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HEREDITARY ABSENCE OF OTOLITHS IN THE HOUSE MOUSE

By MARY F. LYON*

From the Institute of Animal Genetics, Edinburgh

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The recessive gene *pallid* (symbol *pa*) in the mouse (*Mus musculus* L.) produces pink eyes and a pale coat colour but is genetically distinct from the well-known pink-eyed dilution of mice bred as pets (Roberts, 1931). In addition, the author has observed abnormalities in the behaviour of pallid mice, affecting the postural reflexes, and has investigated the inheritance and anatomical basis of these defects. The anatomical basis proved to be absence of otoliths from the utriculus and usually also the sacculus of one or both ears. In inheritance the behavioural abnormalities could not be separated from the pallid gene and must be assumed to be caused by it; they were, however, varied in type and some pallid mice appeared completely normal. Correlation of the various types of behaviour with the anatomical changes proved of considerable physiological interest, and the present paper therefore gives a description of these abnormalities in behaviour and of their correlation with the changes in the inner ear.

MATERIAL AND METHODS

Material

The mice used were taken from a closed stock which had been derived from one pair two to three generations previously. Where possible each pallid mouse was compared with a non-pallid control from the same litter, but some animals were the offspring of pairs homozygous for the pallid gene and were therefore not accompanied by a control. The behaviour of several hundred young mice was observed as part of a study of the inheritance and the animals whose anatomy was studied were a sample of these, thirteen controls and fifty pallids, which were killed after the usual examination of their behaviour.

Histological methods

The preliminary observations were made on animals of various ages, but thereafter mice were killed in the fourth week of life. They were then fixed by injection of Bouin's fluid into the aorta, a portion of the skull bearing the labyrinth was excised, further fixed in Bouin's fluid, decalcified in 2% nitric acid in 70% alcohol and embedded by Peterfi's methyl benzoate method of double embedding, using a 2% celloidin solution (Carleton & Leach, 1938). They were then sectioned at 10 μ. and stained with Delafield's haematoxylin and eosin or orange G. Peterfi's double embedding method seemed the most suitable for this investigation. It gives much better support for the tissues than paraffin wax alone and, while possibly inferior to celloidin embedding for old mice, has the decided advantages that it gives serial sections and requires only an ordinary rotary or rocking microtome.

* Member of the Medical Research Council's scientific staff.

RESULTS

Behaviour

The abnormalities covered three aspects of the postural reflexes of the mouse: posture, response to position change, and response to linear acceleration.

In the mouse these postural reflexes develop post-natally and it was thus possible to compare the development of behaviour in pallid and non-pallid litter-mates and determine the stage at which the pallids first became abnormal. Since the investigation was intended primarily to elucidate the inheritance of the defects a large number of animals had to be examined. For this reason the methods chosen for observation of the behaviour were selected for their speed and for the reliability with which animals could be classified as behaving normally or abnormally.

Response to position change

The head downwards position was used to test the response to position change, the mouse simply being held up by the tail. The response of the normal young mouse to this is a stereotyped one which develops and increases in strength from the age of 2 days. The spine is flexed dorsally, the head is raised and the forelimbs are protracted and extended (Pl. 2, fig. 4). Some pallid mice never developed this response but, from the same age, flexed the spine and neck ventrally and retracted the limbs (Pl. 2, fig. 5). These mice also showed a clumsier gait than normal animals of the same age, were somewhat less active, and had an abnormal posture; the head was held with the snout pointing downwards and the hindlimbs were retracted and abducted. In the third week of life the normal response changes in character, becoming less exaggerated. The abnormal response changed little in the third week but in the fourth week it too became less exaggerated so that in the adult the difference, though still present, was very slight. Similarly, the posture and gait were almost normal after 4 weeks, though some animals passed through a transitory phase, in the fifth or sixth week, in which the posture was the antithesis of that in the younger mice of this type. The snout was raised, as in sniffing, and when the animal attempted to run it frequently sprang vertically instead of moving forwards.

Response to linear acceleration

A downward acceleration was used to test the response to linear acceleration, the mouse being held by the tail and the supporting hand then suddenly lowered. Once more the reason for this choice was that a normal mouse gives a stereotyped response, which develops in the third week of life and consists in a protraction and extension of the forelimbs. Mice which responded abnormally to position change failed to develop this reaction, and it was also

lacking in some mice with the asymmetrical posture described below and rarely in those which showed no other sign of abnormality.

Posture

The posture of pallid mice with an abnormal response to position has already been described. Some other young pallids showed an asymmetrical posture. This was sometimes visible at an age of a few days but could not be classified with certainty until the third week. Mice of this age held the head and forepart of the body tilted constantly to one side, whether resting or walking (Pl. 3, fig. 11). The tilt might be in either direction but the direction was constant for a given animal; the angle varied considerably from almost 0° to about 30°. The forelimb of the up-tilted side tended to be abducted but the hindlimbs seemed symmetrically disposed. Lateral bending of the body, rolling and circling movements did not occur and the animals walked in a straight line. These symptoms were at their peak in the third and fourth weeks of life. After 4 weeks the asymmetrical posture was frequently lost and a response to acceleration developed, so that the animals appeared completely normal.

A sample of 3-week-old pallid mice was tested for the ability to swim. Head-tilting mice, when placed on the surface of water, swam in a straight line in a posture similar to that on land, but when submerged sometimes failed to right themselves sufficiently to get the nose clear of the water. Spiral movements did not occur. Mice which failed to respond to position change were unable to maintain the normal belly-down posture in water and eventually turned over and became submerged, when they swam in any plane, sometimes in circles.

A slight head tremor was sometimes shown by adult pallids of the type which failed to respond to position change, but the author was unable to confirm the observations of Keeler (1947) who stated that pallid mice showed 'head-weaving' which he thought to be due to nystagmus.

Since the disorders affected the postural reflexes and apparently no other aspect of the behaviour, an anatomical defect of the labyrinth was suspected as the cause, and accordingly a histological examination of the labyrinth was made.

Histological findings

The significant defect found in all the abnormal animals was absence of otoliths. The abnormal maculae were normal in size and shape and normally innervated but the otoliths were completely lacking. The cells of the neuro-epithelium appeared normal and the otolithic membrane could be observed lying on the tips of the sensory hairs but it bore no granules (Pl. 1).

The expression of the defect was variable. Otoliths might be lacking in both sacculus and utriculus, in the utriculus only, or in neither, but no ear has yet

been found in which the utriculus was normal and the sacculus defective. Moreover, the two ears of the same animal might be symmetrical or asymmetrical. In some cases a reduced number of otolithic granules were present but it was more usual for them to be either normal or absent.

In the stained sections a further defect was present. The ampullae, utriculus and, to a lesser extent, the sacculus of ears which lacked otoliths were collapsed as though fluid had escaped by osmosis, while those of ears with otoliths present appeared normal. Since there was no sign of histological degeneration and since the behaviour did not suggest ampulla defects in life it was suspected that this collapse might be an artefact. Accordingly ears of twenty freshly killed mice were dissected under the binocular microscope, and the observations shown in Table 1 were made on the superior ampulla and utriculus.

TABLE 1. The state of turgor of the superior ampulla and utriculus of freshly killed mice

Type of ear	No. of ears	Collapsed	Somewhat dented	Normal	Damaged during dissection
Control	4	.	1	3	.
Pallid:					
Otoliths present	18	.	5	10	3
Otoliths absent	16	.	5	9	2
Otolith in sacculus only	2	.	.	1	1

In no ears were the ampulla and utriculus completely collapsed, as in the sections, and those ears described as 'somewhat dented' occurred with equal frequency in all types of ear, the dents being probably the result of slight damage during dissection. From this it was concluded that the collapse seen in the sections was indeed an artefact. Possibly the difference between ears with and without otoliths in this respect was due to the fact that when otoliths were present decalcification occurred in both the endolymphatic and perilymphatic spaces, and when otoliths were lacking it occurred in the perilymphatic space only.

No other defect was found either in the vestibular apparatus or the cochlea of pallids and the control animals showed no variations. An attempt was therefore made to correlate the type of otolith defect with the various types of behaviour defect.

Correlation of anatomical changes with behaviour

Table 2 presents in tabular form the correlation between the anatomical changes and the type of behaviour defect and the following information can be obtained from it.

Response to position change

All those animals which lacked all otoliths failed to respond to position change (Pl. 2, figs. 6, 7) and all those in which some otoliths were present

responded normally, including five in which the only otoliths present were those in the sacculus of one ear (Pl. 2, figs. 8, 9).

TABLE 2. The type of otolith defect of pallid mice with various behaviour defects

(+ = normal; − = absent; N.B. left = left side *uppermost*.)

Behaviour				Otoliths				
				Left ear		Right ear		
Response to position	Tilt	Response to acceleration	No.	Sac.	Utr.	Sac.	Utr.	No.
−	None	−	8	−	−	−	−	8
+	Left	−	10	−	−	+	−	2
				−	−	+	+	8
+	Left	+	4	−	−	+	+	3
				+	−	+	+	1
+	Right	−	6	+	−	−	−	1
				+	+	−	−	5
+	Right	+	6	+	−	−	−	1
				+	+	−	−	5
+	None	−	6	+	−	−	−	1
				+	+	−	−	3
				+	+	+	+	2
+	None	+	10	−	−	+	+	1
				+	+	+	+	9

Posture

All animals with asymmetrical posture showed asymmetry of otolith defect, the head being tilted with the more affected side *uppermost* (Pl. 3). In four animals the asymmetry involved the sacculus only, in one the utriculus only and in twenty-one both sacculus and utriculus, and in each case this rule concerning the direction of tilt held good. As far as could be judged from this small number of animals, the angle of tilt was no greater when the asymmetry involved both sacculus and utriculus. Indeed, in four of the five animals in which there was no tilt although the ears were asymmetrical this asymmetry involved both maculae.

Response to linear acceleration

It has been mentioned that response to linear acceleration was absent in animals which responded abnormally to position change, that is, those which lacked all otoliths, and in some of those with asymmetrical posture. Among the animals with left tilts there is a suggestion that those animals with normal response to acceleration had more normal maculae. However, the small number of animals makes this difference entirely without significance, and since the animals with right tilts showed no such difference there must be considered to be no evidence for a correlation between the number of normal maculae and response to acceleration.

The correlation of anatomical changes with behaviour is thus not complete. The variation in angle of tilt and the presence or absence of response to linear acceleration among asymmetrical mice remain without explanation, together with the disappearance of tilt with increasing age, since the anatomical picture

did not change with age. The positive results, however, concerning the response to position change and the asymmetrical posture seem sufficiently good to justify the conclusion that absence of otoliths was indeed the cause of the abnormal behaviour and that no other defect need be sought. Table 3 gives a summary of these positive results.

TABLE 3. The type of behaviour defect produced by various patterns of otolith defect, neglecting response to acceleration

(+ = normal; − = absent; N.B. left = left side *uppermost*.)

Otoliths				Behaviour		
Left ear		Right ear		Response to position	Tilt	No.
Sac.	Utr.	Sac.	Utr.			
−	−	−	−	−	None	8
+	−	−	−	+	Right	2
				+	None	1
−	−	+	−	+	Left	2
+	+	−	−	+	Right	10
				+	None	3
−	−	+	+	+	Left	11
				+	None	1
+	−	+	+	+	Left	1
+	+	+	+	+	None	11

DISCUSSION

The question now arises what picture can be formed of the physiology of these abnormal ears and what light this throws on the physiology of the normal labyrinth.

Experimental work on the labyrinth has suggested that the utriculus is concerned with the maintenance of muscle tone and with responses to tilting, unusual position and linear acceleration (Tait & McNally, 1934). The sacculus, from its structure, would be expected to have a function similar to that of the utriculus and the earlier workers considered that this was so. Versteegh (1927), however, failed to find any effect on the posture or reflexes after destruction of the sacculus in the rabbit. Similar negative results were obtained in a number of vertebrate animals (Löwenstein, 1936) and in the frog and elasmobranchs the sacculus appears to be sensitive to vibration (Ashcroft & Hallpike, 1934; Löwenstein & Roberts, 1948). In pallid mice, however, the sacculus has a definite effect on the response to position change. All animals which lacked all otoliths failed to respond, but the presence of the otolith in the sacculus of one ear resulted in a normal response. These animals with an otolith in one sacculus possessed an asymmetrical resting posture, showing that the sacculus also affects posture in these mice. This seems to prove that, contrary to supposition following the work of Versteegh, the sacculus does have an equilibratory function in the mammal.

De Kleijn & Magnus (1921) attributed special groups of reflexes to the two otoliths (utriculus and sacculus). Pallid mice, however, provide no evidence for this division of function, since the head was tilted in the same way whether the otolith asymmetry involved the sacculus, the utriculus, or both.

It has been pointed out that in mice with asymmetry the direction of tilt was such that the more affected side was held uppermost. This direction is opposite to that of the tilt resulting from unilateral ablation or denervation of the utriculus which produces loss of tone on the operated side. Denervation and lack of otoliths must therefore produce different effects on the macula. In an attempt to formulate some hypothesis as to the way in which a macula without otolith differs from normal one may first consider the behaviour of a normal macula. Adrian (1943), recording impulses from the vestibular nucleus of the cat, found that receptors reacting to slow tilts showed a resting discharge. This has also been recorded from the cristae and maculae of the elasmobranch labyrinth and seems to be characteristic of labyrinthine sense endings (Löwenstein & Sand, 1940; Löwenstein & Roberts, 1950). After denervation of a macula this discharge cannot reach the central nervous system but it is likely that maculae without otoliths do contribute to the stream of afferent vestibular impulses. A similar conclusion was reached by Magnus & de Kleijn (1922) concerning maculae from which the otoliths had been removed by centrifuging.

The next question concerns the frequency of the impulses emitted. Mice which lack all otoliths show a reduced tonus in the neck muscles, and this seems to indicate that their maculae are emitting fewer impulses than normal. In the unilaterally affected mice one may assume the asymmetrical posture to be due to an attempted equalization of the impulses from the two sides. If the maculae without otoliths are emitting fewer impulses than normal this assumption leads to the conclusion that the lowering of the normal labyrinth results in a decrease in its discharge activity. This represents a direct contradiction of the classical interpretation of the effects of unilateral labyrinthectomy and also of Adrian's (1943) electrophysiological results on the cat. It is interesting in this connexion that Löwenstein & Roberts (1950), recording from the utricular macula of the elasmobranch labyrinth, found that lowering of the labyrinth produced, if anything, a decreased discharge activity. In discussing their results they pointed out this very discrepancy from the findings of classical labyrinth physiology and left its interpretation an open question.

The other alternative, that lack of otoliths leads to a permanently increased activity of the macula, would satisfy the findings of classical labyrinth physiology but appears to conflict with the observation of a decreased neck muscle tone in pallid mice lacking all otoliths. Thus neither alternative fits all the observed facts and this question of the frequency of impulses emitted by a macula lacking otoliths must remain unanswered.

This mutant illustrates with more than usual clarity the value of the study of inherited abnormalities in laboratory animals. Grüneberg (1947) emphasized the importance of such studies in the investigation of hereditary diseases in man. The pallid gene provides a good example of that rarer but more important type of defect which can give information concerning normal physiology, since it has led to the conclusion that the sacculus has an equilibratory function in the mammal.

SUMMARY

1. Some mice homozygous for the gene *pallid* showed defects in the postural reflexes.

2. Some of these mice failed to respond to change of position and linear acceleration and others showed an asymmetrical posture with or without failure to respond to linear acceleration.

3. The histological basis of these defects was unilateral or bilateral absence of otoliths from the maculae of the utriculus and usually also the sacculus.

4. The conclusion that, contrary to recent opinion, the sacculus does have an equilibratory function in the mammal was derived from the fact that mice with otoliths in the sacculus of one ear differed from those which lacked all otoliths, in both the response to position change and the posture.

5. Asymmetrical animals tilted the head with the more affected side uppermost. This led to the conclusion that maculae without otoliths emit a resting discharge but the question of the frequency of this discharge was left unanswered.

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EXPLANATION OF PLATES 1–3

PLATE 1

Sections of the utricular macula, ×230. H = sensory hairs, M = otolithic membrane, N = neuro-epithelium, O = otolith, U = utricular nerve.

Fig. 1. Macula of a non-pallid control mouse born in the same litter as mouse of figs. 2 and 3.

Fig. 2. Macula from the left ear of a pallid mouse. Otoliths lacking.

Fig. 3. Macula from the right ear of the same mouse as in fig. 2. Otoliths normal.

PLATE 2

Figs. 4, 5. The response to head downwards position of 6-day-old litter-mates, a normal mouse (fig. 4) and a pallid mouse showing the abnormal response (fig. 5).

Figs. 6, 7. Sections of the left and right ears respectively of a pallid mouse which failed to respond to position change. All otoliths lacking. ×70. S = sacculus, U = utriculus.

Figs. 8, 9. Sections of the left and right ears respectively of a mouse with normal response to position and a head-tilt with the right side up. Otoliths present in the sacculus of the left ear only. ×70.

PLATE 3

Figs. 10, 11. Two 23-day-old pallid litter-mates, one with a normal posture and one with a left side up-tilt.

Figs. 12, 13. Sections of the left and right ears respectively of the mouse shown in fig. 10. All otoliths present. ×70.

Figs. 14, 15. Sections of the left and right ears respectively of the mouse shown in fig. 11. Otoliths lacking from the left ear. ×70.

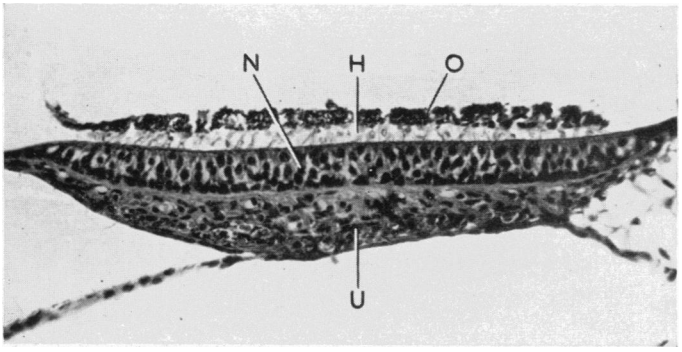
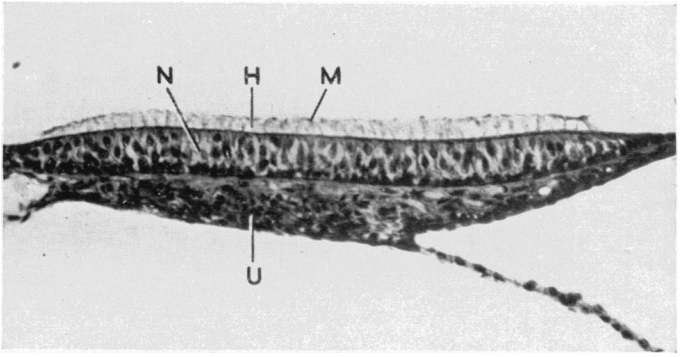


Fig. 1.



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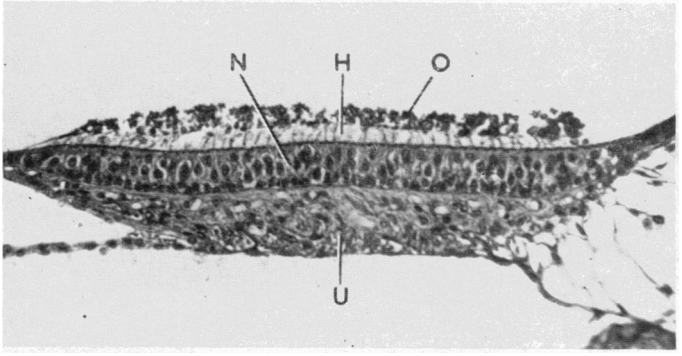

Fig. 3.

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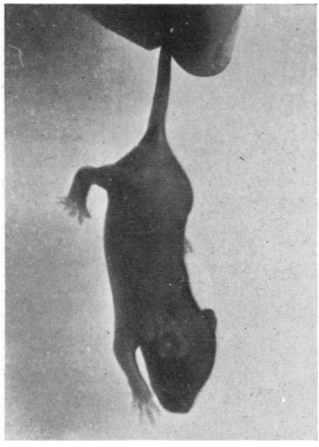

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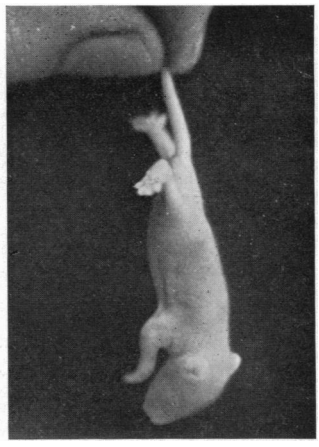

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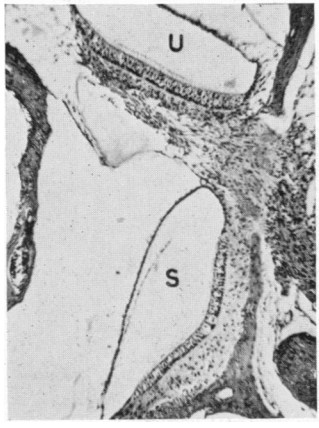

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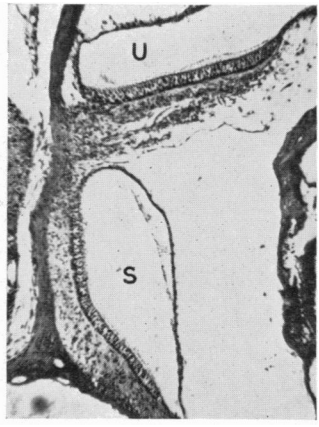

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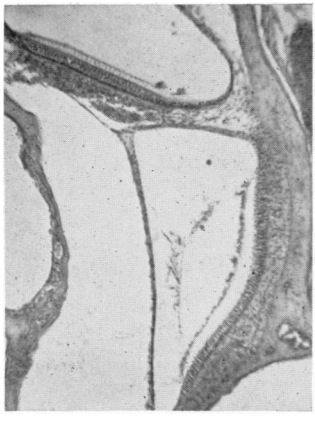

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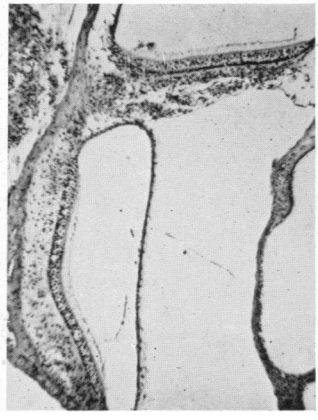
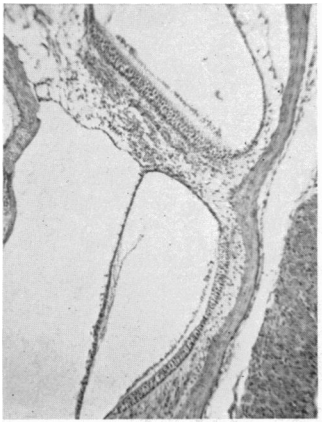


Fig. 9.

Fig. 10.

Fig. 11.



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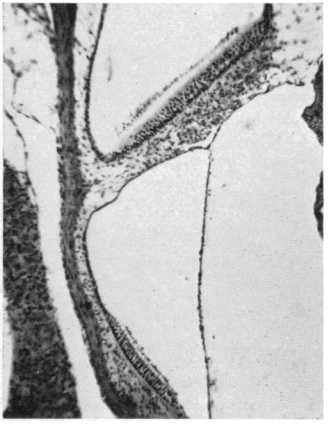

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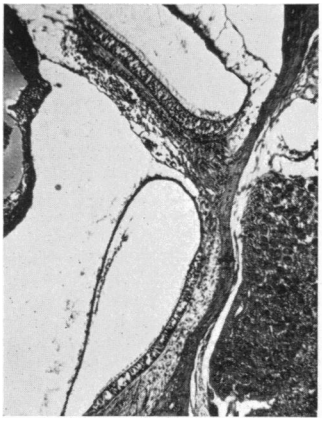

Fig. 14.

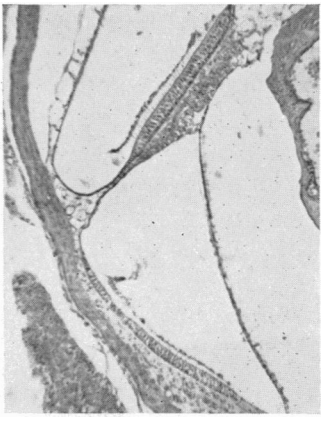

Fig. 15.