PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 156, 155–157 (1977)

Use of Lissamine Green for Micropuncture: A Comparison of Two Methods (39896)

HOWARD H. BENGELE, EILEEN R. McNAMARA, AND EDWARD A. ALEXANDER

Thorndike Memorial Laboratory, Renal Section, Department of Medicine, Boston City Hospital and Boston University School of Medicine, Boston, Massachusetts 02118

"Lissamine green" (LG) has been used regularly in kidney micropuncture experiments to localize specific tubular segments since its introduction by Steinhausen in 1963 (1). In addition, Wright (2) has emphasized the usefulness of LG in determining distal tubular length. Several authors have criticized the continued use of this substance because of effects on sodium and water reabsorption found in both *in vivo* and *in vitro* studies (3–7), but not all have concurred (8).

Presumably to circumvent these effects some laboratories have adopted a policy of using LG to locate puncture sites and then waiting about 30–60 min after dye injection before beginning tubular collections (9, 10). Since the effect of LG on renal function is still controversial and since no studies are available with specific regard to the effect of this dye on distal tubular function, we have compared whole kidney function and proximal and distal tubular collections obtained immediately after injection of LG with data obtained by waiting at least 30 min after LG injection.

Methods. Twelve male rats obtained from Charles River Laboratories, Wilmington, Massachusetts, weighing between 260 and 360 g, were fed with Purina Lab Chow and given tap water to drink. The animals were anesthetized by intraperitoneal injection of inactin (25 mg/kg body weight) and ketamine hydrochloride (85 mg/kg body weight) and maintained at a body temperature of 38° on a heated operating table. After tracheotomy, polyethylene catheters (PE 50) were placed in the jugular veins for infusions. A femoral artery was catheterized (PE 50) for blood sampling and the catheter was connected to a Hewlett-Packard blood pressure transducer and recorder to monitor blood pressure continuously. The left kidney was prepared for micropuncture as previously described (11). The upper third of the left ureter was catheterized (PE 50) and the urine was collected in weighed tubes. Fifty to sixty microcuries per hour of [^{14}C]carboxyinulin (New England Nuclear Corp.) in saline was infused at a rate of 0.03 ml/min. After 1 hr of inulin infusion, micropuncture collections were begun. During micropuncture, four urine collections were obtained, one every 20 min, with accompanying midpoint blood samples. Two to six end proximal and early and late distal tubules were identified by lissamine green transit time. The transit time to point of puncture divided by the earliest distal transit time as described by Wright (2) was used to distinguish early and late distal tubules. A 5% solution of lissamine green SF dye (Chroma-Gesellschaft Schmid and Co., Stuttgart-Unterturkheim) in water was used in all studies to identify nephron sites. The osmolality of the lissamine green solution was 387 mosm/kg H_2O, the sodium concentration was 258 mequiv/liter, and the potassium concentration was < 0.5 mequiv/liter. The solution was not buffered and the pH was 2.5.

In six rats (Group I), lissamine green was injected (0.05–0.1 ml) every 5–10 min. A nephron segment was identified and tubular fluid was collected (11) 60–90 sec after the lissamine green had cleared. A total of 0.5 to 0.75 ml of 5% lissamine green solution was used in the course of the 90-min experiment. In six other rats (Group II), lissamine green solution was injected (0.05–0.1 ml) every 2–3 min and the appropriate nephron segments were identified and mapped. A total of 0.5–0.75 ml of lissamine green was used. Tubular fluid collections were begun at least 30 min after the last lissamine green injection. To calculate renal plasma flow, inulin extraction was measured in all experiments from duplicate blood samples ob-

ISSN 0037-9727

tained by puncturing the left renal vein with a 25-μm tip diameter glass pipet during the last clearance period.

Tubular fluid volume was determined using a capillary of constant bore glass which was previously calibrated. Plasma, urine, and tubular fluid radioactivity were determined by liquid scintillation counting. Tubular fluid sodium and potassium concentrations were determined with an Aminco helium glow photometer. The coefficient of variation of samples of known sodium and potassium concentrations was less than 5%. Plasma and urine sodium and potassium concentrations were measured on an IL flame photometer.

Calculations were made in accordance with formulas previously reported (11). Standard statistical techniques were used and results are presented as the mean ± SE. Comparison between Group I and II was determined using the unpaired *t* test.

Results. A comparison of the values obtained from the two groups of rats studied is found in Table I. No differences were noted in whole kidney or nephron GFR, renal plasma flow, sodium or potassium excretion, or urine volume. Similarly, no significant differences were seen in sodium, potassium, or water reabsorption throughout the nephron. In both groups the ratios of tubular fluid to plasma inulin, sodium, and potassium were comparable in collections from the late proximal and early and late distal tubules. No statistical difference was noted whether the means of means from each animal were compared or whether all values from each collection site were combined and compared.

Discussion. We have compared two regularly used methods of identification of renal tubular puncture sites utilizing lissamine green SF dye. No differences were noted when tubular collections were made immediately after dye injection or after a delay of at least 30 min. The latter collections were made well after dye was no longer visible in either blood or urine, 30–120 min after the last dye injection. No differences were noted in the group between early and late collections or in whole kidney function at the beginning or end of the experiment. These data support the work of Parekh *et al.* (8) who failed to find an effect on inulin clearance, urine volume, or sodium excretion after dye injection or infusion. Our results are different from those of Heller (3), Roch-Ramel and Jotterand (7), and Elmer and Leyssac (5). In clearance studies all these authors noted increased urine volume and sodium excretion after LG administration. In a micropuncture study, Lynch *et al.* (12) studied the effect of LG in dogs. They noted a small increase in sodium excretion after renal artery infusion but not after multiple injections of the dye. Nephron GFR increased after infusion but alterations in proximal reabsorption were not found. These authors (12) concluded that LG might decrease distal nephron sodium ab-

TABLE I. EFFECT OF LG ON RENAL FUNCTION[a]

		Group I	Group II
Number of rats		6	6
GFR (ml/min)		1.41 ± 0.08	1.34 ± 0.09
Renal plasma flow (ml/min)		3.78 ± 0.33	4.20 ± 0.27
Urine volume (μl/min)		4.8 ± 0.9	5.5 ± 0.7
Sodium excretion (μequiv/min)		0.25 ± 0.08	0.32 ± 0.03
Potassium excretion (μequiv/min)		1.73 ± 0.14	1.46 ± 0.09
Nephron GFR (ml/min)		38.3 ± 2.8	33.5 ± 1.6
Proximal $[(TF/P)_{In}]$		2.29 ± 0.13 (15)[b]	2.21 ± 0.08 (19)
Distal $[(TF/P)_{In}]$	E	4.85 ± 0.33 (14)	4.67 ± 0.30 (16)
	L	13.3 ± 1.1 (14)	16.0 ± 1.5 (13)
$[(TF/P)_{Na}]$	E	0.36 ± 0.03	0.34 ± 0.02
	L	0.34 ± 0.03	0.35 ± 0.04
$[(TF/P)_{K}]$	E	0.67 ± 0.09	0.65 ± 0.07
	L	1.72 ± 0.31	2.37 ± 0.32

[a] Values are mean ± SE. GFR, Clearance of inulin; $(TF/P)_{In}$, ratio of tubular fluid to plasma inulin concentration; $(TF/P)_{Na}$ or $_{K}$, ratio of tubular fluid to plasma sodium or potassium concentration; E early, L, late.
[b] Number of tubules given in parentheses.

sorption after renal artery infusion but had no discernable effect when delivered by multiple injections, the method utilized in micropuncture experiments. In rats Brenner and Troy (13) also noted an increase in nephron GFR after LG. Allison *et al.* (14) in rat micropuncture studies noted lower filtration rate, plasma flow, and urine flow in rats not given a combination of LG, aldosterone, and vasopressin. The specific effect of LG in these experiments, therefore, cannot be defined. Alterations in potential differences and short circuit current have been demonstrated by Dörge and Nagel (4) and Christensen and Frederiksen (6), but were not duplicated by Parekh *et al.* (8).

Commercially available LG is a family of compounds which have different chemical structures, contain different impurities, and are utilized as solutions of different osmolality and ion concentrations (15). Parekh *et al.* (8, 15) have suggested that the previously demonstrated effects of LG on renal function may in part be related to the differences among dyes called LG. Regardless of the reason for the differences of results among investigators, our study strongly supports the conclusions of Parekh *et al.* (8, 15). Our data demonstrate that lissamine green SF as utilized in rat micropuncture experiments has no discernible effect on whole kidney or tubular renal function. This is particularly important with regard to distal collections (2). Hence, tubular collections made shortly after dye injection are not different than those collected at least 30 min later.

Summary. The effect of LG-SF dye on whole kidney function and proximal and distal tubular sodium and water reabsorption was studied in rats. In one group tubular samples were obtained immediately after injection of LG while in the other group collections were begun at least 30 min after the last dye injection. No differences between groups in tubular reabsorption of sodium, potassium, water, or whole kidney function were noted. We conclude that all aspects of renal function measured immediately after injection of LG-SF or 30–120 min later do not differ significantly.

The authors greatly appreciate the secretarial help of Mrs. Mary Shea.

These studies were supported by National Institutes of Health Grants HL 17458 and HL 18318.

1. Steinhausen, M., Pflügers Arch. Ges. Physiol. **277,** 23 (1963).
2. Wright, F.S., Amer. J. Physiol. **220,** 624 (1969).
3. Heller, J., Pflügers Arch. **323,** 27 (1971).
4. Dörge, A., and Nagel, W., Pflügers Arch. **313,** 11 (1969).
5. Elmer, M., and Leyssac, P. P., Acta Physiol. Scand. **87,** 296 (1973).
6. Christensen, P., and Frederiksen, O., Pflügers Arch. **331,** 160 (1972).
7. Roch-Ramel, F., and Jotterand, N., Experientia (Basel) **26,** 683 (1970).
8. Parekh, N., Popa, G., and Steinhausen, M., Pflügers Arch. **364,** 77 (1976).
9. Stein, J. H., Osgood, R. W., Boonjarern, S., and Ferris, T. F., J. Clin. Invest. **52,** 2313 (1973).
10. Knox, F. G., Mayo Clin. Proc. **49,** 775 (1974).
11. Alexander, E. A., Bengele, H. H., and McNamara, E. R., Amer. J. Physiol. **231,** 1421 (1976).
12. Lynch, R. E., Schneider, E. G., Strandhoy, J. W., Willis, L. R., and Knox, F. G. J. Appl. Physiol. **35,** 169 (1973).
13. Brenner, B. M., and Troy, J. L. J. Clin. Invest. **50,** 336 (1971).
14. Allison, M. E. M., Lipham, E. M., Lassiter, W. E., and Gottschalk, C. W., Kidney Int. **3,** 354 (1973).
15. Parekh, N., Popa, G., Galaske, R., Galaske, W., and Steinhausen, M., Pflügers Arch., **343,** 1 (1973).

Received March 3, 1977. P.S.E.B.M. 1977, Vol. 156.