Renal Excretion of Lanatoside C. in the Rat.* (19429)

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Previous studies(1,2) have been performed to determine, by means of the embryonic duck heart method, the urinary excretion of digitoxin in the rat. It was felt desirable to determine in a similar fashion, the excretion of Lanatoside C. in the urine of the rat in order that the rate of excretion of both drugs might be compared in this species.

Methods. Various known amounts of Lanatoside C. (10, 20, 30, 40, and 60 μg) were added to 24-hour urine collections of rats. The entire sample was evaporated to approximately 2 ml, adsorbed on 5 g of diatomaceous earth and dried at 70°C *in vacuo* for 12 hours. The glycoside was eluted with 50 ml re-distilled dioxan for 2 minutes in a Waring Blender, filtered and evaporated to dryness, then taken up in 50 ml of Tyrode's solution by shaking for 1 hour. The embryonic duck hearts were then exposed to this latter solution and the time of "digitalis effect"(1) observed. A quantitative differentiation could be made between these various urine samples containing different amounts of added Lanatoside C. As little as 10 μg in a 24-hour urine sample could be detected. After these standards had been prepared, 28 male rats (Long-Evans strain) whose average weight was 209 g. were given 1 μg Lanatoside C. per g of body weight by intravenous injection. Their urine then was collected for 24 hours by placing them in plastic cages(3) specially designed for collection of urine. The urine samples of 11 of these rats were also collected on the second day following injection. All urine samples were extracted and tested on the duck hearts as described above.

Results. Twenty-eight rats after the average injection of 209 μg of Lanatoside C. excreted an average of 35 μg (Range: 10-60 μg) of Lanatoside C. or 17% of the injected amount, in the first 24-hour urine. No Lanatoside C. (*i.e.* less than 5 μg) however, was detected in urine samples collected during the second 24-hour period after injection of the drug.

Discussion. The present results indicate that the rat excretes almost 6 times as much Lanatoside C. as digitoxin(2) in his urine during the first 24 hours after injection of the glycoside. The marked difference in the excretion of the two drugs might have been expected in view of the previously discovered differences in the behavior of the two drugs in the blood of this animal. Thus, although digitoxin is strongly bound to plasma albumin, Lanatoside C. is not(4,5), perhaps explaining why the latter disappears so rapidly from the blood(6) and appears in greater quantity in the urine.

Despite this *comparatively* large renal excretion of Lanatoside C., the actual fraction disposed of in this way is only about 17% of the administered dose. The greater part of the dose then is excreted or destroyed in some other manner than by simple renal excretion of unchanged Lanatoside C.

Conclusions. A method for the quantitative determination of minute amounts of Lanato-

* This work was aided by grants from The Life Insurance Medical Research Fund, and The U. S. Public Health Service.

TABLE I. The Renal Excretion of Lanatoside C.

No. of rats	Avg amt Lanatoside C. inj., μg (1 μg/g body wt)	Urine (0-24 hr) Vol, ml	Urine (0-24 hr) Lanatoside C., μg	Urine (0-24 hr) % excreted	No. of rats	Urine (24-48 hr) Vol, ml	Urine (24-48 hr) Lanatoside C., μg	Urine (24-48 hr) % excreted
28	209	12	35	17	11	12	N.D.*	—
Range:	(137–265)	(5–17)	(10–60)	(5–29)	—	—	—	—
S.E. mean:			± 2.7	± 1.2	—	—	—	—

* None detected.

side C. in rat urine is described. The renal excretion of Lanatoside C. in the rat is considerably greater than that of digitoxin.

The authors wish to express their thanks to Tadashi Ishida for his valuable technical assistance.

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Received February 25, 1952. P.S.E.B.M., 1952, v79.

Complement-Fixation Studies with Poliomyelitis in Humans.* (19430)

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Specific complement-fixing (CF) antigens have been processed from cotton rat tissues infected with Lansing poliomyelitis virus (1-3). The CF antigens prepared by Casals and Olitsky(4,5) from MEF_1 infected tissues of new-born mice have appeared to cross somewhat with serum from Brunhilde-immune monkeys. More recently(6), specific CF antigens have been processed from nerve tissues of rhesus monkeys infected with Brunhilde and with Leon prototype poliomyelitis viruses. The latter antigens have been tested thus far only with serums from monkeys immunized with virus and the adjuvant recommended by Salk(7). The chronological antibody response of Lansing-immunized cotton rats appeared to follow a pattern in which the CF test was transitory, whereas the neutralizing property of their serum was of longer duration(1,2). The exact duration of the latter effect is uncertain but is thought to last for years. Some evidence has indicated that immunized monkeys may demonstrate a similar chronological antibody pattern.

When human cases of poliomyelitis recover from the infection, specific neutralizing antibodies rapidly appear in the blood(8,9). There has been suggestive evidence that such infection may elicit a non-specific rise of Lansing neutralizing antibody, even though the infection was of non-Lansing type(9). Lansing CF antibody has been demonstrated in human serums, but their specificity and clinical interpretation have not been studied extensively(1,5). The possibility of a non-specific rise in Lansing CF antibody during the course of infection with non-Lansing virus has not been studied. It is the purpose of this paper to describe the Lansing CF antibody response elicited in the human following infection with poliomyelitis virus.

Methods. Antigen. CF antigens were processed from Lansing poliomyelitis-infected nerve tissues of mature cotton rats (LCR) by two technics: One by precipitation of clarified nerve tissue suspensions with methanol and use of the final eluate as antigen(2); and secondly, by sedimentation of the virus in the ultracentrifuge and use of the resuspended sediment as antigen(3). The antigens were tested for specificity with antiserums prepared against Brunhilde and against Leon poliomyelitis, as well as against rabies and St. Louis encephalitis viruses. The LCR CF antigens processed by the ultracentrifugation technic appeared to be superior to those prepared by methanol precipitation because of the constancy of results and because such antigens were sufficiently sensitive to detect CF antibodies in immunized monkeys. *Serum.* Serum specimens were collected aseptically from 131

* Aided by a grant from the National Foundation for Infantile Paralysis.