Urinary Bladder Sterols.* (23350)

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The ratio of Δ^7-sterols to cholesterol is much higher in rodent urine than in blood, and preliminary analyses indicated the urinary bladder to contain sterols of the Δ^7-type (1). The present study deals with the nature of these sterols, the amounts found in various species, their distribution within the bladder, and their syntheses from acetate by bladder slices *in vitro*.

Methods. Bladders were dissected out, cut open, rinsed in cold isotonic saline, and subjected to acetone extraction for 24 hours in a Soxhlet apparatus. The fat-free tissue was then dried and weighed, and the acetone extract analyzed for cholesterol and Δ^7-sterol (2). For identification and measurement of 7-dehydrocholesterol, the sterol was precipitated with digitonin, the digitonides were split with pyridine(3), and the free sterol was dissolved in ethanol and the absorption spectrum read in a Model 11 Cary spectrophotometer.‡ For separation of mucosa from the muscle layer, bladders were soaked in N/3 NH_4OH (4) for 10 minutes, rinsed in distilled water, and the gelatinous mucosa scraped off with a dull scalpel. Dog bladders used for incubation with radioactive acetate *in vitro* were taken from beagle pups 7 to 8 weeks old; mouse bladders and skin, from Sutter stock mice 13 to 15 weeks old. After dissection, bladders were rinsed in Krebs-Henseleit bicarbonate buffer (pH 7.4), blotted, weighed, cut into slices and incubated for 3 hours at 37°C with $CH_3C^{14}OONa$ in 50 ml flasks (1 g of tissue/flask) containing 6 ml of buffer (5). The gas phase was CO_2:O_2(1:19). Following incubation, the contents of the flasks were combined, saponified with ethanolic KOH and the non-saponifiable fraction treated with digitonin. The digitonide was split and aliquots of the sterol fraction were examined for 7-dehydrocholesterol, total Δ^7-sterol, and cholesterol as described above. Carrier Δ^7-cholestenol was then added, and p-phenylazobenzoyl ("azoyl") esters of the sterols were prepared and chromatographed on silicic acid - Celite columns(6). After development, the columns were cut, the azoyl esters eluted, measured by their absorbance at 450 mμ in benzene(7) and aliquots plated on copper planchets from a solution of polyvinylpyrrolidone in chloroform and counted in a thin-window Geiger-Müller counter. In the case of the Δ^7-sterols from mouse bladder the azoyl esters were saponified and the 7-dehydrocholesterol removed by forming the maleic anhydride adduct(8). Counts remaining in this fraction were then attributed to Δ^7-cholestenol.

Results. Urinary bladders of mice, rabbits, chinchillas, rats, guinea pigs, dogs, pigs, sheep and cattle were analyzed for cholesterol and fast-acting sterols. Bladders of rodents showed the highest contents of fast-acting sterol, the dog intermediate amounts, and livestock very little (Table I). Guinea pig, chinchilla, rabbit and mouse bladders contained more cholesterol than did those of other species (Table I). As previously reported for rats and mice(1), bladder sterols of all species were unesterified(9).

In rodents and in the dog most (60-84%) of the fast-acting sterol was 7-dehydrocholesterol (Table I). Recrystallized bladder sterol was analyzed for 7-hydroxycholesterol by the method of Sobel *et al.*(10), but in no instance was any of this sterol detected. The other fast-acting sterol in bladder was therefore assumed to be Δ^7-cholestenol. The major sterol was identified as cholesterol by the melting point of its azoyl ester, the spectrum of its Tschugaeff chromophore prepared by the method of Hanel and Dam(11), and the shape of the curve showing L_{620} mμ versus time after treatment with the Liebermann-

* Published with approval of Director of the Wisconsin Agri. Exp. Station. Supported in part by grant from N.I.H., U. S. Public Health Service.

† P.H.S. Research Fellow of Nat. Cancer Inst.

‡ Procedure for analysis and calculation of the amounts of sterols in the mixture found in bladder is described in detail in reference(9).

TABLE I. Sterols in the Urinary Bladder of Various Species.

Species	mg/100 g FFDW ± S.D. Cholesterol	Δ^7-Sterol*	$\frac{7D}{(\Delta^7\text{-Sterol})\dagger} \times 100$	No. samples
Mouse	1260 ± 160	142 ± 20 (129)	69	3
Rabbit	1310 ± 120	117 ± 22 (106)	60	3
Chinchilla	1450 ± 15	79 ± 10		2
Rat	790 ± 140	70 ± 12 (62)	84	6
Guinea pig	1340 ± 95	54 ± 7 (49)	61	3
Dog	815 ± 240	24 ± 9 (22)	71	5
Pig	895 ± 20	12 ± 4 (12)	0	3
Sheep	965 ± 40	10 ± 2 (10)	0	4
Ox	685 ± 120	3 ± 1 (3)	0	4

Abbreviations: FFDW = fat-free dry wt; S.D. = stand. dev.; 7D = 7-dehydrocholesterol.

* Calculated as Δ^7-cholestenol; values in parentheses represent mixture of Δ^7-cholestenol and 7-dehydrocholesterol present in the species.

† Mixture of Δ^7-cholestenol and 7-dehydrocholesterol.

Burchard reagent(2).

In dogs, pigs, sheep and cattle, analyses of separated mucosa and musculature indicated a 3- to 5-fold concentration of cholesterol in the mucosa compared with the muscle layer (Table II). Δ^7-Sterol, on the other hand,

TABLE II. Distribution of Sterols within the Urinary Bladder.

Species	Tissue	mg/100 g FFDW ± S.D. Cholesterol	Δ^7-Sterol*
Dog	Mucosa	2970 ± 390	610 ±320
	Muscle	930 ± 105	9 ± 2†
Pig	Mucosa	5040 ±1580	220 ± 75
	Muscle	915 ± 30	10 ± 4
Sheep	Mucosa	3090 ± 10	60 ± 15
	Muscle	870 ± 90	5 ± 2†
Ox	Mucosa	2220 ± 330	8 ± 1†
	Muscle	635 ± 100	3 ± 1†

Abbreviations: FFDW = fat-free dry wt; S.D. = stand. dev. for 4 samples.

* Calculated as Δ^7-cholestenol.

† Values below 10 mg/100 g cannot be regarded as reliable.

showed a concentration gradient of up to 60-fold in this respect. In no case did the Δ^7-sterol exceed 1.1% of the bladder muscle sterol, values generally being too low to be regarded as reliable (Table II). The mucosa contributed from 1 to 2% of the fat-free dry weight of bladder(9).

Radioactive acetate was incorporated into both the Δ^7-sterol and the cholesterol zones of bladder, but much more actively into the former, by a factor of 35 to 150 times (Table III). The actual difference may have been greater still, since high counting companions arising from tissue slice incubations can accompany cholesterol on the azoate column (5). Mouse skin, included for comparison, was only slightly more active as a site of Δ^7-sterol synthesis than was mouse bladder (Table III). Since little of the Δ^7-sterol resulting from dog tissue incubations was 7-dehydrocholesterol, no conclusions can be drawn regarding the synthesis of this sterol in dog bladder. In mouse bladder, however, where 7-dehydrocholesterol constituted 64% of the Δ^7-sterol, specific activities of 7-dehydrocholesterol and Δ^7-cholestenol were 2700 and 1530 counts/minute/mg, respectively, a ratio exactly equal to that of the amounts of these 2 sterols.

Discussion. In a preliminary communica-

TABLE III. Synthesis of Sterols from $CH_3C^{14}OONa$ by Bladder Slices.

Species	Tissue	Run No.	Counts/min./mg sterol Cholesterol zone	Δ^7-Sterol zone	$\frac{7D}{\Delta^7} \times 100$
Dog	Bladder	I	115	6890	0
	"	II	140	4870	12
Mouse	Bladder	III	14	2090	64
	Skin	III	101	2500	0

Abbreviations: 7D = 7-dehydrocholesterol; Δ^7 = total Δ^7-sterol.

tion Heaton *et al.* reported the presence of 7-dehydrocholesterol in rat bladder(12) and the present results show this compound to exist in the bladders of several other species accompanied by smaller amounts of another fast-acting sterol, probably Δ^7-cholestenol. In species in which separation of the bladder mucosa and muscle layers was possible, the sterols, and especially the fast-acting sterols were found to be concentrated in the mucosa. This parallels the observation that Δ^7-sterols are concentrated in the mucosa relative to the muscle of intestine(13) and in the epidermis relative to the dermis of skin(14,15).

The more active incorporation of labeled acetate into Δ^7-sterols than into cholesterol by bladder slices is also analogous to the situation observed in skin. Both in mouse bladder and in rat skin(5) 7-dehydrocholesterol had a higher specific activity than Δ^7-cholestenol. 7-Dehydrocholesterol has been found in vagina, submaxillary glands(12), intestine, blood(13), placenta, pancreas(16), ovaries, stomach(17), and preputial and anal glands (18)—all sites which would not be expected to receive ultraviolet irradiation. This distribution has led Glover *et al.* to suggest a biological function for 7-dehydrocholesterol other than its role as provitamin D_3(13). Such a suggestion is strongly supported by our finding that the synthesis of 7-dehydrocholesterol from acetate was more active than that of the other sterols in mouse bladder.

Summary. 1. Urinary bladders of many species were analyzed for cholesterol and "fast-acting" (Δ^7) sterol. Rodents showed the highest content of Δ^7-sterol, the dog an intermediate level, and livestock very little. In rodents and in the dog 60-84% of the Δ^7-sterol was 7-dehydrocholesterol. All bladder sterol was unesterified. 2. The sterols were found to be more concentrated in the mucosa of the bladder than in the muscle layer, the Δ^7-sterol showing a greater concentration gradient than cholesterol. 3. Tissue slices of dog and mouse bladder incubated with $CH_3C^{14}OONa$ *in vitro* incorporated label into both the Δ^7-sterol and the cholesterol fraction, but the specific activity of the former was much (35-150X) higher.

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Received June 14, 1957. P.S.E.B.M., 1957, v95.