

TABLE II

Substance	Unabsorbed Serum	SSS1	
		Absorbed Serum	A Absorbed Serum
A	1:4,000,000	1:4,000,000	no reaction
SSSI	1:4,000,000	no reaction	” ”

It is clear that the A substance of Enders and the SSS1 are closely related. It would seem possible that the A substance more closely approximates the Type I pneumococcus haptene as present in the living cell, and that the SSS1 carbohydrate precipitated at its iso-electric point, pH 3.4, is an hydrolytic product of the A carbohydrate. In the procedure of Heidelberger, Goebel and Avery⁶ for the preparation of SSS1, there can be little doubt that the A substance is destroyed at the stage where it is precipitated with Ba(OH)₂. In the short method for preparing SSS1, (Heidelberger and Kendall), much A is always present, carried down by the SSS1 when the latter is precipitated with acetic acid.

The question as to the true nature of the pneumococcus carbohydrates is an important one, if they are to be used in evaluating the strength of antisera. Since in many cases unabsorbed rabbit Type I antisera contain no precipitins for SSS1 whatsoever, yet precipitate heavily with A, the SSS1 is obviously of no value in estimating the strength of rabbit antisera.

The theoretical and practical implications of these observations are evident, but cannot be properly discussed until studies now in progress have been completed.

6985 C

Action of Digitalis on the Kidney.*

A. HOWARD SHANBERG, ROBERT HENNER, AND LOUIS N. KATZ.

From the Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, Chicago.

It has never been clearly established whether or not digitalis has a diuretic action on the kidney aside from the secondary diuresis that occurs as a result of improvement in the circulation in congestive heart failure. A number of workers failed to obtain any direct diuretic action. Schwartz¹ was unable to produce diuresis in normal individuals with digitalis; Cushny² and Cohn³ failed to ob-

* Aided by the Frederick K. Babson Fund of the Michael Reese Hospital.

tain any increase in urinary flow in cardiac patients without edema, and Miller⁴ found that digitalis had no effect on renal edema. Cushny and Lambi⁵ concluded from their studies that the members of the digitalis group produced no diuresis in normal animals. However, Phillips and Bradford,⁶ Pfaff⁷ and Gremels⁸ claimed to have obtained a diuretic action in animals with small doses of digitalis. Recently Bartram⁹ has also reported that digitan has a direct diuretic action, following its injection into one renal artery.

The discrepancy in previous results led us to reinvestigate the subject. In anesthetized animals, conditions vary from time to time sufficiently to alter the urinary flow, so that results ascribed to the action of an injected drug might be coincidental. It seemed to us that the direct action of a drug on the kidney might be more readily estimated by injecting the drug directly into one renal artery and determining the urine flow of that kidney for a short period of time. Any indirect action or any spontaneous change could be eliminated by using the urine flow of the opposite kidney as a control for comparison.

Fourteen medium-sized male dogs were used. No food was given the animals for 12 hours preceding the operation, but water was urged. The animals were lightly anesthetized with nembutal intravenously (25 mg. per kilo) and this was supplemented when necessary during the course of the experiment with further injections of 5 mg. per kilo. The animal was kept on an electrically heated operating table, and all undue exposure avoided. Five hundred cc. of warm normal saline solution were injected into the jugular vein at the onset of the experiment to assure proper diuresis. The ureters were catheterized individually about their middle third, through a mid-line abdominal incision. Ureteral pyelography catheters, No. 5 were inserted and held in place by silk thread. The lower ends of the ureters were tied off and the ends of the catheters brought out of the abdomen through stab wounds in the groin. The left renal artery was then isolated as it enters the superior posterior

¹ Schwartz. (Quoted by Sollmann, T., *A Manual of Pharmacology*. W. B. Saunders & Co., Philadelphia. 4th edition, 1932.)

² Cushny, A. R., *Am. J. Med. Sci.*, 1911, **141**, 826.

³ Cohn, A. E., *J. Pharm. Exp. Therap.*, 1915, **6**, 606.

⁴ Miller, J. L., *Trans. Assn. Am. Phys.*, 1912, **27**, 137.

⁵ Cushny, A. R., and Lambi, C. G., *J. Physiol.*, 1921, **55**, 267.

⁶ Phillips, C. D. F., and Bradford, J. R., *J. Physiol.*, 1887, **8**, 117.

⁷ Pfaff, Franz, *Arch. f. Exp. Path. and Pharmacol.*, 1898, **32**, 1.

⁸ Gremels, H., *Arch. f. exp. Path. u. Pharmacol.*, 1928, **61**, 130.

⁹ Bartram, E. A., *J. Clin. Invest.*, 1932, **11**, 1197.

pole of the kidney. An identifying loose ligature of silk thread was placed about it. The abdomen was then closed with hemostats and covered. (The abdominal approach is no more difficult than the more commonly employed approach from the back and less operative trauma is incurred.)

The urine from each catheter was collected and measured for a control period of 30 minutes. The abdomen was then reopened, the left renal artery picked up and the digitalis (or other) solution slowly injected into it by a syringe with a fine, short needle. If the puncture wound in the artery bled, the bleeding was controlled by a small gauze pack left *in situ*. The abdomen was closed again with hemostats and the urine flow from each ureter again collected for 30 minutes and measured. In several animals, a second control and post-injection period were run using a different strength solution of digitalis or another drug in the latter period. In some experiments no intermediate control period was run.

The position of the ureter, the absence of obstruction in the catheters and the absence of leak around them were verified in each animal antemortem and postmortem. In addition, the injected renal artery was examined for thrombosis in its lumen or wall. In none of the experiments reported were these complications present, except that occasionally intramural hemorrhage was found, which encroached slightly on the lumen.

The results are summarized in Table I. The relative urinary flow of the left (injected) kidney as compared with the right (control) kidney is given in the fifth column in percentage; and the change in this ratio following the injection of the drug is given in percentage in the sixth column. The variability in the relative and absolute urinary flow during the control periods is very marked and emphasizes the need of comparing the urinary flow of the 2 kidneys before making deductions concerning the direct action of drugs.

Using this comparison, it was found that digalen and digitalone in large doses, viz., $\frac{1}{2}$ cc. of the drug (equivalent to $\frac{1}{2}$ cat unit) inhibited the urine flow of the injected kidney, viz., Exp. 1, 2, 3, 6, 9, 10, 12, and 13. Ouabain (Exp. 12) had a much less striking effect even though its strength was 3 times greater ($\frac{1}{2}$ cc. = $1\frac{1}{2}$ cat units). Some of the inhibition in the case of digalen might have been due to the alcohol used as a vehicle since it was found that alcohol in the amount and concentration used in the digalen solution caused a similar decrease in 2 experiments (Exp. 7 and 8). Even in more dilute solutions digitalone (viz., made up in a dilution of 1 to 100, equivalent to $1/200$ cat unit) exhibited a direct inhibiting action on the urine flow (Exp. 10, 11, 13, and 14).

As controls, normal saline, caffeine, and salyrgan were injected in the same way. It was found that 0.5 cc. of normal saline when injected into the left renal artery had no effect (Exp. 8) or else a

TABLE I

Exp. No.	Drug injected	Urine flow in each period (30 min.)		Ratio of urine flow on two sides L/R. %	Effect of drug on relative urine flow of (change in L/R ratio.) %*	Wt. of dog Kg.
		right kidney cc	left kidney cc			
1	control	4.9	5.5	110		15
	½ cc Digalen**	3.9	2.3	60	-55	
2	control	2.2	1.8	81		7
	4/10 cc Digalen	12.0	0.9	7	-90	
3	control	12.0	6.6	55		17
	½ cc caffein sod. benz.	8.4	10.0	120	+120	
	1 cc Digalen	10.8	6.1	56	-45	
(also 200 cc of isotonic sodium chloride intravenously)						
4	control	16.5	6.8	41		9
	1 cc Salyrgan	40.0	0.0	0	-100	
5	control	10.0	17.0	170		17
	½ cc Salyrgan	4.0	16.0	400	+135	
	½ cc Digalen	1.2	0.0	0	-100	
6	control	9.0	13.2	136		20
	2/10 cc Digalen	10.8	9.9	91	-30	
	3/10 cc Digalen	8.4	3.8	45	-50	
7	control	29.9	16.2	54		11
	½ cc alcohol (8%)	46.7	12.7	27	-50	
8	control	30.3	34.5	113		19
	½ cc isotonic NaCl	22.9	22.3	97	-15	
	½ cc alcohol (8%)	35.2	25.3	72	-25	
9	control	20.2	30.4	150		17
	½ cc Digitalone	41.4	13.4	35	-75	
10	control	9.5	21.0	221		21
	½ cc of 1:100 Digitalone	4.5	4.8	106	-50	
	½ cc Digitalone	2.9	0.0	0	-100	
11	control	6.3	33.3	530		20
	½ cc isotonic NaCl	4.5	45.0	1000	+90	
	½ cc of 1:100 Digitalone	3.5	28.1	800	-20	
12	control	29.0	33.1	114		19
	½ cc Ouabain	13.8	9.9	71	-40	
	300 cc of isotonic saline injected intravenously at this time					
13	control	50.0	61.4	122		22
	½ cc Ouabain	36.4	41.1	112	-10	
	control	7.6	7.3	96		
14	½ cc of 1:100 Digitalone	8.8	6.1	69	-30	22
	control	8.8	15.2	173		
	½ cc Digitalone	4.3	0.5	12	-90	
14	control	36.4	23.7	66		14
	4/10 cc of 1:100 Digitalone	32.5	2.6	8	-90	

*0 to ± 15% = no change; ± 20 to ± 35% = slight change; ± 40 to ± 60% = moderate change; greater than ± 60% = marked change.

** 1 cc of Digalen (Hoffmann & La Roche, Inc.) and of Digitalone (Parke, Davis & Co.) = 1 cat unit.

1 cc Ouabain = 0.25 gm. = 2.5 cat units; 1 cc Caffein sodium benzoate = 0.25 gm. caffein.

1:100 Digitalone = a 1 to 100 dilution of the digitalone with isotonic saline.

stimulating action (Exp. 13). Similarly, caffeine (Exp. 3) and salyrgan (Exp. 5) had a stimulating action, although the latter when used in larger quantities (Exp. 4) actually inhibited the urine flow of the injected kidney.

Our results show that while caffeine and salyrgan have an immediate direct diuretic action, digitalis in the forms used never did. Usually digitalis had an immediate and direct inhibitory effect on urine flow. Bartram's data⁹ for the first 30 minutes after injection of digitalis (his Exp. 71, 72, 73, and 74) if analyzed as we have done, shows a similar inhibition. The constant saline infusion and the partial recovery from the anesthetic which he used probably explain the progressive bilateral diuresis he observed.

In our experiments small amounts of digitalis were used, about .025 to .065 cc. of digitalone, digalen and ouabain per kilo; the toxic dose according to the figures given by Cohn and Stewart¹⁰ would be about 1.0 cc. per kilo for the former 2, and 0.5 cc. for the last. The amounts used were made small so that the drug available to the injected kidney would be minimized. If each kidney is assumed to weigh 50 gm., then the maximum amount of drug taken up by the injected kidney would have been 10 cc. per kilo. When the undiluted drug was used this would be equivalent to 10 times the toxic dose, but when the 1/100 dilution was used this would be equivalent to 1/10 the toxic dose.

Summary. The immediate effects of digitalis preparations, digalen, digitalone and ouabain, on urine secretion were studied in anesthetized dogs by comparing the urine flow of the kidney into whose renal artery the drug was injected with the urine flow of the opposite kidney. In no instance did the digitalis preparation produce a diuretic action, in fact in most cases an inhibition of urinary secretion was noted.

¹⁰ Cohn, A. E., and Stewart, H. J., *J. Clin. Invest.*, 1928, **6**, 79.