FURTHER OBSERVATIONS UPON THE CHANGES IN THE ELECTROLYTES OF THE URINE FOLLOWING THE INJECTION OF PARATHYROID EXTRACT [1]

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From previous studies (1, 2, 3, 4, 5) it now seems clear that one of the most striking changes found in the urine after the injection of parathyroid extract is the immediate increase in the absolute amount and in the concentration of inorganic phosphate. Goadby and Stacey (6) have also emphasized the increased excretion of phosphate after the administration of parathyroid extract. In agreement with our experience, these observers did not find at any time an increase in the level of inorganic phosphorus in the plasma, but occasionally noted a fall of that level after the excretion of increased amounts of inorganic phosphate in the urine.

They were unable to prevent the rise in excretion of urinary phosphorus by lowering the level of inorganic phosphate in the plasma with glucose administered at the time of the parathormone injection. The work of Goadby and Stacey, together with ours, serves to direct attention to the behavior of the kidneys in response to parathyroid extract.

In 1934, while studying the phosphate excretion by the kidney, one of us (5) observed that, when urine specimens were taken hourly before and after the administration of parathyroid extract, the pH showed a tendency to shift definitely to the alkaline side during the first hours after the extract was given. In order to examine this effect further it seemed advisable, although the bicarbonate of urine may be roughly estimated from the pH, to determine accurately the bicarbonate content of the urine before and after giving parathyroid extract and to compare the magnitude of any change found with that of the inorganic phosphate.

At the same time estimations of pH, sodium, potassium, ammonium, phosphate, and chloride ions were made. As previous estimations have shown no significant alteration in urinary calcium, in experiments of the duration of the present ones, urinary calcium determinations were not done.

EXPERIMENTAL

The subjects of the experiment were four male patients; three (G., S., K.) were convalescent from respiratory infections and ready for discharge from the hospital, the fourth, M., was a patient who had been operated upon for hyperthyroidism and who had developed hypoparathyroidism after operation. All patients were fasted and kept in bed for 12 hours before and throughout the duration of the experiment. Each patient was given 100 cc. of water by mouth each hour. Urine specimens in the three convalescent subjects were collected under oil, those of M. were not. The specimens were collected hourly for three hours before and four hours after the intravenous injection of 4 cc. of parathyroid extract (Lilly).

The analyses were made by standard methods as follows: pH by the quinhydrone electrode (13); CO_2 content by the Van Slyke method (14); chloride by the Volhard-Harvey titration (15); ammonium by the Van Slyke and Cullen method (16); potassium by the Kerr modification of the Kramer-Tisdall method (17); phosphorus by the Fiske and Subbarow method (18); sodium by the Butler and Tuthill application of the method of Barber and Kolthoff (19).

RESULTS

In 1931, Albright, Bauer and Aub (7) made observations of the total acid-base balance in an individual receiving parathyroid extract. Their studies were made in three-day periods and their data include intake and output balances of many

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of the electrolytes. They found an increase in water, chloride and total base of the urine during the periods of parathormone administration, as well as a decrease in the titratable acidity of the urine. In the periods studied by them, however, they concluded that the fluctuations, other than those of calcium and phosphorus, were probably not great enough to be " fundamental," although the decrease in water, total base and chloride excretion upon cessation of the administration of parathormone. This is a well-known effect. It does not occur invariably by any means (5), but is very often found. The period of diuresis in the four subjects was followed by a period of decreased urine volume.

Inorganic phosphorus. As in previous experiments, the urine specimens were collected hourly. Our results are shown in Table I. It will perhaps be advisable to discuss separately below, the various observations.

Water. In all four subjects there was a distinct diuresis during the first and second hours after the injection of parathormone. This is a

TABLE I

Urinary constituents in hourly specimens

	Volume				Total inorganic phosphate ‡												pH			
Subject*	G.	S.	K.	M.	G.	S.	K.	M.	G.	S.	K.	M.	G.	S.	K.	M.	G.	S.	K.	M.
Hourly specimen																				
	cc.	*cc.*	*cc.*	*cc.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mM.*	*mM.*	*mM.*	*mM.*	*m.eq.*	*m.eq.*	*m.eq.*	*m.eq.*				
1	105	78	77	100	23.8	12.2	21.0	52.5	0.76	0.39	0.54	1.4	0.8	0.6	0.9	2.2	5.5	6.8	6.4	6.4
2	125	128	68	100	32.7	12.9	30.2	50.5	1.05	0.41	0.78	1.3	1.2	0.6	1.2	2.5	5.8	6.8	6.1	6.9
3	122	120	163	70	39.4	9.2	57.6	35.0	1.26	0.29	1.47	0.9	1.5	0.5	2.4	1.9	6.0	7.1	6.4	7.2
	Parathyroid extract 4 cc. i.v.																			
4	235	185	250	150	84.7	44.0	88.2	100.2	2.72	1.40	2.25	2.6	4.6	1.9	5.1	6.2	7.1	7.2†	7.4	7.9
5	185	112	140	100	80.9	60.0	138.5	100.3	2.61	1.90	3.55	2.6	4.3	3.2	7.8	6.2	7.0	7.3	7.3	8.1
6	50	70	39	50	66.0	46.7	78.0	74.8	2.14	1.50	2.00	1.9	3.8	2.5	3.5	4.6	7.4	7.0	6.7	7.8
7	50	28	50	15	68.4		74.0	30.9	2.20		1.90	0.8	4.0		2.7	1.9		6.6	5.9	

	Total chloride				Total CO_2			Total HCO_3^- ¶			Total Na			Total K		Total NH_4			Titratable acid to pH 8.0
Subject	G.	S.	K.	M.	G.	S.	K.	G.	S.	K.	G.	K.	M.	G.	K.	G.	K.	M.	K.
Hourly specimen																			
	m.eq.	*m.eq.*	*m.eq.*	*m.eq.*	*mM.*	*mM.*	*mM.*	*m.eq.*	*m.eq.*	*m.eq.*	*m.eq.*	*m.eq.*	*m.eq.*	*m.eq.*	*m.eq.*	*m.eq.*	*m.eq.*	*m.eq.*	*cc. N/10*
1	15.2	10.8	12.6	11.8	0.10	0.28	1.0	0.02	0.24	0.67	9.8	8.6	12.1	3.4	6.0	1.5	0.8	2.6	5.5
2	18.5	13.9	10.1	12.2	0.17	0.58	0.3	0.06	0.49	0.15	12.2	7.7	10.2	5.0	4.1	1.1	1.0	1.5	11.7
3	20.5	12.4	11.8	11.4	0.20	0.99	0.8	0.09	0.90	0.53	15.2	9.0	7.2	5.6	5.9	1.0	1.1	1.0	14.0
	Parathyroid extract 4 cc. i.v.																		
4	21.3	11.6	18.7	17.1	6.00	1.70	8.0	5.40	1.60	7.60	20.4	18.0	21.3	11.5	12.7	0.1	0.6	0.4	7.1
5	9.0	10.8	11.4	8.5	3.10	2.00	4.8	2.70	1.90	4.50	9.8	12.8	14.7	7.4	10.3	1.0	0.9	0.1	2.0
6	5.0	8.6	4.0	4.4	0.80	0.60	0.6	0.76	0.50	0.48	5.7	5.4	8.1	5.4	3.4	0.5	0.6	0.4	13.4
7			4.9				0.1			0.04					3.5		1.3		27.0

* No provision made in Patient M. for preventing escape of CO_2 from urine specimens.

† This value not determined; approximate value estimated from total H_2CO_3 and average free CO_2 of 1.9 mM. per liter.

‡ M. eq. of total inorganic phosphate calculated from mM. of total inorganic phosphate and determined pH values.

¶ HCO_3^- calculated from pH and total CO_2 ($HCO_3^- + H_2CO_3$) using chart from Peters and Van Slyke (12).

parathyroid extract was sufficient to cause them to comment upon the fact. Our experiments differ very distinctly from theirs in several ways. Albright, Bauer and Aub did not determine the bicarbonate or the pH of the urine, which con-

ence, a prompt and very definite increase in the excretion of inorganic phosphate followed the injection of the hormone. This is shown in the table in milligrams, millimoles and in milliequivalents per hour. The increase in the 4th, 5th, and 6th hours of the experiment is shown clearly in all forms of recording. As the urine becomes more alkaline the ratio of HPO_4 to H_2PO_4 increases so that, when expressed in milliequivalents, the increase in phosphate excretion appears most striking. In the four experiments, although a diuresis occurred at the same time, the increase in inorganic phosphate was sufficient in nearly all specimens, taken after the extract was given, to produce an increase in the concentration as well as total amount. In former experiments (5), it was shown that even when the urine volume was smaller before the extract was injected than afterwards there was still a great increase in the excretion of phosphorus. This excretion is apparently not simply a washing out of phosphate in the process of diuresis.

pH. In three of the four subjects, there was a very distinct shift of the urine pH to a more alkaline range following the injection of parathormone. In the fourth subject, S., before the extract was given, the pH had risen to 7.1. The urine became slightly, but not remarkably, more alkaline following the administration of the extract. Some unpublished data on individuals with alkaline urine show that when the urine is already alkaline ($pH > 7.2$) it does not usually become more alkaline after the injection of parathyroid extract. In Subject M. no provision was made to prevent the escape of CO_2 from the urine samples. Loss of CO_2 probably accounts in part for the very unusual alkalinity obtained (8).

Bicarbonate. Since it has been shown (8, 9) that alkaline urine contains more bicarbonate than acid urine it would perhaps be anticipated, from the pH changes, that the bicarbonate of the urine would be increased after the injection of parathyroid extract. The bicarbonate of the urine in the three individuals in whom it was studied showed a marked rise particularly in Subjects G. and K. The increase lasted two hours in both of these. The decrease in the third hour following the injection of extract was as abrupt as the rise had been in the first and second hours. When the magnitude in milliequivalents of the bicarbonate excreted is compared with that of phosphate the two are seen to be of the same order. If the increase in excretion of bicarbonate should be found to endure characteristically for two hours, it might be said that the phosphate increase lasted longer. In the four experiments of this report the increase of phosphorus excreted in the urine lasted at least three hours. In other experiments (4) it has lasted five hours or more. The fact remains, however, that during the two hours following the injection of parathormone the excretion of bicarbonate rose and its total quantity in milliequivalents per hour was comparable to that of inorganic phosphate.

Chlorides. The alterations in chloride values in the urine after the injection of parathormone were somewhat indefinite. In the first hour after the injection, in two subjects there was no change; in two, a definite increase over the control hours. In the second hour after the injection all the values were lower than those for the first hour, whereas in three of the four the values for the second hour after the injection of extract were lower than the control values. This decrease in chloride excretion in the second hour following injection was observed in the cases formerly reported (5). Although the chlorides at first glance appear to follow the volume of urine, there is actually considerable variation in the concentration of chloride in the various specimens.

Sodium and potassium. The three subjects in whom observations were made all showed a definite increase in the output of sodium after the injection of parathormone. This was mainly observed in the first and second hours after the administration of the extract. The excretion of potassium (two subjects observed) also increased, but somewhat less markedly than that of the sodium. The concentration of sodium, as well as the total amount, underwent considerable change. The amount of potassium excreted seemed to vary fairly closely with the volume of urine.

Ammonium. In the three subjects studied for the ammonium ions, the excretion in the three hours following the injection of parathyroid extract was always somewhat less than in the control hours. In no specimen did it rise above the control level. There was certainly no evidence

of increased ammonia production by the kidney during the experiments.

When the sum of the cations, ammonium, sodium and potassium, in milliequivalents in any specimen is compared with the sum of the anions, the primary and secondary phosphate, bicarbonate and chloride, it is seen (Table II) that (except for G., hour 5, where the difference is 2.2 m.eq.) the difference of the sums is less than 2.0 m.eq. Without the sulphate and organic acids, magnesium and calcium figures (which are all in the normal individual less than 1 m.eq. per hour) no deductions may be made, but the comparison of the above sums shows that the principal anions and cations balanced roughly.

TABLE II

Sums of principal anions and cations excreted per hour

Hourly specimens	$Na^+ + K^+ + NH_4^+$ M. eq. total		$HPO_4^= + H_2PO_4^- + CL^- + HCO_3^-$ M. eq. total	
Subject	G.	K.	G.	K.
1	14.7	15.4	16.0	14.2
2	18.3	12.8	19.1	11.5
3	21.8	16.0	22.1	14.7
	Parathyroid extract 4 cc. i.v.			
4	32.0	31.3	31.8	31.4
5	18.2	24.0	16.0	23.7
6	11.6	9.4	9.6	8.0

DISCUSSION

Without drawing definite conclusions from the data at hand, certain lines of thought are suggested and some points may bear emphasis. Following the injection of the parathyroid hormone there was a tendency for an acid urine to change abruptly to a more alkaline range. This change in pH was sometimes as much as 1.0 or even more. Accompanying this there was a great excretion of bicarbonate for two hours, such as is commonly found in alkaline urines. The excretion of inorganic phosphate was, as before observed, very much increased after the injection of parathormone. With the change in pH relatively more secondary phosphate ions were excreted, and the amount of phosphate in neutralizing (or base-matching) milliequivalents was very much increased after the injection of the hormone. The chlorides showed a rather indefinite trend. Following the injection of the hormone large quantities of base were excreted, particularly in the first hour, when the chloride excretion was the same or higher, the phosphate and bicarbonate much higher, than in the control hours. The total base was sufficiently in excess of the acid radicles, of course, to make the urine alkaline.

As it has been shown before that calcium excretion does not increase in the first hours after the injection of parathyroid extract (and probably magnesium does not), the increased base excreted might have been any of the ions, sodium, potassium or ammonium. In the present experiments the ammonium ions certainly did not increase. The potassium increased moderately, roughly corresponding to the water, while the sodium showed in one instance a definite increase; in two instances, a large increase.

The foregoing observations give rise to two suggestions regarding the behavior of the kidneys after the injection of parathyroid extract. The first is that increased amounts of acid might be *primarily* excreted as a result of the action of the hormone. It has already been suggested by one of us (4) that the renal threshold for phosphate might be lowered by the hormone. The present experiments indicate that if the renal threshold is lowered for phosphate, it must also be lowered correspondingly for bicarbonate. Against the suggestion that the parathyroid hormone causes a primary increase in the excretion of phosphate and bicarbonate are two points. One of these is that the primary excretion of acid usually results in an acid urine, whereas the urine specimens containing the increased amount of phosphate and bicarbonate in the present instance were alkaline. In fact, the actual presence of large amounts of bicarbonate in the urine is very much against a primary acid excretion. The other point very much against it is that an increase in the excretion of acid is, as a rule, accompanied by an increased production of ammonia by the kidney. The ammonia production in the present experiments decreased, in fact, after the parathormone was given and during the hours when bicarbonate and phosphate were excreted in larger amounts.

The second suggestion that arises from the present and previous experiments is that admin-

istration of the parathyroid hormone might result in an increase *primarily* in the excretion of fixed base, particularly sodium, and that bicarbonate and phosphate accompany secondarily the increase in sodium excretion. This is a very tempting hypothesis, as it would explain the alkaline urine, the decrease in ammonia production, the bicarbonate and secondary phosphate excretion.

In view of the recent work of Loeb, Atchley, Benedict and Leland (10) and of Harrop, Soffer, Ellsworth and Trescher (11) showing that sodium is lost through the urine when the animal is deprived of adrenal cortical hormone, the suggestion might be made that the parathyroid hormone may have an effect opposite to that of the adrenal cortical hormone, in producing an increase in the output of base, particularly sodium, in the urine.

SUMMARY

1. The effect of the injection of parathyroid extract upon certain electrolytes of the urine was observed in four human subjects.

2. There was a tendency for the urine to become more alkaline after the injection of the extract.

3. There was not only an increase in the excretion of inorganic phosphate, as noted previously, but a marked excretion of bicarbonate as well, after the administration of the hormone.

4. There was a slight decrease in ammonium ions in the urine whereas the excretion of potassium and particularly of sodium ions was very much increased after the injection of parathormone.

5. The theoretical implications of these observations is briefly discussed.

BIBLIOGRAPHY

1. Greenwald, I., and Gross, J., The effect of long continued administration of parathyroid extract upon the excretion of phosphorus and calcium. J. Biol. Chem., 1926, **68**, 325.
2. Albright, F., Bauer, W., Ropes, M., and Aub, J. C., Studies of calcium and phosphorus metabolism. IV. The effect of the parathyroid hormone. J. Clin. Invest., 1929, **7**, 139.
3. Albright, F., and Ellsworth, R., Studies on the physiology of the parathyroid glands. I. Calcium and phosphorus studies on a case of idiopathic hypoparathyroidism. J. Clin. Invest., 1929, **7**, 183.
4. Ellsworth, R., Studies on the physiology of the parathyroid glands. V. Action of parathyroid extract on the renal threshold for phosphorus. J. Clin. Invest., 1932, **11**, 1011.
5. Ellsworth, R., and Howard, J. E., Studies on the physiology of the parathyroid glands. VII. Some responses of normal human kidneys and blood to intravenous parathyroid extract. Bull. Johns Hopkins Hosp., 1934, **55**, 296.
6. Goadby, H. K., and Stacey, R. S., On the action of parathormone. Biochem. J., 1934, **28**, 2092.
7. Albright, F., Bauer, W., and Aub, J. C., Studies of calcium and phosphorus metabolism. VIII. The influence of the thyroid gland and the parathyroid hormone upon the total acid-base metabolism. J. Clin. Invest., 1931, **10**, 187.
8. Marshall, E. K., Jr., The effect of loss of carbon dioxide on the hydrogen ion concentration of urine. J. Biol. Chem., 1922, **51**, 3.
9. Gamble, J. L., Carbonic acid and bicarbonate in urine. J. Biol. Chem., 1922, **51**, 295.
10. Loeb, R. F., Atchley, D. W., Benedict, E. M., and Leland, J., Electrolyte balance studies in adrenalectomized dogs with particular reference to the excretion of sodium. J. Exper. Med., 1933, **57**, 775.
11. Harrop, G. A., Soffer, L. J., Ellsworth, R., and Trescher, J. H., Studies on the suprarenal cortex. III. Plasma electrolytes and electrolyte excretion during suprarenal insufficiency in the dog. J. Exper. Med., 1933, **58**, 17.
12. Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry. II. Methods. Williams and Wilkins Co., Baltimore, 1932, p. 295.
13. Clark, W. M., Determination of Hydrogen Ions. Williams and Wilkins Co., Baltimore, 1928, 3rd ed.
14. Reference 12, p. 290.
15. Harvey, S. C., The quantitative determination of chlorides in the urine. Arch. Int. Med., 1910, **6**, 12.
16. Van Slyke, D. D., and Cullen, G. E., The determination of urea by the urease method. J. Biol. Chem., 1916, **24**, 117.
17. Tisdall, F. F., and Kramer, B., Methods for the direct quantitative determination of sodium, potassium, calcium and magnesium in urine and stools. J. Biol. Chem., 1921, **48**, 1.
18. Fiske, C. H., and Subbarow, Y., The colorimetric determination of phosphorus. J. Biol. Chem., 1925, **66**, 375.
19. Butler, A. M., and Tuthill, E., An application of the uranyl zinc acetate method for the determination of sodium in biological material. J. Biol. Chem., 1931, **93**, 171.