A re-examination of two skeletal mutants of the mouse, vestigial-tail \((vt)\) and congenital hydrocephalus \((ch)\)

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**SUMMARY**

The development of two skeletal mutants in the mouse has been re-investigated. In vestigial-tail \((vt)\), a simple syndrome is traceable to an anomaly of the primitive streak. In congenital hydrocephalus \((ch)\), the complicated events have been followed to a level at which the most fundamental gene action detected (or, more accurately, postulated) is no longer demonstrable by conventional histological methods. It involves the mesenchyme and some of its direct derivatives (meninges, skeletal blastemata, mesonephros), but also organs of different origin (nasal glands, ureteric bud, certain ganglia) for which the mesenchyme is the environment. Failure of the meningeal mesenchyme to form the subarachnoid drainage system for the cerebrospinal fluid leads to hydrocephalus which, by essentially mechanical means, is responsible for a multitude of subordinated effects.

**INTRODUCTION**

In long-continued research programs, earlier contributions are inevitably based on less experience than later ones. On first approaching the analysis of a mutant gene, one tends to concentrate on the more conspicuous parts of a syndrome, whereas the significance of less spectacular features is sometimes not realized until later. The discovery of new facts also often shows that an earlier hypothesis requires modification or has become untenable. For these reasons, the opportunity has been taken to re-examine the development of two skeletal mutants in the mouse which have been the object of previous studies. The results are presented in this paper.

*Vestigial-tail*

Vestigial-tail \((vt;\) linkage group VII; Heston, 1951) causes absence of the tail except for a small filament, or the tail is a short stump with irregular ankyloses between sacral and proximal caudal vertebrae; on some genetic backgrounds, tail reduction is less extreme. More anteriorly, the bodies of lumbar vertebrae tend to have bilateral centres of ossification (Grüneberg, 1957)

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Table 1. The posterior end of the body of 17 +/+ embryos (from CBA $\times$ C57BL F$_2$) and 13 vt/vt embryos, nominal age 9 days

Sections 7.5 $\mu$m thick. The cross-sectional area at the level of the cloacal membrane was determined planimetrically from projection drawings made at magnification $\times$ 250.

<table>
<thead>
<tr>
<th>CRL (mm)</th>
<th>Number of sections from</th>
<th>Transverse section at level of cloacal membrane (cm$^2$)</th>
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<td></td>
<td>cloacal membrane to end of body (A + B)</td>
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<td>cloacal membrane to end of cloaca or tail gut (A)</td>
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<td>end of gut to end of body (B)</td>
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<tr>
<td>+/+</td>
<td>2.26</td>
<td>37.19</td>
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<tr>
<td>vt/vt</td>
<td>2.25</td>
<td>38.54</td>
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<td>A/N*</td>
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* Abnormal/Normal.

which is generally a sign that the vertebral bodies are reduced; and there is a reduction in the number of presacral vertebrae (Grüneberg, 1955). Minor tail anomalies are not rare in +/vt heterozygotes (Michie, 1955; Grüneberg & McLaren, 1972).

In 9½-day vt/vt embryos (CRL 3.0 mm or a little less), two anomalies appear at about the same time (Grüneberg, 1957). In the caudal and sometimes in the lumbo-sacral region, neural tissue is hived off ventrally on the neural tube; this rounds up, acquires a secondary lumen; and branches to form multiple 'neural tubes' in the tail; the appearance of a constriction coupled with regressive processes soon leads to its loss. The other anomaly involves the Ventral Ectodermal Ridge (VER) of the tail (Grüneberg, 1956), a transitory structure of more general occurrence in vertebrates. The VER is a region of columnar epithelium near the tip of the tail not unlike the Apical Ectodermal Ridge (AER) of the limb-buds which is believed to be a stimulatory organ for limb growth. As the VER is situated where the tail grows by addition of new segments and as it disappears when this process is complete in the 12-day embryo, it may be a stimulatory organ for tail growth, and indeed in vt/vt embryos it is considerably reduced. On the other hand, its reduction might simply be a sign that tail growth is disturbed. The VER arises in 9-day embryos in the cloacal membrane where entoderm and ectoderm are in direct contact with each other; as the tail grows out, the columnar epithelium spreads along its ventral surface, but it soon becomes a separate structure which is no longer in contact with the cloacal membrane.

As neural anomalies and the reduction of the VER appear at about the same time, it is not clear whether one is the cause of the other or whether both are traceable to an earlier common cause. In any case, the VER cannot be respon-
sible for anomalies which must have arisen before the VER, such as neural anomalies in the sacral and vertebral anomalies in the lumbar region; nor for the reduction of the number of presacral vertebrae (i.e. the craniad shift of the posterior limb-buds which turns vertebra 26 from a lumbar into the first sacral vertebra). As the causal relations of the vt-syndrome are thus far from clear, its development has been subjected to a re-examination.

As vt/vt mice are viable and fertile in both sexes, known vt/vt embryos can be compared with known +/+ embryos before the major pathological changes have come into being (Table 1). Though the length of the posterior end of the body (A+B) is still normal, the cloaca or tail gut does not seem to extend as far into it as in +/+; the difference (A/N = 0.83; t_{27} = 1.469) is not formally significant but probably real. The distal region beyond the gut is correspondingly longer (A/N = 1.22; t_{27} = 3.795; P < 0.001). Whereas the overall length of the bud is about normal, its cross-sectional area at the level of the cloacal membrane is significantly reduced (A/N = 0.79; t_{28} = 4.858; P < 0.001).

Mammalian embryos grow in an antero-posterior direction. Hence the fact that vt/vt embryos are thinner at the level of the cloaca is clear evidence that growth anomalies were present before that level was reached. Hence the
involvement of the lumbar region and the cranial shift of the pelvic girdle now fit into the general picture.

In normal 9-day embryos (Fig. 1), the cloacal membrane is deeply indented where the anus will ultimately be formed. In vt/vt embryos, this dimple is much shallower and sometimes all but absent. This is the region where ectoderm and
entoderm are in direct contact with each other and where the VER originates. It also appears that in *vt/vt* embryos, the columnar layer is less thick, as is the case in the VER later on.

The *vt/vt* embryos (Fig. 2) have a reduced primitive streak which distally fails to fill the surrounding epidermis. This was previously interpreted as an oedema (Grüneberg, 1957; Fig. 19, plate 8) in somewhat older embryos (CRL 3-2 mm) whose tail anatomy was already grossly abnormal, and this is probably the reason why the true significance of the observation was not recognized. It now appears to be the key to the whole *vt*-syndrome. In 9-day *vt/vt* embryos, the notochord emerges from the primitive streak less firmly knit than normal, and nodules of notochordal origin (between notochord and neural tube) are present in 3/13 of them in the cloacal region. Similar nodules in the lumbo-sacral region were previously observed in older embryos (Grüneberg, 1957). The paraxial mesoderm is more loosely knit than normal, and this is evidently the reason why the posterior region is reduced in calibre, and why sclerotome derivatives such as the lumbar vertebrae are affected.

As in other axial mutants such as brachyury (*T/+*) and Danforth’s short-tail (*Sd/+*, *Sd/Sd*), the anomaly of the primitive streak gets worse posteriorly, and in the tail itself there is a complete breakdown. The irregular masses of tissue which pile up organize themselves as neural structures with lumina, etc., and eventually the tail regresses more or less completely.

The tail gut of 9-day *vt/vt* embryos does not seem to extend as far posteriorly as in the normal. Presumably the cause of the trouble is the primitive streak anomaly immediately behind it. The anal pit is less marked and the epidermal epithelium of the cloacal membrane less highly columnar. Presumably this is the main reason why the VER is similarly affected; its development may also be hindered by the fact that it is not properly supported by the mesenchyme. It is also conceivable that the anomaly of the primitive streak itself influences the VER. In the limb buds, a reciprocal relationship seems to exist between the AER and a mesenchymal Maintenance Factor (MF) which keeps the AER in a functional state. If the VER functions like the AER, its function might depend on an MF produced by the primitive streak. However, this is pure speculation.

In summary, it is evident that without forcing the facts, all known aspects of the *vt*-syndrome can be accounted for on a unitary basis, a disturbance of the primitive streak. Perhaps ultrastructural studies might give a hint as to its nature.

Brachyury (*T/+*, *T/T*) probably also affects the primitive streak in the first instance and the notochord only secondarily. On the other hand, Danforth’s short-tail (*Sd/+*, *Sd/Sd*) may well affect the notochord in the first instance. For a fuller discussion see Grüneberg (1958a, b).
Congenital hydrocephalus

This recessive condition \((ch; \text{chromosome 13; linkage group XIV; Grüneberg, 1943, 1953})\) is lethal at birth. The immediate cause of death is probably failure to inflate the lungs. The syndrome includes extensive abnormalities of the skeleton, particularly of the chondrocranium, the cervical vertebrae, the larynx and the thorax. The hydrocephalus was believed to be due to the shortening of the skull already present at the 12-day stage; but absence of a subarachnoid space in the neighbourhood of the foramen of Magendie was regarded as a subsidiary cause in later stages. The hydrocephalus, in turn, by overstretching the osteogenic membrane and the overlying skin, interferes with the formation of the calvarium and the closure of the eyelids; it was also held responsible for certain anomalies of the sensory hairs (secondary vibrissae). The syndrome was thus interpreted in terms of a skeletal anomaly, with the hydrocephalus as a secondary and its consequences as tertiary effects.

Meanwhile, many additional effects of \(ch\) have come to light. Green (1970) traced hydrocephalus and lack of a subarachnoid space to the 11-day stage. At the 10-day stage, there is excessive development of the mesonephros, but the coeliac ganglion is reduced; accessory ureteric buds are often present. Grünberg (1971) discovered osseous fusion between maxilla and mandible, abnormal position of molars and reduction of the masseter muscle. The parotis and the Harderian gland are absent, but in the nose certain glands are hypertrophic, others are accessory and still others reduced, as is the Chievitz organ. A ganglion on the nasal septum which belongs to the system of the N. terminalis tends to be increased (Grünberg, 1973); and several other new effects of \(ch\) will be described in this paper.

The original interpretation of the \(ch\)-syndrome is, of course, inadequate to accommodate all the new facts. The question arises of whether a new unitary interpretation can be devised. This would require the demonstration that all the pleiotropic effects now known are co-ordinated or subordinated to each other.

\((1)\) The hydrocephalus and its consequences

Normally, the cerebrospinal fluid on leaving the brain drains into the subarachnoid spaces and is eventually secreted into the venous sinuses of the dura mater. As now shown by Green (1970), in \(ch/ch\) the subarachnoid drainage system fails to form in the 11–12-day embryo and thus the pathway of the cerebrospinal fluid is blocked. This is the obvious and sufficient explanation of the hydrocephalus. Though the early shortening of the basicranium is a fact, it does not seem to be the cause of the hydrocephalus, as originally assumed (Grünberg, 1943, 1953).

In the 12-day embryo, early hydrocephalus and failure of subarachnoid space are clearly present together. But Green (1970) holds that in the 11-day embryo,
Fig. 3. The region of the sinus sagittalis superior in a normal and a ch\ch embryo (13\(\frac{1}{2}\) days old litter mates). The strand of mesenchymal tissue (arrow) in the ch\ch embryo corresponds in the normal to the fibrous layer dorsal to the sinus which will give rise to both calvarium and dura mater. Bouin. H & E. 7\(\frac{1}{2}\) \(\mu\)m. Magnification \(\times 130\).

'The timing of these two events probably does not coincide exactly, the bulging of the cerebral hemispheres appearing first'. To judge from her Fig. 4, the cerebral hemispheres do not seem to be enlarged by excess liquid; they seem to bulge anteriorly because the basicranium is shortened. A direct relationship between blocked drainage and hydrocephalus thus seems to be compatible with the observations.

Laterally and dorsally, the meninx primitiva of ch\ch is closely attached to the brain except in the region of the sinus sagittalis superior (Fig. 3). Here, from the 12-day stage onwards, it forms a dense strand of tissue which seems to interfere mechanically with the formation of the sinus, though to a variable extent. If cerebrospinal fluid were to reach this neighbourhood, reduction of the sinus could become a subsidiary cause of hydrocephalus; however, this does not seem to be the case.

Dorsally and laterally, a calvarium is completely missing. Ventrally, in the region of the cisterna magna, bone is formed, but it stops short at the brain. At this point, the subarachnoid space comes to an end and the meninx is in direct contact with the brain: which of these two interferes with further ossification is a moot point. In any case, failure of the calvarium does not seem to be a mechanical consequence of the hydrocephalus, as originally (Grüneberg, 1943) suggested.

There is no reason to doubt that the hydrocephalus is directly responsible for the failure of the eyelids to coalesce; the latter may be due to pull on the skin or to greater bulging of the eyes, or to a combination of the two. The anomaly of the eyelids in turn gives rise to a subordinated effect. The Harderian gland which is situated behind the eyeball arises in normal development on day 14 from the nictitating membrane. In ch\ch, it is regularly missing except for a ghost gland consisting of loose connective tissue and melanocytes (Grüneberg, 1971). As the nictitating membrane is already deformed in 13\(\frac{1}{2}\)-day ch\ch
embryos, it is reasonable to regard the failure of the Harderian glands as secondary to the involvement of the nictitating membrane.

The parotid gland of \( ch/ch \) is nearly always completely absent, and with it the gl. parotis accessoria which arises later from the ductus parotideus near its origin (Grüneberg, 1971). Occasionally a rudimentary ductus parotideus is formed. In normal development, the ductus parotideus arises in 12½-day embryos just behind the angulus oris (commissura labiorum) and in accurate alignment with the commissure (Fig. 4a, b). In \( ch/ch \), the upper lip is shorter (Fig. 4c) and consequently the angle of the commissure droops much less. The failure of the parotid duct to form is evidently due to the anomalous angle of the commissure. The anomalous configuration of the upper lip is apparently a mechanical consequence of the hydrocephalus which leads to many and profound deformations of the head.

In normal development, the Chievitz organ arises behind the ductus parotideus and in the same accurate alignment to the buccal epithelium. Its involvement
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Fig. 5. Transverse sections through the heads of a normal (a) and a ch/ch embryo, (b), 14½ days old litter mates. Meckel's cartilage densely stippled, bone hatched. In the ch/ch embryo, note the premature movement of the palatal processes and the presence of an ossified zygomatic process on the right; that on the left has coalesced with the mandibular ossification. Camera lucida drawings.

in ch/ch (Grüneberg, 1971) is obviously due to the same deformation as that which leads to the failure of the parotid.

This is the place to re-assess the place of the anomalies of the secondary vibrissae in the ch-syndrome (Grüneberg, 1943). The postorbital vibrissa is usually (52/60) absent, but the postoral vibrissa is usually (51/60) duplicated and occasionally triplicated. Normally, the postoral arises from the coalescence of two neighboring colliculi piliferi. These anomalies were interpreted as mechanical consequences of the hydrocephalus: in the immediate vicinity of the hydrocephalic bulges the postorbital tends to be suppressed, but behind the labial commissure the twin colliculi are separated from each other, whether by simple pull or other forms of deformation. It has now been discovered that in ch/ch, each of the two postorals forms two vibrissae. Apparently, in this vicinity, a colliculus piliferus forms two vibrissae regardless of how it has come into being, and the fact that the single postoral colliculus of the normal forms two vibrissae is not a consequence of its composite origin. Whereas the new fact is thus compatible with the original interpretation, doubts arise from other considerations. Duplications of postorals were found in 4/75 normal litter mates of ch/ch, once bilaterally (Grüneberg, 1943), but not in 140 unrelated normal embryos. As it is now known that ch is not quite recessive (Green, 1970), it begins to look that the duplications in the normal litter mates may be heterozygous manifestations of ch. If so, they cannot be the result of the hydrocephalus which is absent in +/ch. One would have to assume that duplication and reduction of vibrissae is analogous to that of glands, etc. (see below) and hence a more direct effect of ch; the occasional triplications of postorals would fit better into a picture of genuine excess formations.

In normal development, the palatal processes turn from the vertical into the horizontal position in the 15-day stage. In ch/ch, this may happen prematurely (Fig. 5), either hemilaterally or bilaterally. Later, the fusion between palatal shelves and septum may be irregular (e.g., Fig. 6), and the cheek pouch on the
affected side(s) may get flattened out more or less completely. In the process, the dental laminae are pulled from their normal position with the result that the molars \((m_1\text{ and } m_1)\) may come to stand side by side in a common lateral crypt instead of facing each other crown to crown (Fig. 6 on the left), as briefly mentioned previously (Grüneberg, 1971). There is much variability as between individuals, and many \(ch/ch\) embryos are quite normal in this respect. The disturbance is thus evidently marginal and, though details are so far lacking, it is presumably due to some deformation of the head as the result of the hydrocephalus which is fully developed at this stage.

The anomalies discussed above are essentially mechanical consequences of the hydrocephalus and thus subordinated (secondary, tertiary, etc.) gene effects; others no doubt remain to be discovered. The rest of the syndrome, so far as it is known, involves the skeleton, various nasal glands, the mesonephros, and certain ganglia. These will now be dealt with in turn.

(2) The skeleton

Cartilage and membrane bones arise in similar (and indeed in some cases in common) mesenchymal condensations, and both categories of skeletal elements are involved in the \(ch\)-syndrome. With the possible exception of the failure of the calvarium, all of them appear to be co-ordinated effects. As described in detail previously (Grüneberg, 1953), most cartilages appear somewhat later and are (and remain) smaller than normal. However, the manubrium sterni tends to be larger than normal though of anomalous shape; and the tuberculum anterius
Fig. 7. Transverse sections through the anterior region of Jacobson's organ; in B, C and F, the anterior gland is visible. Its anomalous orientation in ch/ch is probably a consequence of the adjacent skeletal anomaly. A, B and C, normal; D, E and F, ch/ch. A and D, 13½ days; B and E, 16 days; and C and F, 18 days old. Bouin. H. & E. A, B, D and E, 10 μm; C and F, 12½ μm. Magnification ×86.
atlantis, if it chondrifies at all, tends to behave similarly. There is complete failure of chondrification of sternebrae, parts of the larynx and several other skeletal elements, particularly of the chondrocranium. It was shown for the arcus anterior atlantis and for the tracheal cartilages that the blastemata are reduced in size, and it was assumed that, below a critical size, chondrification can no longer take place. This is undoubtedly true, but it may not be the whole story. In the case of the organum vomero-nasale (Jacobson’s organ), there is a large mesenchymal condensation (Fig. 7 A) which surrounds the organ ventrolaterally and which gives rise to its skeletal support. This consists (B, C) of a cartilaginous cradle in the immediate neighbourhood of the organ, and ventromedial to it, of the vomer which arises in the same blastema without a cartilaginous model. The \( ch/ch \) blastema (D) does not differ much in size from that of the normal, but it fails to form the cartilaginous cradle (E, F); by way of contrast, the osseous vomer is more massive than normal. The lack of the cartilaginous cradle cannot convincingly be ascribed to a reduction in size of the blastema. One gets the impression that there is a rather more specific lack of response to the normal stimulus for chondrification.

Certain skeletal blastemata of \( ch/ch \) embryos which give rise to membrane bones appear earlier and/or are larger than normal. This is particularly clear for those of the mandible and of the zygomatic process of the maxilla; a more detailed study would probably show a similar tendency in others. A distinct blastema for the zygomatic process is detectable in 13-day \( ch/ch \) embryos, a full day ahead of the normal. Massive ossification has taken place by the 14½-day stage (Fig. 5) when none is present yet in the normal. Similarly, mandibular ossification is more advanced and more massive, and soon, in the 15–16 day stage, mandible and maxilla undergo a solid osseous fusion (Fig. 6). This starts in the neighbourhood of the proc. coronoides and of the proc. zygomaticus respectively and is generally present on both sides. It is evidently part of the blastemal disturbance of \( ch \). How closely this osseous fusion is related to the various cartilaginous ones in hyoid, thoracic basket, carpus and tarsus (Grüneberg, 1953) is a moot point. The massive zygomatic process was noticed early on (Grüneberg, 1953), but the fusion not until much later (Grüneberg, 1971).

The reduction of the masseter muscle in \( ch/ch \) is secondary. In the 14½-day stage the mesenchymal condensation of the masseter-temporalis group (Rayne & Crawford, 1971) is normal or nearly so; similarly, in the 15-day \( ch/ch \) embryo, the three components of the masseter can be distinguished and do not differ appreciably from the normal. However, in the 16-day stage, the masseter complex is much reduced and particularly the anterior superficial masseter is almost non-existent. Evidently, the fusion which has now taken place interferes with adjacent muscles. This is shown by occasional cases where fusion is less complete and the muscle less reduced. Also, masticatory muscles away from the site of fusion like the temporalis and the pterygoidei are not affected, which also shows that the regression of the masseter cannot be due to inactivity atrophy.
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As the temporalis (which is immune) arises from the same mesenchymal condensation as the masseter complex, it is also unlikely that the regression is due to an intrinsic fault in the blastema.

The question arises of whether the skeletal blastemata themselves are abnormal, or whether they are only indirectly affected by some anomaly of the surrounding mesenchyme. Direct information may eventually come from reciprocal transplantation experiments or similar procedures. At present, indirect evidence only is available. Among the earliest known effects of ch is the delay of closure of Rathke’s pouch (Green, 1970) which seems to be related to the shortening of the head as shown in Green’s Fig. 4 (see also Grünenberg, 1953). This suggests that the closure of the pouch is normally assisted by growth pressure from the mesenchyme in front and behind. As the delay occurs before there are any visible condensations, the growth of the mesenchyme as such seems to be responsible. Also, an intrinsic defect of the blastemata would probably lead to more uniform skeletal anomalies whereas an interaction between the blastemata and their environment would give more scope for local differences.

(3) Glandular, mesonephric and ganglionic anomalies

There are rather disparate anomalies of certain nasal glands, some of them constant and others variable (Grünenberg, 1971). The normal mouse has four pairs of glandulae nasales mediales, I and III more dorsally, II and IV more ventrally on the nasal septum. A small accessory pair near glands I and III is regularly present in ch/ch, and less regularly there is another accessory gland not far dorsally in the septal corners. By contrast, gland IV is often absent or rudimentary. All three variants occur sporadically in otherwise normal mice. Nasal glands of the normal complement (medial, lateral and infraseptal) may form ‘variable massive and often bizarre lumps of glandular tissue; one variant is the invasion by glandular masses of the medial aspect of the nasoturbinals, which is normally free of glands’.

All these anomalies are obviously co-ordinated effects. Thus the presence of accessory glands in the septal corners and on the nasoturbinals is clearly the same phenomenon. In the same category is the more variable overgrowth in glands of the normal complement. Though going in the opposite direction, the tendency to reduction of the gl. nasalis medialis IV is probably part of the same situation.

As described by Green (1970), in 10-day ch/ch embryos, excess mesonephric tubules fill the whole region between the normal mesonephros and the kidney. Also, there is a tendency to form accessory ureteric buds. It can scarcely be doubted that these excess formations represent essentially the same phenomenon as those in the nasal glands. Indeed, it seems reasonable to suggest that this is also the case as regards the reduction of the coeliac ganglion (Green, 1970).
MESENCHYMAL DISTURBANCE

Shortening of basicranium

Anomalies of skeletal blastemata

Failure of subarachnoid space to form

Anomalies of nasal glands, mesonephros, ganglia and ? of secondary vibrissae

Delayed closure of Rathke's pouch

Anomalies of membrane bones (incl. mandibular-maxillary fusion)

Anomalies of cartilages and cartilage bones

Failure of calvarium

Regression of M. masseter

Hydrocephalus

Open eyelids.
Disturbance of membrana nictitans

Failure of Harderian glands

Premature movement of palatal processes

Displacement of molars

Disturbance of labial commissure

Failure of parotis.
Reduction of Chievitz organ

Fig. 8. Pedigree of causes of the ch-syndrome.

and the increase of the ganglion in the N. terminalis system (Grüneberg, 1973) which is situated in close proximity to the gl. nasalis medialis I.

If it is accepted that all these anomalies, increases as well as decreases, are co-ordinated effects, we have once again to ask the question of whether the various structures are intrinsically abnormal. They are of very different origin: the mesonephric tubules are mesodermal; the nasal glands probably all ectodermal; the coeliac ganglion is a neural crest derivative, and the N. terminalis ganglion is probably placodal though a neural crest contribution is not excluded. The group is thus highly heterogeneous as to origin, histological structure and function, and it is not easy to visualize a process which is common to all its members and which sets them apart as a target for the direct action of ch. On the other hand, they all grow and differentiate in a mesenchymal environment, and a disturbance of that environment might be the common denominator.

Apart from the meningeal defect which is responsible for the hydrocephalus and all its consequences, we thus have two groups of anomalies both of which are clearly systemic, those of the skeleton and those of glands, mesonephros
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and ganglia. The question arises of whether a further simplification is possible. It would appear that, with the exception of the hydrocephalus, etc., all known effects of \( ch \) can be accounted for by the assumption that there exists a disturbance of the mesenchyme which affects structures of mesenchymal (meninges, skeletal blastemata, mesonephros) and of different origin (glands, ureteric buds, ganglia) which are embedded in it. If this single unifying hypothesis is accepted, the syndrome remains, what it was claimed to be originally (Grüneberg, 1943), ‘a case of spurious pleiotropism’ (Fig. 8), not very surprising, perhaps, in these days of single-base replacements and of single-amino acid substitutions.

The original interpretation of the \( ch \)-syndrome (1943, 1953) implicated the skeleton as such. It now appears more likely that the blastemata are only indirectly affected along with glands and ganglia, and that the real culprit is the mesenchymal environment in which they differentiate and grow. On this interpretation, \( ch \) is no more a ‘skeletal gene’ than it is a ‘glandular gene’, etc. There is as yet no clue as to whether the postulated disturbance of the mesenchymal environment is cellular or humoral in nature. Ultrastructural studies may eventually reveal cellular changes beyond the reach of the light microscope (quite apart from what may still have been overlooked). Also, mesenchyme can be grown in vitro, and differences between normal and \( ch \)-mesenchyme may be detectable and capable of further analysis.

Current trends notwithstanding, there is no reason to regard the analysis of gene action in higher organisms as impracticable. Nor can the challenge be ignored indefinitely, in the hope that eventually an understanding of simpler systems may make the effort unnecessary: progress on one level of enquiry does not make work on other levels redundant.

Announcement

In order to make the collections of the senior author accessible to other workers, the whole sectioned embryological material will in the near future be handed over for safe keeping to the Hubrecht Laboratory of the Royal Netherlands Academy of Sciences and Letters (International Embryological Institute) in Utrecht, Netherlands. The collection includes the mutants \( bp, Cd, ch, gl, Os, Ph, pu, Sd, sm, sy, T, r^e, Ta, tk, un, vt \) and \( Xt \), together with their appropriate normal controls; material on \( Pt \) and \( hy-3 \) collected by Dr R. J. Berry; and material of Dr M. S. Grewal (inbred strains CBA, C57BL and crosses between them, collected for the study of third molars). In addition, there is the material of Grüneberg & Huston on bovine syndactylism.

Similarly, the entire skeletal material (papain maceration preparations) of the Department of Animal Genetics, University College London, will be handed over to the British Museum (Natural History). It includes nearly 15000 mouse skeletons of various mutants (\( Bn, bp, Cd, cr, ct, dw, fi, gl, hy-3, mi, Mi^{wh}, or, pg, Ph, Pt, pu, Sd, se, Str, sy, Ta, tk, Ts, un \) and \( vt \)), of some fusion chimaeras.
involving \textit{se}, \textit{vt} and \textit{Ta}, of inbred strains and crosses between them (A, CBA, 10 sublines of C57BL, C57BR, C57L, BALB/c, C3H, R III, AK, DBA, AKR, CE, NZB and the Q-strain) and of wild mice from London, Surrey ricks, Wales, Scotland, Delhi, Peru and the U.S.A. The material also includes over 1000 skeletons of \textit{Rattus rattus} from Delhi and Kerala, and small samples of various other wild rodents.

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\textbf{REFERENCES}


\textit{(Received 31 May 1973)}