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The origin of the acoustic ganglion and effects of the gene dominant spotting (W^v) in the mouse

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SUMMARY

The gene dominant spotting in the mouse affects coat colour. The heterozygotes for the viable allele (W^v) have one or two white spots and a general greying of the coat. The homozygotes are entirely white. There is evidence that the gene causes abnormalities of pigmentation by affecting the neural crest.

The inner ears of 33 W^v/W^v, 23 $W^v/+$ and 6 $+/+$ mice were examined. Marked abnormalities were found in the cochlea of all W^v/W^v mice and in the saccule of many. They also occurred, though in a restricted form, in a small part of the cochlea of a few old $W^v/+$ mice. The $+/+$ animals were all normal.

These findings may be interpreted as follows. The neural crest contributes to the formation of the acoustic ganglion, and as it is abnormal in W^v/W^v mice in its undifferentiated state, both the melanoblasts and the ganglionic primordia are affected. The abnormality of the primordium of the acoustic ganglion manifests itself as pathological changes in the inner ear.

This interpretation is supported by the results of an earlier study on the mutant piebald-lethal (s^l). But as there is considerable evidence that the otic placode also contributes to the formation of this ganglion, it probably has a dual origin. Since the vestibular part of the inner ear in both W^v/W^v and s^l/s^l mice appears to be unaffected, it may be assumed that the placodal moiety innervates this region, and the neural crest moiety the cochlea and the saccule.

The mode of formation of the acoustic ganglion suggested here should also be applicable to man, the dog, the cat, the mink and the deer mouse, if not to all mammals. In the species named above inner ear abnormalities have either been observed or been inferred from the behaviour, and when examined they have been found to be extraordinarily similar to those discovered in the mouse mutants.

INTRODUCTION

An investigation of the mutant piebald-lethal (s^l) in the mouse indicated that the neural crest played a part in the formation of the acoustic ganglion (Deol, 1967). It was based on two earlier studies: Mayer (1965) had shown by means of grafting experiments that the piebald allele of this gene caused abnormalities of pigmentation by affecting the neural crest, and Lane (1966) had discovered that all s^l/s^l mice had megacolon associated with a reduction of the myenteric plexus. As this plexus is known to originate in the neural crest (Yntema & Hammond, 1954), it appeared that the neural crest was affected

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before its differentiation into the melanoblasts and the plexus primordium. On the assumption that if the neural crest contributed to the formation of the acoustic ganglion this ganglion might be abnormal in s^l/s^l mice, and that the extraordinary complexity of the inner ear structure might bring this abnormality to light, the inner ear of these animals was examined. It was found to be invariably abnormal, and this was taken as an indication that the acoustic ganglion originated at least in part in the neural crest.

It seemed desirable to verify this conclusion by examining another mutant. The viable allele of dominant spotting (W^v: Grüneberg, 1952) appeared to be the most suitable gene for this purpose. W^v/W^v mice have almost normal viability, and while the retina is pigmented the coat is entirely white. No melanocytes can be identified in the hair follicles (Silvers, 1956). Although there had been no report of any deficiency of hearing in these mice, this did not necessarily mean that the inner ear was wholly normal. At the time the present study was begun nothing was known about the manner of action of this gene, but since then Mayer & Green (1968) have published a report on this mutant which clearly implicates the neural crest. They grafted into chick embryos pieces of the neural tube and the skin from normal and mutant mouse embryos in various combinations. The neural tube was taken from the region of the hind limb-bud of 9-day embryos, at which stage the undifferentiated neural crest is still in position. The skin was taken from the sides of the trunk of 11-day embryos, at which stage it has not yet received any melanoblasts. The W^v/W^v genotype being sterile, the mutant embryos came from matings between heterozygotes, and as the W^v/W^v embryos cannot be identified at the stages used, embryos from segregating litters were taken at random and the results interpreted statistically. Mayer & Green found that when both the tissues came from normal embryos the grafted skin developed pigment in all cases. The same happened when the neural crest came from normal and the skin from mutant embryos. But when the neural crest came from mutant and the skin from normal embryos pigment failed to develop in 61 % of the grafts. This was taken to mean that the neural crest in the mutant embryos was abnormal in its undifferentiated state. However, if this were the case with only the W^v/W^v genotype the incidence of unpigmented grafts should have been only 25 %. The discrepancy was explained by assuming that some of the $W^v/+$ embryos gave the same results—a not unwarranted assumption in view of the milder but clear-cut defects of pigmentation found in the heterozygote. Additional evidence for the involvement of the neural crest in W^v/W^v mice comes from the lack of dendritic melanocytes in the choroid (Mayer & Green, 1968), these cells being known to originate in the neural crest (Markert & Silvers, 1956). Nothing is known about the incidence of megacolon in these mice.

MATERIAL AND METHODS

This report is based on serial sections of the inner ear of 33 W^v/W^v and 29 control mice. The control animals, which in all but 3 cases came from the same litters as the mutant homozygotes, were of two kinds with regard to genotype: $23 W^v/+$ and 6 $+/+$. It was thought desirable to use controls of these two types because the possibility that the W^v gene affected the ear even in the heterozygous state could not be excluded. The age of the animals ranged from 14 days, when the fine differentiation of the organ of Corti is just complete, to 381 days. In all, there were 6 W^v/W^v, 7 $W^v/+$ and 3 $+/+$ animals over 300 days old, and 6 W^v/W^v and 5 $W^v/+$ animals under 20 days old. Of the 23 $W^v/+$ animals used 12 had a white spot of variable size in the middle of the head. The remainder had only the patch of white on the belly and a general greying of the coat, which are the characteristic features of this genotype. The genetic background of the stock was kept heterogeneous to obtain the maximum range of gene expression. In view of the impending examination of the organ of Corti accurate hearing tests were considered unnecessary, but the majority of W^v/W^v animals were certainly not totally deaf.

In addition, 10 albino animals, which are known to have melanocytes but no pigment (Billingham & Silvers, 1960), were also examined. Of these 6 came from the inbred strain A/Gr and 4 from the tail-short stock kept at this laboratory. The tail-short mutation originally occurred in the BALB/c inbred strain, and although the stock has not been outcrossed since then, no attempt has been made to adhere to the brother-sister mating rule of inbred-strains. None of the 4 animals used carried the tail-short gene itself.

All animals were fixed intravitally with Witmaack's fluid. The head was then cut off, decalcified in 1 % nitric acid, neutralized in 5 % sodium sulphate and washed overnight in running water. The inner ears, along with adjoining parts of the brain, were then removed with a razor blade, and double-embedded in celloidin and paraffin. The sections were cut at 10 μ in a plane parallel to the modiolus in the cochlea, and stained with Ehrlich's haematoxylin and orange G containing a trace of erythrosin.

RESULTS

Histological abnormalities were found in the inner ear of all W^v/W^v animals. They appeared confined to the cochlea and the saccule, and apart from a possible increase in the utricular otoliths no clear and consistent changes were observed in other parts. The cochlea was invariably affected, although the abnormalities were seldom uniform throughout the cochlear duct. They varied a great deal, without any detectable pattern underlying the variability. The two ears were frequently unlike each other. The saccule was often normal in appearance on one or both sides. When abnormal, the whole sensory organ

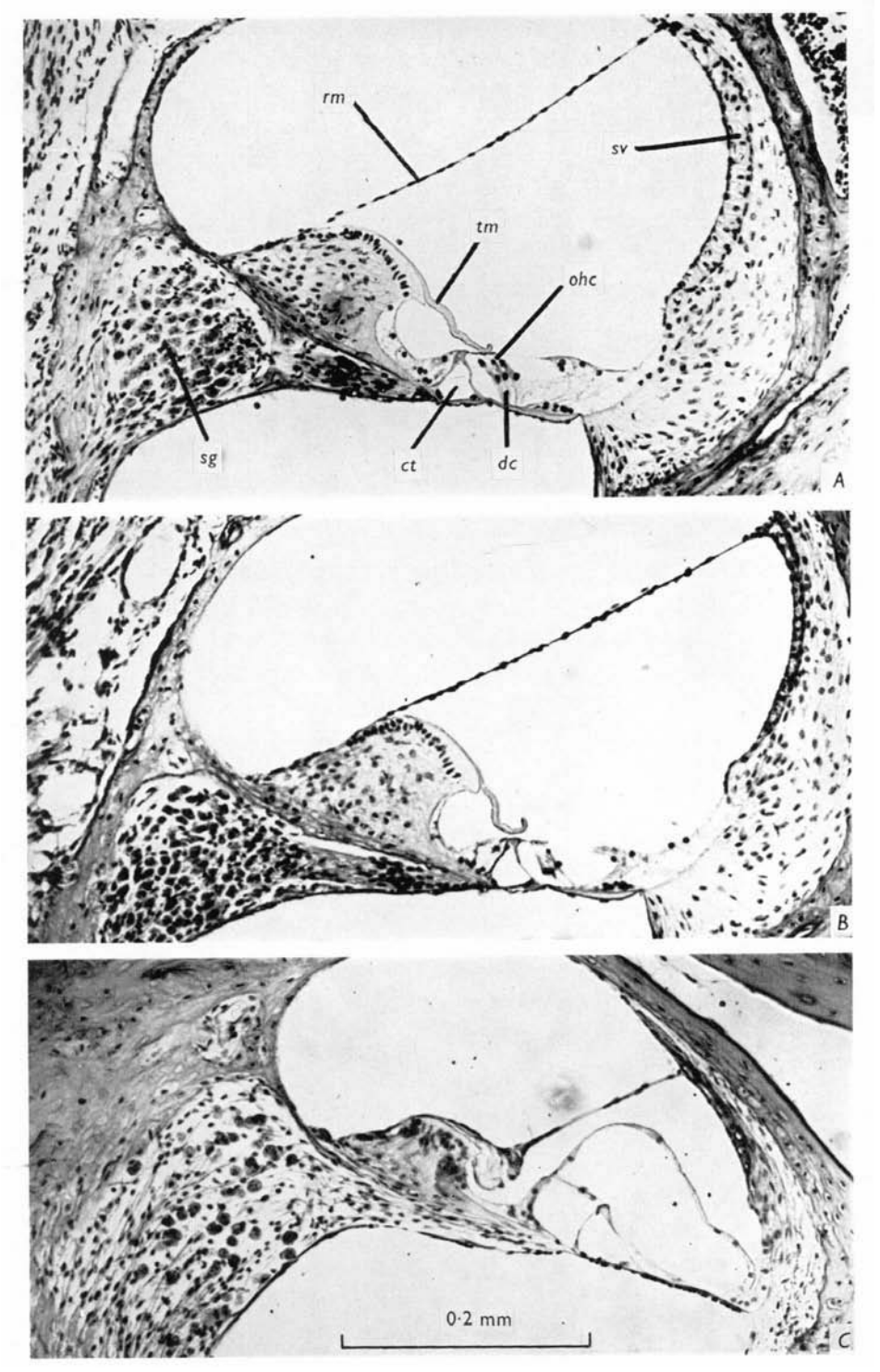

Fig. 1. (*A–C*). Transverse sections of the cochlear duct of a 61-day-old normal mouse (*A*), a 61-day-old W^v/W^v mouse (*B*) and a 320-day-old W^v/W^v mouse (*C*). *ct* = Corti's tunnel; *dc* = Deiter's cells; *ohc* = outer hair cells of Corti's organ; *rm* = Reissner's membrane; *sg* = spiral ganglion; *sv* = stria vascularis; *tm* = tectorial membrane.

was affected in much the same way. As to the magnitude of the abnormalities, they were mild in all animals between 2 to 4 weeks of age, and severe in all animals older than 300 days. Between these ages the degree of severity varied considerably.

In the typical adult W^v/W^v mouse the most striking abnormalities were seen in the organ of Corti and the stria vascularis (Fig. 1*A*, *B*). The outer hair cells in the organ of Corti were mostly missing or malformed. The inner hair cells seemed to be more resistant to degeneration. The supporting cells had lost their highly regular arrangement, and had frequently dedifferentiated, although there was no evidence of actual cell loss among them. The tectorial membrane was distorted and no longer in contact with the organ of Corti. In 8 animals (unilaterally in 5 and bilaterally in 3) a small region of the organ of Corti appeared to be normal. This was always situated at the base of the modiolus. The tectorial membrane was in contact with the hair cells in this region. The stria vascularis was much thinner than normal, and its vascularity was greatly reduced. Along its apical border it often tended to be continuous with Reissner's membrane, as if the distal end of the membrane had differentiated into the stria. In those animals in which the basal end of the organ of Corti was normal the stria was also normal in the same region.

Unlike the organ of Corti and the stria vascularis the spiral ganglion was clearly affected in only about half the animals. The ganglion cells had an immature appearance and tended to occur in clumps in certain regions. In addition, the density of the cells was reduced, especially in the apical part, and this reduction was so severe in most animals over 140 days old that the spiral canal in which the ganglion is lodged was virtually empty. An interesting aspect of this severe degeneration of the ganglion was that it was most in evidence in a region where the hair cells in the organ of Corti tended to survive much longer than elsewhere.

The saccule was less severely affected than the cochlea, and sometimes appeared to be quite normal. In affected cases some of the hair cells in the macula had been lost, and many others had become enlarged and spherical (Fig. 2*A*, *B*). There was usually an increase in the amount of otoliths, and the otolith granules were larger and had a greater affinity for haematoxylin. In some cases there was also a cellular proliferation, originating in the macula or the free wall of the saccule, which tended to fill the saccular cavity (Fig. 2*C*).

In young W^v/W^v animals, only 2 or 3 weeks old, the foregoing abnormalities were much less pronounced. The hair cells in the organ of Corti were present, although they might show slight changes in form. The supporting cells, especially Deiter's cells, lacked their characteristic arrangement of nuclei. The reduction in the thickness of the stria, although unmistakable in all cases, was less marked. The density of the ganglion cells was unaffected, but the immature clumping of cells was sometimes noticeable. The saccular macula appeared to be normal.

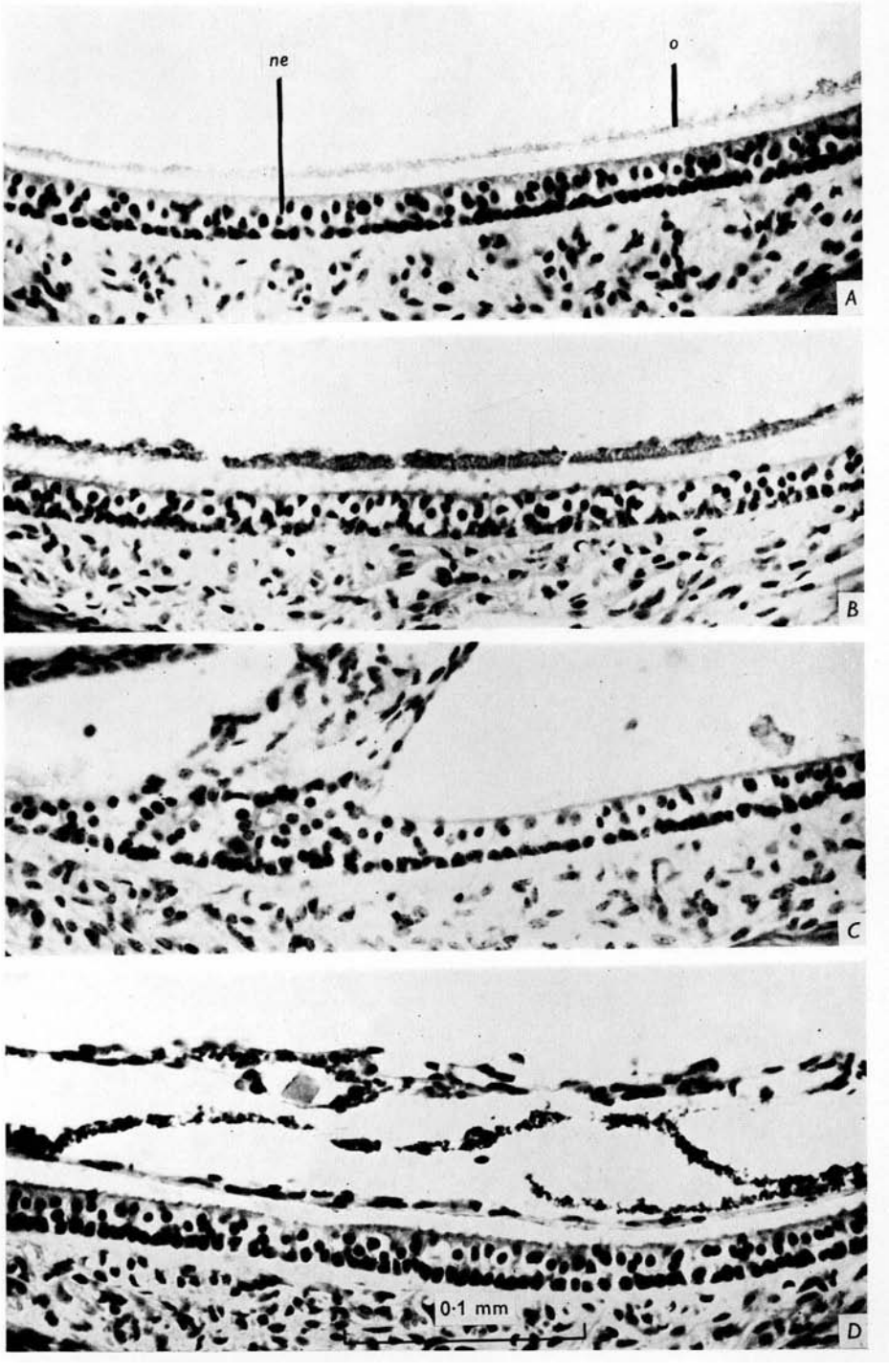

Fig. 2. (*A–D*). Transverse sections of the saccular macula of a 64-day-old normal mouse (*A*), a 64-day-old W^v/W^v mouse (*B*), a 150-day-old W^v/W^v mouse (*C*) and a 381-day-old W^v/W^v mouse (*D*). *ne* = neural epithelium; *o* = otoliths.

In old animals, older than 300 days, the abnormalities of the cochlea and the saccule were very severe. Dedifferentiation and cellular movements in the cochlear duct had advanced so far in some cases that the form of the organ of Corti was completely changed, and no recognizable structure remained except for the tunnel (Fig. 1 *C*). The tectorial membrane had grown thicker, and was usually attached to Reissner's membrane. The lumen of the duct was reduced as a result of these changes. The degeneration of the spiral ganglion was even more severe, and had spread to the middle half-turn. The amount of otoliths in the saccule was increased still further, and the cellular proliferation at times virtually filled the entire cavity (Fig. 2 *D*). In such cases the otolith layer was broken up, and the fragments scattered all over the saccule.

Although the coat of W^v/W^v mice was invariably white the inner ear was frequently pigmented. The distribution of pigment in the normal labyrinth is not uniform, but follows a characteristic pattern, occurring largely in the vestibular part. In the cochlea it is confined to the stria vascularis, and the saccule is on the whole free of it. The free wall of the utricle is heavily pigmented, and so are certain well-defined areas in the semi-circular ducts. Pigment is also present around all cristae, and among the nerve fibres, especially near their external endings. In the stria it appears late, and occurs in comparatively small quantities, while in the vestibular part it appears early, and is very dense. Of the 33 W^v/W^v mice examined 7 had no pigment in the inner ear on either side, 7 had it only on one side, and in 19 animals both sides were pigmented. The distribution of pigment in these 45 pigmented ears was never normal. The stria was always unpigmented except for a small region in the basal part in those few cases in which the basal stria was normal (see above). The area around the crista of the posterior semi-circular duct also lacked pigment in 6 animals unilaterally and in one bilaterally. Quite possibly there were other differences in the density and distribution of the pigment as well, but they could not have been determined without a laborious examination.

With the exception of 4 $W^v/+$ mice, all control animals of both genotypes had normal inner ears. These 4 mice, all over 140 days old, showed some of the abnormalities to a limited extent. There was a degeneration of the spiral ganglion and a reduction of the stria vascularis in the apical region. The organ of Corti was on the whole normal in appearance. Moreover, 4 $W^v/+$ animals ranging in age from 14 to 381 days, only one of which had the limited abnormalities mentioned above, lacked pigment in the area around the posterior crista on one side.

As to the albino mice, minor irregularities were found in a few ears, but they were in no way reminiscent of the abnormalities observed in W^v/W^v mice. There was naturally no pigment.

DISCUSSION

The results of Mayer & Green's (1968) studies described earlier suggest that the neural crest in W^v/W^v mice is abnormal in its undifferentiated state. In view of this the most plausible explanation of the inner ear defects in this mutant is that the neural crest contributes to the formation of the acoustic ganglion in the mouse. The inner ear abnormalities, which mainly occur in the neural elements, can then be viewed as direct or indirect consequences of the origin of this ganglion from a manifestly abnormal source. When considered with similar evidence from the mutant piebald-lethal (Mayer, 1965, 1967; Deol, 1967) this conclusion becomes inescapable. But there is at the same time considerable evidence that points to the otic placode as the source of the acoustic ganglion (Halley, 1955; Batten, 1958). In all probability, then, the ganglion is of dual origin. The evidence from these two mutants is compatible with the assumption that the contributions of the neural crest and the otic placode remain largely separate, and that the neural crest moiety innervates the cochlea and the saccule, and the placodal moiety the remaining parts. It would be of great interest to examine a mutant in which the cranial epidermis is abnormal from the earliest stages, but no such mutant is available at present.

There remains at least one other possible explanation of the association between abnormalities of pigmentation and the inner ear. No satisfactory hypothesis has ever been put forward to account for the presence of large quantities of pigment in the inner ear, and it is conceivable that the pigment or the pigment cells perform some unknown but essential function. It would then mean that the effects of the W^v and s^l genes on the ear might be only the reflections of their effects on the pigment cells. This possibility is not ruled out by the finding that pigment was present in most W^v/W^v ears, for this was true only of the vestibular part, which in any case is not involved. On the contrary, it appears to be supported by the finding that in those few W^v/W^v animals in which the stria was normal in the basal part of the cochlear duct it also tended to be pigmented. To check this possibility 10 albino mice were examined. They all proved to have normal ears. This ruled out any role of the pigment as such, but it remained possible that the supposed function might be an attribute of the melanocytes not connected with their capacity to form pigment (albino animals have amelanic melanocytes). However, there are certain considerations that weigh heavily against this view. Why, for instance, should the saccule be involved at all when even in normal animals it has little or no pigment? The same applies to the spiral ganglion, the degeneration of which cannot be of a secondary nature, for it often takes place independently of the loss of hair cells. Again, when the whole ear is without pigment, why is the vestibular part not abnormal as well? Moreover, although it is conceivable that degeneration may occur as a result of some malfunction of the melanocytes, cellular proliferation and other structural abnormalities cannot so easily be ascribed to the same cause.

As to the correlation between normality of the stria and its pigmentation in the basal region observed in some W^v/W^v mice, its explanation probably lies in the facts that the abnormalities of the stria appear very early and the pigmentation of the stria normally takes place rather late in life. Pigment cells simply do not spread into the abnormal part of the stria, or do not form pigment in the abnormal stria.

The inner ear abnormalities in W^v/W^v and s^l/s^l mice were not exactly alike, but the differences were of a minor kind. No degeneration of the spiral ganglion was observed in s^l/s^l mice, but this was almost certainly the result of the very early death of these animals. Cellular proliferation and dedifferentiation occurred to a much greater extent in this mutant. This may have been an effect of the genetic background or been related to the greater severity of the whole syndrome, one consequence of which would be the very early death. Yet another result of this severity may have been the finding that no pigment occurred in the inner ear in s^l/s^l mice, whereas it was observed in the majority of W^v/W^v animals.

In contrast with the present observations, Mayer & Green (1968) found no pigment in the inner ear of W^v/W^v mice. This discrepancy may have its root either in the different genetic background of their stock or in the small size of their sample, for pigment was found to be lacking in some animals even in the present study. The second explanation seems more likely, because extraocular pigment in this mutant has been observed before in other stocks. Grüneberg (1939) found pigment in the outer ear of W^v/W^v mice on one or both sides. Quite possibly there is a correlation between the presence of pigment in the outer and inner ear, but unfortunately no records of the outer ear pigment were kept in the present study.

The existence of pigment in the ear makes it difficult to understand clearly the nature of the neural crest abnormality in W^v/W^v mice. It is possible that different parts of the crest are affected to a different extent. As there is no clear correlation between pigmentation of the inner ear and its abnormalities, one may assume that the region of the neural crest producing melanoblasts for the inner ear is different from that which produces the primordium of the acoustic ganglion. This assumption is not implausible, for the ganglion is formed in a region anterior to the otic vesicle, and the melanoblasts may well be derived from the region corresponding to the vesicle. However, the manifestation of the mosaic nature of the neural crest may be overlaid by variations in the extent to which different tissues or different regions of the same tissue favour the differentiation of melanoblasts, this variation being a normal phenomenon in no way affected by the W^v gene. The concept of such a variation is supported by Mayer's (1965) finding that the gene *s* in the mouse reduced pigment in different tissues to a different extent, there being no reduction at all in the inner ear. The observations on the W^v/W^v mice are in agreement with this as far as they go: whenever there was any pigment in the animal outside the

eyes (retinal pigment is believed to have a different origin) it occurred in the inner ear. According to this view the entire neural crest in W^v/W^v mice forms melanoblasts, largely abnormal, but some normal or approaching normality, and these latter can differentiate in the inner ear or certain parts of it. The study of the piebald-lethal mutant (Deol, 1967) had suggested that white spots probably had amelanic and morphologically altered melanocytes rather than none at all. It appears now from Mayer's (1967) work on pigment cell migration that melanoblasts are indeed formed, reach their destination in the area of the spot, but then fail to differentiate. Their establishment in their normal locality for a limited period may account for the fact that there is no invasion by the normal melanoblasts from the surrounding areas.

The milder but distinct effects of the W^v gene in the heterozygote, described earlier, are paralleled by the presence of some of the abnormalities in the ears of a few old heterozygotes. These abnormalities are not correlated with the head spot. This could mean that when they occurred without the head spot the corresponding 'spot' was in fact in some unobserved, internal region or, more likely, the number of abnormal melanoblasts produced in the acoustic ganglion region of the neural crest was not large enough to form a spot. Similarly, when the ear was normal in spite of a head spot, it could have been that the abnormal melanoblasts were produced by a region of the neural crest that took no part in the formation of the acoustic ganglion. It is also possible that the inner ear abnormalities of the heterozygotes are in some way related to the general dilution of the coat rather than the head spot. It is hoped that an examination of certain genotypes with head spots but without general dilution will throw light on the problem.

Whatever view one takes of the origin of the acoustic ganglion in the mouse in the light of these studies it must also be extended to certain other species, if not all mammals. Abnormalities of the inner ear, either observed or inferred from deafness and such features, are known to be associated with defects of pigmentation of the spotting type in inherited syndromes in man, the dog, the cat, the mink and the deer mouse, and whenever they have been examined they have been found to be extraordinarily similar to those observed in the mouse mutants (Searle, 1968; Deol, 1968).

The gene W^v also causes sterility and anaemia. The sterility has been traced to a severe deficiency of the germ cells (Mintz & Russell, 1957). The anaemia, of the macrocytic type, has been traced to an abnormality of the haemopoietic tissue (Russell & Bernstein, 1966). No explanation can be offered for the association of abnormalities of the neural crest with either of these features.

RÉSUMÉ

L'origine du ganglion acoustique et les effets du gène dominant 'tacheté W^{v}*' chez la souris*

Le gène dominant tacheté chez la souris affecte la couleur du pelage. Les hétérozygotes pour l'allele viable W^v ont une ou deux taches blanches et une coloration grise générale du pelage. Les homozygotes sont entièrement blancs. Il est évident que le gène cause des anomalies de pigmentation en affectant la crête neurale avant sa différenciation en mélanoblastes.

Les oreilles internes de 33 W^v/W^v, 23 $W^v/+$ et 6 $+/+$ ont été examinées. Des anomalies marquées ont été observées dans la région cochléaire de toutes les souris W^v/W^v et dans plusieurs saccules. Elles se présentèrent également, bien que sous forme restreinte, sur une faible partie de la région cochléaire de quelques souris $W^v/+$. Les souris $+/+$ étaient toutes normales.

On peut donner l'interprétation suivante: La crête neurale participe à la formation du ganglion acoustique, et comme elle est anormale chez les souris W^v/W^v à l'état indifférencié, les ébauches du ganglion et les mélanoblastes sont affectés. Les anomalies de l'ébauche primordiale du ganglion acoustique se manifestent par des changement pathologiques de l'oreille interne.

Cette interprétation est appuyée par les résultats d'une étude précédente sur le mutant léthal '*bigarré* s^l'. Mais comme il est fortement évident que la placode otique contribue à la formation du ganglion, ce dernier a probablement une origine double. Puisque la partie vestibulaire de l'oreille interne chez W^v/W^v et s^l/s^l n'est pas affectée, on peut admettre que la moitié placodiale innerve cette région, et la moitié provenant de la crête neurale innerve la région cochléaire et la saccule.

Le mode de formation du ganglion acoustique suggère son application à l'homme, au chien, au chat, à la loutre sinon à tous les mammifères. Ches lez espèces nommées, les anomalies de l'oreille interne ont été noteés ou déduites de l'étude du comportement et l'examen a montré qu'elles étaient semblables à celles découvertes chez les souris mutantes.

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