J. Physiol. (1981), **314**, *pp.* 531–545
With 8 text-figures
Printed in Great Britain

AN ANALYSIS OF THE REGULATION OF SODIUM EXCRETION DURING INDUCED CHANGES IN PLASMA SODIUM CONCENTRATION IN ANAESTHETIZED DOGS

BY D. GORDON, F. S. NASHAT* AND C. S. WILCOX

*From the Medical Unit, St Mary's Hospital Medical School, London W2 1PG, and *Department of Physiology, Middlesex Hospital Medical School, London W1P 6DB*

(*Received 1 July 1980*)

SUMMARY

1. Renal sodium excretion ($U_{Na}V$) was studied during acute changes in plasma sodium concentration (P_{Na}) induced by altering the concentration of a sodium chloride infusion in anaesthetized dogs.

2. The change in P_{Na} induced other changes, notably in blood pH, the degree of blood dilution, and in glomerular filtration rate and renal plasma flow. No attempt was made to restrain these changes and their effects on $U_{Na}V$ were assessed using factor analysis. This defined new variables ('factors') that are linear functions of the primary variables. Four factors were defined, two of which were related to $U_{Na}V$.

3. Factor 1 was strongly correlated with $U_{Na}V$ and P_{Na}: its other correlations described the acidosis and plasma dilution of hypernatraemia. Further examination of these inter-relationships by regression analysis and data selection showed that the increase in $U_{Na}V$ with rising P_{Na} depended upon a fall in both the blood pH and P_{CO_2}.

4. Factor 2 was negatively correlated with $U_{Na}V$: its correlations with plasma protein concentration, haematocrit, blood pressure, glomerular filtration rate and renal plasma flow implied that it represented post-glomerular plasma protein concentration and its influence on sodium reabsorption.

5. Thus, two independent processes may regulate $U_{Na}V$ during acute salt loading: one is initiated by changes in P_{Na} and plasma dilution but requires a concomitant acidosis and ventilatory adjustment for its full expression; the second relates $U_{Na}V$ to several variables whose influences could be understood by changes they produce in the peritubular capillary plasma protein concentration.

INTRODUCTION

The causes of the increase in renal excretion of sodium ($U_{Na}V$) following sodium loading have not been established. It has been attributed to changes in the concentration of aldosterone and to the release of specific natriuretic hormones (De Wardener, 1977). Alternatively the natriuresis has been ascribed to measurable changes in non-hormonal variables, notably plasma sodium concentration (Kruhøffer, 1960), glomerular filtration rate and plasma protein concentration (Thompson & Pitts, 1952; O'Connor, 1962). It has also been related to changes in plasma

0022-3751/81/4870-1058 $07.50

concentrations of hydrogen ion (Nashat, Tappin & Wilcox, 1976) or potassium (Nizet, 1967), in haematocrit (Nashat, Scholefield, Tappin & Wilcox, 1969; Schrier & Earley, 1970), intravascular pressures (Gauer & Henry, 1963; McDonald & De Wardener, 1965) and renal plasma flow (Earley & Friedler, 1965). A full understanding of the renal response to sodium loading therefore requires an assessment of the simultaneous contributions from a number of variables.

Conventional experimental design demands that each variable should be isolated as an unique signal, the specific response to which must be defined. However, this is not always attainable when studying the response to sodium loading, because many or all of the variables noted above alter simultaneously in response to the sodium load. This approach, moreover, produces only one type of information, i.e. that the variable isolated by the experimental design is, or is not, an absolute requirement for natriuresis: the information is therefore necessarily incomplete.

It may indeed be desirable to allow variables affected by the sodium load to alter in response to the load, and then to study the resultant effect on the renal excretion of sodium by suitable methods of analysis; for it is likely that many of the changes produced form part of the signal to the kidney, and that their containment may modify the natural response to the application of the stimulus. The physiological relevance of a stimulus may depend on the facility with which it can create the environment necessary for the full development of its action. A change in a single variable, sufficiently subtle to be denied any physiological significance in its own right, could assume much greater importance if accompanied by simultaneous changes in other variables.

Nizet (1972) first demonstrated the value of multivariate analysis by using partial correlation to quantitate the influence of a number of non-hormonal blood factors on the control of sodium excretion. He showed that the effect of many of these could be related to changes produced in post-glomerular plasma protein concentration. Useful information may, however, have been lost with this approach, because the predictor variables were correlated with each other as well as with $U_{Na}V$. One variable may have eliminated another from the analysis because the relationship of the second variable with sodium excretion was similar to the relationship of the first. Both variables may have been important physiologically even though one did not appear in the regression equation. Furthermore, this method and multiple linear regression analysis are predictive methods, in which an attempt is made to describe change in $U_{Na}V$ as a function of changes in other variables, without necessarily contributing to an understanding of the underlying physiological processes. A more appropriate method of analysis might be by definition of new variables, allowing simultaneous examination of the inter-relationships of the putative predictor variables and of their relationships with the criterion available ($U_{Na}V$).

Factor analysis is such a method: it defines new variables (factors) by analysis of the matrix of correlations between the primary variables (Lawley & Maxwell, 1971). The factors may define physiological processes that are related to the primary variables, and are linear functions of the primary variables.

This paper describes the analysis of experiments in which a number of variables have been allowed to alter in response to an acute change in plasma sodium concentration. This provided a useful model in which many other important variables

changed. Regression analysis showed that $U_{Na}V$ could be predicted from the primary variables but factor analysis contributed to the understanding of the data, without loss of predictive power, by defining new and independent variables that could be interpreted as distinct physiological processes.

METHODS

Experiments were performed on fifty-two greyhounds anaesthetized with pentobarbitone sodium. Experimental methods and analytical techniques were similar to those used in series 3 of Nashat *et al.* (1976). The dogs received their usual diet of mixed meat and cereal (10–20 mmol d^{-1} of sodium) until *ca.* 12 h before the experiments. Saline was infused continuously at 0·1 ml kg^{-1} min^{-1} into a systemic vein (ten experiments) or through a needle in one renal artery (forty-two experiments). Plasma sodium concentration was altered by abruptly changing the sodium concentration of the infusate from 0·154 M (isotonic) to 0·077 M, 0·616 M or 1·232 M. All dogs were studied during 0·154 M-saline infusions; twelve dogs received 0·077 M-saline in addition, ten received 0·616 M-saline and forty-eight received 1·232 M-saline. Clearly, some animals were subjected to more than one change in the sodium concentration of the infusate. The duration of the hypotonic or hypertonic infusions was 30–60 min: thereafter, the infusion was returned to isotonic saline. Half the animals breathed spontaneously, and the remainder were artificially ventilated to give an initial arterial carbon dioxide tension (P_{CO_2}) of *ca.* 35 mmHg. Data for left and right kidneys were collected separately. Thus the experiments were designed to allow the measured variables to assume a number of different relationships with each other, so that these relationships could be seen in relief.

The following measurements were made: arterial plasma and urine sodium concentrations and plasma potassium concentration (P_{Na}, U_{Na}, P_K) by flame photometry; arterial pH and P_{CO_2} with glass and membrane electrodes (Radiometer); arterial haematocrit (Hct) using a Hawkesley microhaematocrit centrifuge; arterial plasma solids concentration (PS), used as a measure of the concentration of colloids in plasma, by desiccating a weighed plasma sample to constant weight and subtracting the weight of crystalloids calculated from P_{Na}. P_{Na} was measured in systemic arterial plasma but was corrected for concentration or dilution by the infusate when this was administred into the renal artery. These computed values were often verified by simultaneous measurements on renal venous plasma. Such correction was not necessary for the other variables because the rate of infusion into the renal artery was very small compared with renal blood flow. Arterial blood pressure (BP) was measured using a pressure transducer (Bell and Howell) and urine volume by timed collection from ureteric catheters. Glomerular filtration rate (GFR) was measured as the renal clearance of [^{51}Cr]EDTA and renal plasma flow (RPF) as the clearance of [^{125}I]sodium hippurate, with correction for an extraction of 0·85. This extraction was obtained from direct measurements, corrected for uptake of [^{125}I]sodium hippurate by red cells: it was independent of changes in P_{Na}.

Plasma hydrogen ion concentration, plasma [H^+], was calculated from pH; GFR, RPF, and the rate of renal excretion of sodium ($U_{Na}V$) were expressed per kg body weight. Filtration fraction (FF) was calculated as GFR/RPF. The concentration of colloids in the plasma in the efferent arteriole leaving the glomerulus (post-glomerular plasma solids: PGPS) was calculated as $PS/(1-FF)$.

Data were excluded from analysis when GFR was below 15 ml min^{-1} per kidney, or when urine flow rates were changing rapidly. Data available for analysis then comprised 357 sets of observations from either kidney when measurements of $U_{Na}V$, P_{Na}, PS, Hct, plasma[H^+], RPF, GFR, BP and P_K were available. These included 200 sets of observations when measurements of P_{CO_2} were made. The 357 sets of observations that we studied were obtained in fifty-two dogs with between four and twelve observations in each animal. Thus, the matrix of correlations between the variables that we analysed is not free from the constraint of repeated observation in the same animal. This, however, did not introduce any important bias in our conclusions, for similar results were obtained in the analysis of a subgroup of data formed by the selection at random of one set of observations from each of the fifty-two animals (see Appendix).

The data were analysed on the CDC 6000 series computers at the University of London Computer Centre, using the Statistical Package for the Social Sciences (Nei, Hull, Jenkins, Steinbrenner & Bent, 1975). When product moment correlations were calculated and simple linear regressions

performed a scatter diagram of the pairs of observations used in the calculations was examined to ensure that the result was not distorted in a misleading fashion by a single exceptional point outwith the general group of points. The relationship between two variables was also examined when appropriate by a 'rolling' data selection process. The observations were divided into groups according to the values of the putative predictor variable, with the values of the predictor variable in each group overlapping with those in the preceding and succeeding groups, allowing examination of possibly non-linear relationships (see Figs. 3 and 4 for an example of this method). Multiple linear regression analysis was performed using a hierarchical method, incorporating into the regression equation at each step the remaining variable that most reduced the residual sum of squares. Regression equations were written with standardized variables and beta coefficients, i.e. variables adjusted to a common mean and variance, allowing direct comparison of the magnitude of regression coefficients.

The correlations of the other variables with $U_{Na}V$ and with each other were examined using factor analysis, which defines new variables (factors) that are linear function of the primary variables. Factor analysis examines the matrix of correlations between the variables by extraction of the latent roots, or eigenvalues, and latent vectors of the matrix of correlation coefficients. A number of methods of factor analysis are available: we used principal factoring, with iteration after elimination of factors with eigenvalue < 1. This was followed by varimax rotation, defining factors which tended to have either strong or weak (but not intermediate) correlations with the primary variables (Lawley & Maxwell, 1971; Nei *et al.* 1975). Variations in the method of factor analysis (canonical or image factoring; selection of different critical eigenvalue; quartimax or oblique rotations) either yielded a very similar solution, or led to factors that we could not interpret in physiological terms. The basic model of factor analysis assumes a multivariate normal distribution. Some of the variables in this study had distributions differing detectably from normal. This is not a bar to the use of the method to describe patterns of inter-correlation that may have useful meaning, provided that emphasis is laid only on correlations with a high statistical significance (Cooley & Lohnes, 1971).

RESULTS

The mean values ± 1 standard deviation (S.D.) of the variables measured or calculated in the 357 cases used for the analysis are shown in Table 1.

Because the primary experimental manoeuvre was to alter the P_{Na}, the relationship between P_{Na} and $U_{Na}V$ was examined (Fig. 1). $U_{Na}V$ increased with P_{Na}, but showed considerable variability at the higher levels. The examination of the variability was complicated by the extensive inter-correlation of the variables measured (Table 2). For example, increasing P_{Na} was associated with increasing $U_{Na}V$ but P_{Na} was itself positively correlated with plasma $[H^+]$, and negatively with the concentration of plasma solids (PS) and haematocrit (Hct). These were in turn related to $U_{Na}V$ and to other variables.

The results of principal factor analysis with iteration followed by varimax rotation are shown in Fig. 2. Four factors with initial eigenvalues $\geqslant 1$ were extracted:

Factor 1 was strongly correlated with $U_{Na}V$ and with P_{Na}; it was also positively correlated with plasma $[H^+]$ and negatively with PS and Hct. This was interpreted as the increase in $U_{Na}V$, the plasma dilution, and the acidosis that occur with rising P_{Na} (Nashat *et al.* 1976).

Factor 2 was negatively correlated with $U_{Na}V$. It was negatively correlated with RPF and positively with GFR, PS, Hct and BP. This pattern of correlation suggested that factor 2 might represent the post-glomerular concentration of plasma solids (PGPS) and its known influence on $U_{Na}V$ (Nizet, 1972; Nizet, 1976), for PGPS is determined by PS and FF, and FF is positively correlated with GFR, and negatively with RPF. Furthermore, haematocrit is a determinant of FF through its effect on

afferent and efferent arteriolar resistances (Myers, Deen, Robertson & Brenner, 1975). In Fig. 2 the partial correlations of PGPS with the primary variables, controlling for factor 1, are shown and the similarities between factor 2 and PGPS are clear.

Factor 3 was positively correlated with GFR and RPF, and was interpreted as representing plasma flow dependence of GFR (Brenner, Troy, Daugharty, Deen & Robertson, 1972).

TABLE 1. Mean values, with standard deviations and numbers of observations, of measured and derived variables

Variable	Mean	Standard deviation	Number of observations
Renal excretion of sodium, μmol min^{-1} kg^{-1} ($U_{Na}V$)	4·10	6·79	357
Plasma sodium concentration, mmol l^{-1} (P_{Na})	152·7	12·8	357
Plasma solids concentration, g per 100 g (PS)	5·31	0·81	357
Haematocrit (Hct)	49·6	9·8	357
Plasma hydrogen ion concentration, nmol l^{-1} [H$^+$]	48·4	12·0	357
Renal plasma flow, ml min^{-1} kg^{-1} (RPF)	3·57	1·22	357
Glomerular filtration rate, ml min^{-1} kg^{-1} (GFR)	1·18	0·39	357
Mean arterial blood pressure, mmHg (BP)	163·2	26·9	357
Plasma potassium concentration, mmol l^{-1} (P_K)	3·52	0·55	357
Filtration fraction (FF)	0·35	0·10	357
Post-glomerular plasma solids concentration, g per 100 g (PGPS)	8·41	2·80	357
Arterial carbon dioxide tension, mmHg (P_{CO_2})	36·3	8·9	200

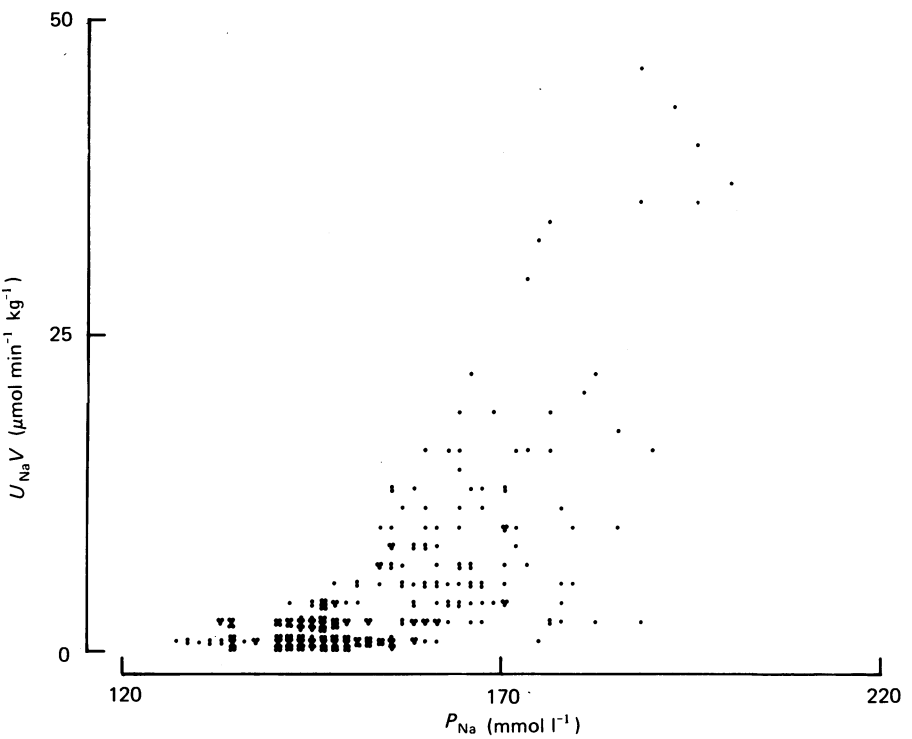

Fig. 1. Renal excretion of sodium ($U_{Na}V$) plotted against plasma sodium concentration (P_{Na}) in the complete group of 357 sets of observations.

TABLE 2. The correlations between the variables measured in the complete group of 357 observations, and used in the factor analysis

$U_{Na}V$	1·000								
P_{Na}	0·711	1·000							
PS	−0·405	−0·523	1·000						
Hct	−0·277	−0·151	0·435	1·000					
[H⁺]	0·371	0·450	−0·248	0·023	1·000				
RPF	−0·011	−0·144	0·283	−0·111	−0·062	1·000			
GFR	−0·130	−0·043	0·169	0·267	0·088	0·510	1·000		
BP	−0·109	−0·090	0·147	0·419	−0·053	−0·030	0·228	1·000	
P_K	−0·031	−0·109	−0·014	−0·066	0·208	0·051	0·060	−0·038	1·000
	$U_{Na}V$	P_{Na}	PS	Hct	[H⁺]	RPF	GFR	BP	P_K

The abbreviations for the names of variables are as in Table 1.

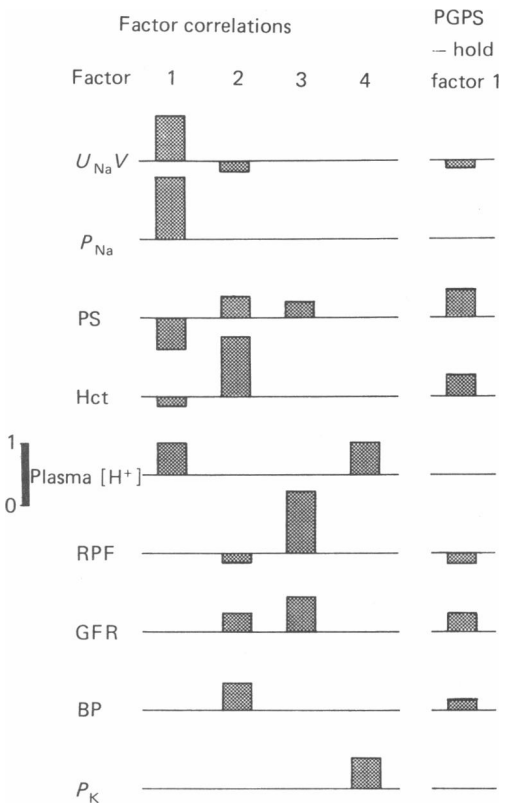

Fig. 2. Correlations of the variables shown in Table 2 with the four factors extracted by principal factor analysis, with iteration following elimination of factors with an eigenvalue < 1, and after varimax rotation. These correlations are presented diagrammatically as the first four columns. The height of each bar represents the magnitude of the correlation coefficient, in relation to the scale of 0–1. Negative correlations are shown below the line. For the sake of clarity, correlations of absolute magnitude less than 0·12 are omitted. On the right-hand side of the Figure the partial correlations of post-glomerular plasma solids concentration (PGPS) with the primary variables, controlling for factor 1, are shown. Note the resemblance of the correlations to those of factor 2 shown in the second column.

Factor 4 showed positive correlations with plasma $[H^+]$ and P_K and was taken to relate to the movement of potassium ions with development of acidosis (Makoff, Da Silva, Rosenbaum, Levy & Maxwell, 1970).

Only the first two factors showed important correlations with $U_{Na}V$. Multiple linear regression analysis showed that:

$$U_{Na}V = 0{\cdot}71 \text{ factor } 1 - 0{\cdot}19 \text{ factor } 2,$$

using standardized variables and beta coefficients. The multiple correlation coefficient

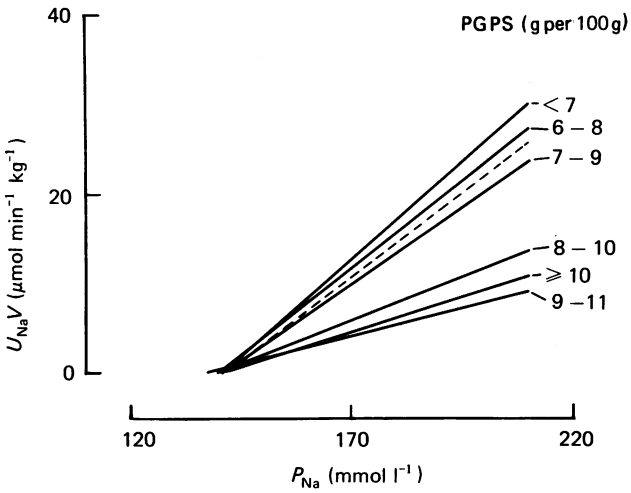

Fig. 3. Sodium excretion ($U_{Na}V$) is shown related to plasma sodium (P_{Na}) as in Fig. 1. The dashed line shows the line of regression for all 357 cases ($U_{Na}V = 0{\cdot}377\ P_{Na} - 53{\cdot}55$; $r = 0{\cdot}71$). The other lines show the same relationship for data selected according to the level of post-glomerular plasma solids concentration (PGPS). Mean values for P_{Na} in each successive PGPS range did not differ significantly. The regression lines depicted are:

PGPS (mean; range)	Regression equation	n	r
6·29; < 7·0	$U_{Na}V = 0{\cdot}431\ P_{Na} - 60{\cdot}50$	76	0·74
7·15; ⩾ 6·0 and < 8·0	$U_{Na}V = 0{\cdot}389\ P_{Na} - 54{\cdot}48$	152	0·69
8·00; ⩾ 7·0 and < 9·0	$U_{Na}V = 0{\cdot}341\ P_{Na} - 47{\cdot}99$	185	0·67
8·83; ⩾ 8·0 and < 10·0	$U_{Na}V = 0{\cdot}192\ P_{Na} - 26{\cdot}59$	152	0·59
9·56; ⩾ 9·0 and < 11·0	$U_{Na}V = 0{\cdot}122\ P_{Na} - 16{\cdot}56$	73	0·43
12·38; ⩾ 10·0	$U_{Na}V = 0{\cdot}157\ P_{Na} - 22{\cdot}36$	35	0·76

of this equation was 0·74. Multiple linear regression analysis using the primary variables led to the equation:

$$U_{Na}V = 0{\cdot}67\ P_{Na} - 0{\cdot}14\ \text{Hct} + 0{\cdot}10[H^+] + 0{\cdot}16\ \text{RPF} - 0{\cdot}17\ \text{GFR} + 0{\cdot}06\ \text{BP} + 0{\cdot}02\ P_K - 0{\cdot}01\ \text{PS},$$

again using standardized variables and beta coefficients. The multiple correlation coefficient for this equation was 0·75, which is very similar to that of the linear regression equation describing $U_{Na}V$ in terms of factors 1 and 2. Thus factor analysis clarified the interpretation of the results of these experiments without loss of predictive power. The regression equation with primary variables, although providing

a unique solution, did not contain as much physiologically useful information as was yielded by the factor analysis.

Factor analysis suggested an influence of PGPS on $U_{Na}V$ which was independent of P_{Na}. The relationship between P_{Na} and $U_{Na}V$ described in Fig. 1 was therefore examined in overlapping ranges of PGPS. Fig. 3 shows that, as PGPS increased from < 7·0 g per 100 g to > 10·0 g per 100 g, so the slope of the line of regression of $U_{Na}V$

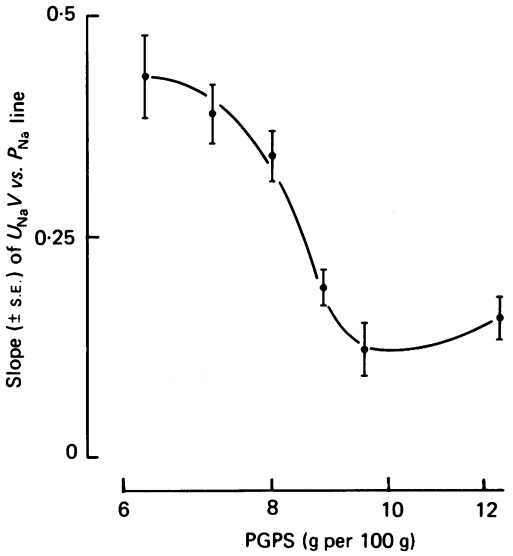

Fig. 4. The slope of each regression line described in the caption of Fig. 3 is plotted (± the standard error of the estimate of the slope) against PGPS (logarithmic scale). The line joining the points was drawn freehand.

on P_{Na} decreased. The relationship between this slope and PGPS is shown in Fig. 4, and demonstrates that as PGPS fell from *ca.* 9 to *ca.* 7 g per 100 g the amount of sodium excreted per unit change in P_{Na} more than doubled. The influence of PGPS on the slope of the $U_{Na}V$ *vs.* P_{Na} relationship was negligible outside this range. Neither FF nor PS (the determinants of PGPS) showed a consistent effect on the $U_{Na}V$ *vs.* P_{Na} relationship.

The correlations of factor 1 suggested that the relationship between P_{Na} and $U_{Na}V$ might require concomitant changes in plasma $[H^+]$ for its full expression. The relationship between P_{Na} and $U_{Na}V$ shown in Fig. 1 was seen to be closely related to plasma $[H^+]$ (Fig. 5). This relationship continued to be seen when the data were divided into two groups according to P_{CO_2}, but two distinct lines were now apparent (Fig. 6*A*). When the data were divided into groups according to plasma $[H^+]$, however, a single line described the relationship at low (< *ca.* 37 mmHg) levels of P_{CO_2}, but the groups diverged at high levels of P_{CO_2} (Fig. 6*B*).

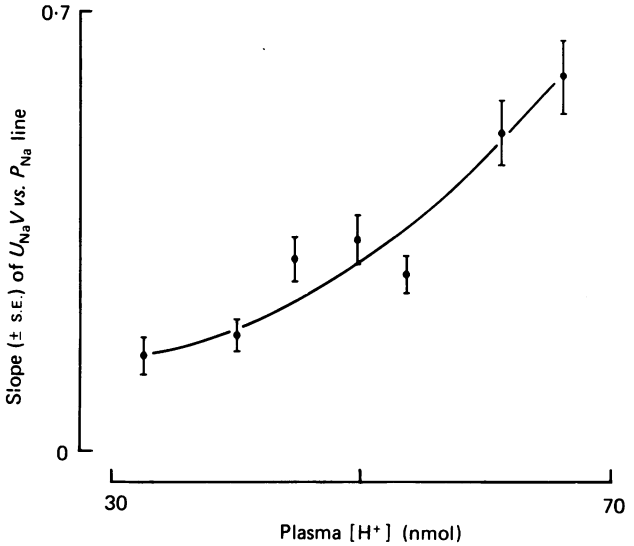

Fig. 5. The slope of the regression line of $U_{Na}V$ on P_{Na} is shown plotted against plasma hydrogen ion concentration (plasma [H^+]). The line joining the points was drawn freehand. The data from which this Figure was drawn are:

Plasma [H^+] (mean; range)	Regression equation	n	r
32·7; $\leqslant 40$	$U_{Na}V = 0{\cdot}150\ P_{Na} - 20{\cdot}66$	69	0·53
40·2; > 30 and $\leqslant 45$	$U_{Na}V = 0{\cdot}183\ P_{Na} - 24{\cdot}95$	144	0·51
44·8; > 40 and $\leqslant 50$	$U_{Na}V = 0{\cdot}308\ P_{Na} - 42{\cdot}90$	148	0·59
49·8; > 45 and $\leqslant 55$	$U_{Na}V = 0{\cdot}334\ P_{Na} - 46{\cdot}91$	105	0·55
53·7; > 50 and $\leqslant 60$	$U_{Na}V = 0{\cdot}278\ P_{Na} - 39{\cdot}36$	72	0·76
61·3; > 55 and $\leqslant 70$	$U_{Na}V = 0{\cdot}503\ P_{Na} - 73{\cdot}35$	76	0·75
66·3; > 60	$U_{Na}V = 0{\cdot}592\ P_{Na} - 87{\cdot}54$	67	0·79

DISCUSSION

In this study we assessed the influence of a number of variables on sodium excretion during the infusion of isotonic, hypotonic and hypertonic solutions of sodium chloride. It was found that sodium excretion was related to two independent processes. These were, first plasma sodium concentration (P_{Na}) and its attendant acid–base and ventilatory disturbances, and secondly the post-glomerular concentration of plasma solids (PGPS). These variables were defined by analysis of data from a large number of experiments in which no attempt was made to dictate a pattern of sodium excretion by the experimental design. Special analytical techniques were then required to examine the possible interactions between the many variables studied. Factor analysis was particularly helpful since it allowed simultaneous description of a number of physiological changes that should properly be considered as appearing in consort.

These results confirm that a rise in P_{Na} is associated with an increase in $U_{Na}V$. This is in accordance with the earlier observations of Kruhøffer (1950), Blythe & Welt (1963, 1965) and Kamm & Levinsky (1965). The magnitude of the change in $U_{Na}V$

produced by unit change in P_{Na} (the slope of the line of regression of $U_{Na}V$ on P_{Na}) is dependent on changes in plasma [H^+] and P_{CO_2}, and in PGPS.

P_{Na}, the concentration of plasma solids (PS), plasma [H^+] and $U_{Na}V$ were all strongly represented in factor 1. This was taken to represent the metabolic acidosis that accompanies hypernatraemia and haemodilution, and which could participate in eliciting the natriuresis (Nashat *et al.* 1976). Indeed, sodium excretion per unit change in P_{Na} was a continuous function of plasma [H^+] (Fig. 5), suggesting that the prevailing acid–base status had a marked influence on $U_{Na}V$ that was independent of P_{Na}.

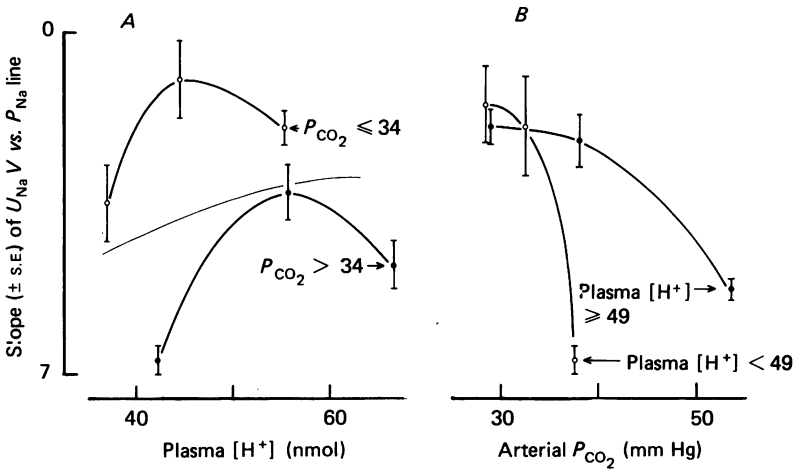

Fig. 6. The slope of the regression line of $U_{Na}V$ on P_{Na} is shown plotted against: *A*, plasma hydrogen ion concentration (plasma [H^+]), in groups of high P_{CO_2} (●) and low P_{CO_2} (○); and *B*, P_{CO_2} in groups of high plasma [H^+] (●) and low plasma [H^+] (○). The third line in *A* shows the form of the relationship depicted in Fig. 5 in the 200 cases in which P_{CO_2} was measured. The lines joining the points were drawn freehand. The data from which this Figure were drawn are:

A	Plasma [H^+] (mean; range)	Regression equation	*n*	*r*
$P_{CO_2} > 34$	42·2; < 49	$U_{Na}V = 0{\cdot}028\ P_{Na} - 3{\cdot}00$	37	0·16*
	55·7; ⩾ 49 and < 61	$U_{Na}V = 0{\cdot}371\ P_{Na} - 53{\cdot}02$	25	0·81
	66·6; ⩾ 61	$U_{Na}V = 0{\cdot}223\ P_{Na} - 30{\cdot}87$	38	0·61
$P_{CO_2} \leqslant 34$	37·0; < 41	$U_{Na}V = 0{\cdot}350\ P_{Na} - 49{\cdot}61$	20	0·73
	44·5; ⩾ 41 and < 49	$U_{Na}V = 0{\cdot}603\ P_{Na} - 87{\cdot}51$	49	0·75
	55·3; ⩾ 49	$U_{Na}V = 0{\cdot}504\ P_{Na} - 72{\cdot}38$	31	0·94
B	P_{CO_2} (mean; range)	Regression equation		
Plasma [H^+] ⩾ 49	29·0; ⩽ 34	$U_{Na}V = 0{\cdot}504\ P_{Na} - 72{\cdot}38$	31	0·94
	38·1; > 34 and ⩽ 41	$U_{Na}V = 0{\cdot}476\ P_{Na} - 68{\cdot}02$	30	0·86
	53·5; > 41	$U_{Na}V = 0{\cdot}173\ P_{Na} - 24{\cdot}33$	33	0·82
Plasma [H^+] < 49	28·4; ⩽ 31	$U_{Na}V = 0{\cdot}550\ P_{Na} - 79{\cdot}50$	38	0·76
	32·5; > 31 and ⩽ 34	$U_{Na}V = 0{\cdot}506\ P_{Na} - 72{\cdot}59$	31	0·68
	37·2; > 34	$U_{Na}V = 0{\cdot}028\ P_{Na} - 3{\cdot}00$	37	0·16*

* The low correlation and slope of the regression line at this point where $P_{CO_2} > 34$ mmHg and plasma [H^+] < 49 nmol/l indicates that there is no detectable increase in $U_{Na}V$ with increasing P_{Na} in this group.

Makoff *et al.* (1970) described the dilution of extracellular buffers, notably bicarbonate, that occurred when hypertonic solutions were infused into dogs. The metabolic acidosis so produced should normally stimulate respiration and thereby alter P_{CO_2} (Fig. 7). We used data selection to investigate whether the dependence of $U_{Na}V$ on plasma $[H^+]$ during sodium loading could be attributed to concurrent changes in P_{CO_2}.

The relationship between plasma $[H^+]$ and the slope of the regression of $U_{Na}V$ on P_{Na}, when divided according to whether the P_{CO_2} was high or low, was described by

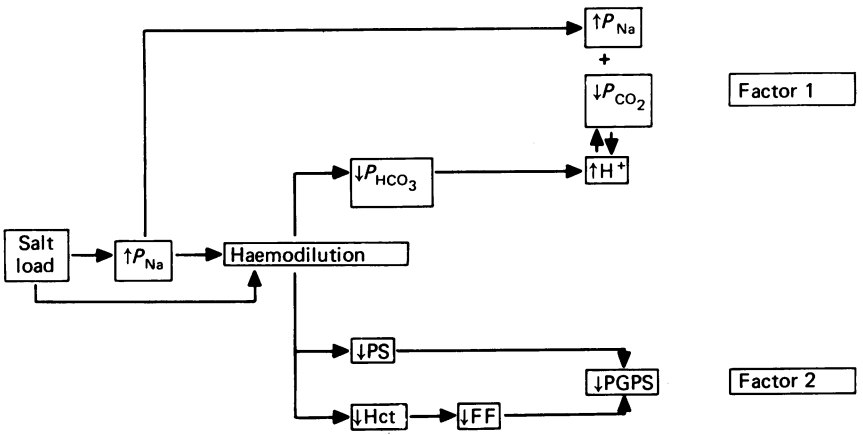

Fig. 7. A diagrammatic representation of the response to salt loading, involving the major interrelationships depicted in the factor analysis.

two distinct lines. This indicates that the level of P_{CO_2} can influence $U_{Na}V$ independently of the measured plasma $[H^+]$. When the slope of the regression was related to P_{CO_2} with the data divided according to whether the plasma $[H^+]$ was high or low, there was no separation between the two groups until P_{CO_2} exceeded *ca.* 37 mmHg. This unique relationship indicates that changes in P_{CO_2} in its lower ranges account for the effects of the acidosis on sodium excretion. A reduction in P_{CO_2} during salt loading, produced by a ventilatory response to acidosis, could play a part in securing the natriuresis. Even in the absence of acidosis, a reduction in P_{CO_2} by hyperventilation in normal man or anaesthetized dogs provokes natriuresis (McCance & Widdowson, 1936; Danielson, Grängsjö, Persson, Ulfendahl & Wolgast, 1973).

The results confirm previous studies which have shown that an acute metabolic acidosis can augment $U_{Na}V$ (Sartorius, Roemmelt & Pitts, 1949). Acute respiratory acidosis, in contrast, has little effect on $U_{Na}V$ (Luke & Levitin, 1966; Danielson *et al.* 1973). Similarly, sodium excretion is augmented by metabolic but not by respiratory acidosis in isolated perfused kidneys (Rostand & Watkins, 1977). These results related enhanced excretion in acidosis to reduced levels of P_{CO_2} or plasma bicarbonate as in the present experiments, and they incidate that the mechanism is intrarenal.

Saline loading causes haemodilution, which will normally diminish PGPS by diluting plasma proteins, as well as by reducing filtration fraction (FF) (Nashat &

Portal, 1967) (Fig. 7). PGPS is a derived value, which may be a final common pathway by which changes in GRF and RPF, their relation to each other (FF), and plasma dilution are manifest at the renal tubule. Neither FF nor PS alone fully describes the relationship of PGPS to sodium excretion. Furthermore, it is interesting that only inverse changes in GFR and RPF, as in factor 2, were found to be related to $U_{Na}V$. When filtration changed in parallel with flow, as in factor 3, no relationship with $U_{Na}V$ was apparent (Fig. 2). Thus, the effects of RPF and GFR on $U_{Na}V$ are best understood through the changes they produce in PGPS. Our results are in accord with those of Nizet (1976), who demonstrated the close inverse relationship between PGPS and $U_{Na}V$ in the whole organism and in the isolated perfused kidney.

Sodium excretion remained a function of P_{Na} in these experiments and, as argued by Nashat *et al.* (1976), the natriuresis observed with increasing P_{Na} is associated with a decrease in the proportion of filtered sodium reabsorbed by the tubule, at least at the higher levels of P_{Na}. The intrarenal mechanism whereby the three main components of factor 1 (P_{Na}, plasma $[H^+]$ and P_{CO_2}) might influence tubular sodium reabsorption cannot be defined by our experiments, but may be related to the entry of sodium from the tubular lumen into the cell (Wilcox, 1980). Those variables represented in the second factor (PGPS) may modulate sodium transport at a later step: the uptake of sodium from the interstitial fluid into the peritubular capillary blood stream (Lewy & Windhager, 1968).

APPENDIX

The usual factor analysis model assumes that a single set of correlated observations is made on each of a number of independent subjects. The application of factor analysis to our data therefore requires justification on two grounds.

The first is that the dogs were subjected to infusions of saline of four different strengths, administered by two different routes. Furthermore, some animals breathed spontaneously and others were artificially ventilated. This might have imposed constraints on our results if it had led to clusters of data. However, the observations made with each strength of saline or each route of administration, or with each manner of ventilation, do not represent a distinct group of observations: rather, this variability in experimental design produced a wide and evenly distributed range of values at the kidney for P_{Na} (Fig. 1) and for the other measured variables.

The second is that 357 sets of observations include a varying number of observations from each of fifty-two dogs, thus potentially introducing bias unless the variability within dogs is similar to the variability between dogs. The possibility of bias due to within-dog variability was examined in the following fashion. One set of observations was chosen at random (using a table of random numbers) from the four to twelve observations made on each dog. This unbiased subgroup of fifty-two sets of observations was then examined, to test whether it led to description of the same physiological mechanisms as had been elucidated in the complete group of 357 sets of observations. Errors introduced by bias due to within-dog variability differing from between-dog variability would lead to a different pattern of inter-correlation between the variables in the complete group of 357 sets of observations compared with this subgroup. Table 3 shows the inter-correlation of the primary variables in this

subgroup. The pattern or inter-correlation is very similar to that found in the complete group (Table 2), and therefore factor analysis yielded very similar factors (Fig. 8) to those previously extracted (Fig. 2). Factor 1 again shows the close relation of $U_{Na}V$ to P_{Na}, with acidosis and plasma dilution concomitant with rising P_{Na}. Factor 2 demonstrates the inverse association of PGPS and $U_{Na}V$ in this subgroup.

TABLE 3. The correlations between the variables measured in the subgroup of fifty-two observations taken at random, one from each dog. Note the similar pattern of inter-correlation to that shown in Table 2.

$U_{Na}V$	1·000								
P_{Na}	0·591	1·000							
PS	−0·454	−0·615	1·000						
Hct	−0·493	−0·061	0·448	1·000					
[H⁺]	0·325	0·443	−0·200	0·035	1·000				
RPF	0·063	−0·228	0·268	−0·049	0·160	1·000			
GFR	−0·065	0·032	0·240	0·361	0·258	0·428	1·000		
BP	−0·131	0·047	0·020	0·345	0·044	−0·141	0·200	1·000	
P_K	0·039	−0·070	0·001	−0·091	0·324	0·055	0·066	0·035	1·000
	$U_{Na}V$	P_{Na}	PS	Hct	[H⁺]	RPF	GFR	BP	P_K

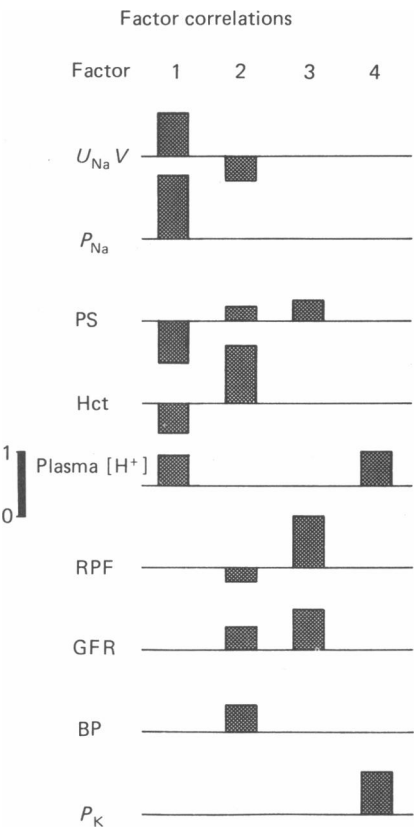

Fig. 8. Correlations of the variables measured in the subgroup of fifty-two observations (Table 3) with the four factors extracted by the same methods as used in Fig. 2. Correlations are shown in the same way as in Fig. 2; for the sake of clarity, correlations of absolute magnitude less than 0·3 are omitted. Note the similar pattern of correlation between primary variables and extracted factors in this Figure and in Fig. 2.

These studies were supported by grants from the Medical Research Council to F.S.N. and the Wellcome Trust to C.S.W. We would like to thank Mrs S. Basu, Miss H. A. Prowse, Miss S. A. Roddis and Mr B. G. Taylor for much expert technical assistance, and Miss G. M. Kearney and Mr J. Thirlwall for advice on computing. We are particularly grateful to Dr J. Fry, Professor F. Hobbiger and Professor W. S. Peart for discussion of this manuscript.

REFERENCES

BLYTHE, W. B. & WELT, L. G. (1963). Dissociation between filtered load of sodium and its rate of excretion in the urine. *J. clin. Invest.* **42**, 1491–1496.

BLYTHE, W. B. & WELT, L. G. (1965). Plasma sodium concentration and urinary sodium excretion. *Trans. Ass. Am. Physns* **78**, 90–96.

BRENNER, B. M., TROY, J. L., DAUGHARTY, T. M., DEEN, W. M. & ROBERTSON, C. R. (1972). Dynamics of glomerular filtration in the rat. II. Plasma-flow dependence of GFR. *Am. J. Physiol.* **223**, 1184–1190.

COOLEY, W. W. & LOHNES, P. R. (1971). *Multivariate Data Analysis.* New York: Wiley.

DANIELSON, B. G., GRÄNGSJÖ, G., PERSSON, E., ULFENDAHL, H. R. & WOLGAST, M. (1973). Renal function in the dog in acute disturbances of the acid–base balance. *Acta anaesth. scand.* **17**, 88–102.

DE WARDENER, H. E. (1977). Natriuretic hormone. *Clin. Sci. molec. Med.* **53**, 1–8.

EARLEY, L. F. & FRIEDLER, R. M. (1965). Changes in renal blood flow and possibly the intrarenal distribution of blood during the natriuresis accompanying saline loading in the dog. *J. clin. Invest.* **44**, 929–941.

GAUER, O. H. & HENRY, J. P. (1963). Circulatory basis of fluid volume control. *Physiol. Rev.* **43**, 423–481.

KAMM, D. E. & LEVINSKY, N. G. (1965). Inhibition of renal tubular sodium reabsorption by hypernatraemia. *J. clin. Invest.* **44**, 1144–1150.

KRUHØFFER, P. (1950). *Studies on Water–Electrolyte Excretion and Glomerular Activity in the Mammalian Kidney.* London: H. K. Lewis & Co.

KRUHØFFER, P. (1960). Handling of alkali metal ions by the kidney. In *Handbuch der experimentellen Pharmakologie*, vol. 13, *The Alkali Metal Ions in Biology*, ed. EICHLER, O. & FARAH, A., p. 233. Berlin: Springer-Verlag.

LAWLEY, D. N. & MAXWELL, A. E. (1971). *Factor Analysis as a Statistical Method.* London: Butterworth.

LEWY, J. E. &WINDHAGER, E. E. (1968). Peritubular control of proximal tubular fluid reabsorption in the rat kidney. *Am. J. Physiol.* **214**, 943–954.

LUKE, R. G. & LEVITIN, H. (1966). The renal and electrolyte response to respiratory acidosis in the adrenalectomised rat. *Yale J. biol. Med.* **39**, 27–37.

MCCANCE, R. A. & WIDDOWSON, E. M. (1936). The response of the kidney to an alkalosis during salt deficiency. *Proc. R. Soc.* B **120**, 228–239.

MCDONALD, S. J. & DE WARDENER, H. E. (1965). The relationship between the renal arterial perfusion pressure and the increase in sodium excretion which occurs during an infusion of saline. *Nephron* **2**, 1–14.

MAKOFF, D. L., DA SILVA, J. A., ROSENBAUM, B. J., LEVY, S. E. & MAXWELL, M. H. (1970). Hypertonic expansion: acid–base and electrolyte changes. *Am. J. Physiol.* **218**, 1201–1207.

MYERS, B. D., DEEN, W. M., ROBERTSON, C. R. & BRENNER, B. M. (1975). Dynamics of glomerular ultrafiltration in the rat. VIII. Effects of hematocrit. *Circulation Res.* **36**, 425–435.

NASHAT, F. S. & PORTAL, R. W. (1967). The effects of changes in haematocrit on renal function. *J. Physiol.* **193**, 513–522.

NASHAT, F. S., SCHOLEFIELD, F. R., TAPPIN, J. W. & WILCOX, C. S. (1969). The effect of acute changes in haematocrit in the anaesthetized dog on the volume and character of the urine. *J. Physiol.* **205**, 305–316.

NASHAT, F. S., TAPPIN, J. W. & WILCOX, C. S. (1976). Plasma sodium concentration and sodium excretion in the anaesthetized dog. *J. Physiol.* **254**, 183–202.

NEI, H. H., HULL, C. H., JENKINS, J. G., STEINBRENNER, K. & BENT, D. H. (1975). *Statistical Package for the Social Sciences*, 2nd edn. New York: McGraw-Hill.

NIZET, A. (1967). Control by plasma potassium concentration of sodium excretion by isolated perfused dog kidney. *Pflügers Arch.* **297**, 162–165.

NIZET, A. (1972). Quantitative influence of non-hormonal blood factors on the control of sodium excretion by the isolated dog kidney. *Kidney Int.* **1**, 27–37.

NIZET, A. (1976). Comparative evaluation of fractional excretion of sodium following saline infusion in transplanted kidneys and in isolated perfused kidneys in conditions of previous high or low sodium intake. *Pflügers Arch.* **361**, 121–126.

O'CONNOR, W. J. (1962). *Renal Function.* London: Edward Arnold.

ROSTAND, S. G. & WATKINS, J. B. (1977). Response of the isolated rat kidney to metabolic and respiratory acidosis. *Am. J. Physiol.* **233**, *F*82–*F*88.

SARTORIUS, O. W., ROEMMELT, J. C. & PITTS, R. F. (1949). The renal regulation of acid–base balance in man. IV. The nature of the renal compensations in ammonium chloride acidosis. *J. clin. Invest.* **28**, 423–439.

SCHRIER, R. W. & EARLEY, L. E. (1970). Effects of hematocrit on renal haemodynamics and sodium excretion in hydropenic and volume-expanded dogs. *J. clin. Invest.* **49**, 1656–1667.

THOMPSON, D. D. & PITTS, R. F. (1952). Effects of alterations of renal arterial pressure on sodium and water excretion. *Am. J. Physiol.* **168**, 490–499.

WILCOX, C. S. (1980). The regulation of sodium excretion during salt loading. In *Advances in Physiological Sciences*, vol. 11, *Kidney and Body Fluids.* Oxford: Pergamon Press (in the Press).