Histologie. — *"On the Histological Structure of Fibrous Substances."* By G. C. HERINGA and Miss H. A. LOHR. (Communicated by Prof. J. BOEKE.)

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In a previous communication some of the reasons were given which led us to regard the cause of the collagenous fibres of the connective tissue as being gelatinization of a fibre-building colloidal solution („Stäbchen Sol" after Szegvari). Further research did but strengthen our conviction. The arguments which support this belief are partly negative, partly positive. As negative arguments serve all the observations of other writers which point to the origination of collagenous fibrils in the absence of cells, and in particular the impossibility of reconciling the architectonics of the fibrillar structure with the arrangement and development of the cells held by others as responsible for the formation of fibres. Especially in respect to this last point the classic observations of VAN EBNER have lost nothing of their value to us, while the better preservation of the structure by means of the gelatin freezing method has assisted our train of thought.

As positive arguments we had : 1. that, especially with the aid of dark-field microscopy and by making use of azimuth effect, we were able to study collagenous fibrils of visibly different thicknesses in the spaces between the cells,which waved in such a way, or even became confused into tangled knots, that there could be no question of a continuous contact with any cell area that might influence the thickness of the fibres individually. On the contrary, the morphological resemblance to the aspect afforded by other colloidal fibres (soap, benzopurpurine, and others) in gelatinization, where certainly no fibroblasts are functioning, is particularly striking. A second and, in our opinion stronger, argument was furnished us by a further similarity, namely, the inclination towards a parallel drawing together of the fibrils. This symptom, which appeared in varying intensity according to the special kind of the connective tissue we examined, proved to be entirely analogous to what SZEGVARI and ZOCHER have described for their substances. In this "bundling process" also specific influence from the cells may be precluded. It was along this path we thought we should arrive at a rational explanation of the bundle-like course of the collagenous fibres so familiar to the histologists of old in their preparations, and which appears again and again in innumerable connective tissue formations, and has hitherto been commonly ascribed to the expression of fibroblastic functioning.

It was naturally of importance now to our train of thought to know whether the same or analogous aspects are to be found again with other substances which, as appears from optical or röntgenographic examination, are related to collagen, i.e. substances which, as appears from double refraction, must likewise be regarded as built up from parallel crystalline micells, and are röntgenographically distinguishable by a 4-points diagram.

Our attention was especially directed to the chitin, and that for a very particular reason. In our examination of connective tissue we had come to the conclusion that the drawing together of the collagenous fibrils leads not merely to the formation of bundles of considerable thickness (which bundles, by-the-way , are never round, but invariably a flattened oval, or ribband-like), but that under certain circumstances dominates the coalescence in one plane in such a way that they must be termed true collagenous membranes (the "lamelles" described by LAGUESSE with so much emphasis, and somewhat over-estimated as to their significance in the construction of the connective tissue). This renders an analogy with the chitin directly obvious ; for there too we find beside chitin tendons, the occurrence of the membranous cuticulae which, to judge from their appearance, would not at all remind us of a "fibre-diagram".

Thanks to the kindness of Dr. HIRSCH, of the Zoological Laboratorium, we had the use of several specimens of chitin, and now looked for a piece that, under the microscope with low power, showed no special structure (pleurite of an astacus).

The examination of this piece in dark field with azimuth lighting yielded the desired effect, finer than we had ventured to expect. It showed us the chitin build up by interwoven bindles of parallelly running fibrillae, differently orientated in consecutive layers ; the latter particular, when the illuminating slit was revolved, yielded, for the azimuth effect, a highly beautiful and charming alternating play of light and shadow.

All previous studies of chitin had already confirmed the supposition long ago expressed as to its fibrous structure. Nevertheless the extreme clearness with which this structure can be seen by our method of investigation is, in our opinion, worth mentioning. But moreover, with reference to the complete accordance in structure, we would touch again upon the analogy with the connective tissue. Whereas in the case collagen a cytological explication of the structure could still, albeit with some difficulty, be based upon the presence of fibroblasts, this was felt to be a greater objection with regard to chitin which, as could not be doubted, makes its appearance as a fluid shapeless mass. In the bulky treatise which BIEDERMANN (Handb. der Vergl. Physiologie, III, 1914) has devoted to this subject, it is curious to note what complicated reasoning is required to make it plausible that some mysterious changes in the function of the chitinous cells must be the cause of the structure observed. (Conf. SCHMIDT. Die Bausteine, etc. Bonn 1924, pg. 195 sequ.)

But we have not arrived by a long way yet at an explanation of the

complicated image of the structure of chitin, no more than of the connective tissue. Only we wish to single out one fact from the total of the phenomena, namely, the common fibrilization of both and the inclination of the fibrils to form themselves into bundles.

These two phenomena, if our assumption of colloidal coagulation be accepted, may be eliminated from the proposition as directly attributable to the micellar properties of the substances. There then remains a second question to be answered separately, viz. that of the factors which govern the course of the bundles and their union in the form of tissue into more complicated systems of a higher order. The observations of colloids in vitro by the colloid-chemici have taught us nothing on this head. At the most they show the result of a mechanical influencing (streaming, cataphoresis) of the preparations by the appearance of structural lines in accordance with the direction of the acting force. That similar influences might well be the cause of polar differentiation in living nature also (according to VAN EBNER's hypothesis of tension) has been irrefutably determined by ROUX' expositions, and by LEVY's experiments on tendon regeneration. Equally certain it is, however, that this explanation absolutely fails in a number of cases. Especially we refer here to the arrangement in layers, with a course intersecting in more or less sharp angles, a peculiarity which, remarkably enough, crops up throughout the entire organism of nature in the most dissimilar fibrous substances. We have just remarked how far-fetched it is to ascribe this phenomenon in the case of chitin, where no cells occur between the layers, to a charge of secretory function. BIEDERMANN is undoubtedly right, however, in saying that a consistent working out of the theory of tension would likewise lead *ad absurdum*. Well, then, the same holds good equally for the connective tissue, as, for example, appears from the fact that in the thin layers of LAGUESSE fine alternating layers are still to be seen.

Here, then, we find ourselves confronted by a still unsolved puzzle. All the more, we ask ourselves whether the alternating layers of fibrous substance, so frequently met with, are caused, like the bundling principle by the micellar properties of the substance itself. Possibly the colloid-chemistry, with its ever-advancing results, may throw some light on this too in the future.