THE PATHOLOGY OF THE LUNGS IN A LETHAL MUTATION IN THE RAT (*RATTUS NORVEGICUS*)

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(With Four Text-figures)

The primary lesion caused by a certain recessive lethal gene in the rat (Grüneberg, 1938, 1939*a*; Fell & Grüneberg, 1939) affects the cartilage, notably of the ribs and the trachea. These cartilages hypertrophy enormously, and the thorax is transformed into an entirely rigid and narrow carapace which is fixed in extreme inspiration. It is compressed from side to side, whereas the dorso-ventral diameter is increased. A marked kyphosis of the thoracic vertebrae develops secondarily in many of the affected animals. The respiration is almost entirely abdominal.

The lungs of the lethals are secondarily affected. The condition observed has been referred to as an emphysema of the lungs in the previous communications. This was concluded mainly from the wide appearance of the air spaces. Emphysema or *volumen pulmonum auctum*, however, comprises not only dilatation of the air spaces, but also a local or general increase of the lung volume. After some time, rarefactions of the walls of the air spaces and obliteration of numerous capillaries develop.

In histological sections such as have been published, the condition found in the lungs of the lethals appears very similar to a genuine emphysema. However, the lungs as a whole are by no means larger than those of normal animals, but decidedly smaller in proportion to the size of the animals. It was pointed out previously (1938, p. 131) that "upon opening the thorax, the lungs are hardly visible from the ventral aspect. They are largely forced behind the heart, whereas in a normal rat the lungs envelop the heart laterally and partly ventrally." In cases of emphysema of the lungs in man, the reverse is true; the dilated lungs tend to interpose themselves with their anterior margins between heart and thorax wall, so that the heart may be entirely invisible when the thorax is opened. Thus in spite of the histological similarity, it seemed doubtful whether the lethal rats suffer from a genuine emphysema, as there are no signs of an increased lung volume. A reinvestigation has

indeed shown that in the lethals, the microscopical and clinical picture of an "emphysema" is produced quite otherwise than in genuine emphysema in man.

In Fig. 1 are shown individual acini of lethals (right) and their normal litter mates (left) at the ages of 2, 7 and 12 days. These lungs were fixed *in situ*, the thorax not being opened before the organs were completely fixed. Comparison of the normal lungs shows that the lungs of very young rats are not yet fully differentiated; the rat behaves in this respect like many animals whose young are rather immature at birth, including man, when prematurely born. The undifferentiated lung consists chiefly of short ductuli the alveolation of which has not yet started. The differentiation of the rat lung progresses speedily, as can be seen from the pictures taken from the 7 and 12 days old animals. In the case of the lethals, however, there is apparently no differentiation at all. The pictures of the 2, 7 and 12 days old lethals are identical in principle and resemble the 2 days old normal. It therefore appears that the difference between normal and lethal lungs is primarily not so much an actual dilatation, but a persistence in the lethals of the wide, undifferentiated air spaces of an earlier stage in development.

On the other hand, some real dilatation of the air spaces undoubtedly takes place in later stages in the lungs of the lethals. For instance, in a section published previously (1938, Pl. II, fig. 9), there are very wide air spaces with rarefaction of the walls; although the volume of that lung as a whole was not increased, the increased volume of some parts is accompanied by collapse in other regions. This actual dilatation is due to the forced respiration of the lethals, which invariably leads to a widening of the air spaces. Under these circumstances, there are two possibilities for the interpretation of the histological picture. The well-developed lung might lose its differentiated shape by overdilatation and the histological picture thus produced might give the illusion of undifferentiated sacculi. On the other hand, the lung might have persisted in its primitive foetal structure and have been over-inflated and distended in this state.

The first assumption does not appear likely as the volume of the lethal lung is very small on the whole, whereas dilatation of the differentiated lung would lead to *volumen pulmonum auctum*, as mentioned above. On the other hand, convincing evidence for the correctness of the second assumption can be obtained from a detailed study of lethal and normal lungs.

The histological feature of the fully developed normal lung is the presence of long and not very wide ductuli which are liberally alveolated.

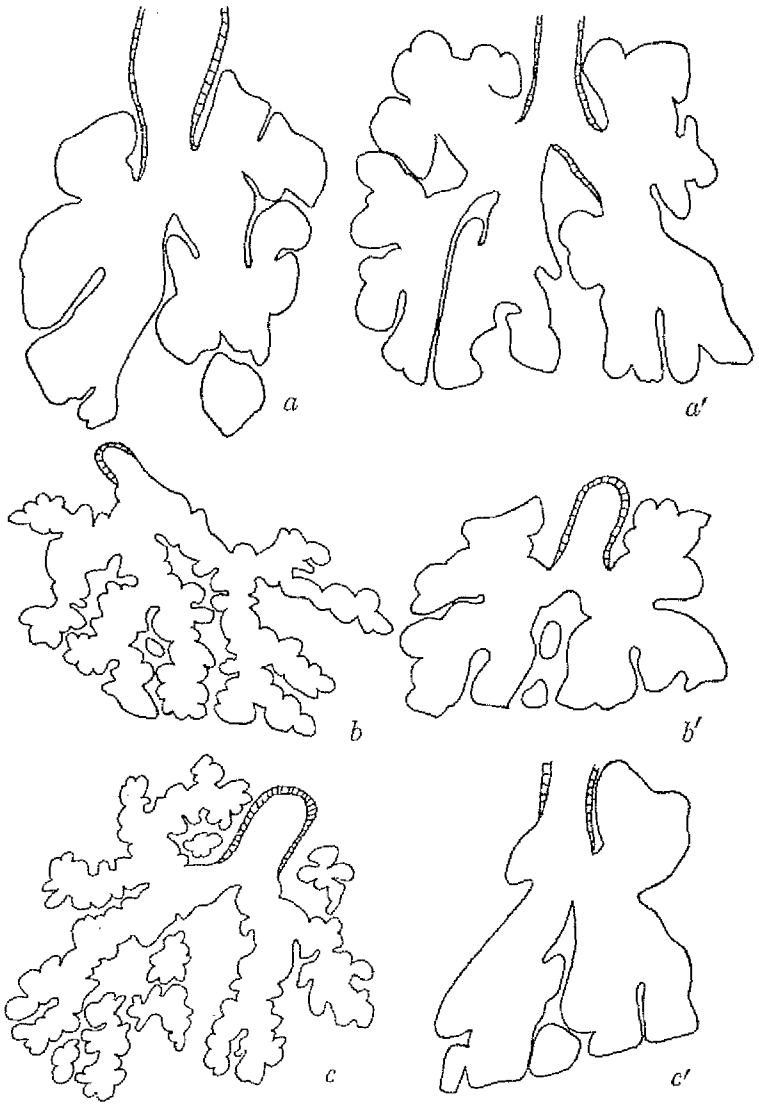

Fig. 1. Camera lucida drawings of acini of the lungs of normals (*a*, *b*, *c*) and lethals (*a'*, *b'*, *c'*), aged 2, 7 and 12 days. The lethals and normals of the same age are litter mates. The lung of the normal 2 days old rat (*a*) shows only short undifferentiated ductuli. The same type of undifferentiated lung is found in all the lethal rats, no matter how old the animals. The lungs of the normal 7 and 12 days old rats present elongated, more or less alveolated ductuli. The differentiation appears still more marked in serial sections (see text). Magnification, 80 times.

The ductuli are so long that one does not succeed in obtaining large parts of an acinus in a single section. The full picture can only be reconstructed from several serial sections in succession.[1] Now there occur in practically every normal lung regions which are more strongly inflated than others. This makes it possible to compare strongly dilated parts of a normal lung with the appearance of the lethal lung. Such pictures, chiefly from transverse sections are given in Fig. 2. They show that when inflated more than normal, the ductuli are widened and the indentation due to the alveoli is somewhat flattened. But the indentations are distinctly maintained even in the case of over-inflation. As such indentations are completely missing in lethal lungs of about the same state of dilatation, it is clear that the lung of the lethals is not simply a normal lung in a state of distension.

Similar evidence is obtained from the lethal lungs. In most cases the air spaces proved to be generally dilated. In one case, however, the extent of inflation happened to vary very much from normal to over-dilatation. This enabled us to recognize what appears to be the "normal" state of the lungs in the lethals. It resembles in no way that of a normal lung. There is no real alveolation, there is no lengthening of the ductuli, that is to say, the two essential criteria of differentiation are lacking. The ductuli are short, clumsy, undifferentiated, bare of real alveoli. This applies naturally still more to the dilated air spaces (see Fig. 3). The shortness of the ductuli is not only demonstrated from their measurable length. In the normal lung the ductuli are so long that it is almost impossible to obtain a complete survey of an acinus from a single section; in the lethals, however, it occurs frequently in the same way as in undifferentiated lungs of newly born rats.

The conclusion must be drawn that the anomaly of the lungs is primarily an arrest of development. The reason for this arrest of development is evidently to be sought in the abnormal configuration of the thorax. The thorax is rigid and remains very small. From observations in man (Engel, 1937, and unpublished data), it appears that in the lung growth and differentiation goes hand in hand. It is, therefore, not surprising that an arrest of lung growth also leads to an arrest of lung differentiation. In their turn, the observations on pathological human material find their confirmation in the unique *experimentum naturae* presented by the lethal rats.

The respiratory surface of the undifferentiated lung is small compared

[1] This is the reason why the difference in complexity between the older normal and lethal lungs is greater than appears in Fig. 1. The acini of the normals are selected for simplicity of appearance in a single section.

with a normal lung. The lethals thus suffer from oxygen want or CO_2 retention or both; as a consequence they resort to forced respiration, and this leads to an over-distension of the primitive, undifferentiated air spaces.

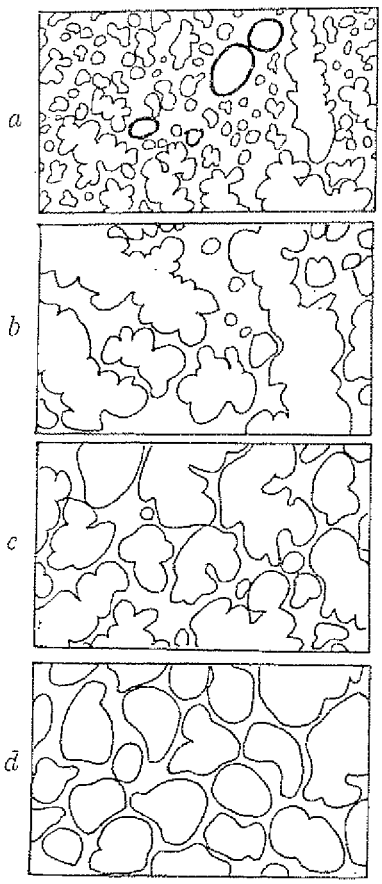

Fig. 2. Camera lucida drawings of air spaces cut transversely and obliquely. *a*, *b* and *c* are taken from the same section of a 14 days old normal rat. In *a* the air spaces are of normal width, in *b* they are slightly and in *c* considerably over-dilated. The three pictures show that the air spaces are well alveolated, although the alveoli are flatter in the strongly over-dilated regions. *d* is drawn from a section of a 13 days old lethal rat. The extent of dilatation is about the same as in *c*. The lack of differentiation, above all of alveolation, is obvious. Magnification, 80 times.

If the arrest of lung differentiation is a consequence of the arrest of lung growth, it is to be expected that those lethals which grow better than the rest have also better differentiated lungs. This seems to be the case. In Fig. 4 are given two acini from a lung of a lethal which reached the

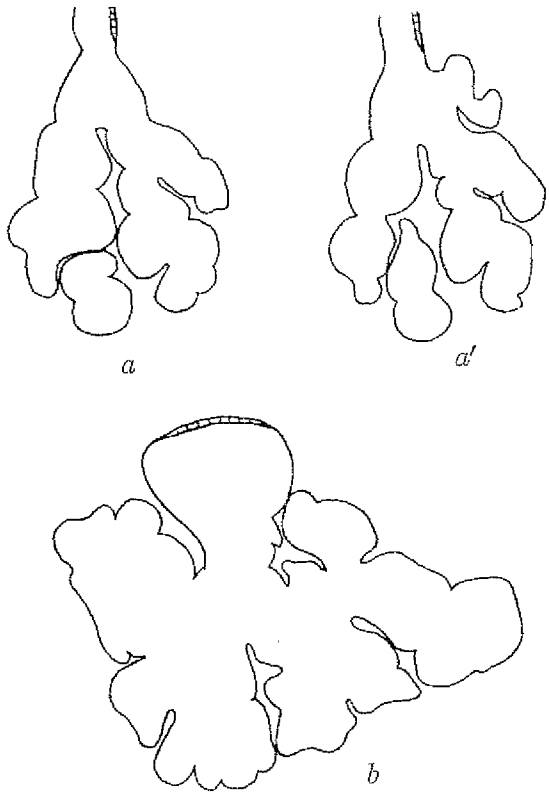

Fig. 3. Camera lucida drawings of acini of a 13 days old lethal rat. *a* and *a'* represent the same acinus in two subsequent sections. The acinus, though not appreciably dilated, is not differentiated; it is short, clumsy and lacks alveoli. *b* is a considerably dilated acinus from the same section. The outlines are still less indented, but they are on the whole similar to those of the non-dilated acinus. Magnification, 80 times.

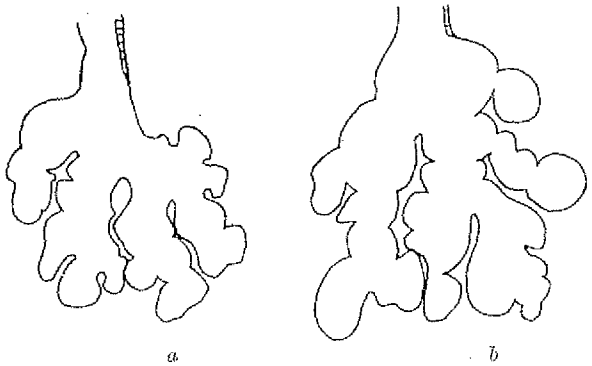

Fig. 4. Camera lucida drawings of two acini of the lung of a 33 days old lethal. *a* is of about normal width, *b* is slightly dilated. Both pictures show a certain extent of differentiation. The alveolus-like extroversions are clumsier and less numerous than in normal lungs. Magnification, 80 times.

exceptional age of 33 days and weight of 21·6 g. (mentioned 1938, p. 134). The differentiation in this case went beyond what is usually found in lethals, though it does not approach that of normal lungs.

The situation observed in the case of the lethal rat lungs resembles the development of an inherited macrocytic anaemia associated with dominant spotting in the mouse (Grüneberg, 1939*b*). In that case, the transition from the megaloblastic blood formation of the foetus to the normoblastic blood formation of the adult is delayed and remains incomplete. The blood picture of the anaemics thus corresponds to that of an earlier developmental stage of the blood picture of the normals. Similarly in the case of the rat, the lung structure of the lethals corresponds to that of an earlier developmental stage of the normal rat. The rat case shows, however, a peculiarity which seems to be of fundamental importance. Whenever the analysis of gene action ultimately leads back to a retarded or arrested process in development, one is apt to regard this "unit process" as the primary result of gene action. It is now clear that such a conclusion, though probably correct in some cases, may be misleading in others. For in our case, the arrest of lung differentiation is clearly a secondary effect of the gene; its primary action is on the cartilage, and the rigidity and narrowness of the thorax thus produced results secondarily in the arrest of the differentiation of the lungs.

REFERENCES

ENGEL, St. (1937). "Postnatal development of the structure of the child's lung." *Acta Paediat.* **22**, 392.

FELL, H. B. & GRÜNEBERG, H. (1939). "The histology and self-differentiating capacity of the cartilage in a new lethal mutation in the rat (*Rattus norvegicus*)." *Proc. roy. Soc.* B, **127**, 257–77.

GRÜNEBERG, H. (1938). "An analysis of the 'pleiotropic' effects of a new lethal mutation in the rat (*Mus norvegicus*)." *Proc. roy. Soc.* B, **125**, 123–44.

—— (1939*a*). "The linkage relations of a new lethal gene in the rat (*Rattus norvegicus*)." *Genetics*, **24**, 732–46.

—— (1939*b*). "Inherited macrocytic anaemias in the house mouse." *Genetics* (in the Press).