Hydronephrosis in Mouse, Rat, and Man

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Evidence has recently been published indicating a hereditary component in the aetiology of congenital genito-urinary malformations in man (Hilson, 1957) and of hydronephrosis in a substrain of Wistar rats (Astarabadi and Bell, 1962). The nature of the hereditary component, particularly in man, is, however, difficult to determine.

It is possible that hydronephrosis is controlled in man by more than one gene with major effect, just as is diabetes insipidus (Martin, 1959; Forssman, 1955). In the mouse, hydronephrosis is known as an occasional effect of several genes whose most consistent effects are elsewhere than in the kidneys. Thus a low incidence is reported by Bagg (1929) in myelencephalic blebs (*mymy*), by Carter (1951) in luxate homozygotes (*lxlx*), and by Green (1951) in short-ear (*sese*). Falconer, Latyszewski, and Isaacson (1964) report hydronephrosis in the severer cases of diabetes insipidus in a stock where the incidence of this condition is high; here oligosyndactyly heterozygotes (*Os*+) have a higher incidence of diabetes insipidus than non-*Os*, but it is not stated whether *Os*+ also have a higher incidence of hydronephrosis.

It is striking that the region most consistently affected by all four of these genes in the mouse, *my*, *lx*, *se*, and *Os*, is the skeleton. Short-ear has a general effect on the cartilaginous skeleton, but its most obvious expression is the reduction in size of the pinna. Although the human cases in Hilson's data include a large variety of urogenital malformations as well as occasional hydronephrosis, it is again striking that these defects were found in patients chosen for examination because they had deformed ears. It is conceivable therefore that some at least of the human cases of hydronephrosis are controlled by a gene or genes, with effects similar to, if not the same as, that of the *se* gene in the mouse.

The present investigation was undertaken to discover some of the factors controlling the incidence of hydronephrosis in mouse strains segregating for

se. The findings in the mouse are compared with those reported for the rat and for man. The possible relations between the skeletal and renal effects of the gene are also discussed.

Known Effects of Gene, *se*

The developmental effects of the short-ear gene on the cartilaginous and osseous skeleton in *sese* mice have recently been reviewed by Gruneberg (1963).

The shortness of the ear, which is symmetrical and occurs in all homozygotes, is mainly due to the near absence of the cartilaginous scapha; the other cartilaginous parts of the ear are reduced. Other multiple skeletal anomalies, including bifurcation or reduction of the xiphoid process of the sternum, reduction in the number of pairs of ribs, and reduction in the size of parts of several small bones in various areas of the skeleton, occur in some individuals and not in others; their incidence depends on the genetic background. The *se* gene is impartial in affecting blastemata of mesodermal or neural crest origin, and has been shown to interfere with the chondrogenic (and apparently also osteogenic) tissue throughout life. The viability of *sese* is reduced on some genetic backgrounds.

A second effect, 'kinky-tail', is frequent in *sese* but occurs occasionally in heterozygotes, +*se*. The condition is apparently neuromuscular, not skeletal, in origin (Keeler, 1927), but it has not received much attention ('Kinky-tail' is not to be confused with 'tail-kinks', *tk*, closely linked with *se* (Falconer and Isaacson, 1966)).

Hydronephrosis is mentioned by Green (1951) in a study of skeletal effects. In her stock, an inbred line, it was confined to *sese*, with an incidence of 16%, though there was one +*se* with atypical kidneys whose hydronephrotic status was doubtful.

Materials and Methods

There were 286 necropsies made from eight stocks containing short-ear, and from one stock homozygous for non-short-ear.

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Two of the stocks were substrains of the highly inbred strain CBA. One was CBA/Cam* $-+se$, in which se was maintained, during sib-mating, as a permanent backcross ($sese$ mated to $+se$); and the other was CBA/Cam $+p$, in which p (pink-eyed dilution) was similarly maintained. se and p had arisen as mutations within CBA (Carter and Phillips, 1950). The substrain containing p , homozygous non- se , may be considered virtually isogenic with that containing se . In all, 22 necropsies (11 of each sex) were made from each.

The remaining 242 necropsies were made from closed colonies, not sib-mated, which segregated for se and several other mutants. All the mutants were quite unlike short-ear and located in different linkage groups (se being in linkage group II). In most of the colonies se was maintained as a permanent backcross, but in some there were also matings of $sese$ by $sese$. The data for these therefore concern somewhat more $sese$ than $+se$, and no homozygous non- se ($++$).

Since the data from individual stocks are small, they are pooled for the main analysis (Tables I, II, III, and V). In Table IV, two of the stocks are treated separately: stock M, or 'Multiplex' (Wallace and Williams, 1965), a long-standing colony representing about 22 generations of slow inbreeding, and stock I/VI (containing markers of linkage groups I and VI), a recently formed colony representing about 5 generations of slow inbreeding.

TABLE I
INCIDENCE OF HYDRONEPHROSIS IN THE MOUSE
ACCORDING TO SEX AND se GENOTYPE

	Normal		Free Hydronephrotic		Constricted Hydronephrotic		Totals
	F	M	F	M	F	M	
$sese$	84	39	9	30	0	9	171
$+se$	39	39	3	3	2	7	93

Note: Of $sese$, 22.8% are free, 5.3% constricted; a total of 28.1% are hydronephrotic.

Of $+se$, 6.5% are free, 9.8% constricted, a total of 16.3% are hydronephrotic.

Of $sese$ females, 9.7% are hydronephrotic; of $sese$ males, 50.0% are hydronephrotic.

Of $+se$ females, 11.4% are hydronephrotic; of $+se$ males, 20.4% are hydronephrotic.

Animals coming to necropsy were reasonably healthy when killed. They were all between 3 weeks and 13 months old, but most of them were between 2 and 8 months. Their kidneys and ureters were examined carefully, and sagittal section of the kidneys was made when necessary to confirm a doubtful diagnosis of hydronephrosis. It became clear at the outset that there were two types of hydronephrotic animal and that the renal vascular system was associated with this distinction. Each animal was therefore classified for these types and

its renal vascular system examined; sketches were made of all unusual cases.

From three $sese$ animals with hydronephrosis, preparations were made of male meiosis. Preparations were also made of corneal mitosis from 10 $sese$ which varied from extreme hydronephrosis to normal.

Results

Of the 22 necropsies from CBA/Cam $-+se$, 7 revealed hydronephrosis, while none of the 22 control from CBA/Cam $+p$ were abnormal. This difference is significant at the 0.001 level of probability.

The data for the 22 necropsies from CBA/Cam $+se$, and for the 242 from the seven stocks containing other mutants besides se , were pooled for analysis (Tables I, II, and III). The two types of hydronephrotic (described fully below) are: those without constriction of the ureter at any point, and those with a sharp discontinuity in the diameter of the ureter at some point in the upper two-thirds of its length. The former are termed 'free', the latter 'constricted.'

Incidence with se Genotype and Sex. Table I presents the number of mice with normal kidneys, and those with each of the two types of hydronephrosis, broken down according to se genotype and sex. Significance tests on trends in these data are given in Table III. The incidence of hydronephrosis is 28% in $sese$, and, somewhat unexpectedly, there is an appreciable incidence in $+se$ also, 16%.

The incidence of constriction among hydronephrotics is 18/63 or 30%. This is affected very much by the se genotype: in $sese$ only 9/48 or 19% hydronephrotics are constricted, whereas in $+se$ 9/15 or 60% are constricted. This difference is very significant (Table III A).

Thus, the se genotype affects both the incidence and type of hydronephrosis.

Sex is a very strong factor: 49/127, or 39%, of males have hydronephrosis, while only 14/137, or 10%, of females have it. This difference is extremely significant (Table III B).

Again, the sex difference is more marked in $sese$ than in $+se$, for in $sese$ 50% of males are hydronephrotic and 10% of females, while in $+se$ these frequencies are: 20% of males and 11% of females (Table I). Thus, the se genotype is a potent and decisive factor in males; whereas in females, though se must be present, it is related to a lower frequency, and the dosage of se ($sese$ or $+se$) is not decisive.

Expression with se Genotype and Sex. Table II presents the variation in expression of hydronephrosis, according to sex and se genotype and in

* Cam is the symbol for substrains maintained at the Dept. of Genetics, Cambridge University. Substrains are sib-mated proliferations of standard inbred strains such as CBA (Snell, Staats, Lyon, Dunn, Gruneberg, Hertwig, and Heston, 1960).

TABLE II
EXPRESSION OF HYDRONEPHROTIC MICE ACCORDING TO SEX AND *se* GENOTYPES

		Females					Males					Totals
		L	L > R	L = R	L < R	R	L	L > R	L = R	L < R	R	
<i>sese</i>	Free	1				8	4	3	15	3	5	39
	Constricted						3	1	3	2		9
+ <i>se</i>	Free	2				1	2				1	6
	Constricted	1				1	7					9
		4				10	16	4	18	5	6	63

Key: L is left kidney and/or ureter affected
 R is right " " " "
 L > R is left " " " " more than right.
 L < R is left " " " " less " "
 L = R is left " " " " approx. same as right.

TABLE III
TESTS OF SIGNIFICANCE OF TENDENCIES OBSERVABLE IN MICE (FROM DATA IN TABLES I AND II)

A			B			C			D		
Hydronephrosis			All			Hydronephrosis			Male		
Free	Constricted		Normal	Hydronephrotic		Unilateral	Bilateral		Unilateral	Bilateral	
<i>sese</i> + <i>se</i> 39 6	9 9	F M	123 78	14 49	F M	14 22	0 27	<i>sese</i> + <i>se</i>	12 10	27 0	
$\chi^2 = 7.61$ $p < 0.01$			$\chi^2 = 27.64$ $p \ll 0.001$			$\chi^2 = 9.07 - 11.31$ $p \approx 0.001$			$\chi^2 = 10.24 - 12.75$ $p \approx 0.001$		

χ^2 = contingency χ^2 with Yates' correction. Where a lower value is given it is a minimal χ^2 taking into account the presence of a nought in the Table.

TABLE IV
COMPARISONS CONCERNING GREEN'S AND OUR DATA ON MICE

A			B			C		
<i>sese</i>			<i>sese</i>			+ <i>se</i>		
Normal	Hydronephrotic		Normal	Hydronephrotic		Normal	Hydronephrotic	
Green's Ours 79 123	15 48	I/VI M	7 22	11 7	Green's Ours	88 77	1? 15	
$\chi^2 = 4.27$ $p < 0.05$			$\chi^2 = 4.96$ $p < 0.05$			$\chi^2 = 11.12$ $p < 0.001$		

χ^2 = contingency χ^2 with Yate's correction.

D Stocks	No. Generations Maintenance of <i>se</i>	<i>sese</i>		% Hydronephrotic
		Normal	Hydronephrotic	
Green's	18 sib-mated	79	15	16
Our M	22 closed colony	22	7	24
Our I/VI	5 closed colony	7	11	61

The heterogeneity $\chi^2 = 16.4$ for 2 d.f.; probability $\ll 0.001$.

FIG. 1. Dissection of *sese* mouse, showing bilateral hydronephrosis without constriction of the ureters. Ventral view.

FIG. 2. X-ray film of *sese* mouse, showing hydronephrosis without constriction of the ureter. 25% urographin was injected into the pelvis of the right kidney. Ventral view.

relation to the two types of hydronephrosis. Expression is described simply in terms of whether one or both kidneys are affected, and in the case of bilateral expression, which kidney is the more affected. Significance tests on trends in these data are given in Table III.

Hydronephrosis in females is exclusively unilateral, whereas males favour bilateral expression, this difference being very significant (Table III C). In females there is a slight bias to the right kidney, but the data are too small to discern significant trends. In males, *+se* expression is always unilateral, while *sese* expression is more usually bilateral, this difference also being very significant (Table III D). In male *+se*, the unilateral expression has a strong bias to the left, and here the largest number are constricted, whereas in male *sese* expression, there is no bias either in bilateral or unilateral expression, except perhaps to the left for constricted *sese*.

Accepting bilateral expression as more extreme than unilateral, it is clear, then, that males are more extremely affected than females, that *sese* have more extreme expression than *+se*, and that there is no

bias to the left or right, except in male constricted cases where the left predominates.

The Two Types of Hydronephrosis. The 'free' or unconstricted hydronephrosis is, as shown above, the more common form. It shows renal enlargement and mega-ureter (Fig. 1 and 2). The size of the kidney may be very great, the renal parenchyma being reduced to a thin shell surrounding a large volume of fluid (up to 2.0 ml.), and in extreme cases the atrophy of renal tissue is so complete that the kidney is transformed into a transparent fluid-filled sac. One animal had died before examination; this revealed that such a sac had recently ruptured. In less extreme cases, the accumulation of fluid is seen as the cause of enlargement only after sagittal section. (The hydronephrotic sac in the mouse is therefore quite different in appearance from that in man, but this is to be anticipated in view of the single pyramid organization of the mouse kidney.)

The ureter is usually enlarged both in diameter and, to a lesser extent, in length. The taper of the pelvo-ureteric junction is exaggerated, as is the taper of the first and middle thirds of the ureter.

FIG. 3. Dissection of + *se* mouse, showing unilateral left hydronephrosis with constriction of the ureter. Ventral view.

There is no evidence of stenosis of the ureter, it being easy to pass a blunt seeker from the bladder to the renal pelvis; nor do the kinks that result from the elongation of the ureter appear to be so severe as to cause occlusion.

In the 'constricted' cases, there is a sharp discontinuity in the diameter of the ureter at some point in the upper two-thirds of its length, and at this point the ureter is constricted (Fig. 3). The kidney presents much the same appearance as has been described above, though we have the impression that the distension is less even. The renal pelvis and the distal region of the ureter may be enormously distended. Although there is no obvious blockage of the ureter, the distension may result in transitory occlusion resulting from kink-formation becoming permanent.

Constriction and the Vascular System. To appreciate the cause of constriction, it is necessary to consider the ureteric vascular system. This is well known in man (Daniel and Shackman, 1952), but no comparable study has been made in the

FIG. 4. Diagram of unconstricted hydronephrosis, showing the usual venation of the renal pelvis and upper two-thirds of the ureter. Ventral view.

mouse. Cook (1965) mentions that the junction of the internal spermatic veins and the inferior vena cava varies between individuals of a random-bred strain of mice, but she shows only the most usual position of the ureteric vein (Cook's Plate III).

In man, Douville and Hollinshead (1955) found that the arteries followed the course of the ureteric veins, and we have confirmed this by examination of a small number of mice. Since the ureteric veins are much easier to see than the arteries, we have used the veins for routine examination.

In the majority of mice (Fig. 4) the most prominent vein opens into the renal vein close to the kidney and appears to drain the renal pelvis, the pelvo-ureteric junction, and the upper third of the ureter; this is also similar to the most common arrangement in man. There are, however, other variations: the ureteric veins may open into any part of the renal vein, into the inferior vena cava, or into the genital vein (ovarian or spermatic: we have not studied the vascular supply of the ureter below the iliac division); similar variations are seen in man.

The occurrence of constriction of the ureter in hydronephrotic mice is always associated with variation in the ureteric blood vessels. In *all* cases of constriction, the point of insertion of the main ureteric vein on the ureter is level with the lower margin of the kidney or below it, and coincident with the point of constriction (Fig. 5). Insertion at these sites also occurs among *sese* and *+se* hydronephrotics without obvious constriction, but is infrequent.

FIG. 5. Diagram of constricted hydronephrosis, showing one of the less usual types of venation of the renal pelvis and upper two-thirds of the ureter. Ventral view.

Mitotic and Meiotic Chromosomes. In both mitotic and meiotic preparations the number of chromosomes was normal and there were no obvious translocations. Small structural changes could have been missed.

Comparisons between Mouse Stocks: Ours and Green's. Table IV compares the incidence of hydronephrosis between Green's data and ours, and between those two of our stocks for which the data are largest, M and I/VI.

The incidence in *sese* of hydronephrosis in Green's strain (16%) is just significantly lower than

the over-all incidence for our stocks (28%) (Table IV A). There is also a marginally significant difference in incidence between our stocks: M (24%) and I/VI (61%)—(Table IV B). In *+se*, Green had one doubtful case, whereas in our stocks the over-all incidence was 16%, a very significant difference (Table IV C).

When the three stocks are arranged according to the number of generations for which they have been closed, it is clear that the most recently closed stock has the highest incidence in *sese* (Table IV D).

Comparisons between Data for Man, Mouse, and Rat. Table V sets out the data for incidence and expression of various kidney defects including hydronephrosis for man, given by Hilson. It also shows data for incidence and expression of hydronephrosis only; these are given by ourselves for eight stocks of mice, and by Astarabadi and Bell for a substrain of Wistar rats. In all cases males are more often affected, this feature reaching almost complete sex-limitation in rats (Table V A).

TABLE V
COMPARISONS BETWEEN DATA FOR
MAN, MOUSE AND RAT

A: Incidence of Kidney Defects in Sexes

	F	M	χ^2	% Male
Man* (with various kidney defects)	7	29	13.4	80.6
Mouse (with hydronephrosis only)	14	49	19.4	77.8
Rat (with hydronephrosis only)	6	104	87.3	94.5

χ^2 tests sex equality. ($p < 0.001$ in all cases.)

B: Expression of Kidney Defects in Males

	Kidneys Affected			Totals
	Left	Both	Right	
Man* (with various kidney defects)	8	15	1	24
Mouse (with hydronephrosis only)	16	27	6	49
Rat† (with hydronephrosis only)	0	Very few‡	Almost all‡	104

* From Hilson (1957). † From Astarabadi and Bell (1962).
‡ No figures given.

When expression is studied in males in all species, it is found to be almost exclusively confined to the right kidney in rats. In mouse and man, however, the 'lateralness' of kidney defects is very similar: thus in Hilson's, as in our data, males more often have bilateral than unilateral expression, and unilateral cases are mainly left (Table V B).

Discussion

Findings in the Mouse.

Hydronephrosis as a Pleiotropic Effect of se. The high incidence of hydronephrosis in the CBA substrain segregating in *se*, and its complete absence in a virtually isogenic CBA substrain lacking this gene, provides definitive proof that the *se* gene is mainly responsible for the condition under study.

The occurrence of hydronephrosis in *+se* might suggest that there were two closely linked recessive genes, of which one controls ear and the other kidney defects, and that in *+se* hydronephrotics these two have recombined. However, the difference in expression of hydronephrosis in *sese* from that in *+se* argues against this—*sese* are mainly bilateral and free whereas *+se* are unilateral and often constricted. Moreover, several attempts have been made, from descendants of *+se* hydronephrotics, to isolate hydronephrotics homozygous for non-short-ear; and these have failed. Again, though the *se* gene in the eight stocks studied came from two independent mutational events, all eight include hydronephrotics; in each stock, too, there have been many generations (the records cover 20 years) in which a linked hydronephrosis gene segregating with *se* could have been lost—by recombination and natural selection against the hydronephrotic animals; but this has not happened. The evidence is therefore strongly in favour of a unit gene, i.e. that hydronephrosis is a pleiotropic effect of the *se* gene.

Ureteric Venation as a Secondary Factor. The higher incidence of hydronephrosis and its more extreme expression in *sese* than in *+se* suggest that, while *se* is of prime importance in initiating the defect, some secondary factors are involved; it seems that these become more important when the effect of the *se* gene is limited by the presence of the normal gene in *+se*. A clue to one of these factors lies in the incidence of constriction in the two genotypes. Cases when the ureter is seen to be constricted occur more frequently in *+se* than in *sese*, and in all these cases there is unusual ureteric venation, the main vein inserting at the point of constriction. It seems likely therefore that, while unusual venation and consequent insertion below the kidneys occurs in *sese* as well, it may there serve merely to exacerbate a condition already strongly promoted by the *se* gene; in *+se*, however, it may enable the limited effect of *se* to cross a threshold dividing incipient hydronephrosis from the overt condition.

The greater incidence and expression of hydrone-

phrosis in males than in females is puzzling. The difference in *kind* of expression between the sexes, however, appears to be partly due again to vascular factors. If males with bilateral expression are ignored, there appears a bias in favour of left in the males accompanied frequently by constriction, whereas in females there is no bias (or a slight one to the right) and very few cases are restricted. There is thus some factor governing the insertion site of the ureteric vein in the male which differs from that in the female.

The biggest difference in venation between the sexes is of course the level of the gonadal veins: the ovarian vein comes across the lower portion of the kidneys and opens high in the posterior vena cava, the spermatic comes well below the kidneys and opens much lower (i.e. more caudally) into the posterior vena cava. Our observation that the ureteric vein sometimes opens into the gonadal suggests that the course of the ureteric veins is in some way connected with that of the gonadal, so that they insert lower in the male than in the female; if this is so, they will more often insert below the kidney in the male than in the female, and thus be in a better position to constrict in that sex. The shorter length of the left ureter than the right (due to the more caudal position of the left kidney) may then be a deciding factor in whether constriction takes place, and thus explain the excess of left constricted male cases.

A full analysis, based on the present 286 necropsies and some further ones, of the relation between the ureteric and the other veins into which it opens, will be published later. It is not, however, expected to account for the over-all greater incidence and expression of hydronephrosis in males than in females, unless there is found to be a sufficient preponderance in hydronephrotics of low insertions accompanying the relation with the spermatic vein; then it may be supposed that, despite the apparent lack of constriction in the majority of hydronephrotics of both sexes, these low insertions do in fact exacerbate in some way the tendency of *sese* and *+se* to hydronephrosis.

Genetic Control of Secondary Factors. The significant variation in incidence of hydronephrosis between the three stocks compared in Table IV indicates genetic control.

On this assumption, the higher incidence in the more recently closed stocks is explicable on the basis of selection of residual genotype. We have the strong impression, from breeding experience, that hydronephrosis impairs female fertility, and it may impair that of the male also; *sese* certainly impairs

the viability of unweaned males in the CBA sub-strain (figures unpublished), and impaired viability of *sese* (sex and age undifferentiated) has been mentioned for other stocks (Gruneberg, 1963). It is to be expected that when the *se* gene is introduced to a new genetic *milieu*, genes which, by their control of hydronephrosis (or of any other effect of *se*) reduce reproductive fitness, will come under strong negative selection pressure, and that this will continue in a closed stock, until either they have been eliminated or their residue has been fixed by inbreeding.

This raises the question: in what way is the hydronephrotic expression of *se* genetically controlled? While the expression of the *se* gene is constant in the ear of homozygotes, it varies in all other respects. The many minor skeletal anomalies are known to depend for their incidence on genetic background; the occasional neuromuscular effect, 'kinky-tail', has not been studied from this viewpoint, but it may do so. It is conceivable that genes which control both these types of expression of *se* may also indirectly affect the incidence of hydronephrosis—indeed they may all be closely related. It might be fruitful to compare variation in these two types of expression, between stocks which differ in incidence and expression of hydronephrosis.

The further analysis of the present investigation will have to be limited to an inquiry into the genetic control of the vascular component of hydronephrosis. Froud (1959) found considerable variation between three inbred strains of mice in the genito-urinary arterial system (and indeed in the whole body). He did not, unfortunately, study the ureteric artery, but it would be surprising not to find that variation in this and in the ureteric vein were also under genetic control.

Comparative Data in Mouse, Rat, and Man.

Comparison of Data for Mouse and Man. Hilson's data for man (1957) are unique in that they show a close association between ear deformities and renal ones (the latter being discovered only because the patients were chosen for examination on account of ear anomalies); and they supply evidence that this complex is hereditary.

It is striking that the simply inherited recessive short-ear in the mouse is also diagnostic for hydronephrosis. Further close parallels between the two are found in the pattern of sex-limitation and in the asymmetry of the urinary tract: in both there is a significantly higher incidence in males; males more often show bilateral expression than females, and unilateral cases are mainly left (Table V). Finally, it has been observed (Gruneberg, 1963), and we

have confirmed that, in short-ear mice, as in Hilson's human cases, many individuals show facial anomalies also, namely an increased width between the eyes and a broadening of the nose. These are among the features first described by Potter (1946) as indicative of renal disorder, and we have seen them in the homozygotes of only one other mouse mutant, brain hernia, *bh* (where, however, *bhhb* have polycystic rather than hydronephrotic kidneys in later life (Bennett, 1959)).

These parallels suggest that in both species a single gene is responsible for ear, facial, and renal defects, and that its action is similar in both species. The parallel is, as might be expected, incomplete. In Hilson's data, the defectives are not all hydronephrotic, the renal complex including absence of one kidney, agenesis, bifid ureters, and various degrees of abnormality of the genitalia. These features, if they occurred at all in our mouse data, were not obvious except in one stock (see below). In Hilson's data also the ears were asymmetrically affected, this asymmetry being somewhat diagnostic of the asymmetry of the kidney defects, whereas in the mouse data the ears are always symmetrically affected though the kidneys are not. It is possible that these differences can be ascribed to the undoubted differences in structure of the kidneys and ears in the two species. It is probable that, as in the mouse, hydronephrosis in man is controlled by more than one gene with major effect, and indeed that these interact. In the one mouse stock with unusual hydronephrotics, these were often cystic or had bifid ureters, and in some cases the internal genitalia were malformed. Many of these were homozygous or heterozygous for the gene *fidget*, *fi*, which has, like short-ear, some skeletal effects (but much less generalized: Gruneberg, 1963) and in some individuals a slightly domed head. It seems likely that the oddness of the hydronephrosis in this stock was due to interaction between *fidget* and short-ear.

In the nature of human pedigrees, it is difficult, particularly where the condition is rare and variable, to discern the action of a single gene. Inasmuch as the short-ear gene does resemble closely the ear-kidney complex in Hilson's data—more closely than does any other skeletal mouse gene with hydronephrosis—it could be useful as an experimental prototype of the human condition. The complete penetrance of short-ear in the mouse and the high incidence of hydronephrosis—with a possibility of improving this by selection—make this perhaps the easiest of such genes to use for a controlled breeding programme.

It is worth noting that, in the hydronephrotics

associated with fidget, constricting ureteric veins were also prominent. Vascular anomalies, certainly important in the aetiology of the hydronephrosis associated with short-ear, may well be important for hydronephrosis associated with other genes.

Comparison of Data for Rats and Mice. The bias towards male-limitation, almost complete in the Wistar substrain of rats (Table V), makes this case similar at first sight to that in mice and men. However, in the rats there is no obvious ear defect and the asymmetrical expression of hydronephrosis is almost confined to the right as against a bias to the left in males of the other two species. This rat substrain is, therefore, of particular interest for research into male-limiting features which are unlikely to be masked by any less specific features operating in short-ear mice.

Aetiology of Hydronephrosis in Mouse and in Man. Leaving aside the urogenital effects described by Hilson, the comparison between hydronephrosis in man, as described by Murnaghan (1958) and Anderson (1963), and this condition as we have found it in the mouse, allows close consideration of its development.

Primary hydronephrosis in man may appear to derive from mechanical causes, e.g. lower polar renal vessels, pelvo-ureteric adhesions, or a high uretero-pelvic insertion, all of which may result in mechanical obstruction to pelvic emptying. Frequently, however, there is no apparent mechanism of stenosis; and it is clear that mechanical obstruction is essentially a secondary exacerbating effect. Murnaghan has provided evidence to show that human congenital hydronephrosis derives from a failure of muscular co-ordination at the pelvo-ureteric junction. Waves of muscular contraction pass from the pelvis to the bladder, being initiated by dilatation of the pelvis, their transmission being myogenic. The muscular coats of the ureter are circular and longitudinal but at the pelvo-ureteric junction of hydronephrotics there is a reduction of muscle bulk, particularly of circular muscle. Anderson has noted in human cases where hydronephrosis is 'obviously functional', that 'where the ureter is freed, the pelvis seldom propels its contents down the ureter with each contraction; in other words, a ureteric contraction does not follow each pelvic contraction . . . and there is often a persistent constriction of the ureter, presumably due to spasm, because there is no demonstrably organic stricture'.

We have not done any work on mice to determine whether they are similar in these respects; however, it may be inferred that their abnormality is, at least

in large measure, of the kidney, renal pelvis, pelvo-ureteric junction, or the upper two-thirds of the ureter, since the 'constricted' cases show comparatively little manifestation below the point of constriction.

Anderson's findings in the 35% of cases where the ureter appears to be compressed by vessels running to the lower pole of the kidneys are interesting; vessels appear to be causal preponderantly in extrarenal types of hydronephrosis, and these cases are usually unilateral. Here 'the segment of ureter above the vessels is dilated, it contracts in sympathy with the renal pelvis, and the contraction stops at the vessels. When the ureter is freed from the vessels, the contractions are propagated down the ureter, and when the ureter is cut across, a spurt of urine accompanied each contraction.' His opinion on the causal role of the vessels is not rigid, as he also regards them as 'a serious aggravating factor, especially in extrarenal hydronephrosis'.

The work on 'constricted' cases in mice, which are also extrarenal and usually unilateral, is still to be done. However, we have observed that in normal anaesthetized mice lacking the ureteric vessels from the renal vessel and having relatively large vessels inserted low on the ureter, there is interruption in the wave of muscular contraction with incipient kink formation; this does not occur in mice with the more usual arrangement of blood vessels. It is possible that this slight incoordination and incipient kinking leads to actual periodic occlusion in mildly hydronephrotic mice. This suggests that the effect of variation in the attachment of blood vessels to the ureter arises not from deficiency of vascular supply but from variation in the mobility of the ureter.

It seems likely, therefore, that the primary cause in man and mouse is some kind of incoordination in the pelvo-ureteric system, and that suitably placed vessels further interrupt the waves of contraction, thereby exacerbating an already established defect. Since not all *sese* or *+se* mice, even with atypical ureteric vessels, show hydronephrosis, it may be that the effect on the mobility of the ureter of any vessels inserted at a given point and with a given course, may depend in part on the number of branches they send to the adventitia.

These considerations make it difficult to ascribe the causation of hydronephrosis in man or mouse to a gene controlling external ear formation. The general effect of the *se* gene in the mouse on the cartilaginous skeleton seems very remote from the genesis of the kidney and ureter and its vascular system. It is possible that an abnormality of the

sacral vertebrae has resulted in damage to the parasympathetic innervation of the bladder, giving rise to hydronephrosis by a mechanism similar to that which results from some accidental spinal injuries; but the specificity of the condition, and the absence of other symptoms that might be expected if this were so, makes this seem unlikely. Clearly, the exact relation of the primary action of the genes controlling ear and kidney defects in mouse and man await a fuller study of the physiological and embryological features of hydronephrosis in both species.

Summary

Hereditary hydronephrosis is described in the mouse, in the rat, and in man. In the mouse, it is associated with the short-ear gene, *se*. Data are analysed from 286 necropsies on mice of several strains segregating in *se*.

There is a significant difference in incidence between three strains that are suitable for comparison. Incidence of hydronephrosis in *sese* also declines the longer natural selection from the more fertile *sese* has been maintained. This indicates genetic control of incidence. Hydronephrosis has appeared in *+se* in those stocks where selection has had least time to operate.

There are two types of hydronephrosis: with and without obvious ureteric constriction. Constriction is associated with the vascular relationship of the ureter and renal pelvis, and occurs significantly more frequently in *+se* than in *sese*: it seems, therefore, that while hydronephrosis is primarily a pleiotropic effect of the *se* gene, secondary factors associated with venation become more important as the effect of *se* is limited by the presence of the normal gene in *+se*.

There is a higher incidence of hydronephrosis in males than in females. Male *sese* more often show bilateral expression than unilateral; male *+se* have almost exclusively left expression and are mostly constricted. Females have unilateral expression only, with an insignificant bias to the right, the incidence being low in both *sese* and *+se*, and constricted cases rare.

In rats (a Wistar substrain), hydronephrosis is almost limited to the male, there is no ear defect, there is a strong bias to the right kidney, and the mode of inheritance is uncertain.

The pattern of sex-limitation and symmetry in mouse is similar to that among cases of urogenital malformations in man which are associated with external ear deformity. This suggests pleiotropy

of a single gene in both species, the gene in each species having somewhat similar action.

The mechanism of hydronephrosis in man and in mouse is discussed. Comparisons indicate that experiments with mice may aid elucidation of cause and control in man.

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