The Influence of Dietary Sodium Chloride on *in Vitro* Renin Release from Rat Kidney Slices[1] (35836)

B. BRAVERMAN,[2] R. H. FREEMAN, AND H. H. ROSTORFER

Anatomy and Physiology Department, Indiana University, Bloomington, Indiana 47401

Recent studies of renin release *in vitro* have shown that glucose may be a necessary component of the media (1, 4). Inhibitors of protein synthesis seem to have no effect on renin release *in vitro* (3, 4). Epinephrine, norepinephrine, and adenosine-3′,5′-cyclic monophosphoric acid (cyclic AMP) have been shown to increase the total renin production in kidney homogenates (11). Aldosterone seems to inhibit renin release *in vitro* (4) while increasing tissue concentrations of sodium chloride stimulate renin release *in vitro* (8). Renin release from surviving rat kidney slices has also been demonstrated by Oelkers *et al.* (14) to decrease with decreasing sodium chloride concentration in the media, and dependency of release on the sodium chloride did not seem to require aerobic conditions.

In the present study, the release of renin from surviving rat kidney cortical slices immersed in Robinson's solution (15) was quantified in Goldblatt units by use of a zero order kinetic assay. Release was determined under aerobic, anaerobic, and metabolically inhibited states for slices obtained from rats on a normal dietary sodium intake and from rats maintained for 14 days on a low sodium diet. The relationship between the renin content of the slice and the amount of renin released on incubation and the relationship between renin release and the sodium concentration in the media were investigated in the two dietary cases.

Methods. Sprague-Dawley rats were maintained on either a control diet or a General Biochemical TD-68503 sodium deficient diet for 14 days. In each experiment, a rat weighing between 250 and 300 g was ether anesthetized and renalectomized. The right kidney was cooled in the isotonic medium on cracked ice. The poles of the kidney were removed and 12 slices, 50 to 70 mg wet weight (approx 0.3 to 0.4 mm thick), were prepared on a tissue microtome and placed in cold Robinson's medium. The medullary and connective tissue were carefully removed and the cortical tissue was rinsed in Robinson's medium, touched to filter paper, and then placed in Warburg vessels, one slice to each vessel, containing 3 ml of Robinson's medium and 0.2 ml of 20% KOH in the center well to absorb carbon dioxide. The vessels were then shaken (100 cycles/min) in the water bath (Bronwill Model UV Warburg apparatus) at 37° and equilibrated with the desired atmosphere for 20 min. After an additional 10-min equilibration the flasks were closed, and tissue slices were incubated for periods of 10, 60, or 120 min with manometer readings taken every 10 min. After incubation the slices were separated from the suspending medium, gently blotted on filter paper, weighed, and then stored frozen at −20° for analysis of tissue renin content and total protein determinations. The medium was centrifuged in a Sorvall RC2-B refrigerated centrifuge at 12,000*g* for 10 min at 5°. The supernatant was stored frozen at −20° for renin analysis.

Assay of the renin content of the incubated slices and of the medium in the incubation flasks, expressed as Goldblatt units/mg of protein, was carried out by means of the zero order kinetic method as previously described by Braverman *et al.* (2). Briefly, a sample of 0.25 ml of diluted extract or undiluted medium was added to 0.75 ml of concentrat-

[1] This work was supported by Research Grant HE 05625 from U.S. Public Health Service.

[2] Present address: Department of Physiology, M-412 Medical Sciences Building, Columbia, Mo. 65201.

TABLE I. The Effect of Excess K in the Media (K-medium) During Cold (2–3°) Treatment Prior to Incubation at 37° in Robinson's Solution on Renin Release (RR) in Comparison with Cold Treatment in Robinson's Media.

Incubation period (min)	RR (GU/mg-protein × 10^{-4})		Q_{O_2} (μl of O_2/mg-protein)		RC (GU/mg-protein × 10^{-4})		%RR (RR/RR+RC) × 100	
Medium (2–3°):	Potassium	Robinson's	Potassium	Robinson's	Potassium	Robinson's	Potassium	Robinson's
10	7.58	9.10	1.84	2.21	133.60	107.00	9.13	8.79
SE	±2.12	±2.02	±0.47	±0.13	±40.6	±22.41	±3.08	±1.53
N	6	6						
60	17.61	18.15	16.40	16.91	95.56	112.20	16.29	15.22
SE	±3.58	±3.40	±0.73	±1.19	±13.92	±16.32	±2.01	±1.73
N	12	12						

No significant differences between any group. RC = renin content of tissue; SE = standard error of the mean; and *N* = number of samples.

ed dog substrate and dialyzed against 0.01 *M* EDTA for 6 hr and 0.02 *M* phosphate buffer (pH 5.3) for 16 hr at 5°. Just prior to incubation at 37°, 0.025 ml of saturated sodium chloride and 0.026 ml of 40 mg/ml diisopropyl fluorophosphate solution (Calbiochem) were added to the sample. Samples were incubated for an appropriate time to produce an adequate amount of angiotensin to measure on the rat bioassay (18). The enzymatic reaction was stopped by placing the sample in boiling water for 10 min. The volume was raised to 2 ml by the addition of buffer (0.04 *M* phosphate plus 0.11 *M* NaCl at pH 8.3). Finally, the sample was thoroughly mixed, centrifuged, and the supernatant was stored frozen at —20° for the bioassay of the angiotensin formed during incubation. Renin concentration was determined by application of the zero order equation $(R) = (\text{angiotensin}/KT)$, where (angiotensin) is the concentration of the angiotensin[3] formed on incubation, K is the rate of angiotensin formed per Goldblatt unit of rat renin[4] at zero order dog substrate concentration and T is incubation time (2).

The slices were homogenized (Tri-R tissue homogenizer), and renin extracts were prepared in distilled water plus 50 mg/100 ml of sodium azide to inhibit mold growth.

Total protein content of the slice was determined on an aliquot of each extract diluted fourfold with 0.1 *N* NaOH and analyzed for protein content by the Lowry *et al.* technique (10) on a Technicon AutoAnalyzer. Similar analyses of soluble protein in the media were performed.

Results. Mudge (12) found that kidney slices in an ice-cold solution lost potassium and gained sodium. Since kidney slices were kept in chilled Robinson's solution before incubation at 37°, the effect of chilling the tissue on renin release was investigated using either Robinson's medium or a buffered potassium medium (potassium = 158 mEq/liter and sodium = 5 mEq/liter), which would

[3] Kindly supplied by Dr. Albert J. Plummer, Ciba Pharmaceutical Co., Summit, New Jersey.

[4] Standard rat renin was kindly provided by Dr. Erwin Haas, the Mt. Sinai Hospital of Cleveland, University Circle, Cleveland, Ohio.

prevent the tissue loss of potassium while the tissue was chilled. Slices from one kidney were divided into two groups, one of which was incubated in 20 ml of the potassium media for 10 min at 2 to 3° and the other in Robinson's media at the same temperature. After initial treatment in the cold, the slices were placed in Warburg vessels in Robinson's medium, one slice to each vessel, equilibrated with 100% oxygen, and incubated at 37°. Slices were then removed after 10 min or 60 min. No significant difference (Student's *t* test) was found in terms of Q_{O_2}/mg of protein, renin release per milligram of total protein (GU/mg of protein) or renin release expressed as a percentage of total renin [%(RR/RR+RC)] (see Table I).

Metabolic aspects of renin release in vitro. The effects of absence of oxygen in the presence of inhibitors of anaerobic glycolysis were determined in a 100% nitrogen atmosphere with chromous sulfate (0.2 ml) added to the side arms of the Warburg vessels to remove any residual oxygen, and sodium arsenate (25 mmoles/liter) and potassium *meta*-arsenite (1 mmoles/liter) added to the Robinson's medium. The results (Fig. 1) show that renin release is inhibited during 120 min of incubation in 100% nitrogen plus arsenate and arsenite. When the inhibitors were left out of the medium and the nitrogen in the vessels was washed out after 90 min of incubation by sparging with oxygen, there was a marked increase in renin release in the following hour; thus, the tissue could still respond after incubation in nitrogen and the renin liberated from the slices sparged with oxygen was twice that liberated from the

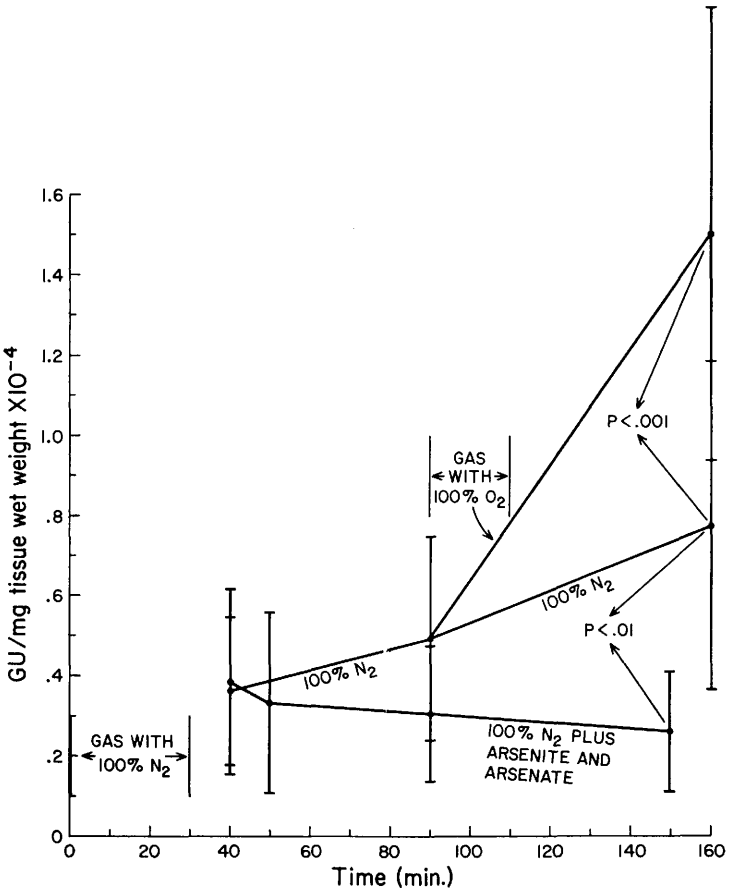

FIG. 1. The influence of anaerobic and aerobic metabolism and metabolic blockade on *in vitro* release of renin from kidney cortical slices taken from rats on normal sodium diet.

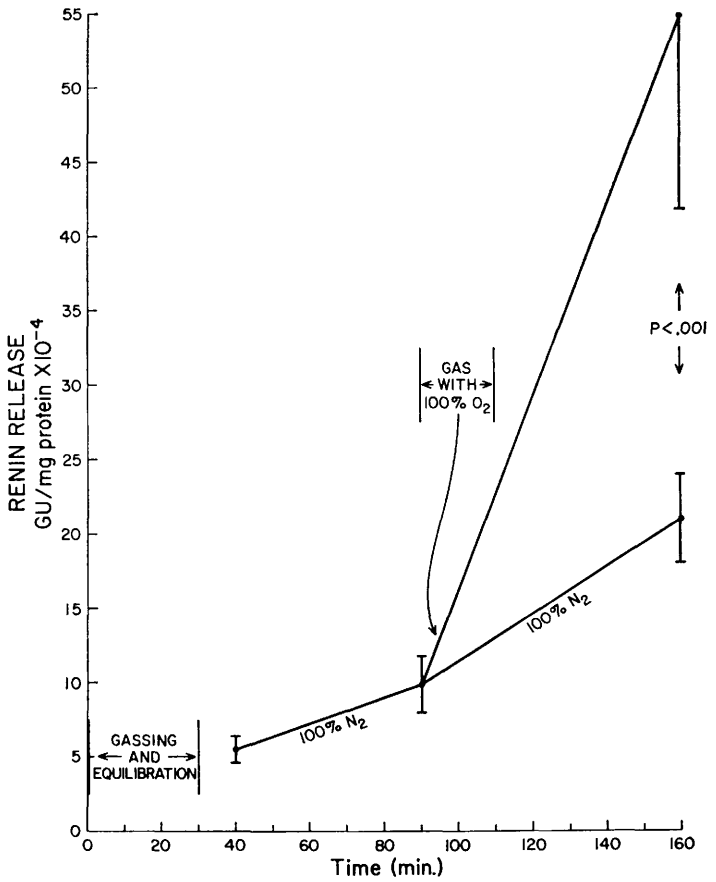

FIG. 2. The influence of anaerobic and aerobic metabolism on the release of renin from kidney cortical slices removed from rats after 14 days on sodium deficient diet.

slices that remained in an atmosphere of 100% nitrogen ($p<.001$).

The influence of magnitude of the renin content of kidney on the behavior of renin release *in vitro* from kidney slices was investigated under the conditions of anaerobic and aerobic environments. Since is is known that rats on a low sodium diet exhibit markedly increased renin content of the kidey (7), rats which were maintained on a sodium deficient diet for 14 days served as donors for cortical slices. The results show that renin release from the cortical slices from rats on a sodium deficient diet (Fig. 2) and normal diet (Fig. 1) behave in the same manner. The percentage of the total renin in the tissue slices which was released into the medium was similar to that for slices from rats on a normal diet under similar circumstances, and slices from the sodium deficient animals behaved similarly when incubated in vessels containing 1 atm of 100% nitrogen and subsequently sparged with 100% oxygen. However, on the basis of GU/mg of tissue protein, the slices from rats on a sodium deficient diet released about three times more renin into the medium on incubation *in vitro* (Table II).

The effects on in vitro *renin release of alteration of the sodium chloride concentration of the media.* Three media were prepared in the same manner as Robinson's medium except that the sodium concentrations were 20, 100, and 160 mEq/liter. The media were adjusted to 300 mOsm/liter with mannitol. Slices from four normal rats and five rats on a sodium deficient diet for 14 days were incubated in 100% oxygen at 37°

TABLE II. Effect of Medium Sodium (Na) on Renin Content (RC), Renin Release (RR), Total Renin (TR), Percentage Renin Released (%RR) and Net Change in Total Renin (ΔTR) for Slices from Rats on Normal and Low Sodium Diets.

Incubation time (min)	Na (mEq/liter):	20				100				160			
		RC	RR	TR	%RR	RC	RR	TR	%RR	RC	RR	TR	%RR
						Normal diet							
60		133	9.9	142.9	6.2	123.5	12.6	136.0	9.1	125.3	13.3	138.6	10.0
	SE	±8.0	±0.97		±0.75	±9.2	±1.3		±1.3	±9.6	±0.93		±0.92
	N	12	12			12	12			12	12		
	p	—	—		—	—	—		—	NS	.02		.02
10		121	3.0	124.0	2.7	94.3	4.5	98.7	3.8	119.0	7.2	126.2	5.5
	SE	±27.4	±0.46		±0.59	±5.3	±0.46		±0.83	±13.9	±1.8		±0.74
	N	4	4			4	4			4	4		
	P									NS	NS		NS
	ΔTR			+18.9				+37.3				+12.4	
	p			NS				.05				NS	
						Low sodium diet							
60		307.5	25.3	332.7	8.4	298.5	33.2	331.7	10.9	293.0	39.4	332.4	13.3
	SE	±29.6	±2.1			±28.4	±3.0			±42.0	±4.1		
	N	15	15			15	15			15	15		
	p	—	—		—	NS	.05		NS	NS	.005		.01
10		331.1	10.9	341.9	4.5	288.3	17.8	306.7	6.4	326.1	23.2	349.3	7.1
	SE	±100.7	±1.9			±48.5	±3.4			±6.69	±5.3		
	N	5	5			5	5			5	5		
	p	—	—		—	NS	NS			NS	NS		NS
	ΔTR				−9.2			+25.6				−17.0	
	p				NS			NS				NS	

p = comparison of data at 100 mEq of Na; liter and 160 mEq of Na/liter to date at 20 mEq of Na/liter; SE = standard error of the mean; RC, RR, and TR = GU/mg of protein × 10^{-4}; *N* = number of samples.

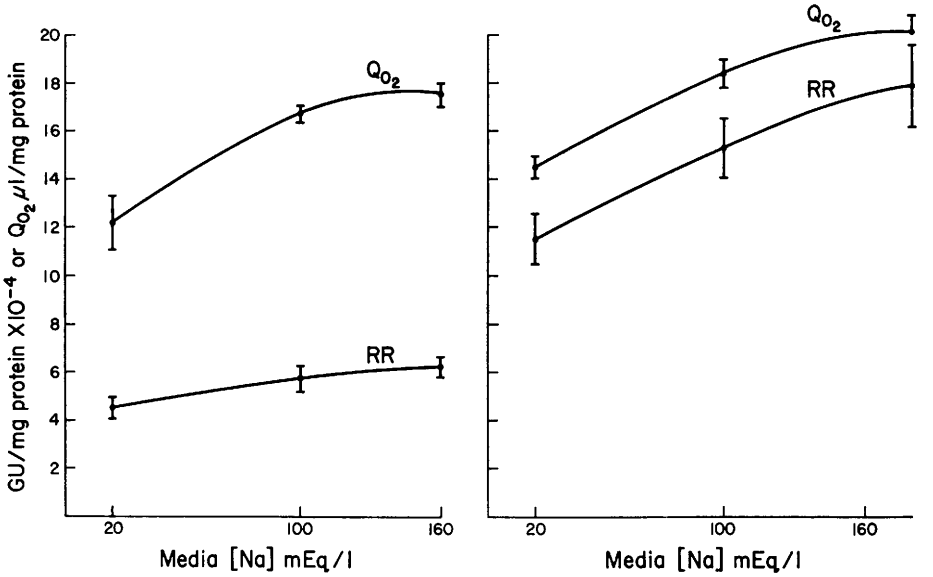

FIG. 3. The relationship between renin release (RR) and Q_{O_2} in rat kidney slices immersed in media at three levels of sodium (20, 100, and 160 mEq/liter) at a total osmolar concentration of 300 mOsm: (left) rats on normal diet; (right) rats on low sodium diet for 14 days. Duration of incubation was 60 min. Differences between values at 20 and 160 mEq of Na/liter are significant, $p<.05$ to $<.001$.

for 10 or 60 min, after equilibration, in each of the three media. Increasing the sodium concentration in the media resulted in an increase in renin release per milligram of protein and percentage of the total renin released after 10 and 60 min of incubation (Table II). The mean values for the percentage renin released and the renin release per milligram of protein for slices from normal rats incubated for 60 min in solutions containing 20 and 160 mEq of Na/liter were significantly different as were the mean values for renin release per milligram of protein for slices from 14-day sodium deficient rats incubated for 60 min at 20 and 100 mEqNa/liter. It should be noted that the Q_{O_2} for the tissues from both groups of rats also increase significantly with increased sodium concentration in the media (Fig. 3).

The amount of renin released from the slices obtained from the sodium deficient rats was nearly three times larger than that from rats on a normal sodium intake. This difference held at all levels of sodium in the media. Despite this fact, the percentage of renin released, RR/(RC+RR) × 100, was insignificantly different; *i.e.*, the amount of renin released per unit of renin in the slices

TABLE III. Comparison of the Percentage of the Total Renin Content and the Total Protein Content of Kidney Slices "Released" into the Media in the Presence of 20 or 140 mEq of Na/Liter During Incubation at 37°.

Medium (Na):	20 mEq/liter		140 mEq/liter	
Incubation time (min)	%RR	%PrR	%RR	%PrR
60	(A) 9.02 ± 3.98	(E) 6.32 ± 0.30	(B) 14.16 ± 4.56	(F) 5.29 ± 0.62
0	(C) 4.68 ± 2.31	(G) 4.68 ± 0.77	(D) 4.39 ± 1.23	(H) 3.68 ± 0.43
Diff.	4.54	1.64	9.77	1.61

$N = 6$ in each case; probabilities: B > A, $p < .05$; C > D, NS; A > C, $p < .025$; B > D, $p < .001$; E > F, $p < .005$; G > H, $p < .005$.

remained constant.

The relationship between the renin released and the protein "released" from tissue slices taken from rats maintained on a normal sodium diet are shown in Table III. Slices were suspended in media containing either 20 or 140 mEq of Na/liter. During the preincubation period (zero time) both renin and protein were lost from the tissues at approximately the same rates with only a slightly higher rate of liberation at the lower sodium concentration in the medium. However, during the following 60 min of incubation only a small additional liberation of protein occurred at each level of sodium; whereas, renin was released to a much greater extent, and the release at 140 mEq of Na/liter was significantly greater than the release at 20 mEq/liter.

The possibility of renin synthesis occurring in surviving tissue is also indicated in Table II in which the gain or loss (ΔTR) in the total renin contents (RC+RR) during a 50-min incubation in 20, 100, and 160 mEq of Na/liter are compared. There was a net gain in renin content for the tissues from rats on normal diet, but significance can be attached only to the results obtained for incubation in 100 mEq of Na/liter.

Discussion. These results suggest that renin release from surviving rat kidney slices is dependent upon energy metabolism of the tissues and therefore is an active process. A comparison of *in vitro* release of renin and *in vitro* liberation of protein indicates that, although there is marked liberation of each during the equilibration period, there is in the subsequent incubation period no correlation between the amount of protein liberated and the amount of renin released. Thus, it would appear that there is some washout of both renin and protein during the equilibration period, while in the following period the renin which is released results from an active process. This is in keeping with the results of DeVito *et al.* (4, 5).

At present an active release of renin under anaerobic conditions cannot be ruled out, but the results indicate that the aerobic release is much larger. When all metabolism has been stopped no release occurs.

An influence of the concentration of the sodium chloride in the media on *in vitro* renin release is clearly evident. A similar relationship has been found very recently by Karsunky *et al.* (8) and Oelkers *et al.* (14). However, this finding may not represent a direct causative relationship between sodium and renin release. In the present study an increase in the oxidative metabolism of the tissue with increasing salt concentration in the media is evident. The work of Vander and Miller (17) and the results presented by Nash *et al.* (13) indicate renin release *in vivo* to be indirectly related to the sodium load presented to the kidney. The *in vitro* studies indicate a direct relationship between sodium concentration and renin release. The *in vitro* and *in vivo* results are not necessarily incompatible with the macula densa theory of the control of renin release, since the sodium concentration in the early distal tubule was unknown in the *in vivo* work cited.

It is clearly shown and has been known for some time (7) that a dietary sodium deficit will cause a marked increase in the renin content in the kidney. However, it will be noted that the slices removed from kidneys taken from rats on a low sodium diet and from rats on a normal diet responded very similarly with respect to the percentage of the renin content which was released. It would appear either that the number of releasing sites increased with the increase in renin content and that the sodium "influence" *in vitro* on these sites was identical, or that this "influence" of the sodium on release was mediated through the metabolic action of sodium on the cell and bears no direct action on renin release. This metabolic influence probably results from increased sodium pumping by the surviving cells with increasing sodium concentrations in the media (9, 19).

The possibility of renin synthesis (+ΔTR) taking place *in vitro* is illustrated in Table II for tissues from normal diet rats. Thus, one must raise the question as to the source of the renin which is released into the media. That is, does the renin released originate from stored renin or is it the result of an immediate synthesis? Since the release is

three times higher from tissue removed from the sodium deficient rats and the renin content is three times higher, the synthesis of renin could be proceeding more rapidly in these tissues. The release of stored renin is not clearly evident since no significant loss in renin (—ΔRC) occurred.

Note that the release of renin *in vitro* represents between 10 and 15% of the renin content in 1 hr of incubation. This, as indicated by DeVito (4), appears to be excessive in comparison to *in vivo* release. Schneider *et al.* (16) measured the metabolic rate of disappearance of renin in the dog and found a half-life of 30 min. One might expect the half-life in the rat to be considerably shorter. Even so, if a half-life of 5 min is taken as the shortest possible time and if the renin content of the two kidneys lies between 3 and 6 Goldblatt units (6) and renin is distributed throughout the extracellular water (16), the renin concentration of the plasma based upon the rate of *in vitro* renin release can be calculated to lie between 0.75×10^{-3} GU/ml, approximately 10 times the concentration found for the rat by Gould *et al.* (6). Since very similar values for renin content of rat kidneys have been found by the method of renin assay used in the present study (2), it would appear that renin release *in vitro* is maximal and is related to the level of metabolism; thus, it is possibly uncontrolled. One might argue that the *in vivo* controls on renin release are largely inhibitory and in the removal or the slicing of the tissues either some inhibitory substance is removed or some anatomical arrangement which is regulative is disrupted.

Summary. In vitro renin release from surviving rat kidney slices, expressed in Goldblatt units/mg of protein or as percentage of the total renin content of the slice, was studied under anaerobic and aerobic conditions and in the presence of arsenite/arsenate blockade. Renin content and renin release from slices from donor rats kept on a sodium deficient diet were three times larger than for slices from rats on a normal diet. The release, expressed as a percentage of the total renin content, was the same. No release occurred under metabolic blockade, and release was much larger under aerobic than anaerobic conditions. Both the quantity and the percentage of the total renin content which was released as well as the Q_{O_2} decreased with decreasing sodium concentrations in the media. Under some conditions an apparent net synthesis of renin occurred. *In vitro* renin release is metabolically dependent, related to the quantity of renin stored in the slice, and in some manner influenced by the presence of sodium chloride. *In vitro* release appears to be much larger than *in vivo* release and is probably always maximal for a given metabolic state.

1. Bozovic, L. J., and Efendic, S., Acta Endocrinol. **60**, 550 (1969).

2. Braverman, B., Freeman, R. H., and Rostorfer, H. H., Proc. Soc. Exp. Biol. Med. **134**, 67 (1970).

3. Bunag, R. D., and Page, I. H., Physiologist. **12**, 187 (1969).

4. DeVito, E., Gordon, S. B., Cabrera, R. R., and Fasciolo, J. C., Amer. J. Physiol. **219**, 1036 (1970).

5. DeVito, E., Cabrera, R. R., and Fasciolo, J. C., Amer. J. Physiol. **219**, 1042 (1970).

6. Gould, A. B., and Goodman, S. A., Lab. Invest. **22**, 443 (1970).

7. Hartroft, P. M., *in* "Angiotensin Systems and Experimental Renal Diseases" (J. Metcaff, ed.), p. 5. Little, Brown, Boston (1963).

8. Karsunky, K. P., Fischinger, J., and Taugner, R., Pfluegers Arch. **312**, R89 (1969).

9. Kramer, K., and Deetjen, P., *in* "Oxygen in the Animal Organism" (F. Dickens and E. Neil, eds.), p. 411. Macmillan Co., New York (1964).

10. Lowry, O. H., Rosenbraugh, M. J., Farr, A. L., and Randall, R. J., J. Biochem. **193**, 265 (1951).

11. Michelakis, A. M., Caudle, J., and Liddle, G. W., Proc. Soc. Exp. Biol. Med. **130**, 748 (1969).

12. Mudge, G. H., Amer. J. Physiol. **165**, 113 (1951).

13. Nash, F. D., Rostorfer, H. H., Bailie, M. D., Wathen, R. L., and Schneider, E. G., Circ. Res. **22**, 473 (1968).

14. Oelkers, W., Molzahn, M., Samwer, K. F., Kreiser, H., and Hutschke, H., Pfluegers Arch. **321**, 67 (1970).

15. Robinson, J. R., J. Biochem. **45**, 68 (1949).

16. Schneider, E. G., Rostorfer, H. H., and Nash, F. D., Amer. J. Physiol. **213**, 1115 (1968).

17. Vander, A. J., and Miller, R., Amer. J. Physiol. **207**, 537 (1964).

18. Wathen, R. L., Kingsbury, W. S., Stouder, D. A., Schneider, E. G., and Rostorfer, H. H., Amer. J. Physiol. **209**, 1012 (1965).

19. Weinschelbaum de Jairala, S., Biochim. Biophys. Acta **79**, 205 (1964).

Received March 11, 1971. P.S.E.B.M., 1971, Vol. 138.