centuated by the trauma of surgery in these animals lacking normal excretory pathways.

Summary. Chlorothiazide (a natriuretic antihypertensive drug), sulfamethoxypyridazine (another sulfonamide derivative lacking antihypertensive effect), and diazoxide (a sodium-retaining antihypertensive thiazide) were given to nephrectomized animals. The effects of these drugs on plasma electrolytes and acid-base pattern did not differ from that of 5% glucose solution alone. No acute extrarenal action of chlorothiazide on plasma electrolytes or acid-base balance was evident.

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Influence of Steroidal Structure on Glucuronoside Formation by Mouse Liver and Kidney.* (27487)

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It has been shown previously that liver and kidney can conjugate Δ^4-3-keto steroids as well as ring A reduced steroids(1,7). The amount of Δ^4-3-keto corticosteroids conjugated by the kidney is quite small(1). Berliner and Dougherty(2) showed that biological half-lives for various steroids and sterols vary according to their molecular structure. The mouse liver and kidney conjugate corticosterone to a greater extent than cortisol(1). It has been postulated that the hydroxyl group at position C-17 in cortisol was influencing its conjugation(3). This study was undertaken to determine whether the presence or absence of the 17α-hydroxyl group will influence the glucuronoside conjugation of ring A reduced steroids and Δ^4-3-keto steroids *in vitro.*

Methods. Normal male CBA mice 10-14 weeks of age with body weights ranging from 20-30 g were used as a source of tissue. The animals were sacrificed by cervical dislocation. Kidneys and livers were removed and placed in iced buffer.

The tissues were finely minced with a razor blade. One gram of tissue was placed in a 125-ml Erlenmeyer with 20 ml of 0.1 M phosphate buffer (pH 7.4). Fifty mμM of each of the following steroids were incubated: cortisol-4-C^{14}, corticosterone-1,2-H^3, pregnane-3α, 17α-diol-11,20-dione-4C^{14} (compound XIII), and pregnane-3α,ol–11,20-dione-4C^{14} (compound X). All the flasks contained corticosterone-1,2-H^3 and one of the carbon-14 labelled steroids. Two flasks contained compound X only and two contained compound XIII only. Using this (double label) technic two steroids can be incubated simultaneously with the same cells in the same flask. The kidney and liver minces were incubated separately for 3 hours at 37.5°C. Four flasks, 2 containing boiled kidney and 2 containing boiled liver, were prepared in the above manner and were incubated for 3 hours. These are the zero controls.

Reactions were stopped by adding 20 ml of acetone to each flask and the contents immediately extracted 4 times with equal volumes

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TABLE I. Conjugation of Steroids by Mouse Liver and Kidney *in vitro*.

Tissue	Steroid*	No. incubations	% of initial incubated steroid remaining as non-conjugated steroid†	P	% of initial steroid rendered free following β-glucuronidase hydrolysis (% steroid as glucuronides)	P
Kidney	Pregnane-3α-ol-11,20-dione[1]	6	63.05 ± 2.5	P <.01	18.34 ± 6.34	P<.01
	Pregnane-3α,17α-diol-11,20-dione[2]	6	93.8 ± 1.8		3.08 ± 1.09	
	Corticosterone[3]	10	97.72 ± 1.97	.8<P<.9	1.01 ± 0.50	.6<P<.7
	Cortisol[4]	2	98.93		0.766	
	Zero incubation control	2	100.20		0	
Liver	Pregnane-3α-ol-11,20-dione	6	70.17 ±10.6	P <.01	10.90 ± 5.78	.01<P<.02
	Pregnane-3α,17α-diol-11,20-dione	6	85.36 ± 3.91		3.57 ± 0.20	
	Corticosterone	12	77.58 ± 2.17	P <.01	6.32 ± 1.73	P<.01
	Cortisol	5	87.61 ± 3.60		2.50 ± 0.636	
	Zero incubation control	2	98.2		0	

* 50 mμmoles incubated. † Avg and stand. dev.

[1] 5.4×10^4 DPM
[2] 5.4×10^4 DPM
[3] 6.82×10^5 DPM
[4] 5.6×10^4 DPM

of warm acetone (40°C). The acetone was evaporated *in vacuo*. The remaining water residue was then exhaustively extracted with equal volumes of ethyl acetate and the extracts evaporated to dryness *in vacuo*. The residue was transferred with $CHCl_3$/MEOH (1:1) to graduated centrifuge tubes and was counted for radioactivity. This fraction contained the free non-conjugated steroids.

A β-glucuronidase hydrolysis was performed on the water fraction containing the water soluble steroids, using Sigma Chemical Co. bacterial β-glucuronidase. The water samples were made up to a standard volume of 20 ml and transferred to a 50-ml Erlenmeyer flask. Fifty mg of ethylene-diamine tetraacetic acid (disodium salt), 80 mg of cysteine, 2 ml of pH 6.5 phosphate buffer (0.75M), 2000 units of β-glucuronidase and one drop of $CHCl_3$ were added to each flask in the order given. The flasks were incubated for 17 hours at 37°C-40°C.

After hydrolysis, the samples were re-extracted with ethyl acetate, as before, and the $CHCl_3$/MEOH fraction was counted for radioactivity.

The difference in beta energies between carbon-14 and tritium makes it possible to count the 2 isotopes simultaneously. The relative amounts of carbon-14 and tritium present are determined by the solution of 2 simultaneous equations(4). These equations were evaluated using a Burroughs 205 Datatron digital computer. The standards used for the experiment were prepared from the stock solutions of steroid that were incubated.

The samples were counted in a Packard automatic liquid scintillation spectrometer series 314A. The counting technics have been previously described(5). Tritium and carbon-14 were used in a ratio of approximately $15H^3$ dpm to $1C^{14}$ dpm. The samples were counted until statistical accuracy was obtained. Only the $CHCl_3$/MEOH soluble steroid fractions were counted. All values used in this paper are percent of the originally incubated steroid present in the organic fraction after the initial extraction and after β-glucuronidase hydrolysis.

Results. The data of Table I show that in the kidney 65% of compound X and 94% of compound XIII remained as free steroid. The kidney conjugated 30% more of compound X than of compound XIII. The value of P indicates that this difference is highly significant. The conjugation of corticosterone and cortisol by the kidney is very small and the value of P shows that there is no difference between them. The percent of total steroid that was conjugated as a glucuronoside also shows that there is a difference between compound X and compound XIII

(Table I). The amounts of corticosterone and cortisol present as glucuronosides are negligible.

The liver shows a similar pattern of glucuronoside conjugation. More compound XIII remained as free steroid than did compound X and this difference is highly significant (Table I). The data indicate that there is also a statistical difference between corticosterone and cortisol conjugation. The difference between the conjugation of compounds X and XIII is reflected in the amount of steroid present as glucuronoside. The liver formed less glucuronoside from cortisol than it did from corticosterone.

Discussion. The liver is the major organ in the animal responsible for complete reduction of ring A and subsequent conjugation of corticosteroids(2,6,9). Compounds reduced in ring A are conjugated to a greater extent and have a shorter biological half-life than non-reduced compounds(2). The kidney is capable of conjugating ring A reduced corticosteroids and negligible amounts of Δ^4-3-keto corticosteroids as glucuronosides.

The biological half-life in blood of various steroids and sterols is dependent upon the number of carbons and oxygen functions present in the molecule(2). Our studies demonstrate that the liver forms glucuronide conjugates from compounds without a 17α-hydroxy group (corticosterone and compound X) to a greater extent than it does compounds containing the 17α-hydroxy group (cortisol and compound XIII). It appears that the presence of the 17α-hydroxy group hinders the glucuronide conjugation of the steroids containing this chemical group. This hindrance of conjugation is present in reduced and non-reduced ring A steroids. The same situation holds true for the kidney.

Inhibition of conjugation by the 17α-hydroxy group is probably a steric hindrance of glucuronosyl transferase. It is not an interference of reduction of ring A, since reduced and non-reduced 17α-hydroxy steroids are conjugated as glucuronosides to about the same extent in liver.

The fact that the mouse is primarily a corticosterone producer could explain why the mouse has the capacity to produce more glucuronosides from corticosterone than it does from cortisol(3). Also, in humans corticosterone has a shorter biological half-life than cortisol although humans are primarily cortisol producers(8).

Summary. Kidneys and livers of male CBA mice were incubated *in vitro* with cortisol-4-C^{14}, corticosterone-1,2-H_3, pregnane-3α-ol-11,20-dione-4C^{14} (compound X) and pregnane-3α,17α-diol-11,20-dione-4C^{14} (compound XIII). It was found that the liver produced more glucuronosides from corticosterone and compound X than it did from cortisol and compound XIII in 3 hours. Cortisol and compound XIII have a 17α-hydroxy group while corticosterone and compound X do not. The kidney was also able to produce more compound X glucuronide than compound XIII glucuronide. However, this organ conjugated very little cortisol or corticosterone. It is shown that the presence of the 17α-hydroxy group in a steroid molecule delays the conjugation of that molecule with glucuronic acid in liver and kidney.

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