

I. "On some Points connected with the Anatomy of the Skin."

By GEORGE THIN, M.D. Communicated by Professor HUXLEY, Sec. R.S. Received November 25, 1878.

[PLATES 2 and 3.]

Rollett, in 1858,* in a memoir on connective tissue, described the results of an elaborate investigation into the structure of the corium. Microscopic examination of leather, and of skin tanned by himself, had shown him that the connective tissue bundles of the corium are made up of smaller divisions, and that these latter are again made up of the previously known minute connective tissue fibrillæ, which are so small that their diameter can only be approximately estimated at 0·0002 to 0·0003 millim. From the connective tissue bundles of the skin of the ox, "treated by lime or baryta water, there can," he states, "be isolated from each bundle a number of component elements which have a considerably larger diameter than the minute fibres known as connective tissue fibrillæ." These elements have, he remarks, in the ox a thickness of 0·003—0·006 millim., and he proposes to call them connective tissue fibres (*Bindegewebsfaser*). In a plate attached to his memoir the bundles and their divisions are shown in a very distinct manner.

This observation of Rollett's has not arrested the attention of anatomists to the degree which might have been expected, and seems, indeed, to have been to a great extent neglected. Two of the latest standard works may be quoted in illustration of this remark. W. Krause, in a volume on "General and Microscopic Anatomy," published in 1876, describes the tissue of the corium proper as being composed of "a network of strong bundles of connective tissue closely interwoven, the bundles being partly cylindrical, partly flattened." There is nothing said about the subdivision of the bundles, as described by Rollett.

The same author, in his chapter on connective tissue, states, "that the ground substance of fibrous tissue consists of closely-packed, very fine, round connective tissue fibrillæ, measuring 0·0002—0·002 millim." The larger of these measurements is inapplicable to the fibrilla of Rollett, and is so near that of the subdivision or "fibre" of that author, that it is evident that Krause does not recognise the distinction between the fibre and the fibrilla established by the former histologist.

In Quain's "Anatomy"† it is stated, "that the corium is made up of an exceedingly strong and tough framework of interlaced connective tissue fibres with blood-vessels and lymphatics. The fibres are

* "Sitzungsbericht der Kaiserlichen Akademie der Wissenschaften," vol. xxx.

† Eighth edition, edited by Dr. Sharpey, Dr. A. Thomson, and Mr. Schäfer; p. 213, vol. ii.

chiefly of the white variety, such as constitute the chief part of the fibrous and areolar tissues, and are arranged in stout interlacing bundles, except at and near the surface, where the texture of the corium becomes very fine." Neither in the above quotation, nor in the sections of the same volume in which areolar tissue and fibrous tissue are described, can I find anything analogous to Rollett's description of definite subdivisions of the bundles as distinct from the fibrillæ.

In a paper presented to the Royal Society in 1875, I stated that in portions of the cutis, macerated for a few days in aqueous humour or blood serum, the tissue is seen to be composed of extremely fine but sharply contoured fibrillæ, arranged in parallel bands, whose breadth approaches the diameter of a human red blood-corpuscle. These bands are the subdivisions (*Abtheilungen*) of the bundles described by Rollett, with whose memoir I was not then acquainted.

During the interval that has elapsed since I wrote the paper referred to, I have been frequently engaged in examining skin affected by various pathological changes, and I have had occasion to observe that the structure of the "bundle" of anatomists, as understood by Rollett, is sometimes seen very clearly in disease. Its recognition is, as I have elsewhere* pointed out, necessary to a right appreciation of some of the appearances seen in cancer of the skin.

It is partly the object of this paper to describe some methods by which this structure of the bundles can be demonstrated, and also to describe some other points in the anatomy of the skin which I have observed whilst studying the tissue by means of these methods.

The nomenclature I shall use is the following: By the term *bundle* or *secondary bundle*, I designate the ordinary bundle of authors, which is more or less conspicuous in all preparations of skin, and which is analogous in structure and size to the bundles as usually described and figured in tendon-tissue. The element described by Rollett as "connective tissue fibre," I shall describe as *primary bundle*, to distinguish it more markedly from the fibrillæ which compose it.

When groups of secondary bundles are isolated, each group being composed of several secondary bundles, I term the group a *tertiary bundle*.

These elements can be isolated by first saturating the corium with chloride of gold solution and then macerating the tissue in acids. Portions of skin, with a thick layer of the panniculus adiposus, were taken fresh from the mamma of a middle-aged woman, which had been removed for a tumour of the gland—the portions of skin chosen being well clear of diseased tissues. The stretched skin was pinned down to a cork board, the under surface uppermost, and then saturated

* "Trans. Roy. Med. Chir. Soc.," vol. lix, p. 189.

with half per cent. chloride of gold solution. From time to time different thicknesses of the fatty layer were removed as the solution had had time to penetrate into the tissue, until finally the deeper layer of the cutis proper was laid bare. The tissue, still extended, was then placed in fresh gold solution for several hours. The object of the manœuvre was to secure the penetration of the fluid through the bundles, whilst these were still extended in their natural condition.

After a due action of the gold, the skin was cut into small pieces, which were then treated by acetic and formic acids in various degrees of dilution. Some of the portions were exposed to sunlight for several days, in water feebly acidulated with acetic acid, and then the strength of the acetic acid was raised to 20 per cent. of the ordinary concentrated acetic acid of commerce. Other portions were treated by formic acid. Some successful preparations were obtained from portions macerated first for a few days in a mixture of one part formic acid, of specific gravity 1·020, and one of water, and then in the undiluted acid for some days longer, but a strict adherence to these strengths was not found necessary.

Portions of the corium thus prepared were teased out in glycerine and examined, directly or after staining by different dyes. Staining by pikric acid I found very advantageous.

I was able to isolate, in a condition favourable for study, the primary, secondary, and tertiary bundles. Generally speaking, although not invariably, the tertiary and secondary bundles were best seen in the tissues macerated in acetic acid, and the secondary and primary bundles in those treated by formic acid.

Numerous elastic fibres were isolated by both methods, the finest fibres more particularly in the formic acid preparations. These fibres were frequently found only partially detached from the bundles, and in such preparations the relations of the fibres to the bundles could be well studied. The primary bundles isolated by these methods were flattened, cylindrical elements, even contoured, homogeneous in appearance, and uniform in breadth over the whole length isolated. The difference in breadth between individual bundles was very slight. By measurement, I found that they were from 0·004 to 0·005 millim. broad. The primary bundles were sometimes seen *in situ*, that is to say, as parts of a secondary bundle, the breadth of the latter being normal. In other preparations the contours of secondary and tertiary bundles were lost, and the microscopic field was filled with a large number of primary bundles, entangled and twisted by the needles used in teasing them out. Sometimes a number of primary bundles, although separated from each other, were yet so placed that I could feel assured that they were the constituent elements of one secondary bundle. Such was the case with the primary bundles shown in fig. 4.

Various methods have been recommended by histologists for the demonstration of the ultimate fibrillæ of fibrous tissue, chiefly with reference to those of tendon bundles.

If I may judge by my own preparations of skin and by the figures published in histological works, the fibrillæ of the cutis bundles are very seldom seen. The appearances usually observed in skin hardened by chromic acid and alcohol are unfitted for a study of the fibrillæ. In such specimens the bundles are more or less broken up, but the individual fibrillæ are not, as a rule, isolated.

I found that they were well shown by the following method:—A portion of fresh skin, with the panniculus adiposus attached, was pinned to a piece of cork, in the manner already described, and treated in the same way, with the exception that this time glycerine, instead of chloride of gold solution, was used for saturation. When the saturated cutis tissue had been laid bare, the whole was placed in glycerine and allowed to remain in it for several days. Small portions were then teased out in glycerine, stained by picro-carminate of ammonia and examined in glycerine. In such preparations the secondary bundles were found isolated, the contours of the primary bundles not being preserved. In the secondary bundles the fibrillæ were seen more or less distinctly, in some of them with perfect distinctness. (See fig. 8.)

In the gold preparations the following facts regarding the disposition of the elastic fibres were noted:—

If a portion of skin is hardened in bichromate of potash, and the sections moderately stained by eosin, all the large elastic fibres are stained much more intensely than the bundles, and it is then observed that they lie on the surface of the bundles, and run parallel to them. In the gold preparations, after maceration in formic acid, further details regarding the fibres can be detected. It is then seen that there is a close network of minute elastic fibres, of which I have observed no traces in eosin-stained bichromate preparations, on the surface of the bundles, and that at certain points the larger fibres give off branches which join this network. At these points the network is so dense over a small defined space that the size of the meshes is nearly equalled by that of the fibres.

Rollett, in the memoir referred to, states that the bundles are embraced by elastic fibres, and that the latter send branches into the substance of the bundles. I am able to confirm this statement, and to extend it. In some of the gold and formic acid preparations, I have observed that the elastic fibres which penetrate the bundles enter between the primary bundles, and that the primary bundles are embraced by the fibres which entwine them very closely. I have never observed an elastic fibre penetrate a primary bundle.

The relation of the elastic fibres to the primary bundles is shown in

fig. 7, but the fibres are in reality more delicate than is shown in the drawing.

The dark very finely granular deposit produced by the reduction of the gold chloride had a special relation to the elastic fibres, which was best observed in portions of skin which had been macerated for a longer period in 20 per cent. acetic acid. This relation will be understood by reference to figs. 3, 6, 9, and 10. Strictly defined narrow strips of this deposit were found investing the fibres, and this so closely that it was only at points where it had been disturbed in the preparation that the fibre itself could be observed.

The appearances reproduced in figs. 3 and 10, in which fibres are seen with deposit still adherent, illustrate this point very strikingly.

In gold preparations large flat oval nuclei are sometimes seen adherent to the surface of the bundle. The nuclei have the characteristic slate colour, and around the nucleus a small ill-defined patch of gold deposit is seen.

This deposit could sometimes be seen to be continuous with that surrounding an elastic fibre. This is shown in fig. 10. There is no reason to believe that in such cases more of the cell than the nucleus has been preserved, or that the gold deposit has any special relation to cellular substance.* The distinctly localised character of the deposit around the elastic fibres supports the idea that the larger ones are surrounded by an albuminous fluid, of a like nature to that shown by gold preparations, to be present between the laminae of the cornea.

Isolated tertiary bundles completely surrounded by elastic fibres (fig. 5), are sometimes seen.

The "spiral" fibre, as I have seen it on the bundles of the skin, is an elastic fibre that encircles the bundles like a ring; and it may continue to do so after the ring has been detached from other fibres, none of which, indeed, may be found in the isolated bundle.

The nature of the spiral fibre is still considered by some histologists as undecided, and Ranvier regards its behaviour under picro-carminate staining as against the view that it is an elastic fibre. In the preparation drawn in fig. 8, which had been stained by picro-carminate, a typical spiral fibre was distinctly stained yellow by the pikric acid, and was not stained by the carmine, behaving in this respect exactly like any other elastic fibre.

Confirmation of Rollett's views as to the structure of the bundles is occasionally found in bichromate of potash preparations of skin. Part of one of the most demonstrative preparations of this kind

* In a paper read before the Royal Society in 1874 ("Proceedings," No. 155, 1874), I followed the view held by some histologists, that the gold deposit in such preparations is indicative of cellular protoplasm, and described and figured (fig. 13) an anastomosis of cells in the skin by means of elastic fibres. As will be observed from the remarks in the text, I now interpret these appearances quite differently.

which I have met with, has furnished the subject of the drawings in figs. 1 and 2.

The specimen is from the skin of the horse, and was thus prepared. A portion of fresh skin, free of panniculus adiposus, was hardened first in weak and then in stronger solutions of the bichromate, and treated by gum and alcohol before being cut. The deep edge of the sections—the part of the tissue that had been in direct contact with the bichromate solution—showed the structure of the bundles best.

The transverse sections of many of the bundles were cut up into a mosaic of somewhat rounded polygonal fields (fig. 1*b* and fig. 2), the measurements across each field varying from 0·0037 to 0·005 millim. Oblique and longitudinal sections of the bundles showed that these fields were sections of primary bundles. The mosaic was not equally distinct in all the bundles, even in parts where the appearance was well brought out. This varying distinctness is seen in fig. 1.

The sections of the primary bundles being rounded there are small angular spaces between them. These have not been successfully shown in the drawing.

In this preparation a delicate connective tissue was found between the bundles of the corium in a well marked form. Its extent relatively to the bundles will be best understood by reference to the drawing. As seen in the preparation it was distinctly fibrillar at parts.

The cells seen in the preparation were in two positions. Some of them were found in the delicate tissue between the bundles; other cells were found in direct connexion with the bundles. Of the latter cells the greater number seen were applied to the surface of the bundles, but others were found in the substance of the bundles between the primary bundles.

These cells were all of the endothelial type. In all of them the cell-contour was clearly marked, and in none of those observed was there a trace of a process, or of ridges and depressions similar to those described by some histologists in tendon. The size and form of these cells is accurately shown in fig. 1, and will be better appreciated by reference to the drawing than by any detailed description which I could give.

EXPLANATION OF THE PLATES.

(All the figures except figs. 8 and 10 are drawn by camera lucida.)

Figure 1. From the corium of the horse, bichromate of potash, gum, and alcohol. Logwood and eosin staining.

(*a.*) Delicate connective tissue between the bundles.

(*b.*) Secondary bundle cut transversely, showing mosaic formed by the sections of primary bundles.

(*c.*) Cells belonging to the inter-fascicular connective tissue.

(*d, f.*) Cells lodged in spaces in the centre of bundles.

(*e.*) Cell applied to the surface of a bundle. × 375.

- Figure 2. Part of a bundle hanging loosely on the free under edge of the same section from which fig. 1 is drawn. The mosaic of primary bundles is unusually well marked. $\times 375$.
- Figure 3. Elastic fibre, with patches of chloride of gold deposit adherent. Isolated from adult human skin. Gold saturation and maceration in 20 per cent. acetic acid. $\times 340$.
- Figure 4. Isolated primary bundles. Human adult skin. Gold saturation and maceration in formic acid. $\times 340$.
- Figure 5. Tertiary bundle entwined by elastic fibres. Human adult skin. Gold saturation; maceration in acetic acid. $\times 340$.
- Figure 6. Gold deposit on a large elastic fibre, and a small elastic fibre on the surface of a bundle almost completely ensheathed in gold deposit. Human adult skin. Gold saturation; maceration in acetic acid. $\times 340$.
- Figure 7. An isolated secondary bundle, in which the contours of the primary bundles are visible. The latter are entwined by minute elastic fibres. Human adult skin. Gold saturation: maceration in formic acid. $\times 340$.
- Figure 8. Bundle showing fibrillæ, and snared by an elastic fibre (spiral fibre). Human adult skin. Saturation with glycerine; picro-carminate staining. (Hartnack, Objective No. 8; Eye-piece No. 3; Tube in.)
- Figure 9. Lines of gold deposit on bundles, following the course of elastic fibres. Human adult skin. Gold saturation: maceration in acetic acid. $\times 340$.
- Figure 10. Elastic fibre with a nucleus adhering to it, and a streak of gold deposit partially detached from the fibre. (Hartnack, Objective No. 8; Eye-piece No. 3.)

II. "On Hyaline Cartilage and deceptive appearances produced by Reagents, as observed in the examination of a Cartilaginous Tumour of the Lower Jaw." By GEORGE THIN, M.D. Communicated by Professor HUXLEY, Sec. R.S. Received November 25, 1878.

[PLATE 3.]

The following paper is written with a twofold object: firstly, as a contribution to the histology of hyaline cartilage; secondly, to illustrate how much the apparent structure of a tissue which is being examined microscopically depends on methods of preparation.

A portion of a large tumour of the lower jaw, believed from its naked eye appearances by two experienced surgeons to be sarcomatous in its nature, was given me for examination. Although I was struck by the peculiar kind of resistance it offered to the knife, I did not imagine at the time, any more than did the surgeons who excised it, that the tumour was cartilaginous. This is to be explained by the fact that the cartilaginous substance which had been growing with extreme rapidity was of a low type.

In order to determine the structure of the growth, I hardened por-