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OBSERVATIONS ON THE CHARACTER OF MERCURIAL DIURESIS

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The very profound effect of mercurial diuretics on the excretion of sodium, chloride and water has been variously ascribed to an action on the proximal (Mudge, Foulks, and Gilman, 1949; Weston, Grossman, and Leiter, 1951) and the distal renal tubule (Duggan and Pitts, 1950). Comparatively little attention has been paid to the other constituents of urine. This paper describes the effect of mersalyl on the excretion of potassium, phosphate, hydrogen ion, ammonia, bicarbonate, uric acid, and creatinine in normal human subjects. Too little is known of the mechanisms governing the excretion of these substances to allow the data to be fitted into any single hypothesis. Nevertheless, the data seem worth recording, as they will have to be taken into account in any final hypothesis concerning the site and mode of action of the drug.

METHODS

The subjects were normal male medical students or doctors. They came to the laboratory in the morning, having taken nothing by mouth since the previous evening. The experiments were begun as near 9.30 a.m. as possible. Urine was collected at 30 min. intervals by voluntary micturition, without a catheter; the collection periods were usually reduced to 15 min. at the peak of the diuresis.

The mercurial diuretic used was Mersalyl, obtained as the solid from British Drug Houses Ltd. The dose was always 0.2 g., dissolved in 20 ml. sterile distilled water and injected intravenously as soon as possible after solution. The injection was given slowly over a period of 10 min. to avoid possible toxic effects on the heart resulting from a momentarily excessive blood level of the drug. No theophylline was given with these injections.

During the experiments the subjects sat in chairs or reclined on couches; their activity was restricted to that necessary for passing urine. They took nothing by mouth, except as stated in the text. Smoking was prohibited.

The chemical methods used were as follows: Potassium, direct reading flame photometer; inorganic phosphate, Fiske and Subbarow (1925); ammonia, Conway and O'Malley (1942); *p*H, glass electrode; uric acid, Benedict and Franke (1922); creatinine, Folin (1914); carbon dioxide, Van Slyke and Neill (1924).

RESULTS

Potassium.—Fig. 1 illustrates a typical experiment. After five preliminary 30 min. periods, 0.2 g. of mersalyl was injected intravenously; the concentration and rate of excretion of urinary

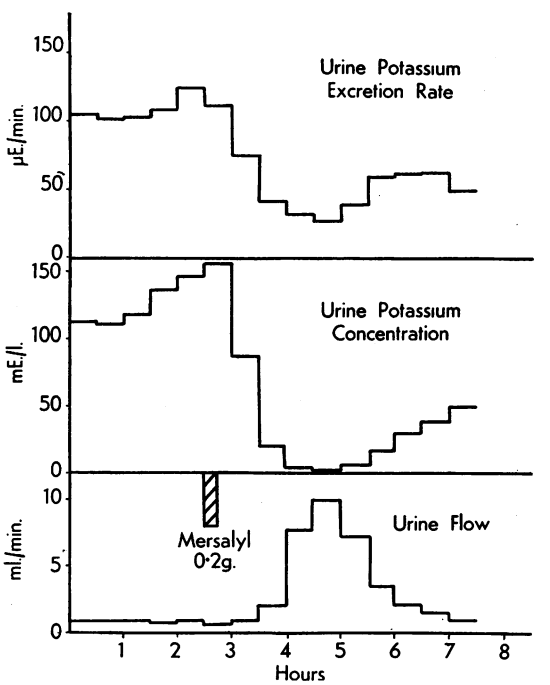

FIG. 1.—Effect of mersalyl on potassium excretion, in a subject taking a normal diet.

potassium fell conspicuously. Table I shows the results of 19 similar experiments in terms of urinary concentration and urinary excretion rate, during the initial control period and at the peak of diuresis. In four of these experiments (marked with an asterisk), the subjects drank 0.9% NaCl solution throughout the experiment at a rate of 200 ml./hr. The object of this was to increase the subjects' reserves of sodium and chloride, in the hope that a more profuse diuresis would result when mersalyl was given. Control experiments without mersalyl had previously shown that drinking saline in this way caused no significant increase in urine flow even if continued for some hours. No increase in diuresis following mersalyl resulted from this procedure; the other results showed no obvious difference from those of the experiments without saline. These four experiments have therefore been grouped with the rest in Table I.

TABLE I

EFFECT OF MERSALYL ON POTASSIUM EXCRETION

Subject	Diet	Urine Potassium Concentration (m.equiv./l.)		Plasma Potassium (m. equiv./l.)	Urine Potassium Excretion Rate (μ.equiv./min.)	
		Initial	Peak		Initial	Peak
R.A.D. ..	N	101	4·0	4·7	91	35
R.E.W. ..	N	119	11·9	4·3	75	44
I.C.S.N. ..	N	125	2·7	4·2	108	27
E.R.D. ..	N	89	6·9	4·0	39	66
B.C.* ..	N	98	1·07	4·4	84	14
R.B.P. ..	N	116	4·5	4·3	150	63
J.S.B.* ..	N	54	10·2	4·6	90	50
T.C.W.* ..	N	85	5·6	4·0	79	48
J.E.G.* ..	N	107	17·8	4·7	103	85
M.B.T. ..	N	93	8·1	4·4	120	55
P.S. ..	N	115	2·2	3·7	111	16
P.A.B. ..	N	179	3·9	3·9	115	47
T.O.W. ..	SP	72	9·7	4·3	30	77
I.C.S.N. ..	SP	62	11·7	4·2	32	97
P.A.B. ..	SP	134	13·2	3·8	63	130
B.C. ..	SP	109	11·9	3·9	52	69
M.H. ..	SP	127	9·1	3·6	87	56
W.R.C. ..	SP	9·1	11·4	3·6	19	192
E.R.D. ..	SP	71·3	19	—	34	70

* Indicates that the subject drank 0·9% NaCl solution, 200 ml./hr., throughout the experiment. N = normal diet. SP = salt poor diet.

In seven of the experiments (marked " SP ") the subjects ate a diet supplying some 0.5 g. of sodium and 1 g. of chloride daily for two or three days before the experiment.

Of the twelve experiments on subjects taking a normal diet, all except one showed a fall both in the concentration and in the excretion rate of potassium. On the other hand, when a salt-poor diet was taken, mersalyl caused a rise in the excretion rate in six out of seven experiments, although the fall in concentration occurred as in the other experiments. Fig. 2 illustrates a result of this type. Although the concentration of potassium in the urine fell in every experiment, there

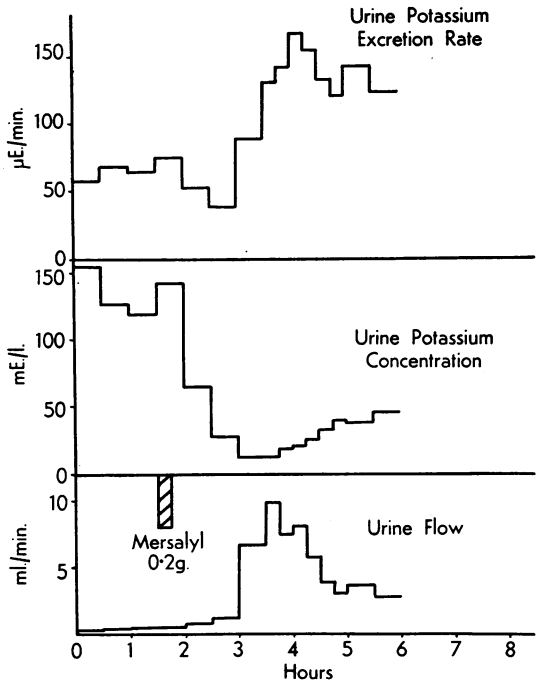

FIG. 2.—Effect of mersalyl on potassium excretion, in a subject taking a diet poor in sodium and chloride. Note the rise in excretion rate, in contrast to the fall seen in Fig. 1.

seemed no real tendency—as had been found with sodium (Dale and Sanderson, to be published)—for the urinary potassium level to approximate that of plasma. Thus, in most of the experiments in Table I, the urinary potassium at the peak of the diuresis greatly exceeded the plasma level; but in three it was much lower.

Inorganic Phosphate.—The results of eleven experiments are shown in Table II. There was a conspicuous fall in the urinary concentration in

TABLE II

EFFECT OF MERSALYL ON THE EXCRETION OF INORGANIC PHOSPHATE

Subject	Urine Inorganic Phosphate Concentration (m.equiv./l.)		Plasma Inorganic Phosphate (m. equiv./l.)	Urine Inorganic Phosphate Excretion rate (μ.equiv./min.)	
	Initial	Peak		Initial	Peak
R.A.D. ..	10·8	0·35	1·53	9·65	3·1
R.E.W. ..	14·9	5·45	1·20	9·5	20·3
I.C.S.N. ..	14·5	1·84	1·77	12·5	18·4
E.R.D. ..	6·4	0·88	1·00	2·75	8·4
B.C.* ..	7·5	1·19	1·19	6·5	15·3
R.B.P. ..	12·1	0·72	1·38	15·3	10·1
J.S.B.* ..	11·9	3·94	1·02	19·6	19·4
T.C.W.* ..	9·8	0·83	1·43	9·1	7·1
J.E.G.* ..	6·7	2·10	—	6·1	10·5
M.B.T. ..	11·7	2·59	—	15·1	17·5
P.S.	7·8	0·67	—	7·5	4·7

* Subject drank 0·9% NaCl.

each. The effect on the excretion rate was variable; there was a rise in six experiments and a fall in five. Weston, Grossman, and Leiter (1951), using thiomerin, concluded that the usual effect was a rise in the excretion rate. In interpreting their results it must be remembered that there is a diurnal change in the rate of phosphate excretion in the normal subject, such that the rate in the afternoon tends to be higher than that in the morning. In Table III are shown the results of ten control experiments, in which the subjects came with the same preparation as in the mersalyl experiments, and produced urine specimens under

TABLE III

DIURNAL CHANGE OF URINARY INORGANIC PHOSPHATE EXCRETION IN NORMAL SUBJECTS WITHOUT MERSALYL INJECTION

Subject	Initial phosphate excretion rate (μ. equiv./min.)	Time	Highest subsequent phosphate excretion rate (μ. equiv./min.)	Time
R.E.W. ..	12·9	9.40 a.m.	21·4	4·40 p.m.
R.A.D. ..	6·3	11.00 „	13·2	4.00 „
R.B.P. ..	6·2	10.10 „	15·6	5.10 „
I.C.S.N. ..	10·3	10.00 „	25·6	5.00 „
M.B.T. ..	14·5	10.10 „	22·1	4.10 „
P.S. ..	23·6	10.15 „	15·8	4.15 „
B.C.* ..	0·7	10.10 „	12·7	4.40 „
T.C.W.* ..	11·0	10.10 „	33·2	4 40 „
J.E.G.* ..	11·5	10.10 „	21·6	5.10 „
J.S.B.* ..	22·2	10.30 „	26·5	2.30 „

* Subject drank 0·9% NaCl

the same conditions, but had no mersalyl. The rate of phosphate excretion in the first timed collection of urine, and the highest subsequent excretion rate, are recorded, together with the times of passing the urine specimen concerned. In all except one subject, the excretion rate rose to a higher level in the afternoon, often to twice or three times, and in one individual to nearly twenty times, the morning rate. Weston *et al.* do not say at what time of day their experiments were done; Figs. 1 and 2 show, however, that an experiment carried on until the rate of urine flow is returning to the preliminary figures is likely to last some six or seven hours. If, as seems likely, Weston and co-workers began their experiments between 9 and 11 a.m., it appears doubtful whether any rise in phosphate excretion they observed can be ascribed to the injection of thiomerin. In fact, it could be argued, from a comparison of Tables II and III, that the action of mersalyl as observed by us was occasionally to convert the normal rise in phosphate excretion into a fall. It is perhaps safest to conclude that mersalyl has no constant effect in this respect.

Hydrogen Ion.—Fig. 3 shows the effect of mersalyl on urinary *p*H. The two lines are the averaged results of twelve experiments with

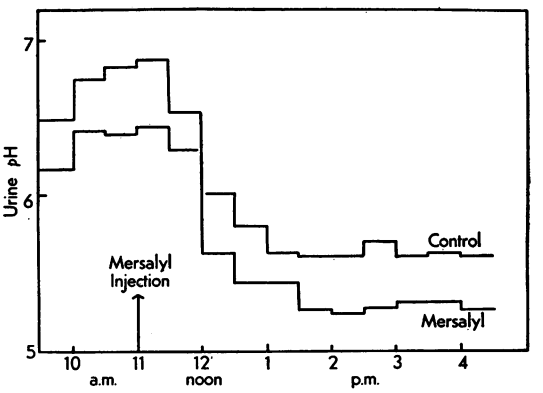

FIG. 3.—Effect of mersalyl on urinary *p*H. The control experiments show a trend towards increased acidity during the course of the day, which is intensified when mersalyl is given.

mersalyl and of eleven control experiments without mersalyl. The time scale is that of the control experiments; in the mersalyl experiments the injection has been taken as the reference point, and the periods have been averaged by arranging them according to their time before or after this. The actual time of injection ranged between 10 a.m. and 12 noon with a mean of 11.07; accordingly, for purposes of comparison, the mersalyl series has been represented with the injection taking place at 11 o'clock. The figure shows the trend towards a more acid urine (which occurs in any normal subject) during the latter part of the day, and the more marked change in *p*H which occurs when mersalyl is given.

Ammonia.—Changes in concentration and excretion rate of urinary ammonia were observed in seven experiments, in three of which the subject took 12 g. NH_4Cl by mouth daily for two days prior to the experiment, with a dose of 2 g. at about 8 a.m. on the morning of the experiment. The results are shown in Table IV. The effect of mersalyl was always to diminish the concentration of urinary ammonia; the effect on rate of excretion of ammonia, however, varied, depending on whether or not the subject had been taking NH_4Cl. The ammonia excretion of those subjects who had not taken NH_4Cl was initially comparatively low (mean of four experiments, 15.2 mE./min.), and rose during the diuresis to a mean value of 46.9 mE./min., whereas in the subjects who had taken NH_4Cl the ammonia excretion, initially high (mean of three experiments, 79.2 mE./min.)

TABLE IV

EFFECT OF MERSALYL ON THE EXCRETION OF AMMONIA

Subject	Date	Concentration (m.equiv./l.) Initial	Concentration (m.equiv./l.) Peak	Excretion (μ equiv./min.) Initial	Excretion (μ equiv./min.) Peak
J.E.G. ..	9.8.51	31·8	11·3	31·4	56·5
M.B.T. ..	24.9.51	6·5	4·65	8·2	31·6
P.S. ..	18.10.51	9·0	7·5	8·2	52·0
P.A.B. ..	8.11.51	20·2	3·9	12·9	47·6
J.G.O.* ..	11.2.52	66·0	7·1	78·5	48·3
T.O.H.* ..	13.2.52	53·1	4·9	83·5	59·1
R.M.L.* ..	21.2.52	75·7	4·4	75·7	59·9

* Subject took NH_4Cl before the experiment.

fell after mersalyl to a mean value, at the peak of diuresis, of 55.8 mE./min. The urinary *p*H values in these latter experiments (which were not included in the compilation of Fig. 3) showed the usual trend towards increased acidity (see Fig. 5), but the initial values were much lower—between *p*H 4.7 and *p*H 5—in all three subjects (compare Fig. 3), and the subsequent change towards acidity extended only as far as *p*H 4.3–4.6, which is probably close to the limiting value for the human kidney (Pitts, 1948). Since we performed no control experiments with NH_4Cl, we are unable to say whether this increase in acidity was due to mersalyl or to the normal diurnal variation; but, in any case, we have in these experiments the unusual situation of an increase in acidity associated with a fall in ammonia output.

Bicarbonate.—From the total CO_2 content of the urine and its *p*H, the bicarbonate concentration was calculated. Fig. 4 shows the changes in *p*H and excretion rate of ammonia and bicarbonate induced by mersalyl in a subject without preliminary medication: Fig. 5 illustrates the same changes in a subject who had been taking NH_4Cl as detailed above. Fig. 4 shows a considerable fall in *p*H and the expected changes in ammonia and bicarbonate output—a rise and a fall respectively. Fig. 5 shows the paradoxical fall in ammonia output noted above, together with a rise in bicarbonate output. It should be noted that the amounts of bicarbonate involved in Fig. 5 are extremely small, so that if plotted on the same scale as Fig. 4 most of the lines would coincide with the zero line. Similar changes in bicarbonate output were found in the other two experiments with mersalyl and NH_4Cl, initial values of 0.061 μE./min. and 0.088 μE./min. rising to 0.34 μE./min. and 1.165 μE./min. at the peak of diuresis. Although the amounts involved are so small, the results are in sharp contrast with the experiments in which NH_4Cl was not given. An attempt to study the effect of mersalyl on bicarbonate excretion in subjects made alkalotic by the

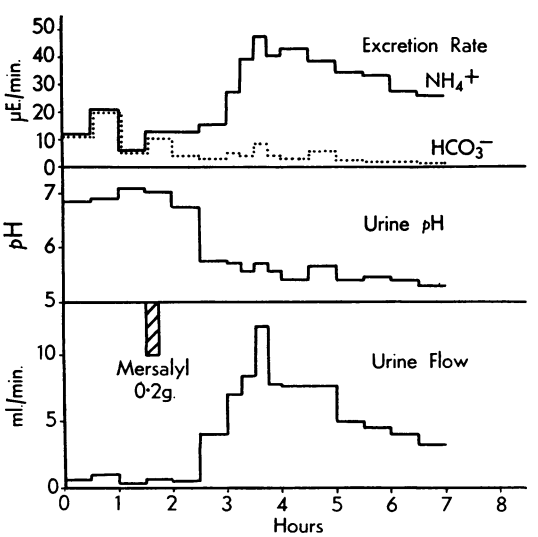

FIG. 4.—Effect of mersalyl on urinary *p*H, and on excretion of ammonia and bicarbonate, in a normal subject.

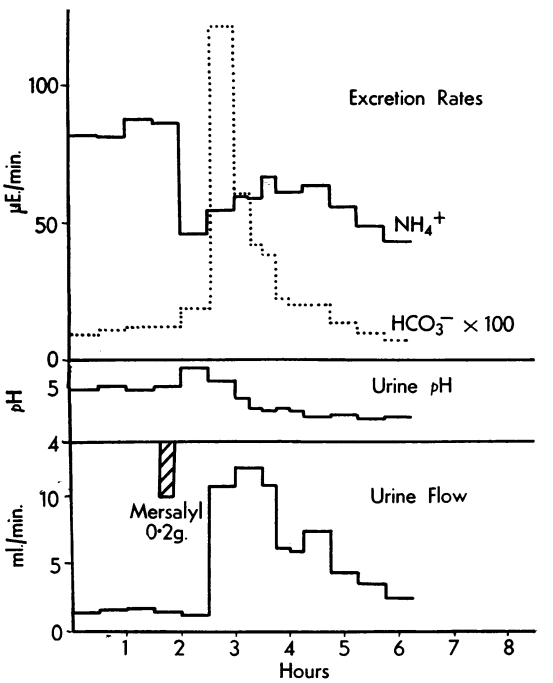

FIG. 5.—Effect of mersalyl on urinary *p*H, and on excretion of ammonia and bicarbonate, in a subject made acidotic by ingestion of NH_4Cl. Note the fall in ammonia output and rise in bicarbonate output, in spite of the usual increase in urinary acidity.

ingestion of $NaHCO_3$ was partly frustrated by the inhibitory effect of this regime on a mercurial diuresis. Three subjects took 8 g. $NaHCO_3$ three times a day for two days before the experiment; two also took 8 g. on the morning of the experiment. In these two subjects, whose plasma CO_2 levels were 36.6 and 33.3 mE./l., 0.2 g. mersalyl given intravenously produced no diuresis whatever; in the third subject, who had no bicarbonate on the morning of the experiment, and whose plasma CO_2 was 31.0 mE./l., urine flow increased from a pre-injection level of 1.35 ml./min. to a peak of 5.73 ml./min. In this subject the pre-injection rate of bicarbonate excretion was 60 μE./min. (cf. Fig. 4), and with the establishment of diuresis this fell to about 1 μE./min. and remained at this level for the rest of the experiment.

In the other two subjects, the initial rates of bicarbonate excretion were much higher (627 and 327 μE./min.) and, although no diuresis occurred, bicarbonate excretion fell, abruptly in the first subject and more gradually in the second, finally reaching rates of 26 and 54 μE./min. respectively.

Uric Acid.—The effect on uric acid excretion was studied in three experiments, with closely similar results in all three. Fig. 6 shows the results of one of these. The considerable increase in uric acid elimination shown here is a well-known feature of the action of mersalyl; similar results have been reported for thiomerin by Grossman, Weston, Edelman, and Leiter (1950). Fig. 6 is included simply to draw attention to an interesting feature of the uric acid response—namely, its relationship in time to the injection of mersalyl. Whereas the rates of water, sodium, and chloride excretion commonly show no change for a full hour after the injection, the concentration and excretion rate of uric acid show a marked increase in the first 30 minutes after the injection. This was also observed in both of our other experiments. It was commented upon by Grossman and colleagues.

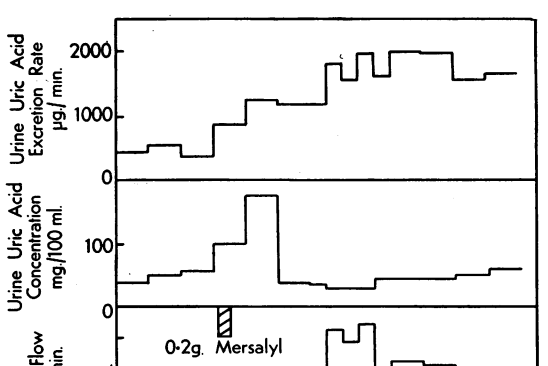

FIG. 6.—Effect of mersalyl on uric acid excretion. Note the immediate effect on uric acid, and the delayed effect on water excretion.

Creatinine.—Fig. 7 shows the mean rate of creatinine excretion in 12 experiments with mersalyl and 11 control experiments. For purposes of comparison, the same method has been used as in the compilation of Fig. 3, that is, the mersalyl experiments are represented with the injection taking place at 11 a.m., although the actual time of injection varied between 10 a.m. and 12 noon. The lines representing control and mersalyl series lie close together except during the last two periods before, and the second, third, and fourth periods after, the injection. When the significance of these differences is tested, "P" for both periods before, and for the fourth period after the injection, is greater than 0.1; for the second and third periods after the injection "P" lies between 0.05 and 0.02, and 0.01 and 0.001, respectively. During these periods there is a real decrease in creatinine excretion.

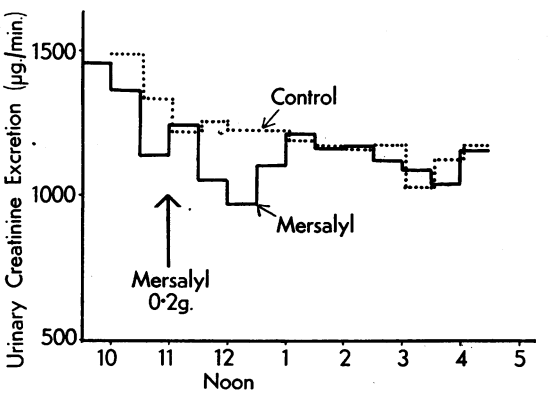

FIG. 7.—Creatinine excretion in control experiments (. . . . .) and mersalyl experiments (———).

DISCUSSION

These results show that the effect of mersalyl on the kidney extends to a wide range of substances in addition to water, sodium, and chloride. Elsewhere (Dale and Sanderson, to be published) we have presented data suggesting that the action of mersalyl on the excretion of water, sodium, and chloride can best be explained as an effect on the distal tubule. The present results, while not neces-

sarily supporting this hypothesis, do not conflict with it. Their interpretation is in some cases relatively straightforward ; in others it will have to await further elucidation of the exact events of the excretory process.

Of the organic solutes studied, creatinine is probably excreted by a combination of filtration and tubular excretion (Smith, 1951) ; the exact significance of the diminished creatinine excretion rate between 30 and 90 min. after the injection of mersalyl is therefore doubtful. However, decreases in inulin clearance after injection of other mercurial diuretics have been observed by Capps, Wiggins, Axelrod, and Pitts (1952) and after injection of mersalyl by ourselves, and it seems likely that the present results are due to transient decreases in glomerular filtration rate. The means whereby this effect is produced is at present obscure.

The behaviour of uric acid suggests the possibility of more than one mercury sensitive mechanism in the tubules—for example, a mechanism for reabsorbing uric acid which is inhibited directly and immediately by mersalyl, and one for reabsorbing water, sodium, and chloride which is inhibited only after a prolonged delay, perhaps as a result of some indirect effect of the drug. The injection of mersalyl and the beginning of the diuresis are usually separated by some 1½ to 2 hr., and the events taking place in the kidney during this time are obviously of the greatest interest and worthy of closer study.

None of the inorganic substances studied undergo such striking changes in excretion as do sodium and chloride, and very much less is known about the sites of their reabsorption. Nevertheless, the clear-cut effects found with some ions constitute a challenge to our ignorance.

The changes in potassium may be related primarily to the excretion rate prevailing before the injection of mersalyl rather than to the excretion rate of sodium or chloride, since Berliner (personal communication) has also observed that mercurials tend to lower a high excretion, and to raise a low excretion, of potassium. There is some evidence (Platt, 1950 ; Berliner and Kennedy, 1948) that two systems exist in the tubules for the regulation of potassium excretion, one being capable of active excretion ; our data would be compatible with the idea that a relatively fixed quantity of potassium was reabsorbed by the first mechanism, and that the second mechanism, located more distally and capable of inhibition by mersalyl, added to or subtracted from the remaining potassium by reabsorption or secretion as required.

Our results indicate that mersalyl causes a fall in the *p*H of the urine. It might be argued from this that the drug's action, which is almost certainly inhibitory, is unlikely to take place in the distal tubule, since this is commonly supposed to be the site of acidification of urine in mammals. However, it should be noted that this idea is based on amphibia (Richards, 1929 ; Montgomery and Pierce, 1937) and birds (Chambers and Kempton, 1933) ; the relevant observations on mammals have not been made, and the hypothesis that acidification occurs in mammalian distal tubules remains without experimental support. Walker, Bott, Oliver, and McDowell (1941) found that urine from the proximal half of the proximal tubule in rats and guinea-pigs was isotonic with plasma, and contained the same concentration of sodium, but about 40% more chloride. They pointed out that this implied the proximal reabsorption of some anion other than chloride, and suggested that this might be bicarbonate. Calculation shows that, when allowance is made for the Donnan equilibrium, the observed ratios could be accounted for by an almost complete reabsorption of bicarbonate. This is more than just a theoretical possibility ; McMaster and Elman (1928), investigating the intracellular *p*H of various tissues in rats and guinea-pigs by the intra-vital administration of erythrolitmin, found the indicator in its acid form in all the cells of the renal tubule, except those of the upper end of the proximal tubule, where it was in the alkaline form. The extent of this alkaline region could be varied by feeding acids or alkalis: acids caused it to extend a lesser, and alkalis a greater, distance from the glomerulus.

There would seem to be some evidence, therefore, for the reabsorption of considerable amounts of bicarbonate in the proximal tubules of mammals. (It may be noted at this point that no such evidence exists for amphibia ; the proximal tubule chloride U/P ratio averages 1.1, which is what would be expected in glomerular filtrate if the Donnan factor were 0.95.) It is very unlikely that any bicarbonate reabsorbed in this way would be accompanied by equivalent amounts of CO_2, since the latter is highly diffusible and apparently passes with ease across cell boundaries. It may be presumed, therefore, that the tubular fluid has a partial pressure of CO_2 of the same order as that of the blood, in which event the extensive reabsorption of bicarbonate would cause the proximal tubular fluid to become acid. Although there is no direct experimental proof of this proximal acidification, it is at least compatible with such facts as are known and would explain the increased acidity

of urine caused by mersalyl if the latter acts, as we believe, on the distal tubule.

In normal or mildly alkalotic subjects, the fall in urinary *p*H caused by mersalyl is accompanied by the expected rise and fall respectively in the excretion of ammonia and bicarbonate. In the presence of acidosis, the urinary *p*H falls, probably to a limiting value, but the changes in ammonia and bicarbonate excretion are reversed. In gross alkalosis no diuresis occurs; there is a fall in bicarbonate excretion, but whether this is due to the action of the drug or to the normal diurnal rhythym is not certain.

Too little is known at present about the mechanisms governing the excretion of hydrogen ion, ammonia, and bicarbonate for the interpretation of these data. The most suggestive observations are those made on the acidotic subjects, where the linkage between urinary *p*H and ammonia and bicarbonate excretion, supposed by Pitts (1948) to depend on simple physico-chemical mechanisms, appears to be reversed.

The abolition of mercurial diuresis in patients made alkalotic by ingestion of $NaHCO_3$ was first observed by Saxl and Erlsbacher (1929) and later by Ethridge, Myers, and Fulton (1936). An important related problem is that of resistance to mercurials in patients with cardiac failure, many of whom are alkalotic. The response can be improved by giving NH_4Cl, and it seems probable that the action of these drugs depends in some way on the *p*H of the plasma, or its chloride content. It is conceivable, for example, that the organic molecule is pharmacologically inert, and that release from it of an active constituent—possibly inorganic mercury—is dependent on an acid reaction, either in the plasma or perhaps in the renal tubules. The problem is obviously capable of further experimental study.

It will be clear that the time is not yet ripe for any single hypothesis to link up these data. What is abundantly clear is that the action of mersalyl, if studied in detail, reveals unexpected features which may well provide a key to the further understanding of the complexities of tubular function. Four points, in particular, emerge from the present results as worthy of closer attention: the changes in potassium secretion; the delay in onset of diuresis; the reversal of the usual changes in ammonia and bicarbonate excretion in acidosis; and the abolition of the diuresis in alkalosis. Study of phenomena such as these, no less than of more physiological changes, will help lead to the final goal of an integrated theory of renal behaviour.

Summary

1. The effect of mersalyl (0.2 g. intravenously) on the excretion of potassium, phosphate, hydrogen ion, ammonia, bicarbonate, uric acid and creatinine in normal human subjects has been studied.

2. In subjects taking a normal diet, mersalyl causes a fall in potassium excretion; in subjects taking a diet poor in salt, it causes a rise.

3. Mersalyl has no constant effect on the excretion of inorganic phosphate.

4. Mersalyl causes an increase in urinary acidity.

5. In subjects in normal acid-base balance, mersalyl causes a rise in ammonia excretion and a fall in bicarbonate excretion. In subjects made acidotic by the ingestion of ammonium chloride, these effects are reversed, although the usual fall in urinary *p*H occurs.

6. The increase in uric acid excretion caused by mersalyl occurs within 30 minutes of the injection, whereas the increase in water excretion is usually delayed until more than 60 minutes after injection.

7. Mersalyl causes a transient fall in the rate of creatinine excretion between 30 and 90 minutes after injection.

8. The implications of these findings are discussed.

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