cardia it may provoke other ectopic rhythms. It may, however, be concluded, that when catecholamines are clinically indicated, the presence of a ventricular ectopic rhythm should not be a deterrent.

Summary. Intravenous injection in the dog of epinephrine in the amount of 0.01 cc kg in presence of a ventricular ectopic tachycardia, did not elicit ventricular fibrillation in 54 attempts. The tachycardia was moderately increased in most experiments or slowed down. Only in 4 experiments did the rate rise for more than 50 beats/minute.

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Glucuronic Conjugation of Steroids in the Avian Adrenal Gland.* (25999)

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It is well established that adrenal steroids are conjugated with glucuronic acid as one phase of their inactivation prior to excretion in mammals. Furthermore, the liver is generally regarded as being the primary site of this conjugation, enzymatic control of which has been ascribed to 2 enzymatic systems: UDPG dehydrogenase (in particle-free fraction) (1, 2) and a glucuronosyl transferase (in microsomes) (3,4,5).

During another study, evidence was obtained indicating that a considerable portion of total steroid content of the avian adrenal tissue was in a bound form—conjugated with both glucuronide and sulfate (6). This report presents data which support the previous finding by demonstrating that the avian adrenal gland possesses the enzymatic systems responsible for both conjugation of adrenal steroids with glucuronide and hydrolysis of glucuronide-bound steroids.

Methods. The adrenal glands used to measure glucuronide formation were obtained by dissection from 3-4 month old White Leghorn cockerels immediately after decapitation, weighed and homogenized in ice-cold Krebs-Ringer-PO_4 buffer (pH 7.4) with a Potter-Elvehjem homogenizer.

Replicate fractions of the homogenate were incubated in Warburg flasks at 37°C for 2.5 hours with one of the following steroids† as a substrate: 11-dehydro-17-hydroxycorticosterone (E, cortisone), 17-hydroxycorticosterone (F, cortisol), pregnane-3α,17α,21-triol-11,20-dione (H_4E, tetrahydrocortisone), or pregnane-3α,11β,17α,21-tetrol-20-one (H_4F, tetrahydrocortisol). Each flask contained: 0.5 ml uridine diphospho-glucuronic acid (UDPGA) (100 γ ml); 2.0 ml homogenate (equivalent to 80-100 mg wet tissue); 1.0 ml steroid (100 γ ml $\cong$ .27 μmoles; and 4.5 ml Krebs-Ringer-PO_4 buffer containing 90 mg glucose/ml, making a total volume of 8 ml.

Prior to incubation of the homogenate with substrate, 4 ml was removed from each flask and boiled to inactivate enzymes. These 4 ml portions were left at room temperature to function as unincubated controls.

After incubation, the flasks were placed in boiling water to inactivate enzymes. Both incubated and nonincubated control samples were then extracted 3 times with methylene

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† Cortisone, tetrahydrocortisone and tetrahydrocortisol were generously supplied by Merck Inst. through the courtesy of Dr. H. J. Robinson.

chloride and steroid concentration of the pooled extract determined by the Porter-Silber technic(7) with the use of a Beckman DU spectrophotometer. For each set of determinations from one pooled homogenate, a control flask containing 8 ml buffer was treated similarly to the incubated samples and used to set the spectrophotometer prior to reading the other samples.

Subtracting the amount of free steroid in the nonincubated samples from that of its corresponding incubated sample yielded the amount of free steroid which disappeared during incubation.

The contents of both incubated and nonincubated flasks were next adjusted to pH 5.0 with 0.2 M acetate buffer, 1 ml of β-glucuronidase (*Ketodase*) was added and the samples hydrolyzed at 47°C for 18 hours. Samples were then reextracted with methylene chloride and free steroid determined as before. The difference in free steroid content between the originally incubated and nonincubated flasks gave the amount of steroid liberated in hydrolysis with β-glucuronidase.

To prepare a microsomal fraction, adrenals were homogenized in an icecold suspending medium containing 300 g sucrose, 2 g Versene (disodium ethylenediamine tetraacetate) and 1.0 g Tris (trishydroxy methyl amino methane) buffer in 1000 ml distilled H_2O. The suspension was centrifuged at 10,000 g for 15 minutes in a Servall refrigerated (1-2°C) centrifuge. Nuclei and mitochondria were removed and discarded. After centrifugation at 105,000 g for 1 hour in a Spinco Model L ultra centrifuge, the supernatant was discarded and the microsomes resuspended in the above medium so that 1 ml of the resulting suspension was equivalent to 10 mg protein/ml.

To 1 ml of the microsomal preparation were added 0.7 ml of one of the 4 steroids (100 γ/ml) ≅ .19 μmoles, 0.5 ml UDPGA (100 γ/ml) and 0.8 ml Krebs-Ringer-PO_4 buffer. Replicates of this mixture were incubated at 38°C for 30 minutes(4). Corresponding control mixtures without UDPGA were run concurrently.

Following incubation the tubes were boiled to inactivate the enzymes and extracted with chloroform to remove unbound steroid and the steroid determined by the Porter-Silber method. After adjusting pH to 5.0 the residual material was incubated with β-glucuronidase (*Ketodase*) for 18 hours at 47°C and reextracted with chloroform to remove liberated steroid which was determined by the Porter-Silber technic.

Prior to extraction after incubation, 0.3 ml of original incubation mixture was removed and UDP content determined by measuring the disappearance of reduced DPN in the phosphopyruvate-pyruvate kinase system coupled to the DPNH-lactic dehydrogenase system(8).

β-glucuronidase activity was determined in the adrenals from young White Leghorn cockerels by a slight modification of the method of Talalay *et al.* using phenolphthalein glucuronide as a substrate(9). An aliquot of homogenized tissue was mixed with substrate and buffer at pH 5.5. After incubation for 1 hour at 37°C the tubes containing the mixture were placed in boiling water for 5 minutes to inactivate the enzyme and precipitate protein prior to development of the color. A unit of β-glucuronidase was arbitrarily designated as that quantity of enzyme responsible for hydrolysis of 1 γ substrate per hour under the above conditions.

Results. The results of incubating avian adrenal homogenates with compounds E, F, H_4E or H_4F in presence of excess UDPGA are shown in Table I. In each case except E, a statistically significant amount of the steroid substrate disappeared during incubation as compared with an equal number of unincubated control determinations ($P < .001$ for H_4E and H_4F; $P < .025$ for F). The percent decrease in steroid content for the 3 compounds varied from 13.7% for F to 27% for H_4F. The 2% decrease for E probably can be attributed to experimental error since the statistical probability was = .53.

Following hydrolysis with β-glucuronidase and reextraction, free steroid, representing the steroid previously bound with glucuronide, was recovered (Table I). A discrepancy exists in the case of compound E where twice as

TABLE I. Steroid Conjugation.

Preparation	Substrate	No. determinations	Steroid* disappearing on incubation	Steroid* recovered as glucuronide	UDP* recovered
Total homogenate	E	10	.008	.017	
	H_4E	14	.053	.011	
	F	10	.037	.009	
	H_4F	10	.073	.016	
Microsomal fraction	E	7	0	0	0
	H_4E	17	.040	.020	.021
	F	6	.023	.008	.014
	H_4F	11	.013	.010	.011

* μmoles in total incubated mixture.

much glucuronide-bound steroid was recovered as disappeared on incubation with UDPGA; an adequate explanation of this result is not readily apparent. For the other 3 compounds, the amount of hydrolyzed steroid varied from 20.7% (H_4E) to 24.3% (F) of the amount that disappeared in the original incubation. Of the 4 compounds used, statistically significant results ($P < .025$) were obtained only in the case of F.

Since total homogenates of adrenal tissue undoubtedly contain a vast assortment of endogenous enzymes and steroids other than the added substrate, it is not surprising that the results of incubating microsomal fractions from avian adrenal glands with steroid substrates offered more clear-cut results (Table I). The percentage of the added steroid substrate which disappeared during incubation with microsomes was the greatest for H_4E (21.0%) and least for H_4F (6.8%) and E (0%). After hydrolysis with β-glucuronidase, 34 to 77% of the 3 steroids which had disappeared during the initial incubation was recovered as glucuronide-bound material. Amount of UDP formed during conjugation was measured and found to correspond reasonably well with the amount of recovered glucuronide-bound steroid.

Since in the procedure using microsomes the UDP formed so closely matched the quantity of glucuronide formed, it is probable that the discrepancy between the steroid that disappeared upon initial incubation and that recovered subsequent to hydrolysis is due to a fairly large share of the steroid substrate having been metabolized by enzymatic mechanisms leading to products other than glucuronide formation and/or products other than those reactive to the Porter-Silber technic. This possibility also exists with use of total adrenal homogenates.

It is generally held that glucuronide conjugation of Δ^4-3-keto-steroids is preceded by consecutive irreversible saturation of the 4-5 double bond under the influence of Δ^4-3-keto-steroid hydrogenase(10,11,12) and irreversible reduction of the C_3 carbonyl group by 3-hydroxysteroid dehydrogenase (13). If this generalization holds for the microsomal system used in the present study, the explanation for the fact that F but not E appeared to be conjugated must mean that F was converted to H_4F prior to conjugation. Alternatively, it would have to be assumed that conjugation of F occurred at a position other than C_3(4,14). Unfortunately, our data do not permit a decision on either alternative.

The results of determining β-glucuronidase in avian adrenal tissue (and in liver and kidney for comparison) (Table II), indicate that adrenal tissue contains almost as much of this enzyme as does kidney and 23% as much as does liver. Furthermore this concentration of β-glucuronidase in the adrenal was stable under various chronic treatments (hydrocorti-

TABLE II. β-Glucuronidase Content of Adrenal, Hepatic and Renal Tissue.

Age (days)	No. birds	β-glucuronidase* Adrenal	Liver	Kidney
24	9	1.38 ± .1	5.34 ± 1.1	1.51 ± .1
14	7	1.42 ± .1	5.52 ± 1.1	2.28 ± .4
16	8	1.23 ± .2	6.29 ± 1.5	1.71 ± .3
14	8	1.53 ± .2	6.27 ± 1.6	2.14 ± .1
14	10	1.39 ± .1	5.84 ± .9	1.71 ± .3

* Units/mg tissue. A unit is that amount of enzyme responsible for liberating 1 γ phenolphthalein at 38°C, at pH 5.5, in 1 hr.

sone, cortisone, amphenone, hyypophysectomy, reserpine, hexestrol, O', P'-DDD, restraint) some of which altered the concentration in liver(15).

Although the hepatic enzyme systems responsible for glucuronide formation have been studied extensively in mammals and identified in the pigeon(16), no data are available concerning their presence in adrenal tissue. β-glucuronidase, responsible for hydrolysis of glucuronides, has been reported to have a wide distribution in various organs (including the adrenal) in both mammals and birds. The presence of the enzymes controlling both synthesis and hydrolysis of steroid glucuronides in adrenal tissue, together with the demonstration that a large percentage of glucuronide-bound steroid occurs in the avian adrenal during normal and experimental conditions (6,15), strongly suggests that the steroid glucuronides play a role in adrenocortical metabolism in the bird. The nature of this role remains to be investigated.

Summary. Total homogenates and microsomal fractions of avian adrenal glands were incubated with cortisone, cortisol, hydrocortisone or hydrocortisol in the presence of excess uridine diphospho-glucuronic acid. The resulting data indicate the presence of an enzyme system in the microsomes capable of conjugating all the above compounds except cortisone with glucuronide with liberation of uridine diphosphate. The presence of β-glucuronidase was also demonstrated in avian adrenal tissue. These results, together with the previous demonstration that significant amounts of glucuronide-bound steroids are present in the avian adrenal gland under normal and various experimental conditions, imply that steroid glucuronide conjugation and hydrolysis play some role in the normal physiology of this gland.

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Effect of Warfarin (Coumadin) Sodium Administration During Lactation on Blood Coagulation of Nursling Rats.* (26000)

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The anticoagulant warfarin (Coumadin) sodium has come into wide use for treatment and prevention of various thromboembolic disorders, including those in postpartum patients(1,2). This latter application has raised the question of the drug's effect in lactation, *i.e.,* whether or not anticoagulant therapy for the mother would have an adverse effect on the nursling. This problem has now been investigated by determination of whole blood clotting time by the capillary method in mother rats fed diets containing warfarin

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