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Morphohistological Changes in Some Organs of *Mus musculus* Linnaeus 1758 from a Coal Mine

Zmiany morfohistologiczne niektórych narządów Mus musculus Linnaeus 1758 z kopalni węgla

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I. INTRODUCTION

The aim of the present work is the investigation of morphological and histological changes in some organs of the house mouse in a coal mine.

Papers investigating the influence of darkness, of a high constant temperature or a strong dust pollution on the structure of different organs are not very numerous, up to date. They often give contradictory results.

Observations were carried through as a rule on laboratory animals in artificial environments.

A coal mine is a very special milieu not often investigated, with specific conditions of warmth, light, moisture and dust pollution,

Half darkness (electric light and miners' lamps), a constant high temperature (23—25°C), a considerable relative moisture of the air $(90^{0}/_{0})$, uniformity of food, the lack of seasons are doubtless factors influencing changes in the activity of the animal and its metabolism.

The mice on which investigations were performed dwell in the mine for at least several generations. Kubik (1960) gave a biomorphological analysis of this material. The author demonstrated numerous changes in the mouse from the mine in comparison with specimens from the surface of the earth. He observed smaller dimensions of the body in mice from the mine, a very abundant fat layer, the lack of endoparasites and of a distinct regularity in the course of the moult, another colouring and a different reaction to light. The obtaining of such interesting results induced me to undertake an attempt of a morphohistological elaboration of this material, although what I disposed of was incomplete and accidental. My work therefore is not a histological or endocrinological publication in the strict sense of the word. It only tries to enlarge our knowledge of a very common species that has relatively been but slightly treated.

Acknowledgement. I feel bound to express my sincere gratitude to the Direction of the Mine at Mysłowice, and especially to its Director, Eng. J. Kasperek, for facilitating the catching of the animals.

My best thanks are also due to Dr. I. Dzierżykray-Rogalska for valuable suggestions concerning the material of investigated thyroids.

II. MATERIAL AND METHOD

The investigated material comes from a coal mine in Mysłowice. Collecting of mice was undertaken in different parts of the mine on a level of 350 and 500 m. They were usually caught in the neighbourhood of stores containing miners' equipment, in disaffected corridors and even near places where coal was being mined. The collecting lasted from March 12 to March 18, 1957. For comparatory aims a series of mice from the surface were also collected in the nearest vicinity of the mine.

The material is composed of 40 specimens from the mine and 27 from the surface. The mice were conserved in ethyl alcohol. Material used for histological investigation was cut in sections 6 μ thick and stained with Mayer's hematoxylin and eosin, thymuses and the eyes were weighed on a torsion balance with an accurateness of 0.5 mg. Every organ was weighed thrice in similar conditions. Mean weight was taken for comparison. The technique of weighing is explained in the work of Bazan (1952).

Age of the mice was determined on the base of the degree of wear of the teeth. Three age classes were determined. The state of gonads of the specimens was also investigated .

All data concerning catching, conservation, measurements carried through on investigated specimens, and facts related to conditions in the mine, climatic and nutritional observations, development and breeding data are to be found in the work of Kubik (1960).

III. ANALYSIS OF MATERIAL

1. The thyroid gland.

The thyroid of mice from the mine shows a different structure when compared with the same gland in surface mice.

We can observe on histological sections of thyroids of mice from the mine a considerable number of diminished vesicles containing a small amount of colloid. Larger and not very numerous vesicles, situated mostly on the circumference of the gland can also be seen, their colloid greatly diluted. In many vesicles Aron's resorptive vacuoles appear. We observe a great number of newly forming small vesicles, appearing in larger quantities near the central parts of the gland.

The epithelium of the vesicles is composed of tall cells. The nuclei are large, vesicle-like with a distinctly indicated chromatin stroma. Frequent mitoses can be perceived.

Numerous groups of epithelial cells in the shape of islets are apparent. A great number of blood vessels, frequently dilated, can be observed. The presence of a small quantity of interlobular tissue distinguishes the sections.

On some of the transections atrophy of the vesicular structure of the gland is visible, even on large surfaces.

The thyroids of mice from the surface demonstrate normaly functioning and very active glands. Their structure does not differ from a typical handbook description.

We observe smaller vesicles in the centre of the gland and larger, sometimes even very big ones, on the borders. The vesicles are lined with cuboidal epithelium. The colloid is dense, generally of an identical structure. No Aron's resorptive vacuoles. Blood vessels, empty and narrow, are seldom seen.

* * *

The description of the structure of the thyroid of mice from the mine shows alterations characteristic for a hyperfunctioning gland, independently of age. Similar histological pictures of the thyroid were observed by many authors in experimental conditions.

Sorour (1923) states that rats reared in the darkness acquired in two months' time a visible hyperfunction of the thyroid gland.

The same is stated by: Aschoff (1922), McCarison (1932), Mayerson (1935), Bomskov (1937), Bargmann (1939), Ber (1954), Guzek (1956). This last author carried through his experiments on white rats and Guinea pigs, animals with a different 24-hours' rhythm. He demonstrated that alterations in the thyroids of these animals are identical. The space of time during which the separate experiments lasted (6 months and 1 year) also gave the same results. The author however did not state in the thyroid glands of investigated animals a phenomenon often seen in similar cases — an important increase in the number of blood vessels.

Thyroids of mice from a mine, when compared with those of mice caught on the surface, have a considerably greater number of blood vessels. Ber (1954) encountered a similar phenomenon. He observed a more important vascularization of thyroid glands of laboratory animals bred in the dark.

The fact of a considerable enlargement of the thyroid (Sorour, Ber) is a rather common phenomenon due to its hyperfunction. Ber states that temperature also influences the enlargement of the thyroid. In a colder environment thyroid glands are usually bigger. I suppose that the fact, that in spite of a considerable hyperfunction the size of the thyroid glands of mice in a mine does not augment visibly, can be explained by this observation of Ber.

The well-known sensitivity of the thyroid gland to different external and internal factors permits to conjecture, that this gland is not only subject to the influence of light, which seems to be, in this case, a dominant factor.

The constant and high temperature of a mine might also be an agent which could influence the thyroid gland.

The influence of temperature on the activity of thyroid is discussed by numerous authors, as for example: Ludford & Cramer (1929), Thomas (1934), Bargmann (1943), Burka (1951), Dzierżykray-Rogalska (1952), Ber (1954) and others. Observations carried through often give contradictory results. I shall quote here, as an example, some observations published on the subject. Burka, having transplanted a lobe of the thyroid gland of a rabbit into the ear, in an environment with a lower temperature stated that in the initial stage it displays an abatement of activity. At a later stage this function increases con-

siderably in relation to that of a lobe that had not been transplanted.

Ber when discussing the influence of temperature on the thyroid gland states that animals subjected to refrigeration do not demonstrate an increase in the activity of this gland. Dzierżykray-Rogalska having investigated the thyroid gland of the common shrew did not observe its increased activity in the period of the autumn and winter decrease of temperature. Some authors maintain that animals with a constant temperature of the body show a lowerd activity of the thyroid gland under the influence of a higher temperature of their environment.

The constant high temperature in which mice have lived for several generations in the mine seems to provoke an increased activity of the thyroid gland.

Food is one of the factors that could influence the thyroid gland. The condition of the investigated mice from the mine was good. Big layers of fat were found during autopsy. It can be presumed that this "fatness" ist most probably caused by a complete lack of endoparasites and a diminished diurnal activity. I suppose that the good condition of all the mice in the mine is normal in character and does not seem to be a patological phenomenon provoked by disorders of function of endocrine glands or hypothalamus.

Kubik (1960) admitted that the principal base of the food of mice in the mine consists in the remnants of miners' meals. Carbohydrates in the food are therefore prevalent. An interesting fact cited in the collective work of Skowron & al. (1951) ought to be noted. In animals fed for a longer period on food composed of carbohydrates the thyroid gland has a smaller amount of colloid and the height of cells of the secretional epithelium augments.

One can presume that in investigated animals disturbances caused by avitaminosis from which mice probably suffer in the mine, at least periodically, may play a certain role. Numerous publications concerning the influence of vitamins on the thyroid gland give contradictory results. It is generally considered that the lack of vitamin C provokes symptoms of hyperfunction.

The present interpretation of the influence of a series of factors on the hyperfunction of the thyroid gland differs in so many points, that further investigations ought to be undertaken.

2. The thymus gland.

The thymus of the house mouse is situated in the thorax. It forms thick, fleshy lobes slightly thinner in the peripheral parts of the gland.

The morphological description of the variability of the thymus of this mammal is based on alcohol material in the same manner as in the investigations I carried through on the thymus of Sorex araneus Linnaeus 1758, Neomys fodiens (Pennant 1771) or Sicista betulina (Pallas 1773) — Bazan-Kubiak, 1952; 1955; 1958

I divided the different thymuses in three groups, according to their weight. To the first group belong glands weighing more than 22~g — "heavy" thymuses. To the second one — organs weighing from 21.9 to 9.0~mg — "medium" ones. To the third — thymuses weighing less than 8.9~mg — "light" ones.

Weights of the thymuses concern material preserved in alcohol, they are not therefore actual ones.

The entire alcohol material from mine and surface was also divided into three classes, relatively to age: Ist - young, IInd adult and IIIrd - old adult. Thymuses of animals belonging to the Ist class (the most numerous in the material) are the heaviest. In the mine, as well as on the surface we encounter in this age class only animals with "heavy" and "medium" weight glands, with a prevalence of heavy ones, especially on the surface. In 12 specimens there were 9 "heavy" ones and 3 of "medium" weight. In the IInd class a visible tendency of an advancing reductive process of the weight of the gland can be observed. The mean weight of thymuses from the mine is reduced from 22.6 to 13.9 mg - in those from the surface from 25.6 to 15.5 mg. The IInd age class comprises thymuses belonging to all three weight groups. In this class the relation of the number of thymuses from separate weight groups is different for mice from the mine and mice from the surface. Out of 12 specimens from the mine only 2 have "heavy" thymuses, 5 "medium" and 5 ,,light" ones. Out of 11 thymuses of control mice 1 ,,heavy" thymus, 9 "medium" and 1 "light" one are found. The preponderance of medium thymuses in mice from the surface is therefore considerable.

Class III has the lowest percentage — out of 11 specimens from the mine, 5 had "medium" thymuses and 6 "light" ones. In this

age class all the glands of mice from the surface are of a medium size. There are no animals with "light" glands. In specimens from the mine belonging to the IIIrd age class a further diminution of weight can be observed (mean value 8.6 mg). The same mean value for mice from the surface does not alter and is equal to the mean value of class III. The table below, presenting a list of mean values of the weight of thymuses in separate age classes of mice from the mine and from the surface illustrates these facts:

age class	I	II	III	
mine	22.6	13.9	8.6	
surface	25.6	15.5	15.7	

The difference observed in mice of the IIIrd age class between the mean values for the weight of thymuses of mice from the mine and mice from the surface seems to be partly caused by the fact that specimens from the surface were slightly younger than those from the mine. Determining of the age of mice on the base of wear of their teeth is very difficult, the more so as the food of both investigated groups is undoubtedly of a different kind.

Mice in the mine probably live much longer as they have no enemies whatever, no parasites and are isolated. The small number of specimens from the surface may also influence the mean value for this age class.

The numbers in the table indicate a slight increase in the weight of thymuses of mice from the surface in the Ist and IInd class in relation to the glands of mice from the mine.

The following computation demonstrates the grouping of thymuses of mice from the mine and the surface according to their weight:

weight groups	"heavy"	"medium"	"light"
nine	27.50/0	45.0°/0	27.5º/o
urface	37.0º/o	59.3º/o	3.70/0

Percentage data presented in the table indicate a considerable and gradual decrease of weight in the thymuses of mice from the mine. In control mice changes of the weight of thymuses are contained in much narrower limits. There is a lack of "light" thymuses. In the mine, as on the surface, "medium" glands prevail in a great measure, amounting to $50.7^{0/0}$ of the entire material. $31.3^{0/0}$ of animals with "heavy" thymus and $18^{0/0}$ with "light" one is noted.

No interdependence is stated in investigated specimens between the weight of the thymus and the state of gonads. No correlation exists as well between the body weight and weight of the thymus of investigated mice.

A lack of distinct lobulated structure characterizes the thymus of the house mouse. Every lobe of the gland can be considered as a lobule. A distinct lobulation is very seldom observed, especially in thymuses of mice from the surface. It is evident that in these cases the cortex of the organ is submitted to division into lobules. The medulla is continuous for the whole lobe.

When analysing histological sections of the thymus of a mouse from the mine and of that of a mouse from the surface no essential alterations in the structure of these glands can be noted. There is also a lack of fundamental differences in the structure of thymuses of animals of various ages. In all sections a distinct division of the lobes into the cortex and medulla is visible. The cortex, usually broader, is characterized by a considerable condensation of thymocytes. Differences between the cortex and the medullar part are obliterated only in the lightest thymuses. Cellular elements are, as a rule, equally disposed in the peripheral part. In the essential one, groups of cells divided by blood vessels and cells of the epithelial reticulum are frequently visible. Epithelial elements forming bands can be sometimes observed, not only in the medulla, but even in the cortex. This kind of elements can be seen in thymuses of mice from the mine.

Hassal's corpuscles are specially visible, in rather numerous quantities, in the medullar part of the thymus. In thymuses of the oldest mice these corpuscles appear in a slightly smaller number. In mice from the mine Hassal's corpuscles are larger and more numerous. In all sections a large amount of blood vessels, especially in the thymuses of animals from the surface is always observed. Cell nuclei of the thymus stain in a different manner, but rather intensively. There are no pyknotic nuclei and no mitoses are observed. The capsule of the gland is weakly developed.

As can be seen from the material presented here, a considerable individual variability of weight in every age group is a characteristic feature of the thymus of the house mouse and also of other animals. However, this variability in the different groups is considerably lower than in other small mammals which I observed (especially the *Insectivora*). In view of the considerable variability

of the thymus and its sensitiveness towards different factors, at great amplitude of variability in such an index as the weight of the thymus in the limits of every age group, even physiologically equalised, might be comprehensible.

In spite of rather essential changes in the weight of the thymus no fundamental differences in their histological structure are observed. Thus, the weight of this gland is no essential index of its histological structure. I observed a similar phenomenon in the *Sicista betulina*.

An histological analysis of thymuses of mice does not demonstrate also any structural differences in relation to the age of investigated animals. The thymus of the house mouse is not subject to senile involution. It maintains a juvenile character of structure even in very aged specimens and remains in this stage during the whole life of the animal. Similar facts were observed in other mammals, as for example by Schaffer & Rabl (1909) in the mole, Browmann & Sears (1956) in the mule deer, Bazan-Kubik (1958) in the birchmouse.

In the case of investigated mice, obliteration of the limit between the medullar part and the cortex might be the only index of involution. This phenomenon is more frequent in mice from the mine.

I presume that the thymus of the house mouse is only subject to accidental involution caused by diverse factors. Changes appearing in glands would then be of a reversible character. Such an interpretation might be confirmed by the fact of the existence of a large number of "medium" thymuses, which might be the most "stable" phase of this gland.

The lack of "light" thymuses in mice from the surface is rather difficult to interpret. A similar configuration of thymuses in sundry weight groups, as observed in mice from the mine is often seen in small mammals, especially in summer and early autumn. A corresponding percentage configuration of thymuses of specimens from the surface (see Table) is rarely encountered in small mammals and only in very good thermal and nutritional conditions. The house mouse, living in human settlements (especially in winter) has as a rule a good food base. It probably has during the whole year conditions similar to those which small mammals usually have in the summer period. We encounter in the mine a complex whole of environmental conditions differing from those on the surface. Besides, as I have mentioned already, mice from the surface are probably

younger than individuals from the mine. This might be the cause of a different course of alterations in the weight of thymus in mice from the mine and from the surface.

A lack of a constant and secure food base for mice in the mine has undoubtedly a great influence on the thymus. Works investigating weight decrease of the thymus provoked by hunger, and especially vitamin deficiency, are well known (Janson, 1909; Hammar, 1941; Bargmann, 1943). The kind of food may also have a considerable influence on the thymus (Hoepke & Peter, 1936).

The grouping of epithelial elements appearing in thymuses of mice was also observed in the hedgehog during the summer period, by Peter (1935). Bargmann (1943) discusses the fact that in some sections of mice from the mine the tapelike disposition of epithelial cells joining together is visible. The variability in number and the structure of Hassal's corpuscles differs in many mammals. In the investigated mice Hassal's corpuscles are mostly small and of different types of structure.

There are no connections between the genital apparatus and the thymus. Even processes so expensive for the organism as gravidity and lactation do not seem to induce considerable alterations in the thymus of the mouse. A i m é (1912) when investigating the thymus of tortoises encountered a similar fact. He even observed its regeneration during the breading period of these animals.

Data from literature indicate a lack of interdependence between the weight of the thymus and that of the thyroid (as Scamon, 1921 — concerning man). This author is also of the opinion that a functional interdependence of these two organs cannot be considered.

In animals deprived of the thyroid gland behaviour of the thymus was also observed from different points of view (enlargement, diminution, no changes at all: Gley, 1909; Jeandelize, Lucien & Parisot, 1909).

3. Lungs.

The degree of pollution by coal dust of the lungs of mice from the mine was investigated by analysing histological sections of this organ. For better determining of the degree of this pollution attention was also turned to the lungs of control mice caught on the surface. In the lungs of mice from the mine a considerable amount of coal dust is observed. The histological picture of the sections shows a slight dilatation and hyperaemia of blood vessels. Round the vessels and in their vicinity anthracotic pigment rarely appears. Large deposits of coal in intervesicular spaces can be observed. Granules of dust are taken up by phagocyte cells of different shapes, often changed by the accumulation of coal dust. These cells can mass up in the vicinity of connective tissue. Phagocyte cells may sometimes pass into the light of alveoli. They then became the so-called dust cells (alveolar phagocyte).

A silght increase of cells of the respiratory epithelium accompanies these phenomena. As a rule, no coal dust is observed in the cells of this epithelium. Only anthracotic pigment adhering to the cells appears. Loose accumulations of dust, large, compact and platelike are unfrequent. Coal dust settled on bronchiole connective tissue or attached to the walls of the bronchiole is also visible on the sections.

Anthracotic pigment is equally dispersed.

Lungs of control mice from the surface are also subject to a rather important dust pollution. The fact that these mice live in a close vicinity to the mine is undoubtedly the cause of this phenomenon. In the lungs of these mice cells of rather large dimensions often settle in the light of the vesicles. Considerable accumulations of dust in the bronchiole connective tissue can even be observed, while in the light of the bronchioles single dust cells appear frequently.

Literature concerning coniosis is rather scanty and usually concerns silicosis. Publications connected with anthracosis are seldom encountered.

Grzycki & Staszyc (1954) studied the degree of dust pollution in the lungs of white mice placed in a movable drum in which powdered coal or soot had been pulverized. The animals had been kept in the drum at different periods. Histological analysis of sections of lung of white mice kept for 3 hours in dusty air is in accordance with the results of my observations on the lungs of mice caught in the mine.

Grzycki & Staszyc in their observations did not state any phagocytic capacities in the epithelial cells of pulmonary vesicles. Other authors, as Nowicki (1939) or Paszkiewicz (1957) report that in cells of the respiratory epithelium coal dust may

be observed. Frei (1955) mentions the appearance of dust accumulations especially in the neighbourhood of this epithelium.

When analysing the histological structure of sections of the lungs of mice from the mine, phagocytic specificalness of cells of the respiratory epithelium is not observed as a rule.

The state of the lungs of mice from the mine, as seen from the description above, does not demonstrate a very strong dust pollution. There is a lack of alterations typical for an advanced coniosis and cited by many authors.

A stronger invasion of coal dust exists probably in other sectors of the lungs, as for example in the hilum or the pleura. I do not possess, however, histological sections of these parts of the organ.

The state of dust pollution existing in the lungs of mice from the mine is probably harmless and does not provoke greater and durable disturbances in the organism. It does not alter with the age of the mice.

4. The eye.

K u b i k (1960) when investigating mice from the mine and the surface observed a different sensitiveness of the animals to light stimuli. This observation leads us to suppose that certain differences in the structure of the eye of mice from various milieus may exist. I do not have, unfortunately, any suitable material which could enable me to undertake histological examinations of the eye. Special methods ought to be applied, difficult to realize in the mode of catching mice in a mine.

The mean value of the weight of mice from the mine is approximately equal to that of mice from the surface. It amounts to 10.3 mg for mice from the mine, while it is of 10.4 mg for mice from the surface.

age class	I	II	III
mine	9.0	10.6	13.0
surface	9.2	10.2	13.0

If we divide the entire material into age classes we observe a gradual increase of the mean value of weight of the eyes as the animal grows older. These data are presented in the table below:

IV. SUMMARY

The authoress has carried out a morphohistological analysis of thyroid, thymus and eyes of house mouse, *Mus musculus* Linnaeus 1758 caught in the coal mine and on the surface.

It was stated, as a result of the investigations that alterations characteristic for a hyperfunctioning gland occur in the thyroid of the house mice from a coal mine.

The thymus of the investigated mammal is not subject to senile involution, demonstrating in individuals of different ages a youthful structure. It is only subject to an accidental involution and the alterations thus provoked are of a reversible character. There is no interdependence between the thymus and the gonads and a correlation between the body-weight and the thymus in investigated animals was not observed.

The authoress considers moreover that dust pollution of the lungs of individuals caught in the mine is slight and does not provoke any far reaching alterations in the organ.

The weight of the eyes of mice from the mine is equal to the weight of these organs in control mice caught on the surface. Weight of the eyes increases with the age of the animal.

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EXPLANATION OF PLATE

Plate VIII

Phot. 1. Thyroid of a mouse from the mine, magnified about 110x. Phot. 2. Thyroid of a mouse from the surface, magnified about 110x.

STRESZCZENIE

Autorka przeprowadziła morfohistologiczną analizę tarczyc, grasic, płuc i oczu myszy domowych *Mus musculus* Linnaeus 1758, odłowionych w kopalni węgla kamiennego i na powierzchni.

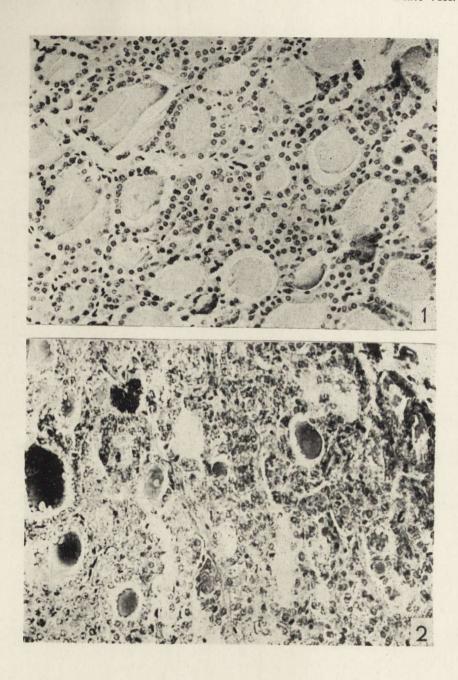
W wyniku badań stwierdzono, że w tarczycy myszy domowej z kopalni zachodzą zmiany charakterystyczne dla gruczołu nadczynnego.

Grasika badanego ssaka nie podlega inwolucji starczej i wykazuje u osobników w różnym wieku budowę młodocianą. Podlega ona jedynie inwolucji akcydentalnej i zmiany w niej zachodzące mają charakter odwracalnych. Brak zależności między grasicą a gonadami, jak również nie obserwuje się korelacji między ciężarem ciała a ciężarem grasicy badanych zwierzat.

Autorka uważa ponadto, że zapylenie płuc osobników odławianych w kopalni jest niewielkie i nie daje daleko idących zmian w narządzie.

Ciężar oczu myszy z kopalni jest równy ciężarowi tych narządów u zwierząt kontrolnych łowionych na powierzchni. Ciężar oczu wzrasta z wiekiem zwierzęcia.

Państwowe Wydawnictwo Naukowe * Warszawa 1961. Nakłal 1500+25 egz. Ark. wyd. 1,1. Maszyn. otrzym. 11.II.1961. Podpisano do druku 10.V.1961 r. Druk ukończono 25.V.1961 r. Papier druk. sat. III kl. 80 gr. Format B-5 Białostockie Zakłady Graficzne. Zam. 699. Cena 6 zł ***



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