made up of cells arrested in the G1 as well as the G2 period. It is seen that as long as 2 days after labeling there are still unlabeled mitoses. Since H3-TdR was present during this entire period, these cells must have been retained for at least 2 days in the G2 period. The fraction of cells delayed in the G1 and G2 periods does not seem to be constant, because in experiments parallel to the one represented in Fig. 2, we obtained a variable amount of cells stimulated from the two periods. It could be that the delay in G1 and G2 during cell cycle inhibition depends on the amount of cells present by chance in one of these periods at the time the critical density is reached, which in turn might be correlated with the size of the original inoculum, the presence of fresh serum in the medium or other unknown factors.

Summary. The influence of cell density on DNA synthesis of human embryonic fibroblasts was studied by measuring the incorporation of tritium-labeled thymidine with autoradiographic techniques. It was shown that as the inoculum decreased an increased proportion of cells entered the S period during the time between subcultivation and confluency. The amount of labeled cells after subcultivation and after induction of division in stationary cultures was measured. Evidence was obtained that cells in crowded cultures are delayed in the G1 and G2 periods.

The skilful technical assistance of Miss Monica Ahlgren is gratefully acknowledged.

1. Macieria-Coelho, A., Pontén, J., Philipson, L., Exp. Cell Res., 1966, v42, 673.
2. ———, ibid., 1966, v43, 20.
3. Levine, E. M., Becker, Y., Boone, C. W., Eagle, H., Proc. Nat. Acad. Sci., 1965, v53, 350.
4. Eagle, H., J. Exp. Med., 1955, v102, 595.
5. Hayflick, L., Moorhead, P. S., Exp. Cell Res., 1961, v25, 585.
6. Santen, R. J., ibid., 1965, v40, 413.
7. Todaro, G. J., Lazar, G. K., Green, H., J. Cell. Comp. Physiol., 1965, v66, 325.
8. Earle, W. R., Sanford, K. K., Evans, V. J., Waltz, K. H., Shannon, J. E., Jr., J. Nat. Canc. Inst., 1951, v12, 133.
9. Kruse, P. F., Jr., Miedema, E. J., J. Cell Biol., 1965, v27, 273.
10. Nilausen, K., Green, H., Exp. Cell Res., 1965, v40, 166.

Received January 20, 1967. P.S.E.B.M., 1967, v125.

Effects of Saline Loading on Distal Renal Tubular Sodium and Water Reabsorption.* (32143)

D. DANNY BERCOVITCH† AND MARVIN F. LEVITT (Introduced by J. Leonard Brandt)
Section of Renal Diseases, Department of Medicine, Mount Sinai Hospital, New York City

There is considerable evidence to indicate that saline loading in the dog results in a depression of proximal tubular reabsorption of sodium and water(1-3). Clearance studies have suggested that there may be decreased distal tubular reabsorption as well(4,5), as demonstrated in rat micropuncture experiments(6,7). However, direct micropuncture observations on distal tubular effects in the dog have not yet been reported.

To elucidate the effects of volume expansion by saline loading in the distal nephron of the dog, stop flow studies performed early and late in the course of a progressive saline diuresis were compared with regard to the sodium/creatinine clearance ratio (U/P Na/U/P creatinine) and U/P creatinine values. The former expression compares the clearance of sodium with the clearance of the glomerular substance creatinine, and represents the fraction of the filtered load of sodium excreted in the urine. The latter expression is an index of tubular water movement without regard to solute movement.

* This study was supported by Nat. Heart Inst. Graduate Training Grant H.T.S.-5505.

† Work performed during tenure of a Postdoctoral Research Fellowship, Nat. Heart Inst. Present address: Renal Laboratory, Jewish General Hospital, Montreal, Canada.

Materials and methods. Twelve stop flow experiments were performed in 5 female dogs and 1 male dog rendered hydropenic by restriction of food and water 15 hours prior to each study. Following anesthesia with sodium pentobarbital the left ureter was isolated through a flank incision and a polyethylene catheter inserted to the ureteropelvic junction and secured with ties. A creatinine prime was given and creatinine was infused in a sustaining solution in amounts appropriate to determine glomerular filtration rate (GFR) during free flow and water movement during stop flow. In the single male dog (Exp. 6) inulin was used rather than creatinine.

The experimental protocol was similar to that outlined by Malvin, Wilde, and Sullivan(8) and modified by Jaenike and Berliner (9). The solution infused was Ringer's Lactate with 0.17% to 0.34% NaCl added. In one study (no. 6) this infusate was superimposed on a constant infusion of 10% mannitol delivered at 5 ml/min. When urine flow rate was about 5 ml/min from the left kidney, a single 8-10-minute clearance was measured. A 1-minute free flow specimen was then collected following which a stop flow experiment (hereinafter referred to as "early stop-flow") was performed by clamping the free catheter tip for 5 minutes (2 minutes in Exp. 6). After the catheter was cut proximal to the clamp, 20 to 30 specimens of 0.6-0.8 ml each were collected in small vials. The saline solution was then infused at gradually increasing rates until urine flow from the experimental kidney was stable at about 8 ml (6.69-9.60 ml)/min. A second clearance was then measured, and a stop flow experiment comparable in all respects to the first one was performed (hereinafter referred to as "late stop flow"). Heparinized blood samples were collected from an indwelling carotid artery needle at the midpoint of both clearance and stop flow periods. All specimens were analysed for sodium and creatinine or inulin. These determinations were done by methods previously described (4,10).

Results. The stop flow patterns for sodium and creatinine obtained with saline diuresis are qualitatively similar to the patterns obtained with mannitol. Fluid in prolonged contact with the distal convoluted tubule is identified by decreasing concentrations of sodium and increasing concentrations of creatinine(9). In stop flow during saline diuresis, as compared with mannitol diuresis, there is greater reabsorption of sodium and water. Consequently only a comparatively small quantity of fluid can be identified in the stop flow pattern as being primarily proximal. In addition, the assessment of proximal samples which have been altered by passage through distal sites after release of stop flow appears to be less reliable than the evaluation of distal samples which enter the collector with a minimum of further modification. For these reasons there has been no attempt to interpret proximal stop flow specimens in the present studies; the presentation of data and analysis which follow have been restricted to the distal portion of each stop flow pattern.

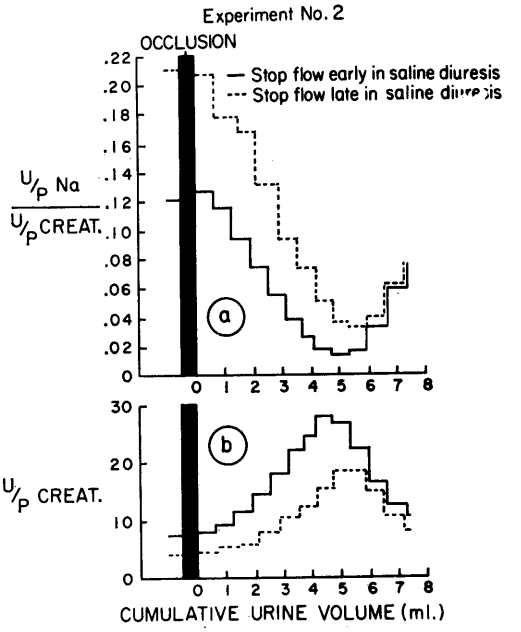

FIG. 1. Distal stop flow patterns obtained in left kidney early in the course of a progressive saline diuresis compared with patterns obtained later in diuresis. Urine volume was 5.10 ml/min prior to early occlusion and 6.75 ml/min prior to later occlusion.

A representative experiment is illustrated in Fig. 1. The free flow sodium/creatinine clearance ratios of 0.121 and 0.210 prior to the

TABLE I. Summary of Experiments in Which Stop Flow Was Performed Early (E) and Late (L) in the Course of a Progressive Saline Diuresis.

Exp No.		Free Flow U/P Na/U/P Cr	Distal minimum U/P Na/U/P Cr	Maximum U/P Cr	GFR, ml/min	V, ml/min	Plasma Na, mEq/l
1	E	.100	.007	42.1	41.2	6.85	150
	L	.215	.021	19.4	28.1	8.32	176
2	E	.121	.013	27.9	38.2	5.10	155
	L	.210	.033	18.4	27.6	6.75	158
3	E	.039	.008	21.9	29.0	4.23	159
	L	.194	.039	9.6	26.0	7.53	175
4	E	.083	.012	62.7	54.5	4.36	143
	L	.149	.015	43.4	60.4	9.60	143
5	E	.081	.033	40.5	64.2	3.88	151
	L	.132	.045	23.0	67.6	8.13	151
6	E	.053	.009	8.7	20.5	3.88	120
	L	.140	.021	8.5	29.9	6.69	127
Mean	E		.014	34.0			
	L		.029	20.4			
	P		$<.02$	$<.01$			

Cr = creatinine; GFR = glomerular filtration rate; V = urine volume.

early and late stop flow collections, respectively, indicate that 12.1% and 21% of the filtered sodium load escaped tubular reabsorption. Similar findings present in all studies indicate that net fractional sodium reabsorption was significantly lower at the time of the late stop flow. Although a distal minimum sodium clearance ratio of 0.013 was obtained in the early stop flow study, a minimum of only 0.033 was observed in the late stop flow. A comparable elevation of the minimum Na clearance ratio in the late stop flow pattern was present in all studies (Table I) and was statistically significant ($P<0.02$). In addition, all specimens from sites distal to the minimum demonstrate a substantial elevation of sodium clearance ratio in the late as compared to the early stop flow (Fig. 1 (a)). This was a consistent finding in all late stop flow patterns. The results obtained were independent of changes in GFR and plasma sodium which may have occurred during the course of the saline diuresis (Table I).

In Figure 1 (a) it is noted that the minimum sodium clearance ratio appears at a cumulative urine volume of 5 ml in the early stop flow as compared to 5.7 ml in the late stop flow. The observation that the minimum clearance ratio appears in a slightly more proximal position in the late stop flow pattern was a constant finding and is probably due to increased pelvic dead space and perhaps greater tubular distention at higher urine flow rates.

U/P creatinine values, illustrating the relative extent of net water reabsorption in early and late stop flow collections, are represented in Fig. 1 (b). A maximum U/P creatinine of 27.9 is achieved in the early stop flow compared to 18.4 in the late stop flow. A reduction in maximum U/P creatinine was a consistent finding in all experiments and was statistically significant ($P<0.01$), (Table I). As well, in all studies there were decreased U/P creatinine values in the late stop flow in all specimens distal to the maximum. The magnitude of the difference between the early and late patterns tended to increase as the site of maximal water reabsorption was approached.

Discussion. The present stop flow studies indicate that saline loading in hydropenic dogs consistently raises the distal minimum sodium/creatinine clearance ratio. This observation provides evidence for an inhibition of fractional sodium reabsorption in the distal convoluted tubule during the course of a saline diuresis. Jaenike and Berliner have suggested that under stop flow conditions during saline diuresis, the collecting ducts (represented in the stop flow pattern as the

most distal samples) are also a site of sodium transport(9). Insofar as this assumption is correct, it appears that the distal convoluted tubule and all sodium reabsorptive areas distal to this site show a decrease in sodium reabsorptive ability during a relatively prolonged tubular contact time of up to 5 minutes.

The striking decreases in stop flow distal creatinine U/P values which occur following saline loading also support the notion of impaired distal reabsorptive capacity for water. Since a portion of the water reabsorbed distal to the ascending limb of the loop of Henle is solute obligated under hydropenic conditions, decreased distal sodium reabsorption would be expected to be associated with diminished water reabsorption. It is possible, however, that at least part of the lowering of distal U/P creatinine in the late stop flow pattern is accounted for by diminished reabsorption of solute free water at the collecting duct. This could arise as a result of an inhibition of sodium reabsorption at the ascending limb with a consequent reduction in medullary solute content. Unfortunately, the ascending limb cannot be identified in stop flow patterns.

The capacity to demonstrate an inhibition in sodium reabsorption during stop flow suggests that the inhibitory process is not a flow rate dependent phenomenon. Moreover, it is likely that an inhibition which is apparent under stop flow conditions in which tubular contact time is greatly prolonged would be even more impressive during free flow. Although the present studies strongly suggest an inhibition of distal tubular sodium and water reabsorption during a saline diuresis, the stop flow technique, for reasons discussed earlier, does not permit a reliable analysis of sodium reabsorption at more proximal sites.

In other reported experiments the administration of pitressin, mineralocorticoids, albumin, and angiotensin did not materially affect the magnitude or character of a saline diuresis (1,11,12). It is therefore unlikely that these factors are of major importance in the present studies.

Finally, it may also be noted that the interpretation of stop flow studies based on the administration of a diuretic agent superimposed on a saline diuresis is hazardous because of the specific alterations in stop flow patterns induced by a progressive saline diuresis alone.

Summary. The stop flow method was used to determine the effects of saline loading on distal renal tubular function in hydropenic dogs. Stop flow experiments were performed in 6 dogs early and late in the course of an increasing diuresis induced by saline loading. Distal tubular stop flow patterns were compared with regard to the sodium clearance ratio (U/P Na/U/P creatinine) and U/P creatinine values. Stop flow patterns obtained late in the diuresis consistently showed elevations of all distal sodium clearance ratios, including the minimum, and decreases in all U/P creatinine values. These data suggest that an inhibition of sodium and water reabsorption develops in the distal tubule during saline loading. This could be characterized only as a fractional rather than an absolute decrease in distal reabsorption. It appeared that the inhibition was not a flow rate dependent phenomenon. Reabsorption at other tubular sites was not evaluated because of limitations inherent in stop flow methodology.

The authors appreciate the helpful participation of Dr. Richard M. Stein in preparation of this manuscript. The technical assistance of Edith Neubert and Sarah Chipoco is gratefully acknowledged.

1. Rector, F. C., Jr., Van Giesen, G., Kiil, F., Seldin, D. W., J. Clin. Invest., 1964, v43, 341.

2. Dirks, J. H., Cirksena, W. J., Berliner, R. W., ibid., 1965, v44, 1160.

3. Watson, J. F., Am. J. Physiol., 1966, v210, 781.

4. Stein, R. M., Bercovitch, D. D., Levitt, M. F., ibid., 1964, v207, 826.

5. Earley, L. E., Friedler, R. M., J. Clin. Invest., 1964, v43, 1928.

6. Giebisch, G., Klose, R. M., Windhager, E. E., Am. J. Physiol., 1964, v206, 687.

7. Lassiter, W. E., Mylle, M., Gottschalk, C. W., ibid., 1964, v206, 669.

8. Malvin, R. L., Wilde, W. S., Sullivan, L. P., ibid., 1958, v194, 135.

9. Jaenike, J. R., Berliner, R. W., J. Clin. Invest., 1960, v39, 481.

10. Levitt, M. F., Levy, M. S., Polimeros, D., ibid., 1959, v38, 463.

11. De Wardener, H. E., Mills, I. H., Clapham, W. F., Hayter, C. J., Clin. Sci., 1961, v21, 249.

12. Levinsky, N. G., Lalone, R. C., J. Clin. Invest., 1963, v42, 1261.

Received January 27, 1967. P.S.E.B.M., 1967, v125.

Effect of Various Hormones on Mammary Gland Growth of Ovariectomized Rats. (32144)

PERIANNA KUMARESAN* AND C. W. TURNER†
(With the technical assistance of Mary E. Powell)
Department of Dairy Husbandry, University of Missouri, Columbia

Study of the hormones which stimulate mammary gland growth has been aided greatly by estimation of the deoxyribonucleic acid (DNA) content of the glands. It has been shown that pregnant rats at the end of pregnancy and ovariectomized rats stimulated with estradiol benzoate (EB) plus progesterone (P) show great variation in the total DNA/100 g bw. The theory has been advanced that part of the variation in the total DNA of the glands of individual rats is due to variation in the secretion rates of various hormones from the pituitary and the placentae of pregnant rats.

In a previous study(1) the mean total DNA of the mammary glands of rats at the end of pregnancy was 7.41 mg/100 g bw. In the present study (Table I) the mean total DNA of a group of ovariectomized rats administered 2 μg EB + 6 mg P for 19 days was 5.57 mg; thus, the pregnant animals showed 33% greater DNA than the rats administered the 2 ovarian hormones. While it is believed the EB + P are of primary importance in stimulating growth of the mammary gland during pregnancy, these data suggest that these hormones may not be able to stimulate increased secretion of other hormones in the absence of the placenta. These other hormones may synergize with EB + P in pregnant animals to increase the DNA of the mammary glands. If the administration of an exogenous hormone has a greater synergistic effect upon DNA in the ovariectomized rats than upon the pregnant animal, it would suggest that the pregnant condition is stimulating the secretion of more endogenous hormone.

In a previous report the effect of various hormones on mammary gland growth of pregnant rats was presented(1). The object of the present report is to present data on the effect of these same hormones in synergism with EB + P on the DNA of the mammary glands of ovariectomized rats.

Materials and methods. Groups of adult ovariectomized rats of the Sprague-Dawley-Rolfsmeyer strain with a mean body weight of 245 g were maintained in a room at 78 $\pm$ 1°F. Purina Lab Chow with an energy value of 4.41 calories per gram and 23.4% total protein was fed during control and experimental periods. Ovariectomized rats were allowed 7 days for recovery and were sacrificed on day 27 after castration.

EB and P were dissolved in sesame oil (USP) and injected daily subcutaneously in 0.2 ml of oil for 19 days. The other hormones were dissolved in alkaline physiological saline solution and were injected subcutaneously daily at approximately the same time: protamine zinc insulin‡ 1 unit/0.1 ml, L-T_4 3 μg/0.1 ml/100 g bw, and GH 1 mg/0.1 ml. Bovine growth hormone (GH)§ was injected in increasing doses of 1 mg from day 1 to 6, 2 mg from day 7 to 12, and 3 mg from

* Present address: Division of Metabolism and Endocrinology, Coney Island Hospital, Brooklyn, N. Y.

† Contribution from Missouri Agr. Exp. Sta. Journal Series 4007. Approved by Director. Aided in part by a USPHS grant.

‡ Kindly supplied by Eli Lilly Co., Indianapolis, Ind.

§ Kindly supplied by NIH, Bethesda, Md. It was reported to contain 1.17 μ/mg GH and contaminated with 0.4 μ/mg of lactogenic hormone.