of $MgCl_2$ to water from Olalla Pond, by a high concentration of Mg. The reduction, as in Hot Lake, of the second zone or sodium "salt" in the presence of a very high concentration of magnesium is possibly due to competition for attachment sites by the ions.

Lakes, then, have a reasonably uniform complement of organic "salts" or perhaps "complexes," the uniformity resting largely on the inorganic ions common to all. High *p*H values or a very high concentration of a single ion may modify the pattern. That the yellow acids are intimately associated with the ionic metabolism of lakes is clear. In addition to the connection of these acids with the major ions, preliminary studies have shown possible involvement with such less abundant, but biologically important, ions as cobalt, iron, manganese, copper, and zinc.

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Effect of Iproniazid on Brain Levels of Norepinephrine and Serotonin

Iproniazid (1-isonicotinyl-2-isopropylhydrazine; Marsilid) was originally introduced as a drug for the treatment of tuberculosis, but was soon found to induce signs of central stimulation. Recent observations (*1*) indicate that the action on the central nervous system may be of value in treatment of depressed mental conditions.

Zeller *et al.* (*2*) were the first to observe that iproniazid inhibits monoamine oxidase, an enzyme which can inactivate norepinephrine and serotonin, substances which may be involved in central regulatory mechanisms (*3*). In a recent paper (*4*) we have presented evidence that monoamine oxidase has a major role in the physiologic inactivation of both monoamines in the brain. The data presented in this report show that repeated doses of iproniazid induce a marked rise in the brain levels of both norepinephrine and serotonin, together with signs of central stimulation.

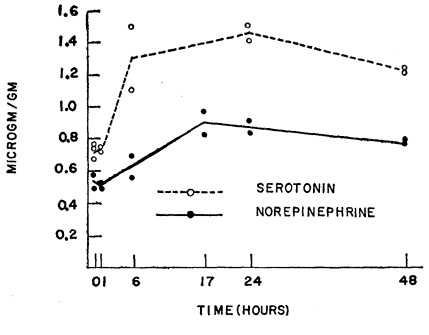

Fig. 1. Effect of a single large dose of iproniazid on serotonin and norepinephrine concentration in rabbit brain stem. Iproniazid (100 mg/kg) was injected subcutaneously. At various times thereafter animals were killed by intravenous injection of air, and the brain stems were analyzed. Each point represents the value from a single animal.

Iproniazid (100 mg/kg) was administered subcutaneously to rabbits, and the concentrations of norepinephrine and serotonin in the brain stem were measured at various times by previously described methods (*5, 6*). As is also reported by other workers (*7, 8*), the serotonin level increased markedly within a few hours after the administration of iproniazid. Norepinephrine levels also increased, but not as rapidly as those of serotonin (Fig. 1). No obvious pharmacological signs were evident.

Iproniazid was given daily in doses of 25 mg/kg subcutaneously to another group of rabbits. Serotonin and norepinephrine levels in the brain stem rose slowly, reaching about twice the normal value in 2 or 3 days (Fig. 2). By the third or fourth day a variable degree of central excitation was evident. Experiments in which the daily dose of iproniazid was 50 mg/kg elicited marked excitement in the animals on about the third day, when serotonin and norepinephrine levels had again risen about twofold. With a smaller daily dose of iproniazid (10 mg/kg), excitation was observed after 4 or 5 days, at which time the brain levels of both amines were again about twice the normal values.

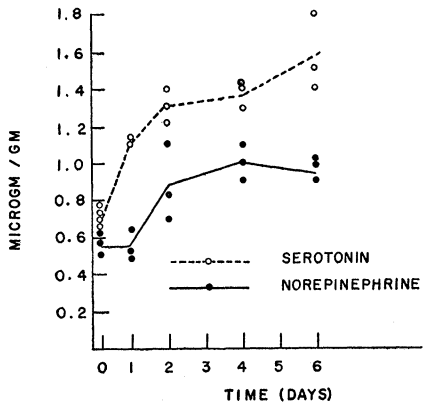

Fig. 2. Effect of daily doses of iproniazid on serotonin and norepinephrine concentration in rabbit brain stem. Iproniazid (25 mg/kg) was injected daily, subcutaneously. Animals were killed at various times by an intravenous injection of air, and the brain stems were analyzed. Each point represents the value from a single animal.

Isoniazid (isonicotinylhydrazine), a congener of iproniazid, is a poor inhibitor of amine oxidase. In daily doses of 50 mg/kg it caused neither a rise in the brain concentration of the amines nor any obvious pharmacologic effect. On the other hand, α-methyl, β-phenylethylhydrazine (JB 516, Lakeside Laboratories) a potent monoamine oxidase inhibitor of a different chemical series, when given daily in doses of 1 mg/kg, induced in 3 to 5 days pharmacologic effects similar to those seen after administration of iproniazid and raised the brain level of the amines about twofold.

It is not possible to conclude from the data given in this report that the central stimulatory effects of iproniazid are causally related to the increase in brain amines. It is noteworthy, however, that the administration of large doses of 3,4-dihydroxyphenylalanine (*9*), a norepinephrine precursor, or of 5-hydroxytryptophan (*7*), a serotonin precursor, causes central excitation which is enhanced by pretreatment with iproniazid.

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Inhibition of Adrenocortical Steroid Secretion by Δ^4-Cholestenone

In the course of studies on Δ^4-cholestenone as an inhibitor of cholesterol biosynthesis it was noted that rats fed high doses of the compound developed striking (six- to eight-fold) hypertrophy of the adrenal glands (*1*). The effectiveness of Δ^4-cholestenone in depressing cholesterol synthesis (*1, 2*) and the demonstrated role of cholesterol as an intermediate in adrenal steroid synthesis (*3*) suggested that the latter might also be depressed. By direct measurement of cor-

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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8. We express appreciation to Dr. W. M. Tullner for helpful suggestions for the technique of adrenal vein cannulation.

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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