THE PATHOGENESIS OF MIXED TUMORS OF THE SALIVARY GLAND TYPE

LOUIS H. HEMPLEMANN, JR., M.D.,

AND

NATHAN A. WOMACK, M.D.

ST. LOUIS, MO.

FROM THE DEPARTMENT OF SURGERY, WASHINGTON UNIVERSITY SCHOOL OF MEDICINE, THE BARNES HOSPITAL, AND THE BARNARD FREE SKIN AND CANCER HOSPITAL, ST. LOUIS, MO.

THE CONTROVERSY concerning the fundamental structure of mixed tumors of the salivary gland type has never been settled satisfactorily. Since 1853, when the tumors were recognized as a clinical entity by Paget,[1] the ensuing years have added much discussion as to their nature. In 1859, Theodor Billroth[2] wrote his classic thesis on salivary gland tumors and surprisingly little of morphologic interest has been added since his excellent description. His interpretation of the tumors as mesenchymal growths (accepted with certain modifications by Virchow) held sway for many years, overshadowing the earlier work of Robin,[3] and others, who suggested that the tumors were epithelial. Toward the latter part of the nineteenth century, Billroth's concept of the tumors was challenged, and since then numerous other theories of histogenesis have been presented. One of the best and most inclusive summaries of these theories is that of Hoepfel,[4] to which the reader is referred. Hoepfel divides the theories of the nature of mixed tumors into three main groups, based on the interpretation of the parenchymal elements of the tumors:

(1) In this class fall all of those conceptions holding that the parenchyma is mesenchymal in origin. Billroth originated this idea. Virchow[5] felt that the epithelium was derived from mesenchyme, due to the ability of the connective tissue elements to undergo metaplasia into epithelial tissues. Others believed that the tumors were composed of endothelial cells, and for many years the tumors were called "endotheliomas."

(2) In this large group fall those theories considering the parenchyma as derived from epithelium. However, while there is agreement concerning the origin of the parenchyma, there are differences in interpretation of the nature of the stroma. These latter opinions may be divided into five subgroups, which regard the stroma in the following ways: (a) The stroma takes origin by displacement of mesodermal germ tissue at the same time in embryonic life as do the ectodermal elements. (b) The stroma is mesodermal tissue which is formed as the result of the improper local "organizer" action of the epithelium on undifferentiated mesoderm. This utilizes the present-day knowledge of experimental embryology. (c) The stroma is a mesodermal tissue which has been formed by metaplasia of the epithelial parenchyma. (d) The stroma is epithelium which has been modified as a result of its secretory products so that it resembles cartilage and other mesodermal tissues. (e) The stroma is a

"hybrid" substance arising through the union of epithelial and mesothelial tissues with their secretory products.

(3) In this small group are included those persistent ideas that the parenchyma possesses epithelial as well as endothelial components. Evidence in support of such a conception has been so vague that little acceptance is given this idea at the present time.

In the American and English literature perhaps the most generally accepted theory held at present is that mixed tumors are true epithelial tumors without mesodermal elements. There are several standard textbooks of pathology that subscribe to this conception. However, during the past decade there has been a trend, particularly in the German literature, to regard the tumors as primary epithelial growths which have induced abnormal differentiation of the undifferentiated mesoderm.

When one examines the evidence for all of these theories mentioned above, it is apparent that they are based on individual interpretations of the morphology of the pleomorphic tumors. One needs only to examine sections of mixed tumors to realize how unsatisfactory and difficult morphologic study may be. All variations within a tissue and gradations between tissues are found. To illustrate the difficulties involved in histologic study of the tumors, let us consider their microscopic appearance very briefly.

HISTOLOGIC FEATURES OF THE MIXED TUMORS

The parenchyma of the tumors is unquestionably epithelial and appears in many variations. The small oval or spindle cells growing in sheet formation represent one of the most common types. They closely resemble the type of cell seen in basal cell carcinomata. Occasionally the cells are larger, with vesicular nuclei. They often form acini, which may contain mucus. Some of the individual cells show evidence of secretion in the form of intracellular droplets of mucus. Epithelial cells with intercellular spinous processes in bulbous formation with typical epithelial pearls are often found. Some of the epithelial cells form pseudorosettes, resembling embryonic ducts more closely than they do adult epithelial structures. Occasionally nests of epithelium show a peculiar type of degeneration of their central portions with the resultant formation of star-shaped cells similar to those seen in ameloblastomata. These cells may be widely separated but usually remain connected by long intercellular processes.

The epithelial cells are separated by a stroma, the appearance of which suggests mesodermal origin. The most characteristic form of the stroma is the chondromyxomatous tissue. The myxomatous tissue bears a striking resemblance to the connective tissue found in the umbilical cord, and that occasionally seen in chondrosarcomata. It is composed of branching cells embedded in a matrix of homogeneous mucoid substance. The cell bodies are usually triangular in shape with long, branching pseudopodia. The amount of cytoplasm enclosing the deeply-staining nuclei is small but the branching processes, of which there are usually two or three, extend far out into the

intercellular substance. Often there are additional more delicate fibrillary processes arising from other parts of the cell bodies. These cells may be widely separated or may form strands of two or three. The cartilaginous or "pseudocartilaginous" tissue differs from adult cartilage only in the absence of the characteristic pattern. Other tissues found in the stroma resemble hyalinized connective tissue, with eosinophilic, dense homogeneous matrix containing a few elongated cell bodies. Adult fat cells and small islands of bone are occasionally found deep within the tumor nodules.

The types of tissue mentioned above are well recognized. Their relationship, however, is interesting and peculiar to this type of tumor. Epithelial tissue of one type appears to change gradually into another type. Myxomatous tissue merges into cartilaginous tissue or into hyalinized connective tissue. Even more puzzling than this, however, is the anatomic relationship between the epithelium and the stroma. Where the epithelium is well differentiated there is a sharp line of demarcation, but in many places there is gradual, apparent transformation of frank epithelium into myxomatous tissue. In these transitional zones the cells cannot be said to be either epithelial or myxomatous.

It is this unusual relationship of tissues which appear to be derived from separate germ layers that is responsible for the confusion regarding the histogenesis and nature of these tumors. It seems incredible that epithelial cells can resemble mesenchymal tissues so closely. Yet if there are two types of tissue how can one explain their intimate relationship and apparent transitions from one into another? A morphologic study of the tissues has failed to provide a satisfactory answer to this question.

HISTOCHEMICAL INVESTIGATION OF TISSUE MUCOIDS OF MIXED TUMORS

In considering a group of mixed tumors of the salivary gland type, the futility of further study of their morphology at once became evident. It occurred to us that a chemical investigation of the mucoid material in the parenchyma and stroma might be of value in determining the nature of the tissues and, accordingly, a microchemical study of epithelial and mesodermal mucoids was undertaken by one of us (L. H. H.[6]). Before describing the results of this study it will be of value to discuss briefly the chemistry of the mucopolysaccharides. For a more detailed discussion the reader is referred to the classic monograph of Levene,[7] as well as the more recent articles of Meyer.[8]

Mucoproteins are complex proteins composed of two radicals, one a protein molecule, the other a carbohydrate complex. It is the latter group which is responsible for the specificity of the molecule and hence its chemical and staining properties. According to Levene, the prosthetic groups are composed of a hexosamine fraction conjugated with sulfuric, glucuronic and acetic acids. They exist as several modifications of one general type, the best known ones being chondroitin sulfuric acid and mucoitin sulfuric acid. These compounds are similar in structure and composition, differing only in the carbohydrate

fractions (which are probably isomeric hexoses) as well as in the attachment of certain side-chains.

Despite the chemical similarity of these mucoproteins, their distribution in nature is widely different. Chondroitin sulfuric acid has been isolated only from mesenchymal tissues such as cartilage, bone, tendon, sclerae, umbilical cord, and the wall of the aorta. The chondroitin sulfuric acid protein complex, therefore, has been said to be the mucoprotein of connective tissue,[8] and is probably responsible for the staining characteristic of the mesenchymal mucoids. Mucoitin sulfuric acid on the other hand has been found in the mucin of salivary glands and gastric mucosa, in serum mucoids, ovomucoid, Wharton's jelly, vitreous humor, and in the cornea. Recently, however, evidence has been presented that the mucoids of Wharton's jelly, and egg white do not contain mucoitin sulfuric acid. The subject of mucopolysaccharides has been further complicated by the identification of sulfate-free mucopolysaccharides in Wharton's jelly and salivary gland mucin.[9] It might be said, however, that the mucoitin sulfuric acid complex is a product of epithelial secretion, while chondroitin sulfuric acid protein is limited to those connective tissues of mesenchymal origin.

Because of the chemical similarity of their prosthetic groups, the tissue mucoids stain alike with basic and metachromatic dyes. For this reason it has not been possible to distinguish between them by ordinary staining procedures. Recently, one of us (L. H. H.[6]) has devised certain microchemical methods by which mesenchymal and epithelial mucoids (presumably chondroitin sulfuric acid and mucoitin sulfuric acid complexes, though the simpler mucopolysaccharides known to exist in epithelial mucus, may play some rôle in the chemical reaction) can be differentiated in fixed tissue sections. One of these is a titration method utilizing the difference in affinity of the protein complexes for very dilute aqueous solutions of the metachromatic dyes. Serial sections of formalin-fixed tissue are stained with increasing dilutions of toluidine blue or polychrome methylene blue. There is a definite range of dilutions (in the case of polychrome methylene blue about 1:200 to 1:1400 depending on the temperature, the age, and the method of preparation of the stock solutions) in which the chondroitin sulfuric acid complexes stain with almost maximum intensity while the epithelial mucoids fail to stain. In the control experiments it has been possible to stain cartilage, chondrosarcomatous tissue and the mucoid degenerative products in the walls of arteries with dyes of the proper dilution while the epithelial glands and secretions of the respiratory, gastro-intestinal and biliary tracts and salivary glands failed to stain unless stronger solutions were used. This is a delicate method and requires considerable experimentation with control tissues to obtain the proper dilution for good differentiation.

Another method of differentiating the mucoids is based on the greater resistance of the mesenchymal mucoids to hot acid. Incubating paraffin cut-sections of tissue with dilute solutions of sulfuric acid changes the tissue mucoids in such a way that they no longer stain with the metachromatic dyes.

This, presumably, is due to the breakdown of the acid complex, as the characteristic color is said to be dependent upon the sulfuric acid ester linkage. The rate of breakdown is different for the mesenchymal and epithelial mucoids. Care must be taken that large enough quantities of acid be used to rule out differences due to disproportionate concentration of mucoid substance.

FIG. 1.

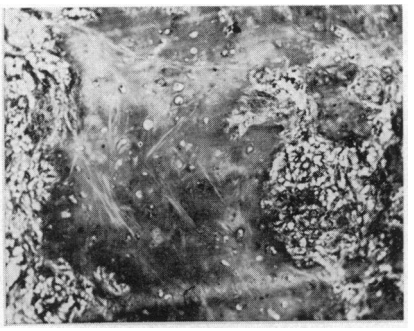
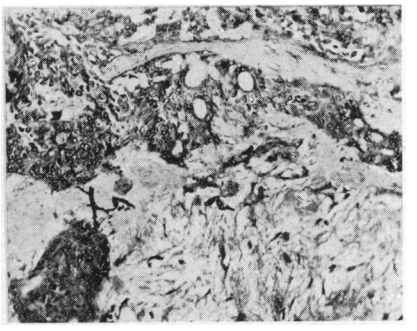


FIG. 2.



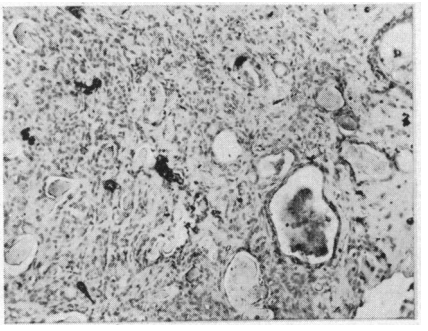


FIG. 3.

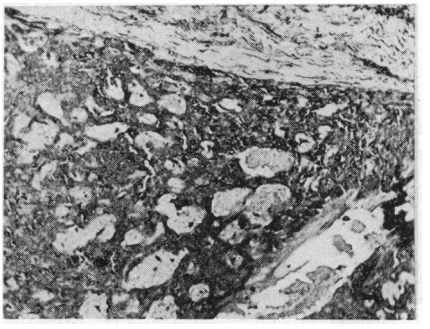


FIG. 4.

FIG. 1.—Mixed tumor of the parotid gland stained with polychrome methylene blue 1:340. The matrix of the cartilaginous tissue stains bright reddish-purple (photomicrograph was taken with a green filter to emphasize this color), while the remaining tissue stains blue or green. Epithelial mucus, as represented by gastro-intestinal biliary tract and salivary glands, failed to stain with this dilution.

FIG. 2.—Mixed tumor of the parotid stained with differential polychrome methylene blue solution described in Figure 1. The wavy strands in the upper right portion of the photomicrograph represent reddish-purple mucus of a myxomatous area. Intra-acinar mucus failed to stain with this dilution.

FIG. 3.—Mixed tumor of salivary gland stained with 1:260 aqueous dilution of polychrome methylene blue. Note the intra-acinar mucus which failed to stain with the differential solution used in Figures 1 and 2. The intracellular mucus in goblet cells of adjacent salivary gland behaved as the above type of mucus.

FIG. 4.—Mixed tumor of the parotid gland stained with 1:260 polychrome methylene blue. The mucus within the acini also failed to stain with the differential solution used in Figures 1 and 2. This type of mucus as well as that in Figure 3 was destroyed by hot sulfuric acid in the same manner as the initial types of epithelial mucus.

While the end-points of this method are not as sharp as they are in the first, the results are consistent, and can be used in the evaluation of the chemical nature of the mucoids.

Both methods show that the mucoid in the myxomatous and cartilaginous areas in mixed tumors of the salivary glands behaves exactly as does the chondroitin sulfuric acid complex in skeletal cartilage, chondromata, chondrosarcomata, and in the walls of arteries showing mucoid degeneration (Figs.

1 and 2). The mucoid within the acini stains exactly as does the mucoprotein complex in the mucin of the salivary gland, gastro-intestinal and respiratory tracts, as well as that in mucoid carcinomata of the intestine (Figs. 3 and 4).

From the results of these experiments, it seems justifiable to conclude that the mucoid substance in the myxomatous and cartilaginous areas is a chondroitin sulfuric acid complex while that secreted by the epithelial cells of the tumors is a different mucoprotein, probably mucoitin sulfuric acid complex as well as perhaps simpler mucopolysaccharides.

Since the mucoid in the cartilaginous and myxomatous areas is a mesodermal mucoprotein, presumably chondroitin sulfuric acid, and since the tissue presents the morphologic appearance of mesodermal structures, it is probable that these tissues are truly mesodermal. It does not seem likely, as Techouyeres[10] suggests, that there is a reciprocal mutation between the chemical forms of mucoitin and chondroitin sulfuric acid. This chemical change, involving a special rearrangement of a molecule and perhaps other changes, has never been shown to occur. Similarly, the metaplasia of epithelial cells into true cartilage and myxomatous tissue after histodifferentiation has taken place is contrary to present embryologic concepts. We conclude, therefore, that there are two types of tissue, mesenchymal and epithelial, in mixed tumors of the salivary gland type.

It is not possible to say whether the myxomatous areas represent phases of rapidly growing tissues or whether they are areas of degeneration or the result of local vascular change. They are usually quite avascular, though one occasionally sees blood vessels within such an area. The similarity between such myxomatous tissues and certain types of chondrosarcoma is striking. The similarity is more than just a structural one. When stained with dilute acid solutions of ortho-Capri blue (an oxidation production of methylene blue*) the branching type of cell structure is demonstrated unusually well. The intercellular substance of myxomatous and chondrosarcomatous tissue fails to stain with the Capri blue, whereas that in adult cartilage stains intensely. With transformation from myxomatous to adult tissue there is a gradual appearance of the stainable substance (Figs. 5 and 6). The chemistry of the intercellular matrix of cartilage has not been worked out well enough to enable us to understand this completely, but it is quite possible that this stainable substance is chondro-albuminoid. Very little is known of this substance except that it is an albuminoid closely related to osseo-albuminoid, and similar in many respects to elastin and keratin. The ground substance of cartilage, the keratin layer of epithelium, the elastic layer of blood vessels, cell nuclei and cytoplasm, hyalin and collagen fibers, serum and egg albumin, epithelial mucoid and serous secretions are all stained intensely by Capri blue. The intercellular substance of the myxomatous areas in the mixed tumors, which stains intensely with mucoid stains and that in the histolog-

* The dye was prepared by boiling an aqueous solution containing several drops of 1:100 o-Capri blue and several drops of dilute hydrochloric acid per 50–60 cc. of water.

ically similar chondrosarcoma, are the only protein substances which have been found not to take the blue stain.

FIG. 5 A. FIG. 5 B.

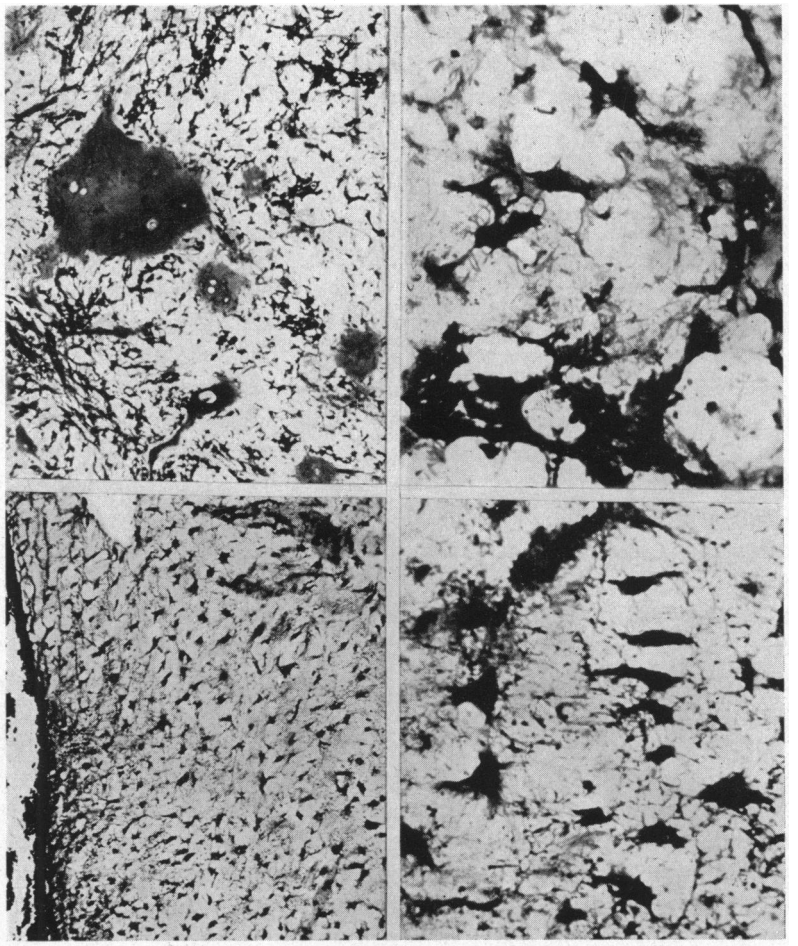


FIG. 6 A. FIG. 6 B.

FIG. 5.—(A) Mixed tumor of the submaxillary gland stained with ortho-Capri blue. Note that the intercellular substance of the myxomatous tissue fails to stain while that of the small island of cartilage is deeply colored.

FIG. 5.—(B) Higher magnification of myxomatous cells showing details of branching structure.

FIG. 6.—(A) Chondrosarcoma of the chest wall stained with ortho-Capri blue. Note that the intercellular substance fails to stain as is the case in the myxomatous tissue of Figure 5. A. Note also the similarity of structure between these cells and those in the myxomatous areas of the mixed tumor.

FIG. 6.—(B) Higher magnification showing similarity to Figure 5 B.

PATHOGENESIS OF THE MIXED TUMORS

If it is accepted that there are two types of tissue in mixed tumors, certain theoretic concepts as to their pathogenesis can be formulated. In view of the presence of two different types of tissue, both of which lack normal differentiation, a failure of normal development seems probable. Evidence continues to

accumulate in support of the fact that normal development depends upon a closely integrated interrelationship between all tissues involved. Functional inadequacy on the part of one tissue at any phase during development may result in structural changes in all tissues concerned subsequently. Such "organizer" or "provocative" action of epithelium on the undifferentiated mesoderm, and *vice versa*, in the formation of certain types of tumors has been suggested before. This view has been proposed by Norrenbrock,[11] after Schürmann and Pflüger's work on the histogenesis of craniopharyngiomata. Such an explanation has been used for mixed tumors of other regions of the body. Schmidt[12] has applied this principle to mixed tumors of the breast, and Möller,[13] and, more recently, Womack and Graham,[14] have used it to explain certain tumors of the lung.

In line with embryologic evidence, the buccal ectoderm of the salivary gland *anlage* probably affects the surrounding buccal mesoderm. In turn, differentiation and development of the ectoderm is probably influenced by the buccal mesoderm. There is considerable evidence that even adult epithelium retains a certain amount of "organizer" influence. Huggins[15] has shown that bladder epithelium is capable of causing differentiation of adult fibrous tissue into bone. This indicates that there are undifferentiated cells in adult fibrous tissue capable of formation of more highly specialized structures. That fibrous tissue is capable of influencing the growth and differentiation of epithelium is shown by the experiments of Drew.[16] He has found that tissue cultures of kidney epithelium and cancer cells from breast carcinomata grow in sheet formation unless fibrous tissue cells are present in the culture. In the latter case, the tumor cells differentiate to form duct-like structures. There is nothing specific in the buccal mucosa which possesses the properties of influencing the differentiation of mesoderm in the manner seen in mixed tumors of the salivary gland type, since the epithelium of the lacrimal gland and skin are capable of similar tumor formation.

The experimental disturbance of tissue environment of the embryo has been shown to lead to structural malformations.[17] It is possible that some such disturbances may lead to development of mixed tumors of this type. The time during embryonic life that such a disturbance occurs, as suggested by Li and Yang,[18] would account for the degree of differentiation which the tissues show. Those occurring earlier in life have the greater potentialities of differentiation.

This interrelationship of tissues has been almost completely ignored in the case of mixed tumors of the salivary gland type, but has been used to explain the development of teratomata.[19, 20] This utilization of the "organizer" conception regards the tumors as a result of primary epithelial maldevelopment, with mesodermal differentiation secondary to this epithelial disturbance. Though the application of this theory to mixed tumors of the salivary glands is not subject to experimental proof at present, it seems to us to be the most rational and is thoroughly in keeping with present-day embryologic tenets.

CONCLUSIONS

(1) The theories of the pathogenesis of mixed tumors of the parotid gland are briefly summarized.

(2) A method is described by which, with special staining technics, mesenchymal mucus can be differentiated from epithelial mucus. Both of these substances are found to be present in mixed tumors of the parotid gland.

(3) In view of the fact that epithelial and mesenchymal mucoids are believed to be identified, it is suggested that two tissue components are represented in these tumors, and that the tumors are, therefore, truly mixed tumors.

(4) It is suggested that the origin of these tumors might be best explained on the basis of embryonic alteration in tissue relationships, in accordance with the "organizer" theory of Speeman.

REFERENCES

[1] Paget, Jr.: Lectures on Tumors. London, Longmans, 1853.

[2] Billroth, T.: Beobachtungen über Geschwülste der Speicheldrüsen. Virchows Arch., **17**, 357, 1859.

[3] Robin and Laboulbene: Memoire sur Trois Productions Morbides non descrites. Compt. rend. Soc. de Biol., **5**, 185, 1853.

[4] Hoepfel, W.: Bau und Wesen der Schleimdrüsengeschwülste am Gaumen und in Mundhöhlenbereich insbesondere in ihren Beziehungen su den sog. Speicheldrüsenmischgeschwülsten. Ztschr. f. Hals-, Nasen-, und Ohrenheilkunde, **41**, 52, 1936–1937.

[5] Virchow, R.: Vorlesungen über Pathologie. Bd. ii, Berlin, Hirschwald, 1863.

[6] Hempelmann, Jr., L. H.: Staining Reactions of the Mucoproteins. Anat. Rec., **78**, 197, 1940.

[7] Levene, P. A.: Hexosamines and Mucoproteins. London, Longmans, 1925.

[8] Meyer, K.: The Chemistry and Biology of Mucopolysaccharides and Glycoproteins. Cold Springs Harbor Symposia on Quantitative Biology, **6**, 91, 1938.

[9] Blix, G., Oldfeldt, C., and Karlberg, O.: Zur Chemie der Mucine und Mucoide. Ztschr. Physiol. Chem., **3**, 234, 1935.

[10] Techouyeres, E.: La Genesis du Tissu Cartilagineux dans les Tumeurs Mixtes des Glandes Salivaires. Ann. d'anat. Pathol., **11**, 905, 1934.

[11] Schürmann, Pflüger, and Norrenbrock: Die Histogenese Ekto-Mesodermaler Mischgeschwülste der Mundhöhle. Georg Thieme, Leipzig, 1931.

[12] Schmidt, I.: Zur Frage der Entstehung der Mischgewäche und Hand von zwei Fällen von Milchdrüsenmischgeschwülsten des Hundes. Virchows Arch., **291**, 491, 1933.

[13] Möller, A.: Zur Entstehung der Lungenmischgeschwülste. Virchows Arch., **291**, 478, 1933.

[14] Womack, N., and Graham, E. A.: Mixed Tumors of the Lungs. Arch. Pathol., **26**, 165, 1938.

[15] Huggins, C. B.: The Formation of Bone Under the Influence of Epithelium of the Urinary Tract. Arch. Surg., **22**, 377, 1931.

[16] Drew, A. H.: Growth and Differentiation in Tissue Cultures. Brit. Jour. Exper. Path., **4**, 46, 1923.

[17] Stockard, Chas. R.: Developmental Rate and Structural Expression. Am. Jour. Anat., **28**, 115, 1920-21.

[18] Li, R. L., and Yang, C. S.: An Inquiry into the Origin of Mixed Tumors of the Salivary Glands, with Reference to Their Embryonic Interrelationships. Am. Jour. Cancer, **25**, 259, 1935.

[19] Nicholson, G. W.: The Histogeny of Teratomata. Jour. Path. and Bacteriol., **32**, 365, 1929.

[20] Willis, R. A.: Structure of Teratoma. Jour. Path. and Bacteriol., **40**, 1, 1935.