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Genetic analysis of physiological homeostasis: glomerular filtration rate following saline loading in mice

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SUMMARY

Saline loading caused an increase in glomerular filtration rate in RAP mice but not in CBA mice. On the basis of progeny testing of F2 hybrids, backcrosses to CBA, and inbred lines derived from backcrosses to RAP, it was concluded that the difference between the strains RAP and CBA was probably largely accounted for by a single gene locus. The use of this gene in physiological investigations of the control of glomerular filtration rate is suggested.

1. INTRODUCTION

It is one of the classical observations of physiology that animals have homeostatic mechanisms for maintaining the composition of their body fluids constant (Cannon, 1929). However, although all animals have effective homeostatic responses to disturbances of their body fluids, there is increasing evidence that the mechanisms of response may be different in different species and even in different strains of the same species. For example, in studies which formed the basis for the present work, it was shown that mice from both strains CBA and RAP responded to saline loading by increasing their rates of urinary sodium excretion (Stewart, 1969). Moreover, this homeostatic response was equally effective in the two strains, so that 70% of the sodium surcharge was excreted within 3 h of giving the load. However, closer physiological investigation revealed that the mechanism of increased sodium excretion was different in the two strains; whereas in strain CBA the mechanism was primarily a decrease in distal tubular reabsorption of sodium by the kidney, in RAP the increased rate of sodium excretion was primarily due to an increase in glomerular filtration rate (GFR) (Stewart, 1970). The work reported here is concerned with one of these components of homeostatic response, the change in GFR. The purpose of this paper is first to confirm that saline loading causes an increase in GFR in RAP but not in CBA mice, and then to provide a genetic analysis of this difference.

2. MATERIALS AND METHODS

(i) *Stomach loading test*

All the mice used in this work were stomach-loaded at 5–6 weeks with 1 ml per 16 g body weight of 1·35% sodium chloride. Urine was collected at measured time

intervals after loading. The volume and sodium concentration of each urine sample was determined. The rate of sodium excretion was calculated for each time interval as $R' = (16/\text{body weight}) \times (\text{volume}) \times (\text{sodium concentration})/(\text{time interval})$. The greatest value of R' was taken as the parameter R, the maximum rate of sodium excretion. Full details of this method are given in a previous publication (Stewart, 1969). In these present experiments both male and female mice were used; when the correction for body weight implicit in the parameter R was made, none of the sex differences were significant. Thus in the results presented below male and female mice are not distinguished.

(ii) *Mice and breeding programmes*

Mice were bred and stored under standard conditions as for the earlier work (Stewart, 1969). Experiments were carried out on male and female mice from the inbred lines CBA/Fa Cam and RAP; the two reciprocal F1 hybrids between CBA and RAP; the four possible backcrosses to CBA (with each of the two reciprocal F1's, and with CBA or F1 as mother), the four backcrosses to RAP, and the four possible F2 hybrids. In fact it turned out that there were no significant differences between any of these reciprocal crosses, so they are not distinguished in the results presented below. The second generation mice (backcrosses and F2) were further progeny-tested as follows. Backcross to CBA mice which had been measured on the stomach-loading test were further backcrossed to CBA mice. F2 mice which had been measured on the stomach-loading test were assortatively mated with other F2 mice. Backcross to RAP mice were mated in pairs as the founder-members of 13 separate lines. Each of these lines was then inbred by sibmating for 10–12 generations. Mice from each of these inbred lines were then measured on the stomach-loading test and selected test-crosses between these inbred lines were made.

(iii) *Measurement of glomerular filtration rate (GFR)*

The glomerular filtration rate in 8- to 12-week-old male mice under Nembutal anaesthesia was measured by inulin clearance as described in another report (Stewart, 1971). After the GFR measurement at a low, resting level of sodium excretion, 0·2 ml of 1·5% NaCl was given intravenously followed by a steady infusion at the high rate of 20 μl/min. This generally produced a saline diuresis within 30 min. 50–60 min after the onset of the high infusion rate, the glomerular filtration rate was measured again. The results below are given in the form of GFR following saline loading, expressed as a percentage of GFR before saline loading.

3. RESULTS

(i) *Effect of saline loading on GFR*

The effect of saline loading on GFR in the strains CBA, RAP and their F1 hybrid is shown in Fig. 1. As suggested in the earlier paper (Stewart, 1970), it is clear that saline loading did not cause an increased GFR in CBA mice. The mean

GFR after saline loading, $90 \pm 5\%$ of that before saline loading was not significantly different from 100% ($t_6 = 2{\cdot}0$; $P = 0{\cdot}09$) and in fact was slightly below the control value. In contrast in RAP mice the mean GFR after saline loading, $127 \pm 6\%$ of that before saline loading, has definitely been increased. Again as

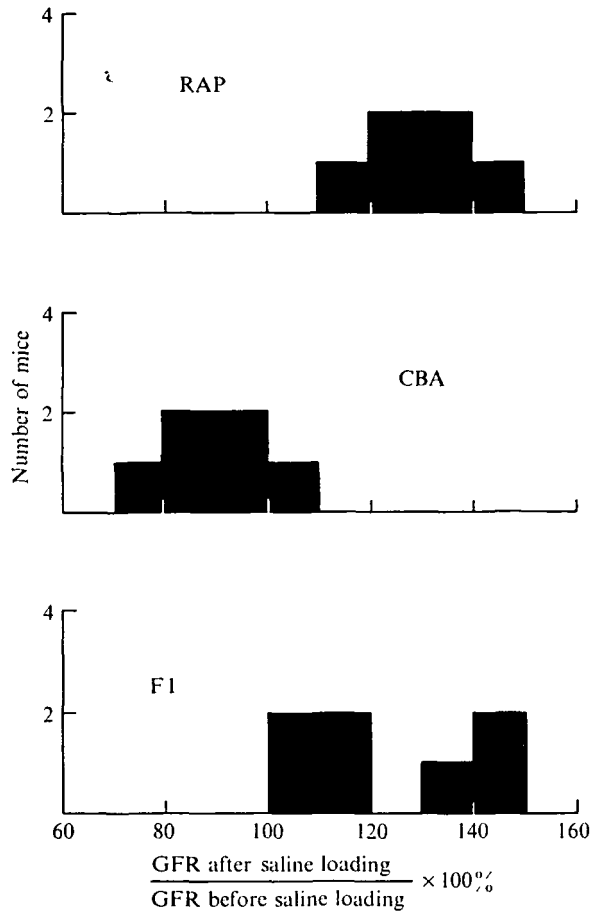

Fig. 1. Effect of saline loading on glomerular filtration rate in RAP, CBA and F1 hybrids.

suggested in the earlier paper (Stewart, 1970), the F1 hybrids are like the RAP parent: The mean GFR after loading, $128 \pm 8\%$ of that before loading, is clearly not significantly different from the RAP mean, but is significantly greater than the CBA mean ($t_6 = 4{\cdot}0$; $P < 0{\cdot}01$).

(ii) *Relationship between GFR and diuretic response to saline load*

It is clear from the above results that the strains do differ in the response of GFR to saline loading. However, the measurements of GFR by inulin clearance were made in terminal experiments on anaesthetized animals so that the mice were not subsequently available for progeny testing. This limited the possibilities

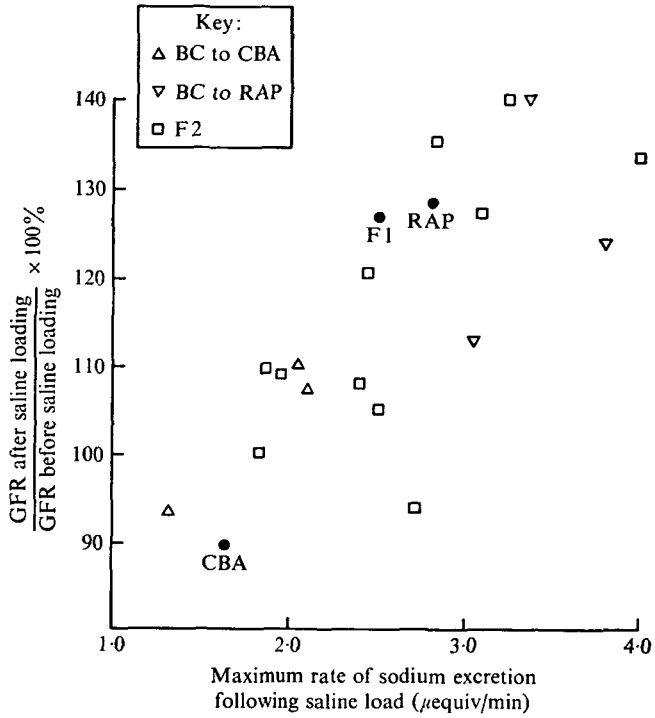

Fig. 2. Relationship between maximum rate of sodium excretion and increase in GFR following saline loading in second-generation hybrids between RAP and CBA.

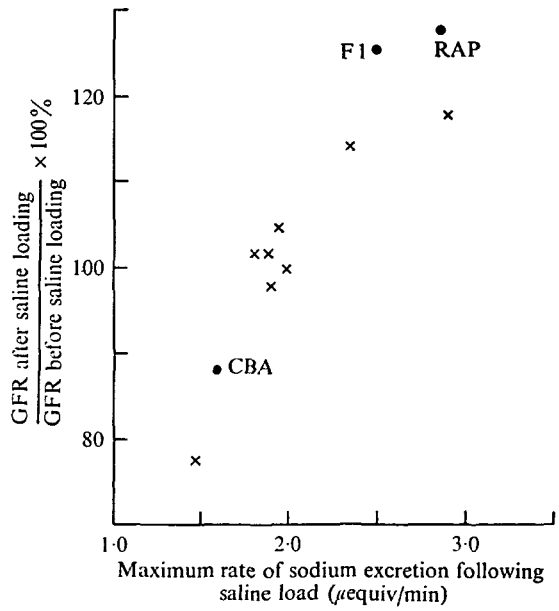

Fig. 3. Relationship between maximum rate of sodium excretion and increase in GFR following saline loading in inbred lines derived from backcross to RAP. Each point represents mean of 3–6 animals.

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for genetic analysis. It was therefore highly desirable to find some more rapid measure of GFR response to saline loading which would not involve sacrifice of the animal. In the previous paper (Stewart, 1970), it was reported that the parameter R, the maximum rate of sodium excretion following stomach-loading with saline, correlated highly with the rate of delivery of sodium to the distal tubule, which in turn was related to the increase in GFR caused by the saline loading. Moreover, this correlation was maintained in a segregating F2 generation. This suggested that the parameter R might provide a convenient indirect measure of the change in GFR caused by saline loading. In order to test this possibility both R, and GFR as measured by inulin clearances, were measured on a number of mice from genetically segregating generations, comprising backcrosses to both RAP and CBA, and the F2 hybrids. The results of these experiments are shown in Fig. 2. The overall correlation coefficient, 0·67, is highly significant ($P < 0{\cdot}01$). Moreover, although the scatter of the points is rather high, no individual observations unambiguously break this overall correlation. It is possible that this correlation was due to genetic linkage between one factor (or factors) affecting GFR, and a separate factor affecting R. Genetic recombination between these two factors may not have been detected in the experiment shown in Fig. 2, either because experimental error obscured the observation of recombinant individuals, or because the hypothetical factors were so closely linked that recombination did not occur. This question was further investigated by measuring both R and GFR on three to six individuals from each of eight sublines derived from backcrosses to RAP after four to six generations of inbreeding. The results of these experiments, taking mean values for each of the lines, are given in Fig. 3. The effect of reducing random experimental error by taking means rather than individual values is seen in the reduced scatter of the points in Fig. 3 compared with Fig. 2. The reduction in scatter is such that even a single observation breaking the overall correlation would almost certainly have been detected. In fact there were no individual exceptions to the overall correlation between R and GFR, and the correlation coefficient of 0·90 was very high. The number of independent sublines investigated, 8, was relatively small. However a certain degree of heterozygosity probably persists for several generations of sib mating (Haldane, 1955) which would increase the opportunities for a rare recombinational event to occur. If the correlation between R and GFR in Fig. 3 was the result of genetic linkage between two separate factors, one affecting R and the other affecting GFR, then genetic recombination between these factors was probably not a very frequent event. It thus appears that under the conditions of these experiments, and for the particular case of the genetic difference between strains CBA and RAP, the parameter R provides a reasonably reliable measure of the change in GFR caused by saline loading. The genetic analysis of the difference between strains CBA and RAP has therefore been carried out on the basis of the parameter R.

(iii) *Genetic analysis: F1, F2, and backcrosses*

The distribution of R in the parental strains CBA and RAP, their F1 and F2 hybrids, and both backcrosses, is shown in Fig. 4. The means, standard errors of

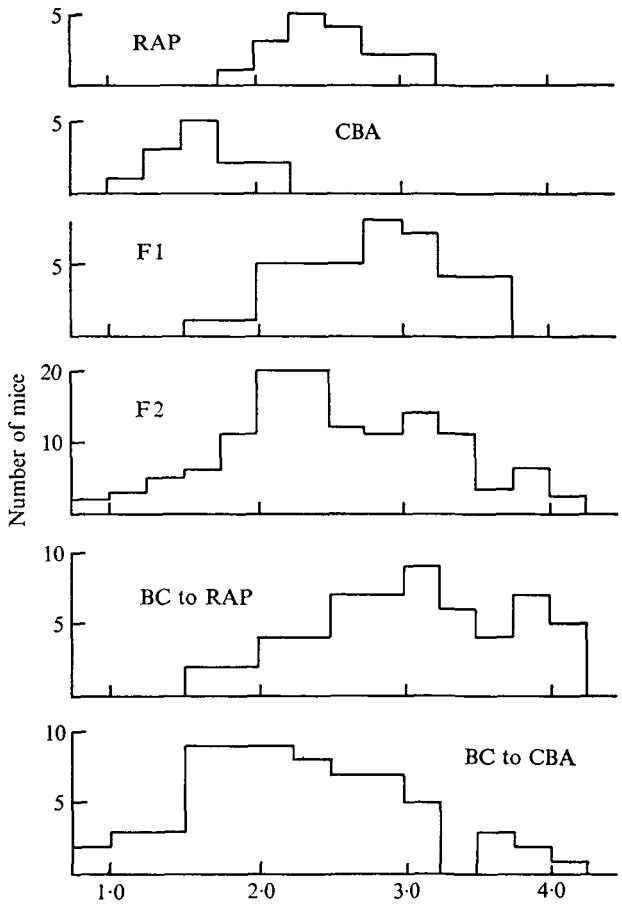

Fig. 4. Distributions of R, maximum rate of sodium excretion following saline load, in CBA, RAP and first- and second-generation hybrids.

the means, and variances of these distributions are given in Table 1. The mean value for RAP is significantly greater than the mean value for CBA ($t_{13} = 3{\cdot}06$, $P < 0{\cdot}01$). The mean value of the F1 hybrids is not significantly different from that of the RAP parent ($t_{17} = 1{\cdot}2$, $P > 0{\cdot}2$) and if anything is a little higher indicating overdominance. The possibility of overdominance is also suggested by the rather high mean of the backcross to RAP. More important, however, is the fact that the variance in the genetically segregating generations, F2 and backcrosses, is consistently greater than in the genetically homogeneous generations, strains and

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F1 ($F_{237,67} = 2{\cdot}69$, $P < 0{\cdot}01$ for the overall comparison). This increased variance not only confirms that the original strain difference was genetic in origin, but implies that the genetic factors responsible for that difference must be limited in

Table 1. *Means (plus or minus standard error) and variances of the parameter R (maximum rate of sodium excretion following saline loading), in the strains RAP, CBA and first- and second-generation hybrids*

Stock	No. of animals	Mean (μequiv/min)	Variance
RAP	17	$2{\cdot}50 \pm 0{\cdot}22$	0·13
CBA	13	$1{\cdot}61 \pm 0{\cdot}20$	0·07
F1	39	$2{\cdot}80 \pm 0{\cdot}12$	0·28
BC RAP	55	$2{\cdot}92 \pm 0{\cdot}11$	0·42
BC CBA	69	$2{\cdot}05 \pm 0{\cdot}09$	0·43
F2	123	$2{\cdot}40 \pm 0{\cdot}06$	0·52

number. Examination of Fig. 1 suggests that one dominant locus might account for much of the variation in R; but since there is considerable overlap between the parental and F1 distributions, the hypothesis of a single locus requires further testing. Three breeding programmes were designed in order to provide such a test.

(iv) *Progeny-testing of F2 individuals*

If genetic variation in F2 was due primarily to segregation at a single locus, then the lower 25 % of the distribution should consist largely of individuals homozygous for the CBA allele at this locus. Thus when individuals from the lower 25 % of the distribution were mated to each other, their offspring would be expected to be uniformly like the CBA strain. If, on the other hand, variation in the F2 was due to segregation at more than one locus, then at least some individuals in the lower 25 % of the distribution should carry some RAP alleles at some of the relevant loci, and so at least some of their offspring should be unlike the CBA strain. Four male and four female mice were taken at random from the lower 25 % of the F2 distribution, and mated in four separate pairs. Six to ten progeny were measured from each mating; the results are summarized in Fig. 5. On the basis of their standard errors, none of the progeny means were significantly different from the CBA mean of 1·61; and the overall mean within-litter variance, 0·26, was low (cf. 0·28 for F1 hybrids). These results are thus in accordance with the hypothesis that a single locus is responsible both for genetic variation in the F2 and for most of the original strain difference.

(v) *Progeny test of backcross to CBA*

If segregation at a single locus was responsible for variation in the backcross to CBA, then there should be only two distinct genotypes in this backcross. Thus the progeny of further backcrossing to CBA should fall into two distinct classes. If, on the other hand, more than one locus was responsible, then there would be more than two different genotypes in the backcross and the progeny of further backcrossing to

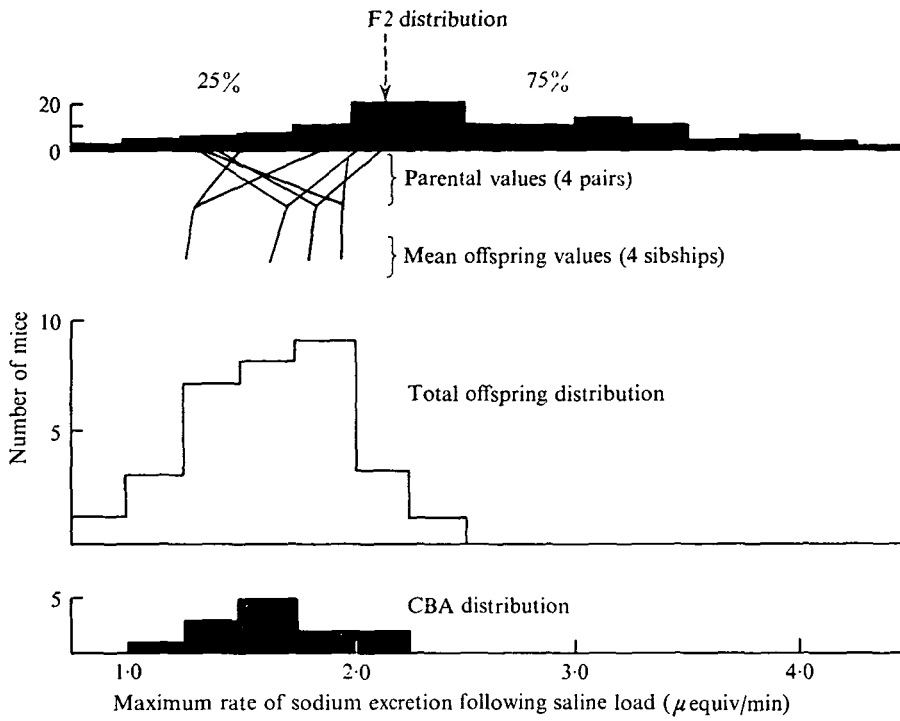

Fig. 5. Progeny tests from F2 hybrids of *R*, maximum rate of sodium excretion following saline loading.

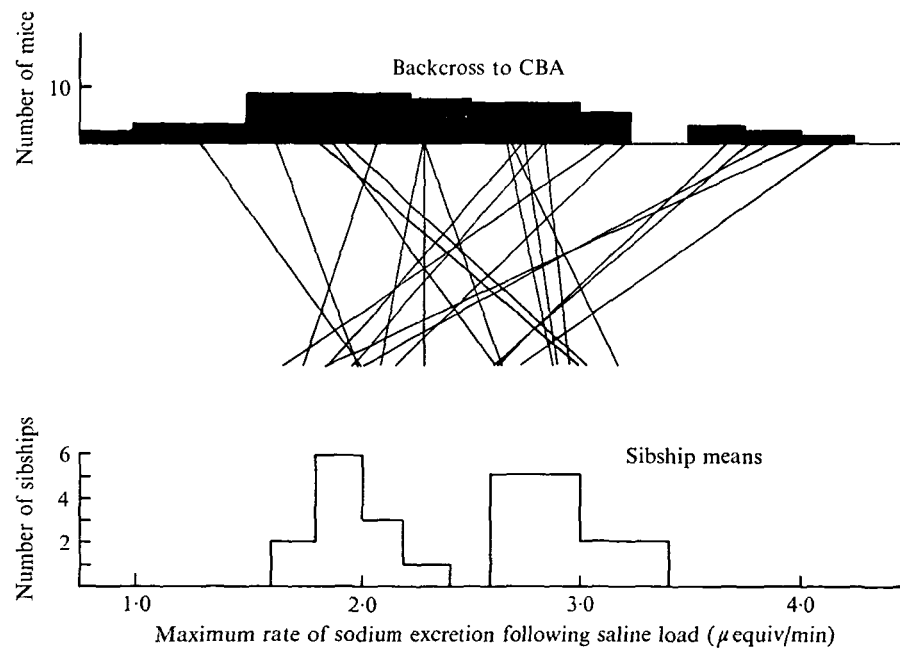

Fig. 6. Progeny tests from backcross to CBA, of *R*, maximum rate of sodium excretion following saline loading.

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CBA would not be expected to fall into two distinct groups. The results of further backcrossing 24 BC to CBA mice to the parental CBA strain are summarized in Fig. 6; an average of 6 (range 3–9) offspring from each mating were measured to provide the 'progeny means'. It is very clear that the progeny means fall into two distinct groups, strongly implying that there were only two effectively distinct genotypes in the first backcross to CBA, and that there was segration at a single locus.

(vi) *Inbreeding of backcross to RAP*

If there was genetic segregation at a single locus in the backcross to RAP, then inbreeding for 10 generations should lead to fixation, i.e. homozygosity for either the CBA or the RAP allele at this locus in most of the lines. The relative frequencies of fixation for each of the two alleles would correspond to the gene frequency in the founding backcross population, i.e. 25% of the lines should run to fixation for the CBA allele, 75% for the RAP allele. This therefore leads to the rather striking prediction that inbreeding of the generally high backcross to RAP mice should lead to the reappearance of some lines with the characteristically low R-values of the CBA strain. This group of 'low' lines should comprise approximately 25% of the total, and should be quite distinct from the remaining 75% of the lines with the higher R-values of the RAP strain. If, on the other hand, there is more than one locus involved, then in the limited number of generations of inbreeding not all the lines would be expected to run to fixation at all loci, and of those that did run to fixation, only a minority would be fixed for the CBA allele at all loci. Thus in general one would expect few of the lines to reproduce the characteristically low R-values of the CBA strain, and the mean R-values of the inbred lines should be spread over a continuum rather than falling into two distinct groups.

The results of inbreeding 13 lines from the backcross to RAP are summarized in Fig. 7. On the basis of mean values, there are clearly two separate groups of lines. One group of four lines, labelled L in Fig. 7, has means in the low range of 1·4–1·8 which is characteristic of the CBA type; the other group of nine lines, labelled H in Fig. 7, has quite discontinuously higher means. There is some evidence that one of the 'low' lines, labelled S in Fig. 7, had not in fact run to fixation. First, the within-line variance for this line, 1·18, was much higher than the overall within-line variance for the other groups, 0·36 ($F_{27,106} = 3{\cdot}3$, $P < 0{\cdot}01$). Secondly, when eight individuals from this line were progeny-tested by crossing to individuals from the other 'low' lines, the offspring means showed a wide variance ('L × S' in Fig. 7). This would be expected if this line was still segregating at a single locus, so that three separate genotypes (both homozygotes and the heterozygote) had been 'sampled' among the eight individuals chosen for progeny testing. The wide variance in progeny means is in itself further evidence that one or at most few loci were involved.

It has been noted above that the CBA allele(s) were recessive to the RAP allele(s), the F1 being like the RAP parent and higher than the CBA parent. Correspondingly, the allele which is presumably homozygous in the 'low' lines is recessive to that in the 'high' lines: when a mouse from each of the three 'low'

lines was crossed to one from the 'high' line, all three offspring means were within the 'high' range (L × H crosses, Fig. 7). When the low lines were crossed among themselves (1 with 2, 2 with 3, and 3 with 1), all three offspring means were 'low' (L × L crosses, Fig. 7). Given that 'low' alleles are recessive to 'high' alleles, this

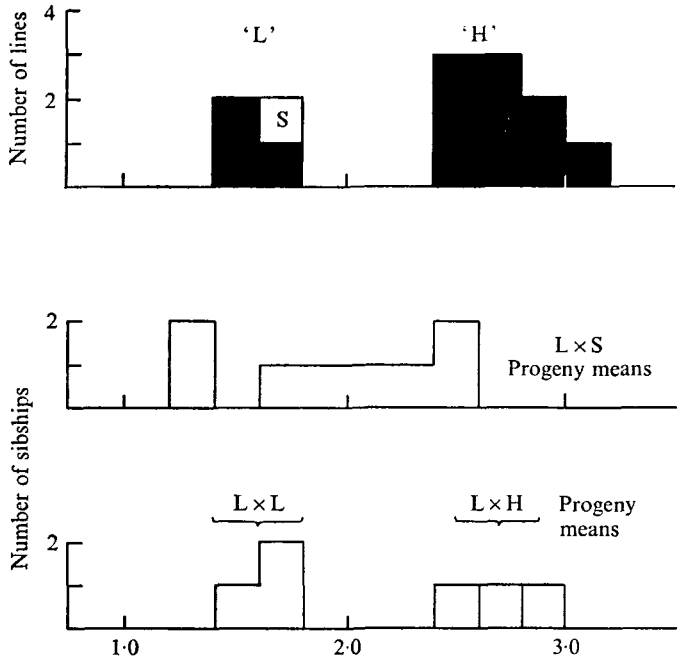

Fig. 7. Progeny tests of inbred lines derived from backcross to RAP, for character *R*, maximum rate of sodium excretion following saline loading.

result means that all three low lines must have been homozygous at the same locus (or loci). The chance of all three lines going homozygous at the same locus if more than one locus was involved would be rather small, $(1/n)^2$ where n is the number of loci; so that this result further confirms that only one locus was involved.

4. DISCUSSION

The results reported above strongly suggest that the strains CBA and RAP differ at a single locus with a large effect on *R*, the maximum rate of sodium excretion following saline load. This was indicated by four separate lines of evidence: examination of the distributions of parental, F1 and backcross generations; progeny testing of the F2; progeny testing the backcross to CBA; and inbreeding from the backcross to RAP. There was also evidence that under the conditions of these experiments a strong correlation existed between *R* and the increase in glomerular filtration rate (GFR) caused by saline loading. The conclusion seems justified that the strains CBA and RAP differ at a single genetic locus

which has a major effect on whether or not GFR increases following saline loading, although it must be noted that a pair or group of closely linked genes would give the same result.

The question as to how this gene or group of genes acts is clearly a matter for further investigation. There are a number of interesting possibilities. For example, it has been shown that nephrons are not homogeneous in their response to saline loading (de Rouffignac & Bonvalet, 1970). Whereas those nephrons with glomeruli near the surface of the renal cortex respond by greatly increasing their glomerular filtration rate, in deep glomeruli the glomerular filtration rate increases less and may even paradoxically decrease (Thurau, 1969). In this respect the CBA kidney as a whole appears to behave like the 'deep' nephrons. Another line for investigation concerns the possible role of the juxtaglomerular apparatus in the regulation of glomerular filtration rate. In any event, particularly suitable material for these or other possible investigations is provided by three 'low' lines resulting from inbreeding of the backcross to RAP. Each of these lines has a genetic background generally similar to that of the RAP strain, but is homozygous for the CBA allele at the locus affecting control of glomerular filtration rate. The probability of all three lines being homozygous for a CBA allele at an unrelated locus is $(1/4)^3 = 1/64$, i.e. very low; so that it would be reasonable to suppose that if all three lines differed from the RAP in some aspect of their renal physiology, that aspect would be related to control of GFR.

Finally, it may be pertinent to raise a rather more general question in the field of population genetics. In the introduction it was stated, as a classical physiological tenet, that all vertebrates have effective homeostatic mechanisms for maintaining constancy of body fluids; and that, at any rate for members of the same species, these mechanisms would be equally effective. The fact that strains CBA and RAP both excreted 70% of an administered saline load with 3 h was offered as an example of their equality. However, although the mechanisms in these strains were equally effective, they were different. Now the conclusion from the above analysis was that the strain difference in at least one of the possible components of homeostatic response, i.e. changes in GFR, was largely due to a single locus. There is no reason to suppose, however, that this same locus also affects the other component of homeostatic response in which these strains differ, i.e. changes in distal tubular reabsorption. In this case, independent assortment in a segregating generation would give rise to mice which could neither increase GFR, nor decrease tubular reabsorption, and would thus have an impaired response to saline loading. Such mice would not have an equally effective homeostatic response. Something of this sort does seem to have occurred. The three 'low' lines, resulting from inbreeding backcross to RAP mice under laboratory conditions, excreted a mean of only 40% of the administered saline load in the three hours following loading. The question for population genetics is whether in natural populations of mice there are any mechanisms, for example racial organization of the breeding structure, or linkage of genes in 'balanced' combinations (Thoday, 1953), which might minimize the production of such animals with a relatively ineffective homeo-

static response. The work described in this paper cannot in itself begin to answer this question; but the genetic analysis of one of the relevant elements may provide one of the tools for a future attack on this problem.

REFERENCES

CANNON, W. (1929). Organization for physiological homeostasis. *Physiological Reviews* **9**, 399–431.

DE ROUFFIGNAC, C. & BONVALET, J. P. (1970). Variations in glomerular filtration-rate of single superficial and deep nephrons under various conditions of sodium intake in the rat. *Pflugers Archiv, European Journal of Physiology* **317**, 141–156.

HALDANE, J. B. S. (1955). The complete matrices for brother–sister and alternate parent–offspring mating involving one locus. *Journal of Genetics* **53**, 315–324.

STEWART, J. (1969). Diuretic responses to electrolyte loads in four strains of mice. *Comparative Biochemistry and Physiology* **30**, 977–987.

STEWART, J. (1970). Genetic variation in patterns of nephron function during natriuresis in mice. *American Journal of Physiology* **219**, 865–871.

STEWART, J. (1971). Renal concentrating ability in mice: a model for the use of genetic variation in elucidating relationships between structure and function. *Pflugers Archiv, European Journal of Physiology* **327**, 1–15.

THODAY, J. M. (1953). Components of fitness. *Symposium of the Society for Experimental Biology* **7**, 96–113.

THURAU, K. (1969). The dependency of intrarenal distribution of single nephron filtration-rates on dietary salt intake (micropuncture studies). *Proceedings of the Royal Society of Medicine* **62**, 1118–1122.