Table 1. Effect of cholestenone on secretion of corticosterone by the rat adrenal. Group B was fed 1 percent cholestenone and group A the control ration.

Animal	Body weight (g)	Adrenal weight (mg)	Adrenal vein flow (plasma) (ml/min)	Corticosterone		
				µg%	µg/min	µg/mg of gland/min
			Group A			
1	235	11.0	0.041	878	0.36	0.033
2	273	13.8	0.179	762	1.36	0.099
3	262	13.0	0.050	1560	0.78	0.060
4	225	9.0	0.053	1042	0.55	0.061
Mean	249	11.7	0.081	1061	0.76	0.063
			Group B			
B-1	212	86.0	0.094	129	0.12	0.0014
B-2	230	83.0	0.061	152	0.09	0.0011
B-3	270	39.0	0.056	300	0.17	0.0044
B-4	212	103.0	0.032	223	0.07	0.0007
Mean	231	77.8	0.061	201	0.11	0.0019

ticoids in adrenal vein blood, it has now been found that the feeding of cholestenone results in a profound reduction of steroid output by the rat adrenal.

Male Sprague-Dawley rats were placed on a synthetic, cholesterol-free diet to which was added 1 percent cholestenone by weight (group B, Table 1). Rats in a control group (A) were pair-fed with the drug-fed rats; they maintained comparable weights. After 43 days, the animals in both groups were anesthetized with ether and Nembutal and heparinized, and left-adrenal vein blood was collected. The plasma content of corticosterone was determined by a spectrofluorometric method (*4*). The results shown in Table 1 indicate that the greatly enlarged glands of the treated animals were secreting corticosterone at a rate per unit weight of gland which was only about 3 percent of that in the controls.

Corticosterone and aldosterone were also determined specifically in pooled plasma samples from the adrenal vein collections by an isotope derivative technique (*5*). The values for corticosterone (in micrograms percent) obtained from the pooled plasmas by this method were: group A 1–2, 720; and group A 3–4, 742. The values in the treated animals were group B 1–2, 109; and group B 3–4, 192. These results were consistent with those obtained by the less specific method, which may be expected to yield somewhat higher values.

The values for aldosterone obtained on the same pooled samples were 5.6 and 6.1 µg percent in the control pairs, and 4.2 and 3.5 in the treated pairs. These data suggest that aldosterone secretion is likewise decreased.

Adrenal vein blood was also obtained from rats fed 1 percent cholestenone for only 12 days. The treated and control groups each contained 5 rats. The mean adrenal weights per unit body weight of the treated animals were 1.7 times those in the controls. In the treated rats the mean plasma corticosterone concentration was 57 percent and the output (per gram of gland per minute) 22 percent of that in the controls. These data indicate that inhibition of steroid output and adrenal hypertrophy both progress with continued feeding of cholestenone.

The general architecture of the hypertrophied adrenal glands from cholestenone-fed animals remains undisturbed, and the hypertrophy is limited to the cortex. The hypertrophied glands show very low concentrations of ascorbic acid and of cholesterol (*6*). On the other hand, the relative sterol content is either unchanged or slightly elevated, and the properties of this nonsaponifiable material suggest that it is largely dihydrocholesterol, known to be a major end-product of cholestenone metabolism (*7*).

There are several ways in which cholestenone feeding may be leading to inhibition of adrenal cortical function. (i) Cholesterol, known to be at least a potential precursor of many adrenal steroids (*3*), may be actually an obligatory precursor. An inhibition of adrenal cholesterol biosynthesis would then be reflected in an inhibition of steroid synthesis. (ii) The marked depression of serum and of adrenal cholesterol concentrations, due in part to replacement by dihydrocholesterol, may deprive the adrenal of cholesterol normally transported to it via the blood, and of its normal reserves of stored cholesterol, for use in steroid synthesis. (iii) Cholestenone, or its product dihydrocholesterol, may inhibit a reaction or reactions in the pathway of steroid synthesis from small precursors, even though that pathway does not necessarily involve cholesterol as an intermediate. The fact that simultaneous feeding of cholesterol partially protects rats against the toxic effects of cholestenone and limits the adrenal changes suggests that one of the first two mechanisms is of importance. These results emphasize the importance of considering the effect upon adrenal function of agents designed to lower plasma cholesterol, particularly through an inhibition of cholesterol synthesis.

The effects of Δ^4-cholestenone upon adrenocortical hormone secretion are being studied in other species and with regard to site of action. The toxicity of cholestenone (*6*) suggests that it will have little clinical application for the production of adrenocortical inhibition. However, these results offer another approach not only to the study of adrenocortical inhibitors but to that of the more basic mechanisms of hormone synthesis.

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16 January 1958

Respiratory Properties of the Hemoglobin of Two Species of Diving Birds

Comparison of the oxygen-hemoglobin equilibrium of three species of diving mammals (*1*) with terrestrial mammals indicates no significant differences. It would be of interest to know whether the respiratory properties of the hemoglobin of diving birds are essentially identical to those of nondiving forms (*2*).

Blood was obtained from the heart of freshly killed *Oidemia deglandi* (white-winged surf duck) and *Aechmophorus occidentalis* (western grebe). Erythrocytes were washed in an isotonic phosphate buffered Ringer's solution and either used immediately for determination of the oxygen equilibrium of erythrocyte suspensions or washed two more

times and then hemolyzed in distilled water (5 volumes of water to 1 of packed red blood cells). Oxygen dissociation curves were determined spectrophotometrically (*3*). The data consist of percentage of oxyhemoglobin, y, as a function of partial pressure of oxygen, p. An approximately linear relationship is attained if log $[y/(100-y)]$ is plotted against log p. The two constants of the Hill equation are obtained from such a linear transformation: n, a measure of the heme-heme interaction, is the slope; p_{50}, a measure of the oxygen affinity, is that value of p at which log $[y/(100-y)]=0$. These two constants have been plotted as functions of pH in Fig. 1; each pair of values of n and p_{50} was obtained from an original oxygen dissociation curve based on six to eight determinations.

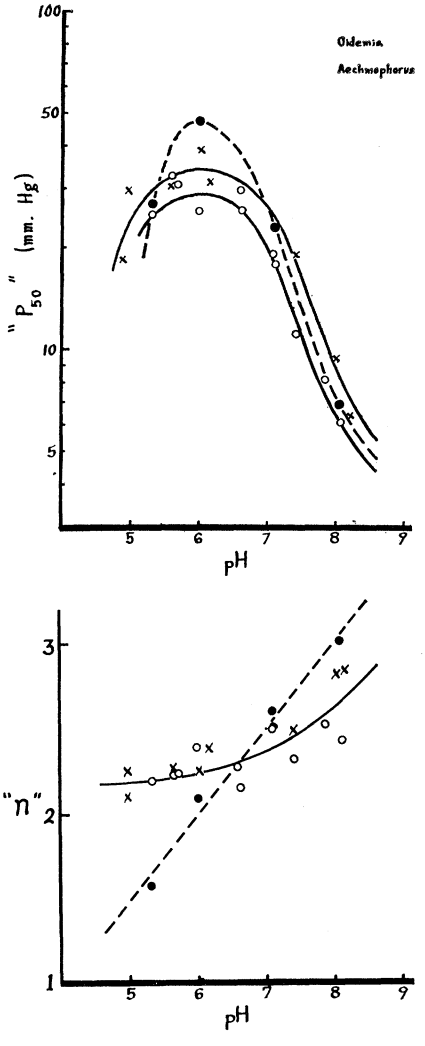

Fig. 1. Oxygen affinity and heme-heme interaction as functions of pH for the hemoglobin of two species of diving birds. Solid line, *Aechmophorus occidentalis* (western grebe); broken line, *Oidemia deglandi* (white-winged surf duck). Circles, 3 percent hemoglobin solution in potassium phosphate buffers; $\Gamma/2$ 0.267. Crosses, erythrocyte suspensions in isotonic sodium phosphate buffered Ringer's solution. Temperature, 24 to 26°C.

Certain biochemical and physiological conclusions can be drawn from the information given in Fig. 1, which includes the first published complete comparison of the Bohr effect of a hemoglobin inside and outside the red blood cell.

For hemoglobin of the western grebe, the oxygen equilibrium is the same inside and outside the erythrocyte with regard to the heme-heme interactions and their pH dependence. The slight increase in p_{50} in hemoglobin solutions as compared with erythrocyte suspensions can be ascribed to the difference in hemoglobin concentration—a dilution effect (*5*).

The Bohr effect—p_{50} as a function of pH—of these avian hemoglobins basically resembles that of horse (*6*) and rat (*7*) hemoglobin in that the bell-shaped curve can be explained on the assumption of two heme-linked groups. However, the reverse Bohr effect (at $p\text{H} < 6$) is much more marked in these avian hemoglobins; the two heme-linked groups are of equal and opposite strength—in contrast to the two mammalian hemoglobins in which the two heme-linked groups are of very unequal strength, a fact that has been made use of with regard to functional structure of the hemoglobin molecule (*6, 8*).

The magnitude of the heme-heme interactions and the oxygen affinity at physiological pH's of hemoglobins of these diving birds are essentially identical to those of the duck (*9*) and, when corrected for temperature, of other birds (*10*).

The equation

$$\phi = \Delta \log p_{50}/\Delta p\text{H},$$

evaluated at pH 7.0 to 7.5, is a useful physiological measure of the Bohr effect (*6*). For rat (*7*) and horse (*8*) hemoglobin, $\phi = -0.60$; for the ordinary dabbling duck, $\phi = -0.67$ (*8*). For the surf duck (see Fig. 1), $\phi = -0.58$; for the western grebe, $\phi = -0.45$. Hence, the Bohr effect is slightly reduced in the surf duck as compared with the ordinary duck; for the western grebe, the reduction is considerably greater.

Perhaps the most salient point, physiologically, in the evolution of diving birds and mammals is the increase in tolerance for lactic acid and CO_2 (*10*). This tolerance ranges from the respiratory center of the brain to—at least for the western grebe—the Bohr effect of the hemoglobin. During diving, such a decrease in the Bohr effect, combined with the well-known decrease in blood flow through the muscles, would prevent a too rapid unloading of O_2, perhaps effecting a saving of that substance for the obligatorily aerobic central nervous system. In addition, during diving and the post-diving acidosis, a large Bohr effect could interfere with the O_2 saturation of blood in the lungs. The finding that the Bohr effect is more reduced in diving birds than in diving mammals (*1*) may be correlated with the findings of Scholander (*11*) that, during diving, the lactic acid of the arterial blood increased relatively more in the duck and the penguin than in the seal. However, Scholander also found that the position of "natural" oxygen dissociation curves of the seals *Halichoerus* and *Cystophora* were shifted much less by changes in CO_2 concentration than were in vitro oxygen dissociation curves of other mammals—including the harbor seal (*1*). The possibility that there is considerable interspecific variation in the phylogeny of physiological adaptations to diving cannot be neglected, especially since the evolution of diving forms has occurred several separate times in both birds and mammals.

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25 November 1957