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Diabetes insipidus associated with oligosyndactyly in the mouse

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1. INTRODUCTION

It was noticed that some mice of a certain stock emptied their water bottles unusually fast, and that their cages became abnormally wet. These 'cage-wetting' mice were then tested for their water intake and it was found that the phenomenon was not due to leakage of the bottles but was a real excess of water intake with a correspondingly high output of dilute urine which contained no sugar. Furthermore, this diabetes insipidus was found to be associated with the dominant gene oligosyndactyly (*Os*), whose principal manifestation is a skeletal defect affecting the hand and foot (Grüneberg, 1956, 1961; Kadam, 1962). The skeletal defects are present in heterozygotes and the gene is lethal in homozygotes. The associated diabetes insipidus was found to be a pleiotropic effect of the *Os* gene, but the severity is subject to modification by other genes. The investigations described in this paper concern the manifestation of the diabetes, and the association with the *Os* gene.

2. MATERIAL AND METHODS

The stock carrying the *Os* gene was a non-inbred one of mixed origin. A subsidiary stock, to be referred to as the DI stock, was established from five individuals showing the 'cage-wetting' abnormality, and was subsequently continued from *Os* heterozygotes with high water intakes. After the removal of the 'cage-wetting' individuals for the establishment of the DI stock, the original stock carrying the *Os* gene was continued for four generations, during which no further cases of 'cage-wetting' were seen. The stock was then discontinued and the *Os* gene was transferred to a new stock, designated stock VII. The main series of experiments concerns the DI stock and the F_1 progeny of crosses between it and three inbred strains, CBA/Fa, C57BL/Fa and JU/Fa. Twenty different animals from the DI stock were used for the cross with CBA, thirteen with C57BL and fourteen with JU. A subsidiary experiment was carried out three years after the conclusion of the main experiments; in the subsidiary experiment the DI and VII stocks and the F_1 between them were compared.

The manifestation of the diabetes was studied from the water intakes of individual mice. For the measurement of water intake, mice were housed singly in cages and supplied with water from an inverted 12-oz. bottle to which was attached a glass tube terminating in a hole about 3 mm. in diameter. The water intake was measured

daily from the depletion of the water in the bottle, and averaged over a period of seven days. Great care was taken to avoid leaks from the bottles, and the losses from evaporation and dripping probably did not vary much because the daily amounts consumed showed a satisfactory regularity day by day. In the subsidiary experiment the method was modified: the glass tubes had a smaller terminal opening of about 1 mm. in diameter, and the depletion of the water was not measured daily, but only once, at the end of seven days. The losses from evaporation and dripping were probably less. Most of the data to be presented refer to mice aged nine weeks, recorded over the period from nine to ten weeks of age, but some records were obtained also from younger and from older mice. Body weights were recorded at the same time as the water intakes.

3. RESULTS

(i) *Water intake and body weight at nine to ten weeks*

The mean daily water intake at nine weeks in the DI stock and the F_1s of the crosses with the three inbred strains are given in Table 1, and the distributions of

Table 1. *Mean daily water intake and body weight (with standard errors) of* Os *heterozygotes* (Os/+) *and non-*Os *mice* (+/+) *of the DI stock and crosses with inbred strains, at nine weeks of age*

	Females		Males	
	Os/+	+/+	*Os*/+	+/+
*Number of mice**				
DI	50	28	55	35
CBA (F_1)	66	61	52	91
C57BL (F_1)	89	109	98	119
JU (F_1)	86	85	80	114
Water intake (ml.)				
DI	22·6 ± *1·32*	11·2 ± *0·44*	18·9 ± *0·90*	10·2 ± *0·54*
CBA (F_1)	12·0 ± *0·30*	8·7 ± *0·14*	11·0 ± *0·26*	8·7 ± *0·12*
C57BL (F_1)	11·4 ± *0·17*	9·3 ± *0·14*	10·8 ± *0·16*	9·0 ± *0·13*
JU (F_1)	13·0 ± *0·24*	10·1 ± *0·14*	12·4 ± *0·22*	10·2 ± *0·13*
Body weight (g.)				
DI	25·5 ± *0·32*	25·1 ± *0·46*	28·7 ± *0·42*	29·5 ± *0·51*
CBA (F_1)	22·0 ± *0·27*	22·3 ± *0·27*	25·8 ± *0·32*	27·1 ± *0·28*
C57BL (F_1)	22·0 ± *0·19*	22·4 ± *0·21*	26·7 ± *0·25*	27·4 ± *0·23*
JU (F_1)	23·6 ± *0·20*	24·5 ± *0·18*	28·2 ± *0·21*	29·4 ± *0·24*

* The numbers of mice tested are not indicative of the segregation of the *Os* gene.

the daily intakes are shown in Figs. 1 and 2. The results are shown separately for males and females, and for *Os* heterozygotes and non-*Os* mice. The following conclusions can be drawn. (1) The *Os* mice had significantly higher intakes than the

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non-*Os* mice in all the groups, which proves that the diabetes is either a pleitropic effect of the *Os* gene or is caused by a gene closely linked with *Os*. Discrimination between these two possibilities would require the demonstration of recombination between the diabetes and the oligosyndactyly. The distributions of water intake give no clear evidence of recombination, but the difference of intake between *Os*

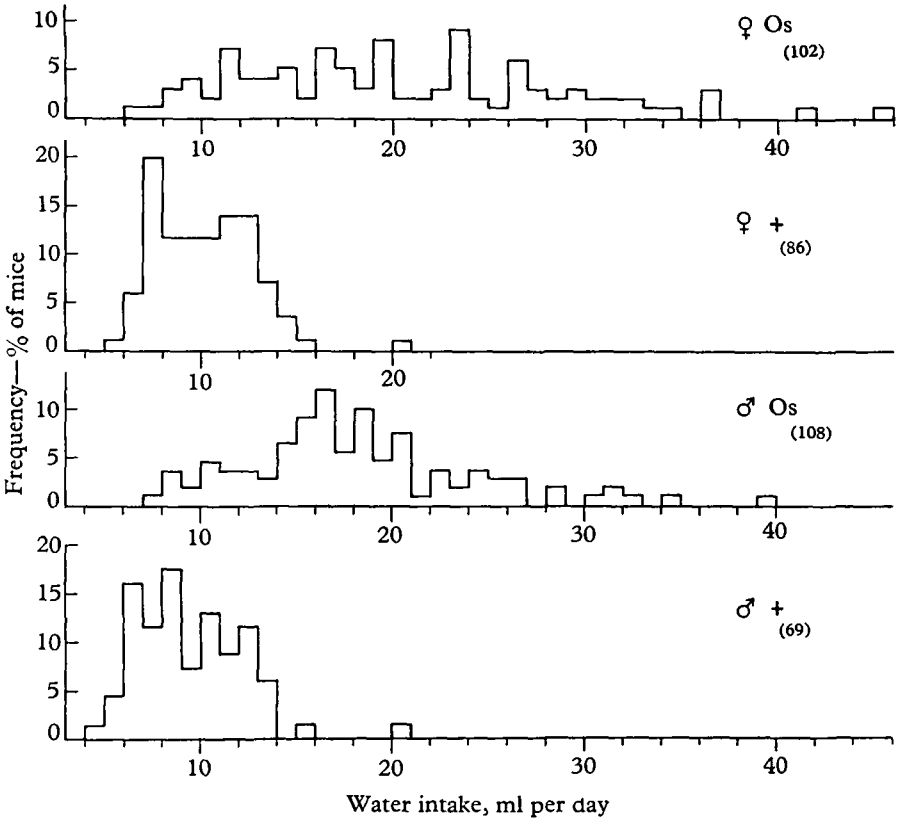

Fig. 1. Distributions of daily water intake of mice of the DI stock, aged nine weeks. The number of individuals in each group is given in brackets.

and non-*Os* mice was not great enough in relation to the variation within each group to allow recombinants to be readily detected. Further evidence against the occurrence of recombination will be given later. (2) The water intake of *Os* females was higher than that of *Os* males, though among the non-*Os* mice females and males drank about the same amount. (3) The four strains—i.e. the DI stock and the three F_1s—differed from each other in their mean intakes, particularly in the intake of *Os* mice.

Before the differences of intake can be properly evaluated it is necessary to find out what adjustment, if any, should be made for differences of body weight. Regressions of water intake on body weight were accordingly computed and these

are given in Table 2. The regressions were calculated separately for females and males and for *Os* and non-*Os* mice. The regression coefficients in these four groups differed significantly from each other in the DI stock but not in any of the three

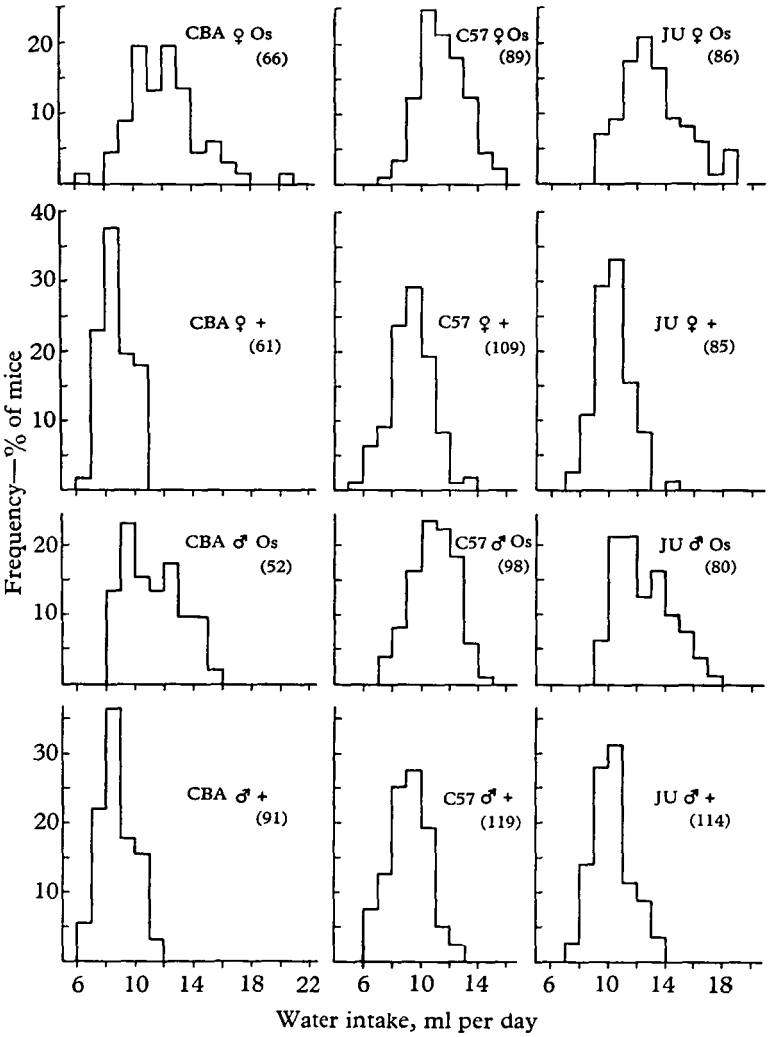

Fig. 2. Distributions of daily water intake, at nine weeks of age, of F_1 mice from crosses between the DI stock and three inbred strains. The number of individuals in each group is given in brackets.

F_1s. The regression lines did not pass through the origin, so the water intake was not simply proportional to body weight, and the intake per gram of body weight would not be a valid basis for comparison. The intakes were adjusted, by means of the regression coefficients, to a standard weight of 26 g. for both sexes, and these adjusted intakes are also given in Table 2. The conclusions based on the simple

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intakes are not materially affected. The differences between *Os* and non-*Os* mice are increased and the greater intake of *Os* females than of *Os* males is more marked. Though among the non-*Os* mice females drank the same amount as males, the adjusted intakes show that females drink more than males of the same body weight. The differences between strains—DI stock and F_1s—were found to be highly significant when the adjusted intakes were compared: analyses of variance showed the differences between strains to be significant at the 0·1% level in all four groups, i.e. females and males, both *Os* and non-*Os*. This proves that the water intake of both *Os* and non-*Os* mice is subject to modification by the background genotype.

Table 2. *Regression coefficients* (± *s.e.*) *of water intake on body weight at nine weeks*; *and water intake adjusted to a standard weight of* 26 *g. The numbers of mice are the same as in Table* 1

	Females		Males		All groups combined
	Os/+	+/+	*Os*/+	+/+	
Regression (*ml./g.*)					
DI	2·36 ± *0·49*	0·41 ± *0·17*	1·10 ± *0·27*	0·61 ± *0·15*	—
CBA (F_1)	0·30 ± *0·13*	0·14 ± *0·06*	0·17 ± *0·11*	0·13 ± *0·04*	0·18 ± *0·041*
C57BL (F_1)	−0·01 ± *0·09*	0·05 ± *0·07*	0·10 ± *0·06*	0·04 ± *0·05*	0·05 ± *0·032*
JU (F_1)	0·08 ± *0·13*	0·20 ± *0·08*	0·33 ± *0·11*	0·14 ± *0·05*	0·17 ± *0·043*
Adjusted intakes (*ml.*)					
DI	23·8	11·6	15·9	8·1	
CBA (F_1)	13·2	9·2	11·0	8·6	
C57BL (F_1)	11·4	9·5	10·7	8·9	
JU (F_1)	13·2	10·4	11·7	9·7	

The most striking difference is, however, that the *Os* mice of the DI stock had much higher intakes than the *Os* mice of the F_1 crosses. This shows that the DI stock carried one or more unidentified genes that greatly increased the severity of the diabetes shown by *Os* mice. Additional evidence about the genetics of the diabetes was obtained from the subsidiary experiment which will now be described.

(ii) *Comparison of DI and VII stocks*

As was explained in the section on stocks, the *Os* gene was transferred to another stock, stock VII. Mice of stock VII and the DI stock were tested for water intake about three years after the conclusion of the main experiments described above. At this time stock VII had been separated from the DI stock by six outcrosses to other strains. The probability that stock VII mice carried genes, unlinked with *Os*, derived from the DI stock ranged from 1/32 to 1/96 in the animals tested, and the length of chromosome linked with *Os* that was expected to have remained intact was about 16 centimorgans on each side of *Os* (Bartlett & Haldane, 1935). *Os* mice from the two stocks were crossed, and F_1 animals were tested at the same time. The mean daily water intakes are given in Table 3, and the distributions are shown

Table 3. *Mean daily water intake (ml.) at nine weeks, with standard errors, of the DI and VII stocks and the F_1. (Subsidiary experiment.) The numbers of mice are given in brackets*

	Females		Males	
Stock	*Os*/+	+/+	*Os*/+	+/+
DI	21·4 ± *1·38* (15)	7·5 ± *0·36* (7)	20·3 ± *1·47* (24)	6·8 ± *0·34* (11)
VII	5·0 ± *0·18* (25)	3·9 ± *0·12* (24)	5·5 ± *0·35* (14)	3·9 ± *0·09* (16)
F_1	8·4 ± *0·54* (42)	4·8 ± *0·22* (19)	8·2 ± *0·47* (36)	5·4 ± *0·16* (21)

in Fig. 3. The intake of the DI stock was about the same as before, though the non-*Os* mice had lower intakes and the difference between *Os* and non-*Os* was somewhat greater than before. The *Os* mice of stock VII had very much lower intakes, but still in excess of the non-*Os* mice. This supports the conclusion that the *Os* gene itself causes mild diabetes, though a linked gene within about 16 cross-over units from *Os* is still not excluded. The great difference of intake between the *Os*

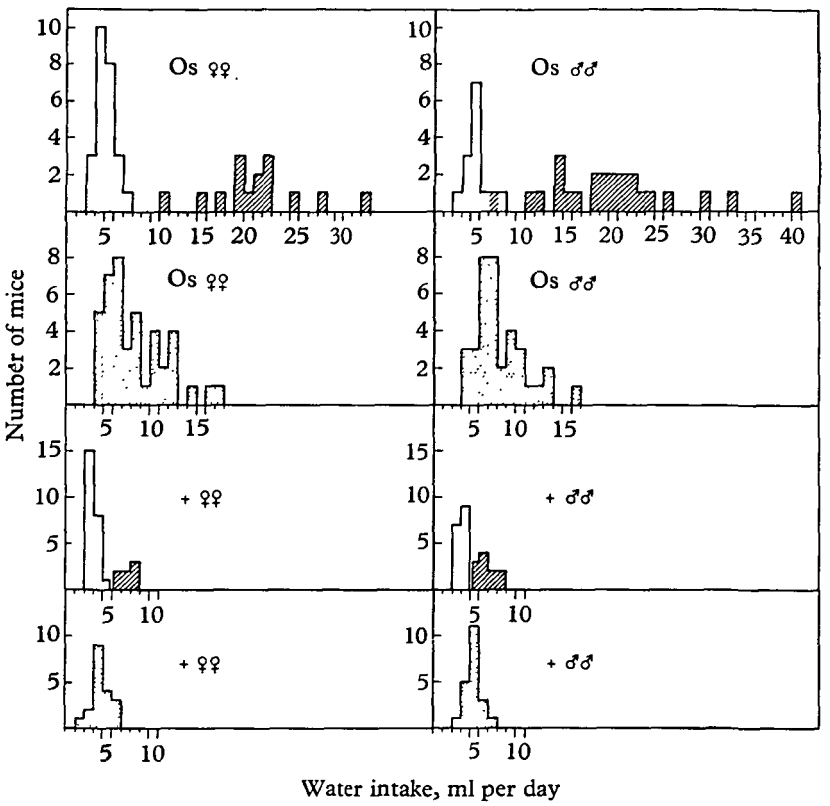

Fig. 3. Distributions of mean daily water intake of mice of the DI and VII stocks and the F_1 between them, at nine weeks of age, measured in the subsidiary experiment. DI stock cross-hatched, stock VII outlined, F_1 stippled. The number of individuals in each group is given in Table 3.

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mice of the two stocks confirms the conclusion that one or more modifying genes enhance the severity of the diabetes. These genes must have been lost from stock VII in the process of outcrossing. One further conclusion can be drawn. The intake of non-*Os* mice was considerably greater in the DI stock than in stock VII; in fact non-*Os* DI mice had substantially higher intakes than *Os* VII mice. This shows that the modifying genes alone cause a mild diabetes. More information on this point will be given later. The intakes of the F_1 animals, both *Os* and non-*Os*, were intermediate between the parent strains, but nearer the lower parent. This shows that the modifying genes in the DI stock were nearly, but not completely, recessive in their combined effect.

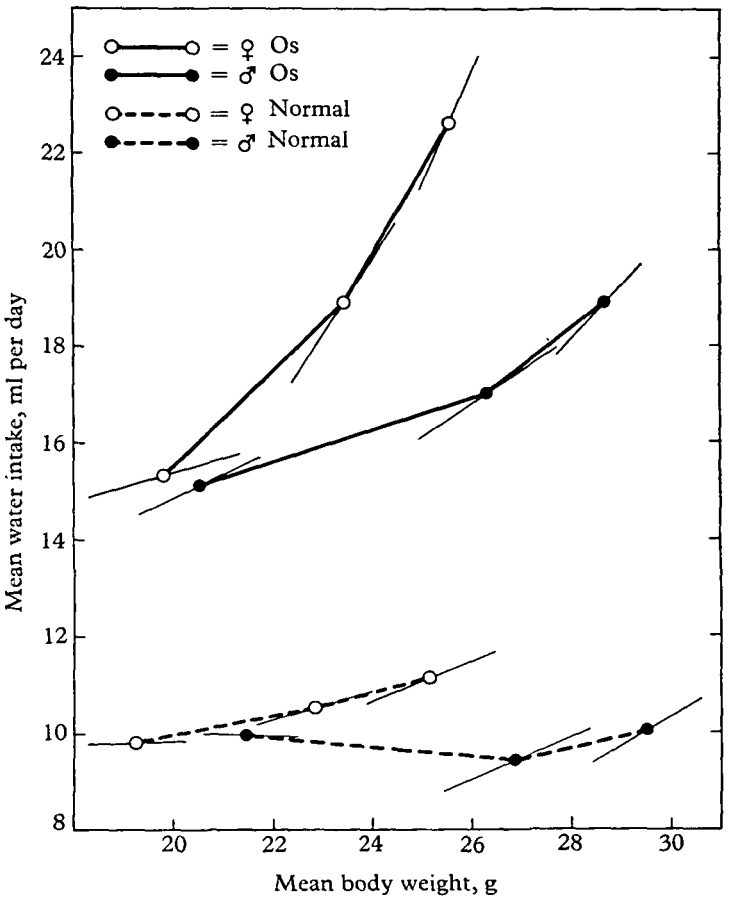

Fig. 4. Mean daily water intake plotted against mean body weight at the ages of five, seven and nine weeks. The thin lines through each point show the regression coefficients of intake on weight at that age.

(iii) *Water intake before nine weeks*

The mice of the DI stock whose nine-week intakes are given in Table 1 were also tested for water intake at the ages five and seven weeks, and the mean intakes at

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the three ages are shown in Fig. 4. The higher intake of *Os* mice was already evident at the age of five weeks, though the difference between *Os* and non-*Os* mice was not as great at the earlier ages as at nine weeks. In order to show the connexion between water intake and body weight, the mean intakes are plotted in Fig. 4 against the mean body weights at the three ages, and the regression of intake on weight at each age is shown by a straight line drawn through each point. The increases of intake from five to nine weeks are probably not greater than would be expected from the relationships between intake and weight at each age. Non-*Os* males, however, did not increase their intake between five and nine weeks although they increased their weight from 21·4 to 29·5 g., and they showed significant regressions of intake on weight at both seven and nine weeks.

(iv) *Water intake after nine weeks*

Some litters of the DI stock and of the cross with JU were tested for water intake at nine weeks and again later, at various ages up to 1½ years. The mean intakes are shown in Fig. 5, plotted against the approximate mean age. The DI stock continued the changes seen from five to nine weeks. The *Os* mice of both sexes increased their intakes enormously, females reaching average daily intakes of 50 to 60 ml. per day at about one year old; this was an average intake of about 1·7 times the body weight. The non-*Os* females also increased their intake after nine weeks, but the non-*Os*

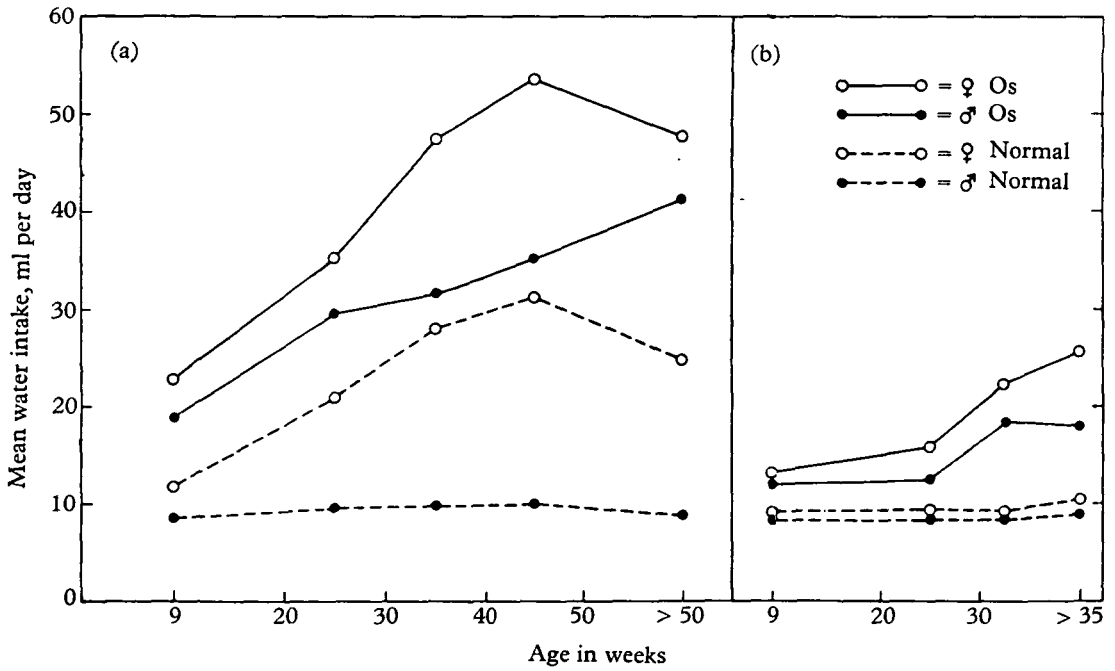

Fig. 5. Mean daily water intake of mice at different ages: (*a*) DI stock, (*b*) F_1 mice of the cross between the DI stock and the JU inbred strain. The means at different ages are mostly based on different individuals, but all the mice on which the nine-week mean is based had measurements at one or more later ages.

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males maintained a constant intake at all ages. The *Os* mice from the cross with JU showed rather less increase of intake, and the non-*Os* females as well as the males maintained a constant intake. These observations show the the diabetes of *Os*

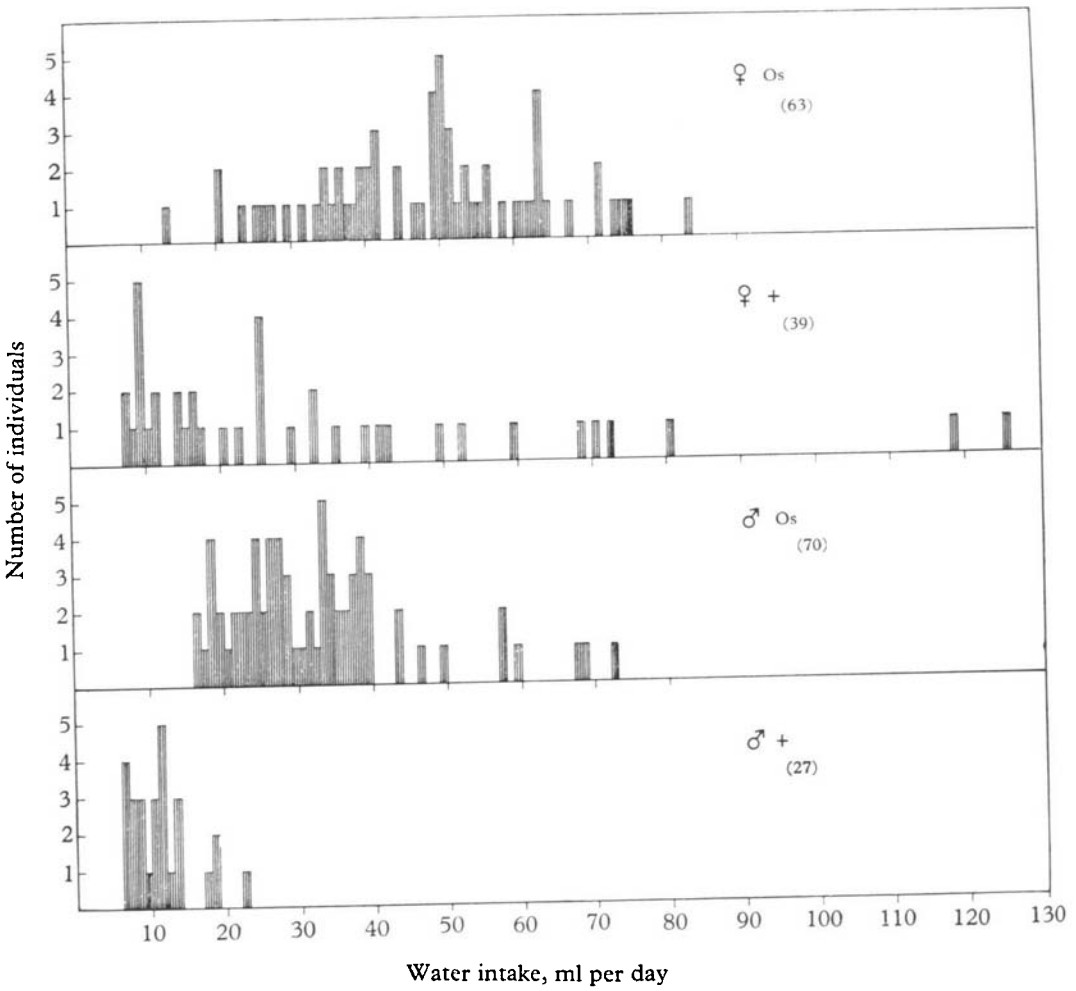

Fig. 6. Distributions of daily water intake of mice of the DI stock aged six months or over. The number of individuals in each group is given in brackets.

mice became more severe with increasing age, particularly in the DI stock. The increase of water intake was much more than would be expected from the increase of weight. The increase of intake with age shown by non-*Os* mice of the DI stock is interesting and was investigated further, as follows.

Figure 6 shows the distributions of water intake of mice of the DI stock aged six months and over. Comparison of these distributions with those at nine weeks in Fig. 1 shows that the increased intakes of *Os* mice of both sexes was by a general

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upward shift of the whole distribution, but the increase of non-*Os* females was due to very high intakes by a few animals. These non-*Os* females with quite severe diabetes are of particular interest because they may seem at first sight to be recombinants between *Os* and a linked gene producing diabetes. There are, however, three reasons for concluding that they were not recombinants: (i) The distributions in Fig. 6 are not consistent with recombination because there were no recombinant

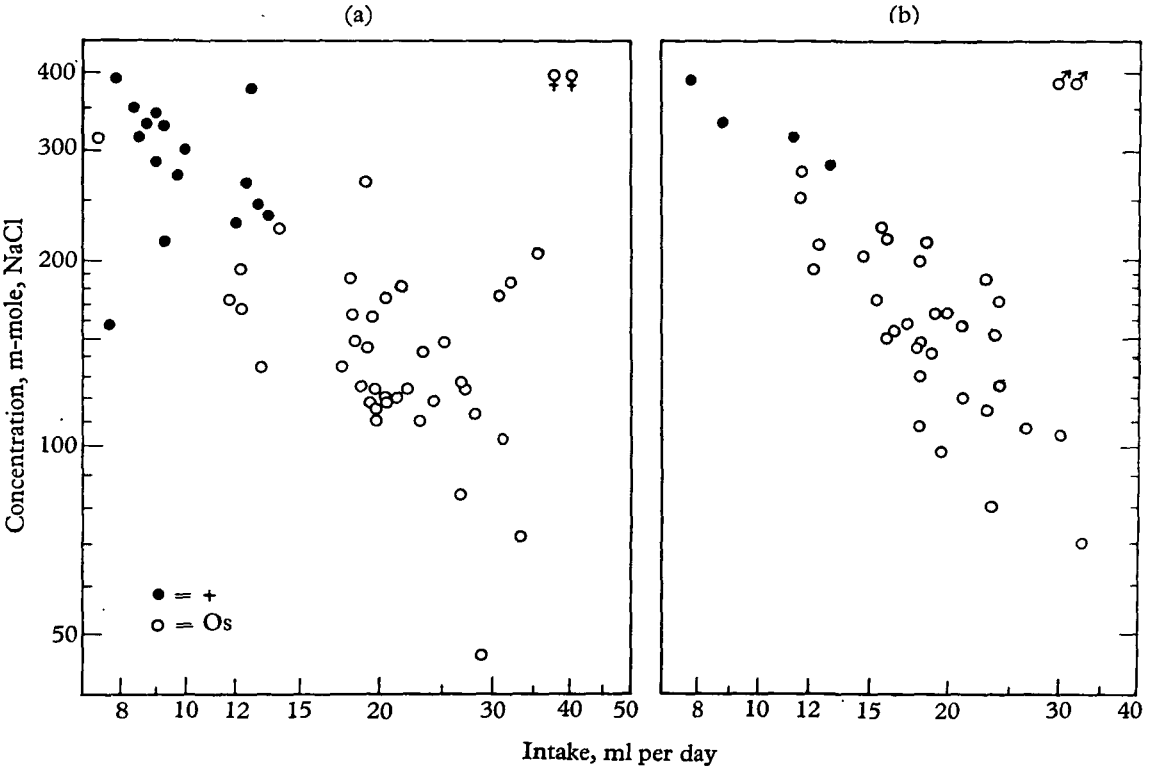

Fig. 7. Salt concentration of urine and daily water intake of mice of the DI stock aged nine or ten weeks. Each point represents one mouse and is the mean of between two and seven determinations on successive days. Open circles = *Os*, closed circles = non-*Os*. Both variables are plotted on a logarithmic scale. 1 m-mole NaCl = 58·5 mg. NaCl per litre.

males, and there is no indication of the complementary recombinants, *Os* mice without diabetes. (ii) The intake of the anomalous females was normal at nine weeks of age. The non-*Os* females with intakes of over 20 ml. in later life had a mean intake at nine weeks of 12·2 ml., whereas their *Os* litter mates had a mean of 22·9 ml.; this difference is significant at the 0·1% level. (iii) Two of the anomalous females were proved by breeding tests not to be recombinants: their non-*Os* offspring all had normal intakes. Furthermore, four of the anomalous females were proved to be non-*Os* by breeding tests, so the high intakes cannot be attributed to misclassification of *Os*.

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It was concluded in a previous section that the 'modifying genes' present in the DI stock themselves produced a mild diabetes in the absence of the *Os* gene. The observations on the older animals show that this diabetes became severe in some females, but not in males. Only a few of the affected females had been mated, so the diabetes was not associated with pregnancy. Many of the severely affected animals, both *Os* and non-*Os*, developed hydronephrosis in later life.

(v) *Urine analysis and other observations*

Samples of urine from a number of *Os* mice with high intakes were tested for the presence of reducing substances and albumen; all were negative.

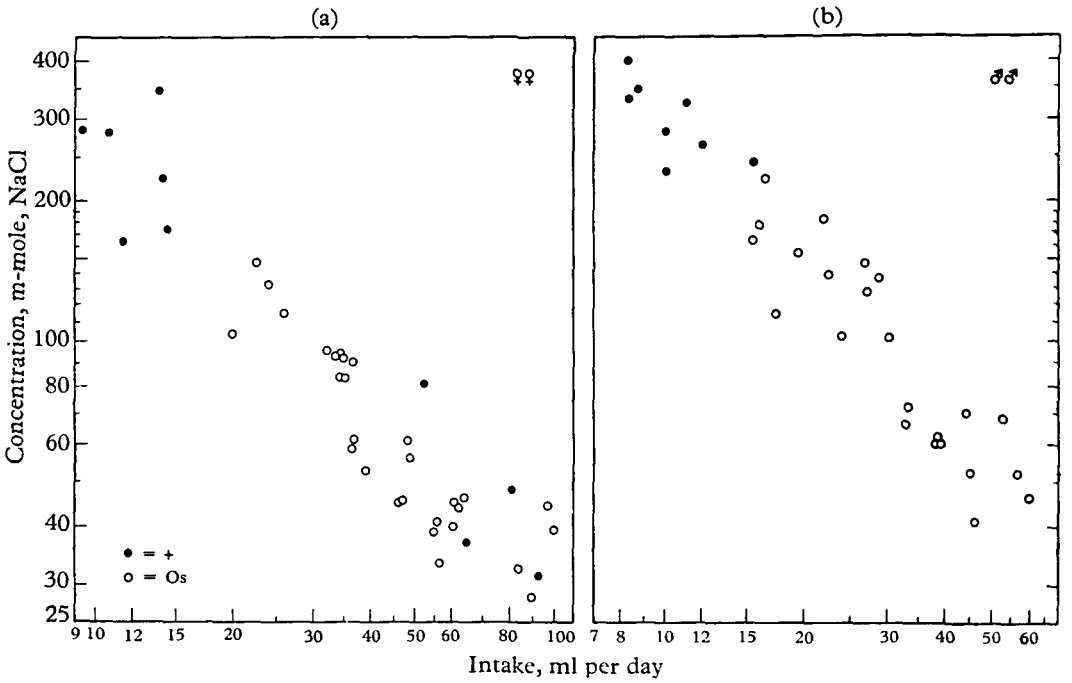

Fig. 8. The same as Fig. 6, but for mice aged between three and twenty-six months.

Determinations of electrolyte concentration of the urine of both *Os* and non-*Os* mice of the DI stock were made by a micro-conductivity cell. The results are summarized in the scatter diagrams in Figs. 7 and 8. Each point refers to one individual, and is the mean of between two and seven determinations of urine concentration and water intake made on successive days. Figure 7 refers to mice aged nine and ten weeks, and Fig. 8 to mice aged three to twenty-six months. The results show that individuals with high intakes produced urine of low salt concentration and that, in general, differences of intake were associated with differences of urine concentration, both between *Os* and non-*Os* mice and between *Os* mice with different intakes. There was some overlap in urine concentration, as in water intake,

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between *Os* and non-*Os*, but there was very little overlap when both factors are taken into account together. The intake together with the urine concentration therefore gives a better means of discriminating between the normal and the abnormal than either factor singly. On this criterion, there was one anomalous animal at nine weeks, an *Os* male with very low intake and high salt concentration. Among the older animals, four non-*Os* females with high intakes were included, and these all had correspondingly low salt concentrations, so that in this respect their diabetes was not distinguishable from that of the *Os* mice.

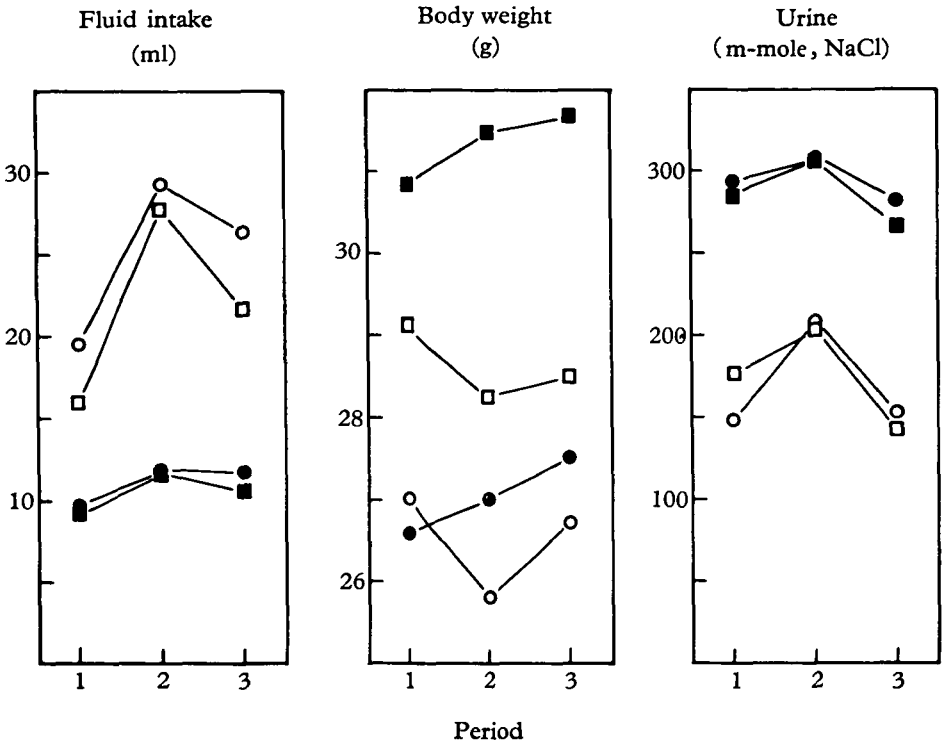

Fig. 9. Effect of giving saline as drinking fluid. Means of daily measurements of fluid intake, body weight, and concentration of urine in three periods of seven days: periods 1 and 3 when given tap-water, period 2 when given N/10 NaCl. Open symbols = *Os* mice; solid symbols = non-*Os* mice; circles = females; squares = males. Numbers of animals: *Os* ♀♀ = 7, + ♀♀ = 6, *Os* ♂♂ = 10, + ♂♂ = 5.

In order to find out if the *Os* mice were able to concentrate their urine further, the effect of giving saline as drinking fluid was tested. Twenty-eight mice from four litters of the DI stock were treated between the ages of nine and twelve weeks as follows. For the first seven days they were given tap-water, then for seven days N/10 NaCl in their water bottles with no other source of fluid, then for the final seven days they were given tap-water again. The fluid intake, body weight, and the electrolyte concentration of the urine were measured daily. The results are summarized in Fig. 9 which shows the daily fluid intake, body weight, and urine

concentration, averaged over each of the three seven-day periods. Both *Os* and non-*Os* mice increased their fluid intake when transferred to saline, but the *Os* much more than the non-*Os*. The *Os* mice did produce more concentrated urine when given saline to drink, but the concentration did not approach the non-*Os* level. Presumably their greatly increased fluid intake was necessitated by their

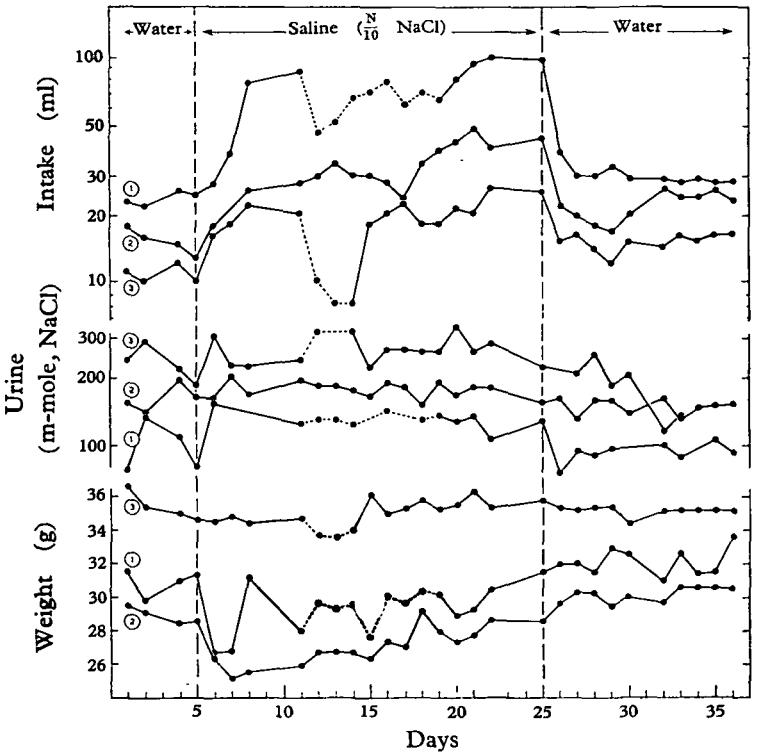

Fig. 10. Effect of pitressin injections on fluid intake, urine concentration, and body weight. Daily records of three individual male mice: 1 = *Os*, treated; 2 = *Os*, untreated; 3 = non-*Os*, treated. The periods of treatment with pitressin are shown by dotted lines.

inability to concentrate their urine above a certain level. The most striking difference between the *Os* and non-*Os* mice was in the changes of body weight. Whereas non-*Os* mice increased their weights regularly through the three-week period, the *Os* mice lost weight during the period when they were given saline. In order to assess the change of weight during the saline period, the average weights on the last two days of the first week (water) and of the second week (saline) were calculated. The difference gave the change of weight, and was as follows (the numbers of mice are given in brackets):

	Os	non-*Os*
Females	−1·87 g. (7)	+0·08 g. (6)
Males	−1·13 g. (10)	+0·62 g. (5)

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The difference between *Os* and non-*Os* mice is significant at the 1% level in both females and males.

The loss of weight by *Os* mice when given saline to drink suggests that they were not able to concentrate their urine enough to eliminate all the salt without excreting more water than they were taking in, and it points to a defect of water absorption by the kidneys as a primary cause of the diabetes insipidus, rather than excessive thirst as in the strain of mice described by Silverstein *et al.* (1961).

In order to find out whether the defect of *Os* mice is renal or hypothalmic in origin, the effect of exogenous vasopressin was tested on a few animals. The mice were given N/10 NaCl as drinking fluid and the daily fluid intake, body weight, and urine concentration measured for six days. Then they were given between three and eight daily sub-cutaneous injections of 1 pressor unit of 'Pitressin tannate in oil' (Parke, Davis and Co.). After a further week tap-water was given instead of saline. Four *Os* and one non-*Os* males were treated in this way. The daily measurements on one *Os* and the non-*Os* are shown in Fig. 10, with one *Os* mouse that was treated in the same way but without the pitressin. The reactions of all the *Os* mice to pitressin were qualitatively the same as the record shown. The fluid intake was reduced immediately after the first injection of pitressin, but thereafter it increased gradually to the original level, and it was never reduced to the level of non-*Os* mice. Despite the reduced intake, however, the concentration of the urine did not seem to have been increased at all by the pitressin. Though far from conclusive, these observations suggest that the primary defect of *Os* mice is renal rather than hypothalamic.

4. DISCUSSION

This work has disclosed two genetically distinct forms of diabetes insipidus in the mouse. The first, which was the main subject of the study, is that associated with the dominant gene, oligosyndactyly. (In what follows this form will be referred to as the *Os*-diabetes.) The second (or non-*Os* diabetes) is caused by one or more nearly recessive genes which were not analysed in detail. Each form separately produces only a mild diabetes, and neither would be readily recognized as an abnormality from the water intake or the urine output of individuals, though the non-*Os* form becomes severe in some old females and would then be easily recognized. Together, however, the two forms produce a severe diabetes, and it was in combination that they were first recognized by the abnormally high water intake and urine output of individual mice.

The manner of origin of the severe abnormality is relevant to the genetic interpretation. The *Os* gene had been maintained in the laboratory for $2\frac{1}{2}$ years but no abnormality had been noticed. The sudden appearance of the severe abnormality in some individuals must therefore be attributed to the gene or genes responsible for the non-*Os* diabetes, which, coming into combination with *Os*, produced the severe abnormality. The non-*Os* genes could have been introduced from another stock since the stock carrying *Os* had only recently been constructed, as a linkage-testing stock, by combination with two other dominant genes, *Re* and W^v. The severe

diabetes first appeared in the progeny of one mating, which is consistent with the nearly recessive nature of the non-*Os* diabetes, and suggests furthermore that the non-*Os* diabetes may well be due to a single gene.

The genetical evidence was all consistent with the *Os*- diabetes being a pleiotropic effect of the *Os* gene. If this interpretation is correct it raises the interesting question of the developmental connexion between the diabetes and the skeletal defects caused by the gene. This problem was not investigated.

Inherited diabetes insipidus has previously been reported in rats and in mice, as well as in man. The condition in rats (Valtin *et al.*, 1962; Sawyer *et al.*, 1964) is recessive and probably due to a single autosomal gene. It responded to pitressin and its hypothalamic origin was confirmed by histological abnormality of the hypothalamus and pituitary. The condition in mice (Silverstein *et al.*, 1961) is genetically determined because it affected the animals of a particular inbred strain, but the manner of inheritance was not determined. Its physiological cause was neither renal nor hypothalamic, but an excessive thirst. Several forms are known in man, which differ both clinically and genetically. One form, which responds to pitressin, is transmitted as a simple autosomal dominant (Martin, 1959). There are two forms transmitted as sex-linked recessives, with slight manifestation in heterozygous females, one responsive and the other resistent to pitressin (Forssman, 1955).

The severe diabetes insipidus of mice described here, being the effect of a combination of two or more genes, may possibly not have a unitary physiological cause. The tests with vasopressin were done on severely affected individuals in which the two genetic causes were combined, and, though not conclusive, they suggest that the defect was renal in origin.

SUMMARY

1. A mild diabetes insipidus is associated with the oligosyndactyly caused by the dominant gene *Os*. No recombinants were observed between the diabetes and the oligosyndactyly so the diabetes is probably a pleiotropic effect of the *Os* gene.

2. There are one or more modifying genes which in combination with *Os* enhance the manifestation and cause a severe diabetes insipidus. This affects both sexes and becomes progressively worse in older animals, the average water intake reaching 50–60 ml. per 24 hours, or 1·7 times the body weight.

3. The modifying gene or genes, in the absence of the *Os* gene, themselves produce a mild diabetes insipidus, which becomes severe in some old females.

4. The severe diabetes insipidus associated with *Os* and the modifying genes together is probably renal in origin.

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Note added in proof. For a study of the effect of the *Os* gene on body weight throughout life see Grüneberg, H., Gray, J. M., & Truslove, G. M. Congenital defects as indicators of lifelong abnormal processes. *Genet. Res.* (in the press).