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7. Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; NAD^+ and NADH, oxidized and reduced nicotinamide adenine dinucleotide; F6P, fructose 6-phosphate; FDP, fructose 1,6-diphosphate; DAP, dihydroxyacetone phosphate; GAP, glyceraldehyde 3-phosphate; DPG, 1,3-diphosphoglycerate; 3PG, 3-phosphoglycerate; 2PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate; PYR, pyruvate; PFK, phosphofructokinase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; PK, pyruvate kinase; and P_i, orthophosphate.
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21. Supported in part by the Department of Energy and the Air Force Office of Scientific Research. A fellowship from the Deutsche Forschungsgemeinschaft to P.H.R. is gratefully acknowledged.

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14 May 1980; revised 6 October 1980

Free and Forced Diving in Birds

Abstract. *Heart rates were measured during free and forced diving on each of two species of aquatic birds: the double-crested cormorant* (Phalacrocorax auritus), *a true diver, and the Canada goose* (Branta canadensis), *a bottom feeder in shallow water. When they immersed voluntarily they showed no bradycardia, but when the same birds were forcibly held under water there was a rapid drop in heart rate to well below that at rest. This decrease indicates that there may be a large component of emotional stress in the heart rate records from previous diving studies where restrained animals were forcibly submerged.*

Experimentally submerged diving birds and mammals have usually shown an abrupt decrease in heart rate (*1*). The reduced circulation indicated by this bradycardia is generally interpreted as an oxygen saving response that allows the animal to extend its diving time. Recent observations, however, on unrestrained animals have shown either a variable degree of this bradycardia or none at all (*2–4*), and intense bradycardia unrelated to diving has been seen in a variety of mammals and birds (*5,6*). We have sought to clarify this relation by looking at the heart rate response to both free and forced diving in the same animal. We used two each of two species of trained and hand-reared seabirds, the double-crested cormorant (*Phalacrocorax auritus*) and the Canada goose (*Branta canadensis*).

The cormorants were taken as 2-week-old nestlings. The geese were hatched from the eggs of wild birds. They were hand-fed in a pen adjacent to our house and were generally treated as pets. After they reached 3 to 4 weeks of age they accompanied us on walks and swims, and even sought our company by invading the house through open doors and windows. This lack of fear they showed toward us allowed us to place instruments on them and work with them at Great Harbor in Woods Hole.

Both species learned to fly at 7 to 9 weeks of age. The cormorants also began diving spontaneously at the same time, and within a day or two were seen to catch fish and eels. But they were always receptive when we offered a fish, making it easy for us to catch them for heart rate observations during forced submersion.

We used frequency modulation radiotelemetry of the electrocardiogram (EKG) from the geese, and also from the cormorants when they were not underwater. The transmitters were thumb-sized cylinders held on the back of the bird by a wire harness and straps. The transmitter's weight was never more than 5 percent of that of the bird. The EKG leads were inserted under the breastbone close to the heart. The wire was sutured at the point of entry and also glued to the feathers.

Since radio signals do not penetrate seawater we used acoustic telemetry from the cormorants while they were on and under the surface. The geese only put their heads and necks under, so that the backpack radio remained in the air. The range of both types of transmitters was a few hundred meters (*7, 8*). The received EKG signals were heard as pitch changes of an audio tone, which was magnetically recorded for later demodulation to the heart rate curves shown (Fig. 1).

The forced dives were not prolonged, and there is little doubt that the birds would have survived for at least a minute. However, the times we used are sufficient to demonstrate the biological unnaturalness of forcing a bird underwater to study the biological significance of the heart rate response to diving.

We were able to observe the heart rates of these tame birds during, for example, feeding, running, flying, swimming, and diving. Both species easily adapted to living in the vicinity of our house and wore transmitters with no observable change in behavior after a few hours. Although both species struggled somewhat during the forced submersion, they returned quickly to voluntary diving when released. This allowed us to make the following heart rate observations.

1) Both cormorants and geese have a steady heart rate of 100 to 120 beats per minute when they are inactive. This basal rate is maintained for many hours at night when they perch quietly in the dark.

2) Increases in heart rate to twice or more of the basal rate are common when there is a disturbance. Such periods of tachycardia suggest that both species respond emotionally to people, sounds, lights, and other birds.

3) Free-ranging geese had heart rates of 140 to 160 beats per minute while swimming slowly. In moderately active cormorants that were swimming slowly, or standing on the dock while drying by gently flapping their wings, the rate was 170 to 230 beats per minute. These rates, significantly above basal, are the normal heart rates to which voluntary diving or underwater feeding rates should be compared.

4) Both of the geese eagerly ate corn (maize) placed at the bottom in shallow water. They repeatedly submerged their heads for 8- to 14-second periods. These were separated by 3 to 5 seconds in air. The heart rate while the head was under the water was the normal active one of 150 beats per minute. As the head started to come up to the surface, the rate rose rapidly and stayed at 250 to 300 until they submerged again. This onset of tachycardia precedes the actual breath (or breaths).

5) In the middle of such a series of voluntary dives we seized a goose and forcibly held it under water. The heart rate dropped immediately to the base

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rate of 100 beats per minute as the goose struggled. Soon the struggling stopped, and the rate decreased further to 50 beats per minute after 20 seconds. Such a heart rate response is characteristic of forced diving experiments on birds.

6) Both of the cormorants showed much the same pattern of heart rate responses. They would voluntarily begin a series of 20 to 30 dives which were broken only if a fish were caught. The pulse rate under water was 180 to 220, roughly the same as when swimming on the surface. During the 5 to 10 seconds between dives there was a tachycardia of 50 percent (280 to 340). Forced dives on a cormorant in the middle of such a series also began with struggling. The heart rate decreased to about the basal rate of 100, as in the goose. When this struggling ceased, the rate also decreased further to 50 beats per minute.

7) Atropine (0.4 mg/kg), injected intramuscularly, completely abolished the bradycardia response to forced submersion in both cormorants and geese. Under the influence of this drug, heart rate remained high and constant throughout several forced submersions, even though the birds struggled more, and the birds did not dive or bottom feed voluntarily.

The major part of the evidence for diving bradycardia has come from observations on restrained animals forcibly submerged. Observations on unrestrained mammals (*5*) and birds (*4*, *6*), along with our records, project a different picture, one in which bradycardia is absent or less marked. Therefore, it is possible to say that all diving is not accompanied by bradycardia.

Many cases of bradycardia in nondiving situations, most of which involve an element of emotional stress to the animal, have been observed in fish (*7*), mammals (*5*), and birds (*6*). This also leads us to conclude that all bradycardia is not associated with diving.

There are, in addition, instances where heart rate reflects intended actions as well as conditioned responses. The first is seen in Kooyman's observation on the Weddell seal diving freely beneath the Antarctic ice (*3*). When the seal begins a dive, it shows a variable degree of bradycardia. The initial extent of the heart slowing indicates how long a dive the animal intends to make. For a long dive, the bradycardia occurs more quickly and is deeper, apparently facilitating the oxygen saving that is considered the reason for its slower heart rate. In our birds, as well as in ducks (*4*), there was a pronounced tachycardia between dives. This is associated with, but not

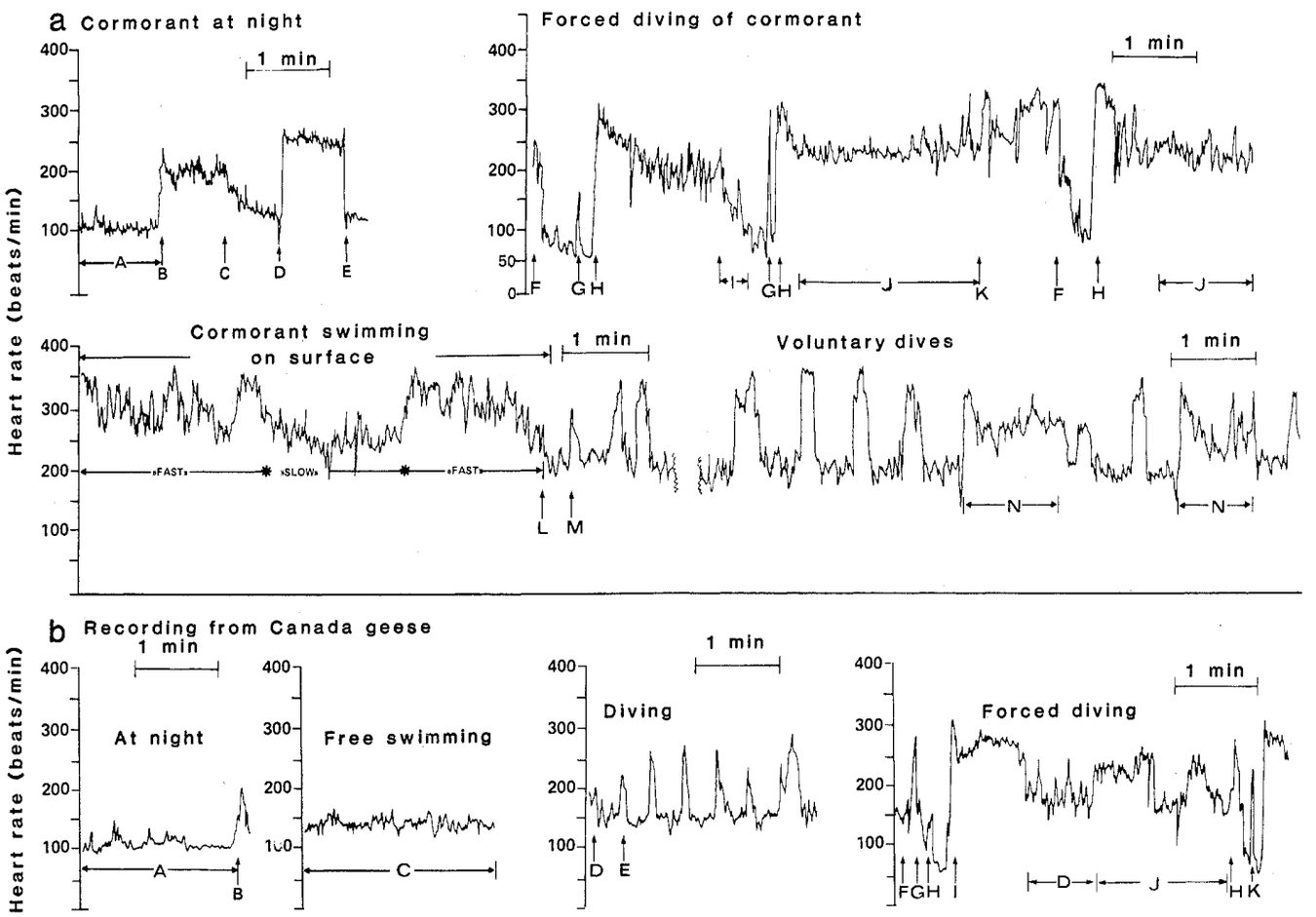

Fig. 1. Heart rate records of two cormorants and two geese. (a) Cormorants: *A*, basal heart rate at night with the bird sitting quietly in the dark; *B*, person entering pen; *C*, person leaving pen; *D*, light pointed at bird; *E*, light off; *F*, cormorant held at surface is forced under water; *G*, double heartbeat while being held under water; *H*, cormorant brought to surface and begins flapping wings; *I*, bird struggling during the first part of another forced submersion; *J*, swimming free on the surface; *K*, bird is caught again preparatory to being held under water again; *L*, after a period of fast and slow swimming free on the surface the cormorant begins a series of voluntary dives; *M*, bird comes to surface; *N*, bird is swimming on the surface eating an eel it has just caught. (b) Geese: *A*, basal heart rate at night; *B*, increase when person walks by the pen; *C*, swimming slowly on the surface; *D*, goose tilts head under water to feed; *E*, head comes to the surface for 3 to 5 seconds; *F*, heart rate before capturing goose; *G*, capturing goose; *H*, forced under water while struggling; *I*, brought to surface and released; *J*, goose is caught again and struggles intermittently before being held under water again; *K*, double heartbeat while under water.

the result of, breathing since it began before the head was above the surface. It would seem to be associated with a planned course of action, not an automatic reflex from the breathing itself.

An example of bradycardia due to a conditioned response is seen in the sea lion, which can be trained to slow its heart whether or not it is diving (*9*). With sufficient training this rewarded bradycardia will develop many times faster than that seen in actual diving. These observations give the impression that heart rate is affected by volitional as well as autonomic factors.

With a few exceptions (*2-4,9*), very few investigators have suggested that either emotion or learned behavior plays any part in accounting for the observed physiological details of diving; in fact, Jones and West (*10*) even suggest that the different heart rate response during free diving is a modification of that seen in forced submergence. We feel that this relation should be inverted. We suggest that from now on the word "diving" be reserved for the voluntary underwater activity of aquatic animals. "Forced submersion" is a more accurate title for studies on restrained animals. Our telemetry results indicate that stress-induced artifacts account for a large part of the "diving" bradycardia reported in laboratory studies.

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15 January 1980; revised 30 April 1980

Exposure of Newborn Rats to Pharmacologically Active Compounds May Permanently Alter Carcinogen Metabolism

Abstract. *Administration of phenobarbital to mother rats during early lactation causes long-term, perhaps permanent, alteration of hepatic microsomal mixed-function oxidase activity and aflatoxin B_1 adduct formation in the adult male offspring. These findings suggest that perinatal exposure to pharmacologically active compounds may be a determinant of cancer risk.*

Environmental factors modulate the microsomal mixed-function oxidase (MFO) enzyme system, which metabolizes xenobiotics, endogenous steroids, and fatty acids (*1*). Modulation of the activity of the MFO system by exposure to foreign compounds occurs primarily through enzyme induction (*1, 2*). This induction generally requires the presence of the inducer, results in an increase in enzyme protein, and is associated with increased synthesis or decreased degradation of the enzyme or both. Since most carcinogens are either metabolically activated or detoxified through the MFO system, the biological activity of these compounds can be altered by factors that modulate their metabolism (*3*). However, for enzyme induction to affect carcinogen metabolism and neoplastic sequelae, temporal coincidence is required between induced enzyme activities and the presence of the carcinogen substrate since enzyme induction is a transient mechanism.

If the basal enzyme activities were more permanently altered, their impact on carcinogen metabolism and tumorigenicity would be more significant. Such would be the case if the perinatal environment were to modify long-term enzyme development. This is supported by the observation that steroid metabolism in the adult male rat is imprinted or programmed by neonatal testicular androgen (*4*).

The present study was designed to determine whether neonatal exposure to foreign compounds confers an altered capacity for carcinogen metabolism later in life. It was surmised that such an alteration might be the result of a mechanism other than that responsible for the transient MFO induction.

Phenobarbital (PB) has a powerful and comprehensive effect on the classical MFO induction process. Therefore, a protocol was designed to examine the influence of PB administration during the neonatal period on (i) the metabolism of the hepatocarcinogen aflatoxin B_1 (AFB_1) in the adult rat and (ii) the adult MFO activities for *N*-demethylation of ethylmorphine. Phenobarbital decreases AFB_1 tumorigenicity (*5*) and macromolecular adduct formation in rats (*6*) but increases the *N*-demethylation of ethylmorphine (*1*).

Mature male and female Long-Evans hooded rats (200 to 225 g) (Blue Spruce Farms) were randomly paired and bred in our facility. Upon procurement, the animals were acclimated for 10 days in a room with controlled temperature and 12-hour photoperiodic cycle. Relative humidity was maintained between 40 and 60 percent. All the animals were given free access to purified AIN-76 diet (Dyets, Inc.) and tap water. During gestation and lactation, the dams were housed in polycarbonate cages containing hardwood shavings (American Excelsior Co.). At parturition they were randomly assigned to a treatment or control group, and the newborn pups were pooled, sexed, and randomly assigned to dams with the same genetic background. Litter size was maintained at ten pups (five males and five females) per dam.

The experimental dams received their first PB treatment within 12 hours after giving birth. They were gavaged with PB (40 mg per kilogram) for seven successive days. It was expected that the pups would receive PB and its metabolites through the milk (*7*). We did not measure the amount of PB or its metabolites which may have been transmitted through the milk to the pups, although we did observe a higher incidence of deaths in these pups than in the control pups. The control dams received an equal volume of 0.9 percent saline. Progeny were weaned at 21 days. Only the data for males are presented in this report.

The effect of PB exposure on AFB_1 disposition in the offspring was examined when they were 37 weeks of age. Before killing the rats, we gave them a secondary treatment of PB (80 mg/kg, intraperitoneally) or 0.9 percent saline daily for 5 days. Hence there were four groups of animals based on the primary neonatal treatment (saline or PB) and the secondary adult treatment (saline or PB). Thus the groups were designated control-control, control-PB, PB-control, and PB-PB. To evaluate the AFB_1 disposition effect, we injected animals in

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