EXPERIMENTAL TERATOMA TESTIS IN THE FOWL

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Teratoid or mixed tumors of the genital glands, i.e., tumors consisting of derivatives of all three germ layers, have long interested oncologists and biologists. Until 1925, however, studies were restricted to spontaneous cases. In that year Michalowsky succeeded in obtaining an experimental teratoma in a rooster following the injection of a small amount of a 5 per cent zinc chloride solution into the testis. The results obtained by Michalowsky were confirmed by Ljvraga (1934) and by Bagg (1936), as well as by workers in our laboratory, where there were obtained, in 1937–38, 10 new experimental zinc teratoid tumors in different stages of development (Falin, Anissimova).

It has also been shown that teratoid tumors may follow the injection of other salts of zinc, notably a 10 per cent zinc sulphate solution (Falin and Gromzewa). With this technic 3 teratomata were obtained quite similar to the tumors produced by the original method of Michalowsky.

Thirteen experimental teratoid tumors of the genital glands of the fowl have been obtained in our laboratory during the last two years. If to these are added the tumors described elsewhere by Michalowsky, the total reaches 23, a figure which considerably exceeds the total number of spontaneous cases recorded (Winokuroff; Schminke; Sheather; Baird; Joest and Ernesti; Kaupp; Troude and Prévost; Cohrs; Montpellier; v. Bornstedt and Röhrer; Mashar).

The testicular tumors obtained by us, as well as those described by Michalowsky, are teratomas of embryonic character, composed of derivatives of all three germ layers. As Michalowsky has given a detailed description of the morphology of these experimental teratomas, we shall devote ourselves to observations on the structure of our more recent tumors.

The stroma of the tumor consists of an embryonic connective tissue—the mesenchyme—which differentiates rapidly in diverse directions. In particular it forms hyaline cartilage, which is often replaced by bone of endochondral origin. Among the mesenchymal derivatives must also be included smooth muscle, developing directly in the mesenchyme or adjacent to glandular formations. These glandular ducts, lined with goblet or columnar epithelium, are derived from the entoderm and are present in large numbers. Mucus is secreted, as a rule, into the lumen of the ducts but in some cases it was observed also in the basal portions of the cells. In such instances, droplets of mucus accumulated between the basement membrane and the base of the cell. Not infrequently the glandular cavities show a peculiar association of diverse epithelial elements, islands of stratified horny epithelium occurring

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Fig. 1 (left) shows a cystic cavity lined with columnar and goblet cells along with stratified squamous horny epithelium. Fig. 2 (right) shows a complex of glandular formations, surrounded by two layers of smooth muscle fibers resembling the wall of the large intestine.

Along with mucus-filled goblet cells (Fig. 1) in some cases these glandular complexes are surrounded by two layers of smooth muscle, the arrangement being quite similar to that observed in the wall of the large intestine (Fig. 2). Finally, in one teratoma we discovered ducts lined with ciliated epithelium and with walls consisting of regularly arranged islands of hyaline cartilage. In structure these ducts closely resembled bronchi (Fig. 3).

Among the elements constituting the experimental zinc teratomas the derivatives of the ectoderm are the most highly developed. Especially noteworthy are cavities and ducts lined with stratified horny epithelium and numerous feather anlagen. In many cases the feathers were macroscopically visible through the wall of the feather follicles. Frequently the latter contained anlagen of several feathers at the same time (Fig. 4). Separately growing feathers were also seen, each of which was enveloped by its own epithelial sheath and possessed well-formed barbs.

In one tumor produced by zinc sulphate solution there was detected a complex of peculiarly twisting epithelial strands whose cells were filled with yellowish-brown pigment granules. In appearance this resembled the external wall or pigmented layer of the eyecup (Fig. 5).

A particular interest attaches to anlagen of the central and peripheral nervous system, which are also constituents of the experimental zinc teratomas. Elements of medullary tissue in the form of single medullary tubes may appear in the earliest stages of tumor development and are often found in the immediate vicinity of the testicular substance from which the tumor arises. In one case young nerve cells were detected inside seminiferous tubules. More frequently medullary tissue is found in the form of distinctly delimited nodules of yellowish-pink color. In younger tumors this medullary tissue consists of slightly differentiated elements constituting the medullary tubes (Figs. 6 and 7). Often a multiple formation of medullary tubes is found in the foci of
FIG. 3. Teratoma produced by zinc chloride: Formation similar to bronchus (ciliated epithelium, hyaline cartilage)

FIG. 4. Teratoma produced by zinc chloride: Multiple formation in one feather follicle
medullary tissue and a picture is produced resembling the medulloblastoma (Cushing) (Fig. 8).

In the course of further differentiation of these medullary tubes with the formation of neuroblasts, the latter apparently migrate beyond the limits of the tubes. Dense foci arise consisting of nerve cells and neuroglia in which only occasional single medullary tubes persist (Fig. 9). In the process of differentiation part of the neuroblasts may migrate far beyond the foci of nervous tissue and enter the connective-tissue stroma of the tumor. Large numbers of isolated neurones are thus to be seen in the connective tissue. Many of these reach a considerable size and structurally often belong to a higher phase of development than the corresponding nerve cells in the foci of medullary tissue (Fig. 10).

With the aid of a special technic (Lawrentjew's modification of the Bielschowsky-Gros method), it was possible to demonstrate in the connective-tissue stroma of the tumor, besides separate neurones, medullated and non-medullated nerve fibers, often so numerous as to give the impression of a "neuroma" (Fig. 11). The formation of these "neuromas" in experimental teratoid tumors can be explained on the basis of the general conception of the growth and regeneration of the nerve fibers given by Prof. Lawrentjew. Nerve fibers, having lost their connection with the periphery or, as in our cases, having not yet established a close and definite connection with the peripheral organ in the form of nerve endings, are deprived of stimuli which inhibit their further development, and thus assume excessive growth. The
**Fig. 6.** Teratoma produced by Zinc Sulphate: Medullary tube in Cross Section, showing many mitotic figures.

**Fig. 7.** Medullary tube in Longitudinal Section.
fact, first discovered by us, of this rich innervation of the teratoid tumors at
the expense of the nerve elements belonging to the tumor itself, presents a
new problem as to the rôle of the nervous system in morphogenetic processes
underlying the development and growth of the teratoid tumors. This will
be the object of further investigation.

To complete this brief morphological outline the extreme diversity of
structure of these teratomatous tumors must be emphasized. In spite of the
presence of a number of general features permitting the classification of all
the tumors as mixed or teratoid, each has its own peculiarities of growth and
structure. Some are characterized chiefly by the development of epithelial
elements, others by nervous elements or by derivatives of the mesenchyme,
etc. Variations are also noticed in the growth rate of these experimental tera-
tomas, as is demonstrated in Table I. As shown in this table, there exists no
correlation between the size of the tumor and its age. While in some cases
during thirty to forty days a weight of only 0.5–1.0 gm. was attained, and
the lesion was not macroscopically visible, in others a tumor weighing as much
as 20 gm. (Rooster No. 4383) was obtained in the same period. Similar
observations were made after longer periods. The tumor in rooster No. 1823
on the 59th day weighed 238 gm., while the tumor in rooster No. 141, killed
150 days after injection, weighed only 162 gm.

The cause of these striking variations in the growth of experimental tera-
tomas is not yet clear. Undoubtedly, they may explain the variations in
structure discussed above.

**Pathogenesis and Histogenesis of Experimental Zinc Teratomas**

Though experimental teratomas have been produced with equal success by
injections of zinc chloride and zinc sulphate solutions, we cannot reach any
conclusion as to the specificity of zinc as an agent in the production of tera-
toid tumors. The fact is that these salts exert on the tissues an almost iden-
tical caustic action, depending not only upon a special relation of the zinc to
albuminous substances, with which it forms albuminates, but also upon an
independent action exerted on the tissue by acids liberated in the process of
this reaction. The effects produced by other substances acting similarly upon
the tissues remain to be investigated.
FIG. 8. **Teratoma Produced by Zinc Sulphate: Multiple Anlage of Medullary Tubes**

FIG. 9. **Teratoma Produced by Zinc Chloride: Multipolar Nerve Cell in Focus of Nervous Tissue (Gros-Bielschowsky)**
Following injections of zinc chloride or zinc sulphate solutions there are found in the testes of roosters more or less limited foci of necrosis. Gradual resorption and organization of these necrotic areas follow, resulting in the formation of a characteristic cicatrix on the surface of the testis. It is in this initial period of resorption of the necrotic areas and in their immediate vicinity that the first tumor cells appear. This close connection between the tumor cells and the necrotic foci was most evident in the early stages of tumor formation, but even in the later cases the tumor, as a rule, was found at the site of earlier necrosis, growing in the form of a triangle, with its apex turned towards the tissue of the testis from which the growth arose, and the base extending far beyond the limits of that organ.

These characteristic features of the growth and form of experimental zinc teratomas are shown in Figs. 12–14, where the teratomas growing in the form of a triangle are represented in section. In the process of its growth the tumor seems to split the testis into two widely separated parts.

This same peculiarity of growth appears in the figures illustrating the work of Bagg (Am. J. Cancer 26: Fig. 6, p. 75, 1936). We are led to assume, therefore, that an important rôle in the genesis of these experimental teratomas is played by the products of disintegration (autolysis) of the cellular elements of the testis formed at the site of the zinc injections. This view is of particular interest in view of the fact that in processes of regeneration, to which such importance is now attributed in tumor production, great significance is attached to the products of cellular disintegration, namely necrohormones, trephones, etc.

It appears probable that the injection of the zinc salts into the testis, producing disintegration of the cellular elements, is followed by liberation of substances acting on the pluripotential testicular cells like the "inductors" or "evocators" in the processes of embryonic development.

This analogy between the processes of embryonic development and those underlying the growth and the differentiation of teratoid tumors, is based not only on morphological resemblances. As shown by investigations of recent years, inductors are contained not only in the dorsal lip of the blastopore, as was first suggested, but they may be detected in an inactive form, long before gastrulation, in any region of the developing embryo, and even in the ovum. These embryonal regions acquire an inductive capacity, however, only after they have been killed by alcohol, chloretone, etc., when an active inductor is liberated.

It is difficult to believe that the process of normal embryogenesis involves such strongly acting factors as those applied in the experimental activation of the inductors. It seems probable that under normal conditions activation of the inductors occurs in some other way. In this connection the recent investigations of Voss (1933), Bieling, and Stockenberg are of interest. In experiments on embryos of amphibia, birds, and mammals they found that the accumulation of yolk in different regions of the embryo, or the appearance of numerous foci of cellular disintegration (chiefly the nuclei), precedes the occurrence of morphogenetic processes. From this it may be assumed (Dorfman 1936) that the products of yolk or cellular disintegration might serve to activate the inductors in the course of normal embryonic development.
FIG. 10. Teratoma produced by zinc chloride: two nerve cells embedded in connective tissue (Gros-Bielschowsky)

FIG. 11. Teratoma produced by zinc chloride: great numbers of nerve fibers in the connective tissue, suggesting neuroma (Gros-Bielschowsky)
Figs. 12 and 13. Teratoid Tumors Produced by 5 per cent Zinc Chloride (above) and by 5 per cent Zinc Sulphate (below)

In each instance the tumor arose in the right testis and the uninvolved left testis is shown for comparison. The tumor shown above is from a rooster (No. 4309) killed 43 days after injection. It grows from the middle portion of the testis in the form of a small triangle, with its base turned outward. The tumor shown below is from a rooster (No. 4383) killed 42 days after injection. It arises from the middle portion of the testis.

Thus, in the processes of embryonic development an association is observed between the disintegration of cells or albuminous substances (the yolk) and morphogenesis. By analogy the salts of zinc, which produce a disintegration of albuminous substances of the cell, may be considered as activators of those inducing substances which even in the normal state are found in the pluripotential cells of the testis (Needham) and are liberated in the process of their disintegration. This, it is true, can be accepted only as a hypothesis. Further investigations will show whether destruction of the cellular elements of the testis by other means is followed by a liberation and activation of these inducing substances or whether the salts of zinc play a specific rôle.

It must also be taken into consideration that such “activation” of inducing substances liberated in the genital glands by the injection of zinc salts or by other means may occur only at a particular season (in spring). This is indi-
cated by the seasonal development of experimental teratoid tumors in the fowl, which is recorded by all investigators.

We have, therefore, to take into account certain internal factors favoring the accumulation of inducing substances (obviously in the inactive form) in the testis and thus creating a certain disposition for the development of teratomas. The essence of this disposition is as yet not quite clear. It may, however, even be said that it need not necessarily imply a fall of the general resistance of the organism, as Fischer-Wasels believes, or a depression of function of the active mesenchyme as shown by the investigations of the academician Bogomoletz and his school. In the case of experimental teratoid tumors of the testis in roosters we have to do with an organism in a flourishing state, in the period of the greatest activity of all vital functions, and especially of the endocrine system. It is obvious, therefore, that in our attempt to discover the essence of tumor disposition in the cases under consideration we must pay special attention to hormonal peculiarities in the roosters at this "critical" (in the sense of the tumor production) period.

Another important problem consists in the establishment of a genetic correlation between elements of the testes and the tumor cells. Even a simple enumeration of the tissue elements and organoid formations (glands, feathers, anlagen of the central nervous system, etc.) constituting the teratomas shows that in the study of the genesis of the teratoid tumors we must look for a pluripotential tumor anlage capable of growth and differentiation in various directions. Here, above all, we may think of embryonic cells or blastomeres separated from the whole embryonic complex and displaced into the region of the developing testis (Marchand, Bonnet). This supposition, however, does not explain the peculiar affinity of the separated blastomeres for the genital glands in which spontaneous teratomas, also, develop most frequently. Nor are observations on more than 20 experimental zinc teratomas obtained by Prof. Michalowsky and by us in conformity with the fact of an occasional
separation of the blastomeres and their displacement into the anlagen of the genital organs. We must look, therefore, for the cells of origin of the teratoid tumors in the genital gland itself or in the peritoneal layer covering the testes (the mesothelium).

Mashar (1932) tried to explain the origin of teratomas of genital glands by the capacity of the peritoneal mesothelium to differentiate in widely diverse directions, and suggested that in the process of injection of the zinc salts solution this mesothelium may be drawn by the needle into the testis where it gives rise to a teratoid tumor. This point of view is in sharp contradiction to the modern conception of the mesothelium as a fully determined tissue unable to undergo further transformation into other tissues (Chlopin and his school). It is difficult, also, to explain in this way the presence in the tumors of ectodermal derivatives, as feathers, anlagen of the central nervous system, etc. Mashar speaks of dedifferentiation of the celomic epithelium with subsequent formation of genital cells from which, according to his opinion, ectodermal elements arise. This view is not confirmed by more recent data concerning the origin of genital cells (Dantschakoff et al.).

As regards the cells of the testis itself, some authors have suggested that spermatogonia might initiate the tumor process, and this was also the opinion of Michealowsky, who believed that in their capacity to give rise to various tissues, the spermatogenic cells of the spermatic tubules approach the ovum. If we consider the spermatogonia from this standpoint, we must assume the possibility of their parthenogenetic development. While not objecting on principle to the possibility of the parthenogenetic development of the spermatogonia, at least in the rooster, we believe that this opinion requires further proof. It is for this reason that special attention has been given to the so-called "transitional zone," where testicular tissue and tumor substance are closely adjacent, and where we may more reasonably expect to find evidence of transformation of the spermatogenic cells into tumor elements. The existence of such zones is confirmed by the investigations of Michealowsky as well as by our own observations. In many cases it is possible to detect a gradual transition from the testicular substance to the tissue of the tumor itself. Sometimes the walls of the spermatic tubules are destroyed and sperm-cells are found beyond their limits. Generally, however, it is not possible to establish the actual differentiation of these cells into tumor elements. On the other hand, cellular elements of the tumor are often detected inside the spermatic tubules, but here again it is difficult to determine whether we have to do with the actual formation of tumor cells at this site or with their penetration into the tubules from the growing tumor.

Another weak point of this theory, as well as of the theory set forth by Mashar, consists in the impossibility of explaining the development of spontaneous teratoid tumors in other regions of the organism than the testes.

These and similar objections may be avoided only if we admit that spontaneous as well as experimental teratoid tumors develop from the primary genital cells, or gonocytes. As shown by investigations of Swift, Dantschakov, and others, the gonocytes appear in the chick embryo very early, long before the formation of the anlagen of the genital glands. They are concentrated at first in the region of the so-called germ crescent, in the germinal disk.
From here they spread throughout the organism and finally enter the testis anlage. It is likely that some of these gonocytes fail to reach their final destination, i.e., the testis anlage, and stop somewhere on the way.

The majority of the gonocytes found in the testis anlage go on to the formation of the genital cells. Some obviously may be preserved in the testis in the postembryonic period (Stschegolew). Apparently, it is these gonocytes which under suitable conditions may be the source of origin of spontaneous as well as of experimental teratoma testis.

This theory allows for the presence of the pluripotential tumor anlagen in the testis (the gonocytes) and thus explains the predominant incidence of teratomas in that organ, at the same time accounting for the production of teratomas in extratesticular regions. The fact that experimental zinc teratoma testis does not develop in all cases, but in only 10 or 11 per cent of the treated roosters (according to our data), leads us to assume that a part of the gonocytes perish with age. It seems, therefore, that in old birds the production of teratomas is much less to be expected than in young ones. Finally this theory gives an explanation of the seasonal production of teratoma testis in the rooster. The formation of a great amount of gonadotropic or other hormone, inducing the testis to active spermatogenesis after the period of winter rest, creates the ground for a further development of the gonocytes preserved in the testis. Under the influence of those inducing substances which accumulate in the testis at this time in particularly large numbers, and are activated by the treatment with zinc salts or possibly other factors, these gonocytes may display a previously latent potency for growth and differentiation.

**Literature**