THE OCCURRENCE OF RETICULUM IN TUMORS *

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Introduction. Since von Kupffer discovered the "Gitterfasern" of the liver in 1876, and Mall (1891, 1896) first distinguished the reticular connective tissue fibers from the collagenous fibers, reticulum has been the subject of considerable investigation; its occurrence in various organs under conditions of health and disease, its relationship to collagenous connective tissue, and its histogenesis (which is still in doubt) have all been studied. The reader may find an extensive bibliography in Russakoff's (1908) article on the pulmonary reticulum. At the time he wrote, the introduction of the Bielschowsky-Maresch silver impregnation technic had given impetus to the investigation of reticulum; Kon (1908) had studied it in the liver, Rössle and Yoshida (1909) in the lymphnodes, while Russakoff examined sections of liver, kidney, pancreas, and lymphnodes as well as lung. Neuber (1912) investigated the cardiac reticulum in health and disease, and more recently Corner (1920) and Miller (1923) have attacked the problem as it applies to the capillaries and the lung.

A survey of this work would lead the reader to conclude that reticulum is the product of cellular activity, that it may be connected with the blood-vessels on the one hand, or serve as a supporting, reticulated membrane for epithelium on the other. It forms practically the sole supporting tissue in lymphnodes. That it is the product of endothelial cells and, occasionally, fibroblasts, seems to be the general belief; some authorities claim the reticuloendothelium, some the vascular endothelium as well, as its progenitor.

One may readily study the distribution of the reticulum in various organs by means of silver impregnations according to Bielschowsky's technic, so that further preliminary discussion of this subject is unnecessary; let it suffice that it is very widely distributed and its

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importance is often overlooked. For example, its widespread occurrence in epithelial organs, where it forms a dense basement membrane, is insufficiently recognized. That it may with time, and under rather uncertain conditions, become transformed into collagenous fibrous tissue is becoming more universally believed. Russakoff, Rössle and Yoshida, and Miller have all stressed this point. That it is the product of cellular activity on the part of endothelial derivatives is, however, becoming a debatable question. Miller has pointed out the close relationship between newly-formed reticulin fibers and neighboring, preëxisting reticulum; Baitsell (1915, 1916) has advanced anew the theory of purely extracellular origin; and one of us (Foot, 1925) has recently stated his belief that we must look elsewhere than to cells for the origin of reticulin fibrils, basing this view on observations on the development of reticulum in experimental tubercles.

As nothing appears to have been done in connection with the distribution and occurrence of reticulum in tumors, a hundred or more have been collected in the past three years' accumulation of laboratory material of this department, and these have been studied with special reference to their reticulum content.

Technic

In most cases Zenker-fixed material is available, but where it is not, formalin-fixed tissue has been used. Several serial sections were cut from each paraffin block, three being stained with the routine Harris' hematoxylin-eosin stain and two by a modification of the Bielschowsky-Maresch technic devised by one of us last year. (Foot, 1924.)

Briefly summarized, the steps are as follows. Sections about five microns in thickness are cut in paraffin. Zenker fixation is superior to formalin, but the technic is the same in either case. After removing the paraffin, treat five minutes with a weak alcoholic solution of iodine, removing this by a brief immersion in 5 per cent "hypo." Wash and treat for five minutes with 0.25 per cent potassium permanganate in water. Wash and place the sections in 5 per cent aqueous oxalic acid. Wash in distilled water and impregnate for forty-eight hours with a 2 per cent solution of silver nitrate in distilled water. Wash in distilled water and treat with Bielschowsky's silver-ammonium oxide solution for one half-hour. Rinse in distilled

water and leave in 5 per cent formalin (2 per cent formaldehyde) for another half-hour and then, after washing in tap-water, tone the sections for one hour in a one per cent aqueous solution of gold chloride, after which a two-minute immersion in 5 per cent "hypo" will remove any superfluity of the silver. The sections may be mounted at this point, but it is better to counterstain for the usual time in Harris' hematoxylin, wash, and stain for forty-five seconds in Van Gieson's picric-acid-acid-fuchsin. Run the sections immediately through ascending percentages of alcohol into xylol and mount in balsam, avoiding water as it decolorizes the fuchsin. After the silver impregnation the sections should be practically colorless, turning brownish-black in the formalin and fading to gray in the gold bath. The "hypo" turns them old-rose and gray. The finished, counterstained sections show the reticulum in sharp black lines, the collagen in vermilion to crimson. The nuclei are brownish, the cytoplasm and muscle substance yellow. The silver impregnation is performed in subdued daylight, not in the dark.

Reticulum Fibers in Tumors

Epithelial Tumors

Surface epithelium. The epidermoid carcinoma is of paramount interest here, the benign epithelial tumors of this type conforming closely to normal histology. In these carcinomata there is a varying amount of reticulum; plentiful in the corium, it forms a network of delicate fibrils about the advancing columns of tumor cells which dip down from the epidermis. Generally speaking, if the tumor be of rapid growth reticulum is plentiful, if slowly growing more collagen than reticulum develops. Usually the latter forms a membrane-like plexus at the base of the epithelial plugs, occasionally it penetrates them superficially. A metastasis from such a tumor is shown in Fig. 1. In the basal cell, or hair-matrix carcinoma the reticulum forms a delicate basket-work around the cell masses, with radiating fibrils that are continuous with those of the corium.

Glandular epithelium. The common adenoma usually shows more collagen than reticulum in its stroma, the various types of fibroadenoma following this rule, but a narrow zone of reticulum is commonly found about the base of the acini, outlining them in black. In a rapidly growing papillary adenoma the stroma is composed al-

most entirely of reticulin fibrils, to the comparative exclusion of collagen. If the tumor acini are closely compressed one observes reticulin fibrils between them; lying almost, if not quite isolated and free from any great admixture of mesenchymal cells. Capillaries carry a sheath of reticulum, but frequently there are but few vessels in this stroma and the reticulum appears to have been produced independently of cellular agency.

The various types of adenocarcinoma and medullary carcinoma all follow the description just given; the cell-masses in the medullary type are usually quite free from penetration by the reticulum, but very rapidly growing tumors, with a tendency to cell dissociation, may show a good deal. Fig. 2 shows a typical adenocarcinoma. If the tumors have metastasized to lymphoid tissue the lymphoid reticulum will, naturally, be found intimately intermingled with their cells. In the colloid type, those cells which remain at the center of the mucoid masses may have small, woolly plexuses of what seems to be reticulum about them, but the production of fibrils resembling reticulin is quite common in necrotic areas in epithelial tumors and it is doubtful if such fibrils constitute true reticulum. Scirrhous carcinoma, as one might expect from its slow, sclerotic growth, shows large amounts of collagen in its stroma and a very variable quantity of reticulum, which is usually scanty. The sarcomatoid type of pancreatic carcinoma, shown in Fig. 3, presents a moderate amount of reticulum in its stroma and there is some penetration of this tissue into the more or less dissociated groups of tumor cells, which is also true of the similar type of carcinoma of the thyroid. The difference in the distribution of reticulum in these tumors and that in lymphosarcomata is, however, so striking as to render a diagnosis comparatively simple in cases that would be quite obscure when stained in the usual way.

Hypernephroma, or adrenal carcinoma, shows a very small amount of reticulum which does not penetrate into the alveolar cell masses. Although this tumor is found in the kidney, an organ rich in reticulum, there is practically none in the alveoli which push the preëxisting fibers aside.

The tumor section photographed for Fig. 4 was kindly sent to us from the Albany Hospital, by Dr. Victor Jacobson, and is worthy of special mention. It is from a medullary carcinoma of the uterus, which differs in no way from any other such carcinoma excepting

that its stroma shows an extraordinary anaplasia to what appears to be a true fibrosarcoma. Thus two malignant tumors are intimately combined in one section. The carcinoma is practically free from reticulum while its stroma shows an abundance of straight, coarse reticulin fibrils exactly analogous to those of the fibrosarcomata to be discussed presently.

Endothelial Tumors

Vascular. Cavernous hemangiomata show a reticulum about their vessels and sinuses quite similar to that normally seen surrounding capillaries. A malignant perithelioma appears to produce very little reticulum, as this is found only in the immediate proximity of the vessels. As we could secure only one specimen, this negative finding should be confirmed.

Reticuloendothelial. Several types of malignant endothelioma were examined, some showing comparatively little reticulum, some a great deal. Of the latter group, the primary lymphnode endothelioma, so closely resembling medullary carcinoma, is a good example. Where the carcinoma shows little reticulum in its alveoli, the fibers usually stopping short at their margin, or simply traversing them in straight, unbranching lines, the endothelioma exhibits an abundant, branching plexus of curving reticulin fibrils intimately associated with the cells of the tumor within its alveoli. The fibrils are distinctly plexiform and anastomotic in this case, which affords a good criterion for the diagnosis of doubtful alveolar tumors of the axillary lymphnodes in women, where primary endothelioma is readily mistaken for the more common metastatic alveolar carcinoma of the breast. We feel this to be the chief contribution of this paper. (Figs. 5 and 6.)

Another type of endothelioma, supposedly originating in the reticuloendothelium of lymphnodes, is composed of polygonal, anastomosing cells, and shows an abundant reticulum intimately related to these cells and following their cytoplasmic processes very closely. A given fibril may, however, course over several cells without any interruption in its substance and the unbroken continuity of these fibrils with those of the neighboring normal tissue is always striking. This tumor (Fig. 7), was recently reported by one of us (Foot, 1924) in detail.

The endotheliomata arising in bone-marrow correspond in the

main with the loosely reticulated portions of the primary endothelioma of lymphnodes, insofar as their reticulum is concerned; but they contain somewhat less and the fibers are far more delicate. One case of diffusely disseminated cells, resembling endothelial phagocytes and occasionally grouped into tumor masses, shows marked reticulum production wherever the cells become so massed.

Dural endothelioma. This tumor (Fig. 8) probably produces more reticulum than any other tumor we have observed; there is a dense felting of reticulin fibrils in its matrix and they are intimately intermingled with an almost equally dense mass of collagen fibrils.

Mesothelioma

A tumor from the pleural cavity, connected with the lung, is composed of squamous cells that tend to keratinize and to form pearls. It resembles an epidermoid carcinoma in every respect, but there is little upon which to base this diagnosis if one considers that the tumor lay in and beneath the visceral pleura, not near large bronchi, and there was no history to suggest bronchiectasia. A similar growth is described by Beitzke, in Aschoff's "Pathologische Anatomie" (1923), as a "pleural cancer." We prefer the term "mesothelioma" and feel security in our diagnosis for the reasons just given. This tumor shows an abundant reticulum between its cell masses and a moderate amount of collagen; it is almost exactly like an epidermoid carcinoma in respect to its stroma. Its cell masses are entirely free from reticulin fibrils.

Fibroblastic Tumors

Fibroma. Both types of fibroma, the soft and hard, are chiefly collagenous; practically no reticulum is seen, save around the blood vessels. (Fig. 9.) A very mucoid fibroma of the external auditory canal, which recurred twice in a year, shows rather scanty collagenous tissue with wide, unstained spaces and an abundance of short, rather straight reticulin fibrils. (Fig. 10.) It is not a true myxoma, for there is no bluish hyaline-matrix, and it is not malignant. Here there appears to be a direct causal relationship between the presence of reticulum and rapidity of growth.

Keloid. Both the keloid and the fibrous granuloma show reticulum and collagen in about the same proportions as occur in normal corium; the total amount of these fibers, however, is greatly in-

creased. The keloid has rather more collagen than reticulum, however.

Giant cell tumors. These are not rich in reticulum and show only moderate amounts of collagen. The giant cells become deeply stippled with silver, as is the case with some endothelial cells and with nerve cells. The tumors are chiefly cellular.

Fibrosarcoma. Two types are recognizable: one producing almost nothing but reticular matrix (Fig. 11), and one an abundance of collagen (Fig. 12). The former is very similar to the reticulo-endothelioma with branching cells, already described. Apparent transitions between the reticular and collagenous types are seen, but the degree of anaplasia and the rate of growth will not entirely explain this difference — for one very anaplastic and rapidly growing fibrosarcoma of the lung shows little or no reticulum, but produces an abundant collagenous matrix (Fig. 13). Generally speaking, however, there is more reticulum in the rapidly growing sarcomata and more collagen in those of slower development.

It is interesting to compare the tumors in our series of fibrosarcoma as regards their reticulum content. One, a sarcoma of the scapula (Fig. 14) of rapid growth, shows an evenly distributed mesh of very fine, short, and curly reticulin fibrils, many of them beaded and interrupted. This indicates very young reticulum, judging from the literature and from personal observation on its formation in tubercles. Very little collagen is present. Then come a sarcoma of the lung, one of the kidney, and a third from the bladder, all of which show coarse, straight reticulin fibers and little collagen. At the other end of the scale we find a sarcoma of the lung and one of the knee, both of which show much collagen and little reticulum. From this one may infer that reticulum is laid down by the more embryonal type of rapidly growing tumor and that it may ultimately be changed into collagen. The very primitive fibrosarcomata and reticuloendotheliomata both revert to a common type, the mesenchymal. Hence their similarity and the comparative uselessness of attempting to classify them by adult-cell standards, or to distinguish them apart.

Other Connective Tissue Tumors

Lipoma. Tumors of this type produce more collagen than reticulum, as one would expect after a survey of normal fat.

Liposarcoma. In one case we find short, wavy, more or less beaded reticulin fibrils and little collagen. (Fig. 15.) In the others, which resemble Fleming's fat organ, there is abundant reticulum and but little collagen.

Leiomyoma. Here much collagen is produced; often more of this is seen than of muscular tissue and a large amount of reticulum closely invests the muscle fibers. Is this reticulum converted into the abundant collagen that gives the clinical name of "fibroid" to the tumor?

Leiomyosarcoma. This shows an abundance of both reticulum and collagen, the latter in the form of short fibers and not at all as profuse as in its benign prototype. Here again we see more reticulum in the more youthful form of tumor.

Rhabdomyosarcoma. One of these, from the endometrium of an old woman, was examined. It is seen to be composed of alveolar septa of collagen, and in the spaces thus formed is a very loosely made network of reticulin fibrils, sometimes short and curly, sometimes nearly straight and much longer, but always wavy in contour. In the smaller spaces between these fibrils lie the rhabdomyoblasts, usually quite isolated. An abundance of cells resembling fibroblasts are also present and the reticulum is more apt to adhere to these than to the muscle cells.

Melanosarcoma. Melanosarcomata are abundantly supplied with collagen, but a good deal of reticulum is found penetrating the alveolar cell masses and running among them almost as freely as in the primary endothelioma of lymphnodes.

Chondroma and chondrosarcoma. Benign chondroma shows practically no reticulum, while chondrosarcoma shows a great deal in its more immature portions, the production of chondromucin later obscuring it. Sometimes the reticulum persists in this matrix and its fibers may be seen through the homogeneous chondromucin.

Osteoma. Aside from the marrow spaces, no reticulum is seen in this form of tumor. We did not obtain an osteosarcoma and cannot report on its reticulum.

Lymphoid Tumors

Lymphoma and Lymphosarcoma. Here one finds practically no fibers other than reticulum, and this may be quite unassociated with any cells resembling reticuloendothelium; in one malignant lym-

phoma of the small celled type the reticulum lies quite free in a mass of dissociated microlymphocytes, and one can see no connection between it and any other type of cell. It appears to have been laid down by some process independent of cellular activity, or borrowed from the lymphoid tissue.

The commoner lymphosarcoma, with its larger type cell, exhibits an abundant reticulum that branches all through the tumor and is in unbroken continuity with that of the lymphnode. (Fig. 16.) Where the tumor penetrates the capsule and invades the surrounding tissue, it forms a new reticulum that is continuous with the older.

Hodgkins' granuloma. The amount of reticulum produced in the Hodgkins node varies a great deal; sometimes there is much, at others (Fig. 17) there is practically no new reticulum to be found.

Tumors of the Nervous System

Glioma. This shows no reticulum other than that of its vascular supply.

Neuroblastoma and Ganglioneuroma. The same is true in this case, which makes it possible to differentiate these neoplasms from other types which might be confused with them. Neuroblastoma often resembles lymphosarcoma in routine sections, but a reticulum stain will readily differentiate the two.

Amputation neuroma and neurofibroma. There is very little reticulum in these, and it is confined to the connective tissue of the epineurium and its prolongations. In these there is much collagenous tissue.

Pituitary struma. One chromophile struma was examined, from a case of well-marked acromegaly. This tumor is so dissociated in its structure that practically the only stroma present is seen in the immediate vicinity of its vessels, in the form of perivascular reticulum.

Mixed Tumors

Mixed tumor of parotid. Several of these were studied, all showing a variable amount of reticulum about the epithelial islands and ducts. Collagen varies, being most abundant in the more mixed, and therefore more mature varieties; with cartilage and heavy connective tissue septa. The more youthful, epithelial type of tumor shows practically no collagen and a very dense reticulum about its ducts and acini. (Fig. 18.)

Teratoma. Several of these, one a malignant embryoma with rather advanced differentiation, were examined. No rule can be laid down for such diversified tumors; if they contain those types of tissue normally rich in reticulum, the latter will naturally be present.

Summary

From what we have said, it is evident that reticulum is a common and widely distributed constituent of the stroma and matrix of tumors, the only marked exceptions being those of the nervous system. Some of the very fibrous tumors show so much collagen that the reticulum is overshadowed and may, indeed, have been replaced by this tissue. In general, there appears to be a definite connection between rapidity of growth and reticulum formation, for this tissue is more abundant in young and rapidly growing tumors than in those of more adult type and slower progress. It is indicated that this would be converted into collagen in time; that this is not an invariable rule is shown by the presence of much collagen in some rapidly growing sarcomata. That anaplasia is not necessarily connected with the production of reticulum is indicated by the coincidence of reticulum and good differentiation in a benign fibroma, several lipomata, and other mature tumors and by its absence in tumors showing marked anaplasia.

One point that cannot be stressed too emphatically is our failure to find any definite relationship between cellular structures and reticulum. Although it is usually near vessels and is undoubtedly more abundant in their vicinity, it often appears to be quite unassociated with cytoplasm. It is often as abundant in the proximity of epithelial cell masses as it is near endothelial or fibroblastic cells, although it is undeniably more abundant in the case of endothelial tumors. It seems that the conditions for its production are more favorable in these tumors but that nothing points directly to the cellular origin of reticulin fibrils. As in the case of tubercle reticulum, there appears to be a closer relationship between reticulum and preëxisting reticulum than there is between that tissue and any cells. It is evident that we must keep our minds unclouded by dogma and further test out the hypothesis of the intercellular, or extracellular, origin of reticulum. The production of these fibrils may prove to be more or less similar to that of fibrin, a process of precipitation and accretion, rather than one of intracellular differentiation.

Conclusions

1. Reticulum is a regular constituent of the stroma of most tumors, excepting those of the nervous system.

2. It is usually most abundant in tumors of rapid growth.

3. It is apparently converted into collagen in the more slowly growing neoplasms.

4. It does not show any constant relationship to cellular constituents of tumors, but seems to be laid down in continuity with preëxisting reticulum in the intercellular fluids or substances by a process independent of cytoplasmic differentiation and analogous to precipitation or crystallization.

5. Silver impregnation of tumor reticulum constitutes a valuable diagnostic method, especially in the case of tumors of endothelial, lymphoid, and nervous-tissue origin.

This work represents the examination of 135 tumors, of which: 23 were adenocarcinoma, 11 fibrosarcoma, 10 lymphosarcoma, 9 epidermoid carcinoma, 8 endothelioma of various types, 7 fibroma, 4 leiomyoma, 4 lipoma, 4 Hodgkins' nodes, and 4 melanosarcoma. The rest were represented by one or two examples, either because it was obvious that they were typical, or because of lack of material, as in the case of neuroblastoma, ganglioneuroma, and rhabdomyosarcoma.

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DESCRIPTION OF PLATES LXVII–LXXI

PLATE LXVII

Fig. 1. Metastasis from an epidermoid carcinoma of the penis. The epithelial alveoli are not invaded by reticulum, which merely outlines them.

Fig. 2. Medullary form of adenocarcinoma primary in stomach. Here there is more dissociation of the cells and consequently slight invasion of cell masses by reticulum.

Fig. 3. The sarcomatoid type of pancreatic carcinoma. There is comparatively little reticulum, which tends to invade cell aggregations. Cf. with lymphosarcoma photomicrograph.

Fig. 4. Medullary carcinoma of uterus, with sarcomatous stroma. Specimen of Dr. Jacobson's. Note the very heavy, coarse reticulum of the stroma, and compare with that of the preceding figures and with that of the fibrosarcomata.

PLATE LXVIII

Fig. 5. Primary endothelioma of lymphnode. Compare this reticulum, which traverses the cell masses in all directions, with that of Fig. 6 and note its extensive branching and its gently curving course.

Fig. 6. Medullary type of adenocarcinoma of breast for comparison with Fig 5. This shows the greatest amount of invasion of the epithelial cell masses by reticulum that we have observed. There is a much straighter, simpler reticulum in this tumor.

PLATE LXIX

Fig. 7. Malignant reticuloendothelioma. The reticulum is very intimately associated with the tumor cells in this case.

Fig. 8. Dural endothelioma. The coarsest, densest reticulum observed in any of our tumors; very few of these fibers are collagenous in nature.

Fig. 9. A fibroma molle of the vulva. The black fibers are reticulum, or partially impregnated collagen, the gray are collagenous.

Fig. 10. A soft, mucoid fibroma of external auditory meatus. No appreciable amounts of collagen, much fine reticulum.

PLATE LXX

Fig. 11. A fibrosarcoma of the lung. The reticulum is fairly coarse and straight, and collagen is negligible.

Fig. 12. A fibrosarcoma of the knee. There is much collagen, which photographs grayish, and rather sparse reticulum.

Fig. 13. A fibrosarcoma of lung with marked anaplasia, but abundant collagen and comparatively little reticulum.

Fig. 14. A very rapidly growing fibrosarcoma of the scapular region, with extremely young and delicate reticulum and little collagen.

PLATE LXXI

Fig. 15. A liposarcoma of the sacral region. This shows very little, young reticulum. Note the cytoplasmic network. Little collagen.

Fig. 16. Lymphosarcoma originating in the thymus. It is typical of the lymphosarcomata we have examined from other localities. Very abundant reticulum, often young and beaded, and usually unassociated with large cells. Cf. Fig. 3.

Fig. 17. A section of lymphnode in Hodgkins' disease. This case shows little reticulum, probably all of it from the original lymphoid reticulum.

Fig. 18. A tumor from the parotid region, which is partly, if not entirely, epithelial in its make-up. It shows a very dense reticulum and well illustrates the relationship of epithelium to that tissue.

All these photomicrographs were taken at ×650 diameters, 4 mm. objective and ×15 ocular, with a short bellows (17 cm.).