

Genetic variation in diuretic responses: further and correlated responses to selection

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1. INTRODUCTION

The use of genetic selection as a tool in the analysis of a physiological character has been discussed by Thoday (1967). It was with the aim of elucidating some of the genetic determinants of a physiological character that we started selection for three parameters of diuretic response to water-load in mice (Stewart & Spickett, 1965). In this paper the further responses to selection in each of the three lines are described.

It is possible to discuss certain features of responses to selection, for example asymmetry of response, in relation to underlying physiological mechanisms (Falconer, 1953). However, such a discussion is necessarily expressed in very general terms and is of limited physiological value. In order to describe a response to selection in terms of the actual genetic determinants of a physiological system, it is necessary to observe correlated responses to selection (Falconer, 1967). For this reason, the correlated responses in parameters of electrolyte metabolism, adrenal weight and renal structure have been observed in one of the selection lines.

2. MATERIALS AND METHODS

(i) *Measurement of diuresis and definition of parameters*

The work of Verney (1958) has shown that the diuretic response of an animal to stomach loads of water can be considered as a reflexion of an underlying mechanism of physiological homeostasis. Details of the method of water-loading and the monitoring of the diuretic response are given in the earlier paper (Stewart & Spickett, 1965). Each mouse was given a stomach load of 1 ml. water/16 g. body weight. The volume and the time after loading of each urination in the consequent diuresis were noted; the cumulative volume of urine excreted was then plotted against time as shown in Fig. 1. This diuretic response to water-load is characterized by three descriptive parameters: diuretic lag, L (time to excretion of 50% of total volume); maximum rate of diuresis, R ; and the ratio of total output to input volume, U .

(ii) *Techniques of selection*

The details of techniques of selection are also given in the earlier paper (Stewart & Spickett, 1965). The selection lines for L , R , and U , were started from the

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heterogeneous base-stock resulting from a four-way cross between the strains of mice CBA/FaCam, A/Cam, SanFrancisco/Cam and Peru. For the parameters U and R simple two-way directional selection has been practised, with a high and a low line of six matings per generation for each generation. For the first six generations of selection the matings were made up so as to minimize inbreeding; for later generations the highest male was mated to the highest female, the second highest male with the second highest female, and so on down to the lowest male which was mated with the lowest female. The purpose of this relative inbreeding was to stabilize the responses to selection.

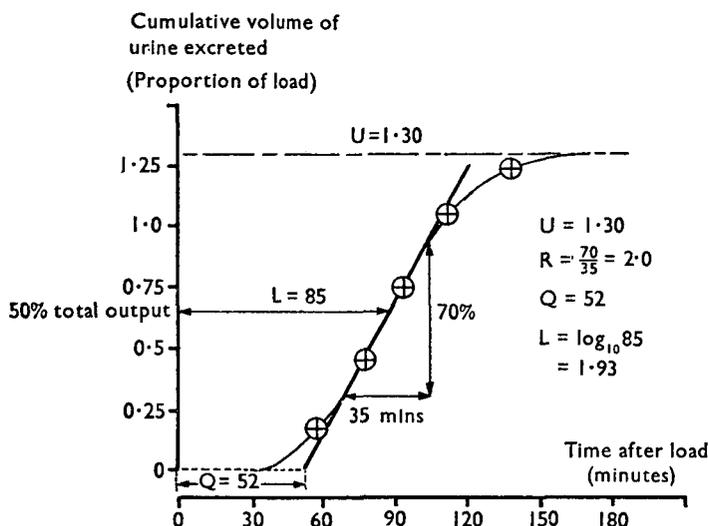


Fig. 1. A typical curve of water-diuresis, and definition of parameters of diuretic lag (L and Q), maximum rate of diuresis (R) and ratio of total output to input volume (U).

The parameter L was measured twice on each mouse, once at 4 weeks (L_4) and once at 8 weeks (L_8). Disruptive selection has been carried out for two combinations of L_4 and L_8 (Stewart & Spickett, 1965).

(iii) Measurement of correlated responses to selection

The weight of the adrenal glands was measured. Mice were killed with ether at 8 weeks of age, the adrenals were dissected, cleaned of adherent tissue, and weighed on a torsion balance.

The structure of the kidney was characterized quantitatively as described in detail by Stewart & Spickett (1967) and Stewart (1966). The following zones of the kidney were distinguished in histological sections: cortex, containing proximal and distal convoluted tubules and glomeruli; outer medulla, containing the pars recta of the proximal tubule and the ascending limb of the loop of Henle; inner medulla, containing thin descending and thick ascending limbs of the loop of Henle; and the

pyramid, consisting principally of the later stages of collecting ducts. The volume of each of these zones was measured by reconstruction of the kidney from the histological sections.

The amounts of Na⁺ and K⁺ excreted in the course of the water diuresis were also measured. The urine from the water diuresis was diluted to 5 ml. with distilled water and the Na⁺ and K⁺ measured in the standard way on a Unicam SP 900 flame spectrophotometer.

3. RESULTS

(i) *Further responses to selection for diuretic lag, L*

The disruptive selection experiment for negative correlation between L_4 and L_8 has been continued. The purpose of this experiment was to select genes acting at 4 weeks but not at 8 weeks, and vice versa, on a genetic background which would

Table 1. *Response to selection for negative correlation between diuretic lag at 4 and 8 weeks (L_4 and L_8) Expt. II*

	Generation					
	1	2	3	4	5	6
Correlation coefficient between L_4 and L_8	0.29	0.42	0.57	0.08	-0.08	0.03
Variance in L_4 * attributable to:						
Offspring-parent regression (genetic variance)	7.2	2.4	2.1	0.7	0.13	0.5
Residual (environmental variance)	1.8	0.6	4.8	17.7	4.1	7.0
Regression coefficient of male offspring on parental mean for L_4 (realized heritability)	0.83	0.63	0.54	0.20	-0.17	0.27

* All values $\times 10^{-3}$.

enhance their effects (Stewart & Spickett, 1965). After six generations of selection the correlation between L_4 and L_8 has fallen to zero (Table 1). Thus the correlation has been reduced, but this is probably due to the decrease in the proportion of genetic variance to random environmental variance (Table 1). This inference is supported by the decrease in heritability. In other words there has been selection against the genes causing variance in L_4 . This means that there genes caused variance in both L_4 and in L_8 ; in other words that there were few or no genes causing variance in L_4 but not in L_8 , or vice versa. For this reason the experiment designed to select such genes has failed. This failure is instructive in one respect. It suggests that there

may be no genes causing variance in L at 8 weeks which were not already active and expressing themselves at 4 weeks. Since the physiological system mediating the diuretic response to water-load is defined by genetic information, this implies that all the elements of this physiological system are present and active by 4 weeks. This is of course what one would expect, since the mice are weaned at this age and therefore need to have a homeostatic system controlling their water balance (Adolph, 1957). This does not mean that no overall developmental changes occur between 4 and 8 weeks: it has already been argued that a genetic difference causes greater phenotypic variation in L at 4 weeks than at 8 weeks (Stewart & Spickett, 1965).

The 'control' line of selection for positive correlation between L_4 and L_8 has continued successfully for six generations. However, the failure of the selection for

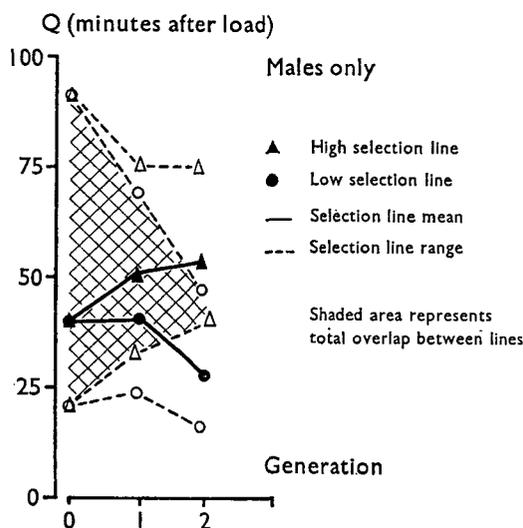


Fig. 2. Response to selection for diuretic lag Q .

negative correlation means that there is no further point in the measurement of L at two different ages or in the complex disruptive selection technique. For this reason both disruptive selection experiments were stopped after six generations and the mice used as the base stock for a simple two-way directional experiment completely analogous to that for the parameters R and U . At the same time the criterion of 'diuretic lag' was changed to a new parameter, Q , which is less obviously correlated with R (Fig. 1).

Selection for Q has been carried out for two further generations. There has been a good response to selection with a rapid decrease in overlap between the lines (Fig. 2). The regression coefficient of male offspring on parental mean for these generations of selection is 0.46 ± 0.12 , which is highly significant and represents a high heritability for this character. This rapid response to selection probably reflects the efficiency of disruptive selection in producing gene segregation on a genetic background enhancing the effects of that segregation (Thoday, 1964).

(ii) *Further responses to selection for maximum rate of diuresis, R*

There has been a good response to selection for *R* (Fig. 3). In the later generations of selection the high and low lines have become discontinuous. The regression coefficients of male offspring on parental mean of 0.85 ± 0.15 for generation 5–6 and 0.63 ± 0.04 for generations 8–9 and 9–10, are highly significant and indicate high heritabilities. There is a marked asymmetry in the selection response: in the low line the generation mean has fallen to a value of 0.9 which is well below the value of 1.4 for the lowest founder strain, strain A/Cam. Thus a new type of mouse has been produced by selection. On the other hand the high line has shown little response to selection and the generation means have not risen above the values of 2.5 for two of the founder strains (Peru and CBA).

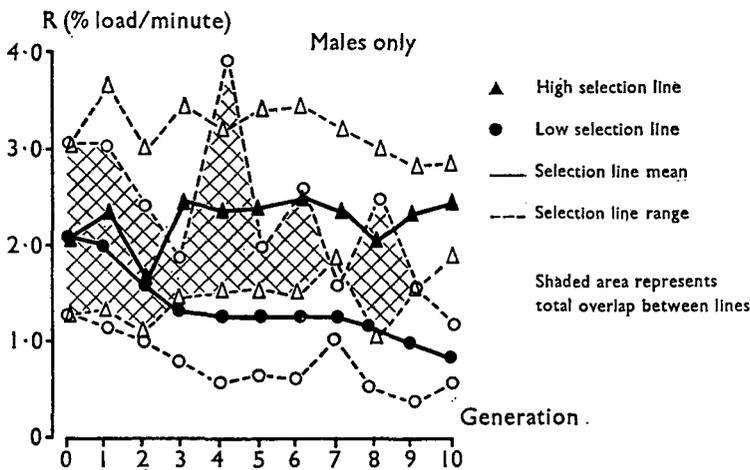


Fig. 3. Response to selection for maximum rate of diuresis *R*.

(iii) *Further responses to selection for ratio of total output to input, U*

The response to selection is shown in Fig. 4. The high and low selection lines diverge significantly in the second and all succeeding generations of selection. The most satisfactory criterion of genetic divergence is the regression coefficient of offspring on parents. The regression of male offspring on parental mean is 0.41 ± 0.04 for generation 5–6, and 0.42 ± 0.05 for generations 8–9 and 9–10. Both these values are highly significant.

For the first three generations of selection, the low line appears to have a lower 'threshold' value of 1.0 that is not significantly exceeded. Further, as the generation mean approached 1.0 the distribution became skewed (Stewart & Sprickett, 1965). In generation 4 and in all succeeding generations of selection, some mice were produced with extremely low values of *U*, less than or equal to 0.5. However, these mice generally proved to be infertile so that although the heritability even in later generations is fairly high, there has been no dramatic response to selection. These results taken together strongly suggest that the value of 1.0 for *U* is a 'natural' lower limit in terms of the mechanism mediating the diuretic response to

water-load. This is not surprising since *a priori* one would expect any homeostatic mechanism at least to excrete the input volume of water. Of course a value of U greater than 1.0 could result: for example if some solute was excreted in the course of diuresis and the homeostatic mechanism was regulating plasma tonicity; or if the response was mediated by a hormone, in which case the response could 'overshoot' the equilibrium position (Stewart & Spickett, 1967). Thus a drop in the value of U to much less than 1.0, achieved in one generation, is likely to represent a failure in some element of the homeostatic mechanism. It is not surprising that a change of this order of magnitude in the physiological organization of an animal should be accompanied by in fertility. In spite of this the generation means have continued to drop steadily to a value of 0.92 for generations 9 and 10. This value is well below the limit of the heterogeneous base stock (1.0) and of the lowest

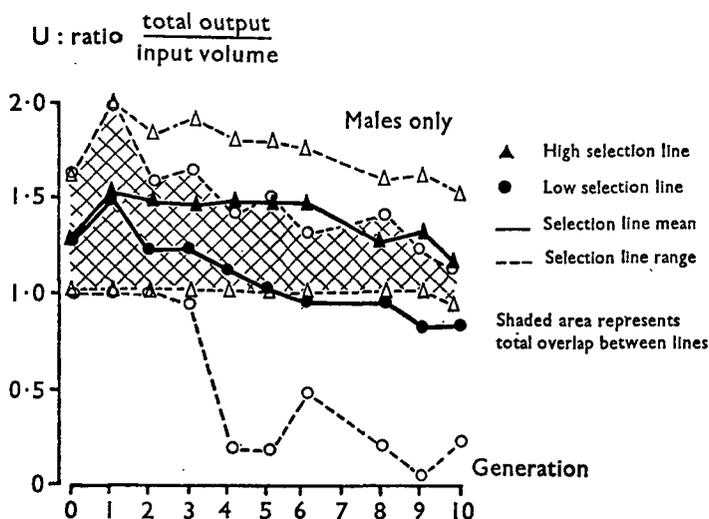


Fig. 4. Response to selection for ratio of total output/input volume, U .

strain, Peru (1.0). Thus again a new type of mouse has been produced by selection. The response to selection for high values of U is less interesting. The generation mean has never risen consistently above the value of 1.30 of two of the founder strains, A/Cam and CBA/FaCam: even this limited response has dropped during the relative inbreeding of later generations.

(iv) *Correlated responses to selection*

The correlated responses to be described below have been observed in the high and low selection lines for U . The selection lines for U were chosen because they showed most evidence of underlying changes in the mechanisms of diuretic response.

(a) *Electrolyte excreted in diuresis*

The amounts of Na^+ and K^+ excreted were measured on the males of generation 5 in the course of the same diuresis as that on which U itself was measured for the

purpose of selection. The results of $\text{Na}^+ + \text{K}^+$ excreted are plotted against U in Fig. 5. The units of 'total electrolyte excreted' are such that a line of slope 1.0 passing through the origin would represent a urine isosmotic with plasma. Within the limits of standard error of the regression of U on electrolyte excretion (0.87 ± 0.13) has a value of 1.0, whereas the regression of electrolyte excretion on U (0.59 ± 0.19) does not. One interpretation of this is that the electrolyte excretion is a basic factor in determining U , rather than vice versa; that enough water is excreted to bring the animal back into osmotic balance. Thus a high value of U will result from

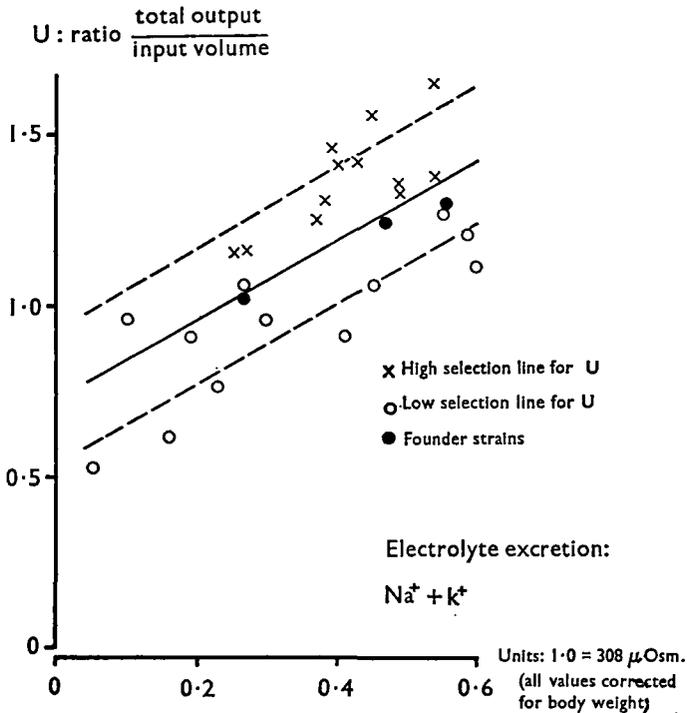


Fig. 5. Correlated response in electrolyte excretion to selection for U .

a high electrolyte excretion. It must be noted that there are certainly other causes of variation in U : all the 'low' selection line mice lie below the regression line of Fig. 5.

(b) Relative adrenal weight

Adrenal weight per gram body weight at 8 weeks of age for the rejected males of the sixth generation of selection is plotted against U in Fig. 6. The relative adrenal weights of the high selection line are discontinuously lower than those of the low selection line for U . The correlation coefficient of -0.67 is highly significant. If the relationship between relative adrenal weight and U is physiologically significant, then selection for adrenal weight should involve a correlated response in U . The selection experiment for relative adrenal weight starting from the same base stock

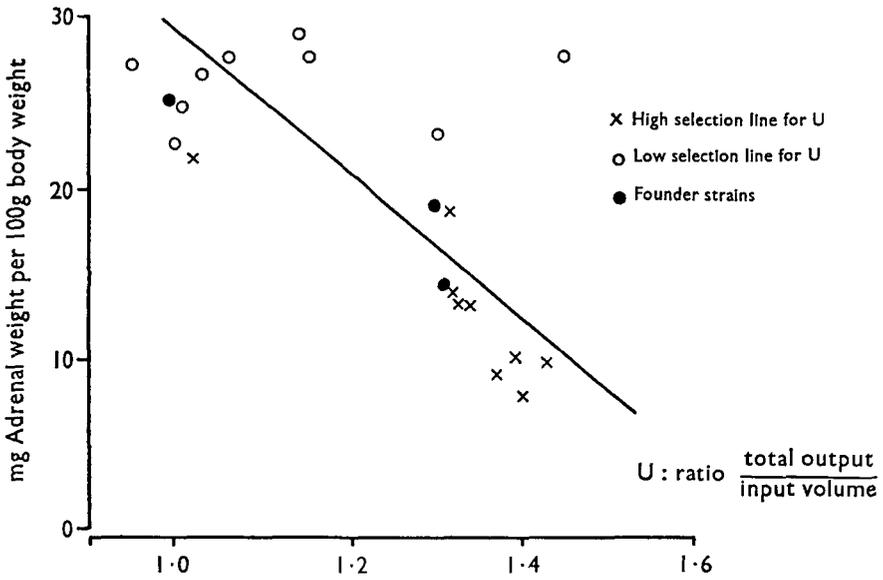


Fig. 6. Correlated response in relative adrenal weight to selection for U .

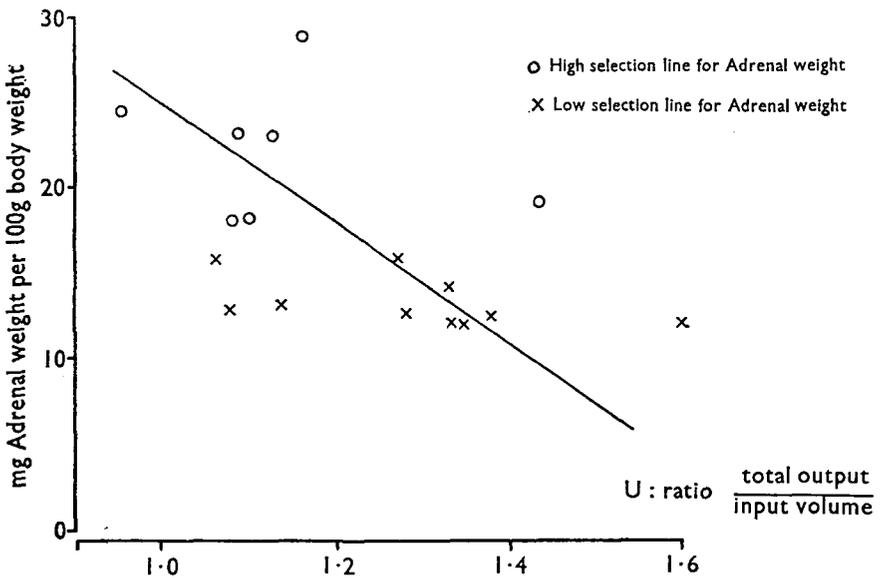


Fig. 7. Correlated response in U to selection for relative adrenal weight.

has been carried out by Badr (1965), and the results of water-loading the males of the eighth generation of selection are shown in Fig. 7. The correlation coefficient, -0.47 , is again negative and significant.

(c) *Renal structure*

Renal structure was measured quantitatively in three high-line and three low-line male mice from generation 7, which was not measured for *U*. The results are shown in Table 2. There are significant differences between the lines in two respects. Firstly, both the inner and outer zones of the renal medulla are relatively large in the selection line for low values of *U*. The part of the nephron common to these two sections is the thick ascending limb of the loop of Henle. Secondly, the zones containing the later stages of the collecting ducts are relatively large in the selection line for low values of *U*.

Table 2. *Renal structure in high and low selection lines for U*

Parameter	High <i>U</i>	Low <i>U</i>
mg. kidney weight per g. body weight	16.4 ± 0.3	15.8 ± 0.4
Total number of glomeruli	$15,400 \pm 500$	$15,000 \pm 500$
Volumes of zones:	% total	% total
1. Cortex	77 ± 3	72 ± 2
2. Outer medulla	12.8 ± 0.5	14.2 ± 0.6
3. Inner medulla	8.9 ± 0.4	10.1 ± 0.4
4. Pyramid	1.0 ± 0.3	3.1 ± 0.3

4. DISCUSSION

The regression coefficient of offspring on parental mean may be regarded as the realized heritability for the character (Falconer, 1960). The fact that each of three arbitrarily chosen descriptive parameters has responded to selection with significant realized heritability is strong evidence of the existence of genetic variation affecting diuretic responses to water-load. Thus the conclusions of the earlier paper are confirmed (Stewart & Spickett, 1965) and the first purpose of the selection experiment has been fulfilled.

The significant correlated responses to selection for *U* have demonstrated that this genetic variation exists both at the functional level of diuretic response to water-load, and at the lower level of some of the elements of the homeostatic mechanisms underlying these diuretic responses.

A significant correlated response to selection implies that gene pleiotropy is involved, i.e. that some of the selected genes that affect *U* also affect other physiological characters. There are two criteria for deciding whether a number of char-

acters are the pleiotropic effects of a single gene. Firstly there is the 'gene as a unit of function'; the characters should be related in a 'pedigree of causes' (Grüneberg, 1938). Secondly, there is the gene as a unit of segregation and recombination: the pleiotropic effects of a single gene must constitute an indivisible unit in breeding experiments.

The pattern of pleiotropic effects of a single gene is a reflexion of the physiological (or developmental) relationship between the pleiotropic characters. Thus in order to analyse the correlated responses in terms of 'units of function', it is necessary to consider the physiological relationships between the various characters involved.

The correlation between total electrolyte excretion and U in the response to selection has been interpreted above on the basis that enough water is excreted to bring the animal back into osmotic balance. This implies that the physiological system mediating diuretic response to water-load is controlled by plasma osmotic pressure rather than by total body water as such. This is in agreement with the well-established view that water-diuresis is caused by a fall in antidiuretic hormone, and that the release of this hormone is inhibited by a fall in plasma osmotic pressure (Verney, 1958). Thus any gene which affects total electrolyte excretion, i.e. salt loss in water diuresis, will also have a pleiotropic effect on U . This would explain the correlated response in salt loss.

It is well known that adrenalectomy or adrenal insufficiency results in salt loss and drastically reduces the ability of an animal to produce a dilute urine (Chester Jones, 1957). Thus a gene which results in increased adrenal size may be expected to have a pleiotropic effect on salt loss, and hence ultimately a pleiotropic effect on U . This could account for at least a part of the observed correlated response in adrenal weight.

The mineralocorticoid hormones secreted by the adrenal cortex stimulate active sodium reabsorption from the thick ascending limb of the loop of Henle in the renal medulla. According to the counter-current theory of renal function (Wirz, 1963; Morel & Guinnebault, 1961), this sodium reabsorption is primarily responsible for the ability to produce an osmotically dilute urine. Thus a gene increasing the size of the renal medulla will increase the ability to produce a dilute urine in water diuresis; it will thus decrease total salt loss in the diuresis and hence have a pleiotropic effect on U . This would explain the correlated response in the renal medulla.

A further correlated response to U concerned the more distal parts of the collecting ducts. It is commonly supposed that water is reabsorbed from the collecting ducts and that this is the site where osmotically free water output is finally controlled (Smith, 1951). Thus a gene increasing the relative importance of collecting ducts will have a pleiotropic effect in decreasing U . This would explain the correlated response in volume occupied by the collecting ducts. Moreover, this effect of collecting ducts on U is relatively independent of final electrolyte excretion, so that such genes may account in part at least for the values of U below those expected on the basis of salt loss in the low line (see above, Fig. 5 and text).

Thus it is possible to account for the observed correlated responses to selection in terms of three 'genetic units of function'.

1. A gene (or genes) increasing adrenal weight, which has the pleiotropic effects of increasing hormone-dependent sodium reabsorption, decreasing salt loss in water diuresis and hence decreasing U .
2. A gene which increases the relative size of the renal medulla which in turn increases the ability to excrete a dilute urine, which similarly decreases U .
3. A gene which increases the volume occupied by collecting ducts, hence increasing free water reabsorption and decreasing U .

These three 'units of function' are of course very much a minimum estimate of the number of genes involved in the responses to selection. Correlation is not necessarily the result of direct cause and effect; some of the correlated responses may even be due to the combined effects of gene linkage and genetic drift. Thus the selection experiment alone cannot positively demonstrate the physiological relationships between the various characters. The above analysis could therefore be usefully extended as well as tested by breeding experiments and the observation of units of segregation and recombination as well as those of function. In the methodological introduction to his original paper (Mendel, 1865), Mendel stressed the importance of starting breeding experiments with two different inbred lines. In this way genetic segregation could be confined to the second generation hybrids (F_2 and backcrosses) and hence clearly observed. The necessity for two different homozygous lines is no less great when the complication of gene linkage is taken into account. For this reason selection has been discontinued and each line is now being inbred by brother-sister mating in order to achieve homozygosity.

If it proves possible to analyse the differences between the selected lines, in terms of single genes and their pleiotropic effects at all levels of observation, the responses and correlated response to selection can be reinterpreted (Thoday & Boam, 1961; Thoday, Gibson & Spickett, 1964; Spickett & Thoday, 1966). The genetic differences between the four strains CBA/FaCam, A/Cam, S.F./Cam and Peru (Badr, 1965; Badr & Spickett, 1965; Spickett, Shire & Stewart, 1967; Shire & Spickett, 1967; Stewart & Spickett, 1967; Stewart, 1966), will have been regrouped in response to selection. The analysis of this regrouping should illustrate the relationship between genetic and physiological integration in mice.

SUMMARY

Three parameters of diuretic response to water-load (diuretic lag, maximum rate of diuresis and ratio of total output to input volume) have each responded to selection from a genetically heterogeneous base stock with significant realized heritabilities. Selection has been continued for ten generations and has produced mice more extreme than any of the founder strains. In the selection lines for ratio of output to input, correlated responses in salt excretion during the course of the diuresis, relative adrenal weight, the relative size of the renal medulla, and the quantity of renal collecting ducts, have been observed. These correlated responses are accounted for in terms of renal and adrenal physiology. The elucidation of the individual genetic determinants of the responses is discussed.

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