure (Fig. 7). This response is no doubt the result of a massive sympathetic discharge to peripheral vascular beds. In the recovery this sympathetic discharge is suddenly released, but the simultaneous onset of a sympathetic drive to the heart results in a tachycardia which gives a transient increase in pressure. A long-lasting hyperemia will likely attend the hypotension that ensues later in recovery.

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