

	Méthode de synthèse	Spectre d'absorption	Chromatogramme sur papier	
			a	b
V	Décarboxylation de IX	Un peu différent de la figure 1	0,25	0,28
VI	Décarboxylation de X	Identique à la figure 1	0,34	0,24
VII	Décarboxylation de XII	Identique à la figure 1	0,54	0,30
VIII	Décarboxylation de XIII	Identique à la figure 1	0,70	0,34
IX	<chem>Nc1nc(N)c(O)c(N)n1</chem> + $\begin{array}{c} \text{CO}_2\text{Et} \\ \\ \text{CO}-\text{CO}_2\text{Et} \end{array}$, hydrolysé par l'alcali	Identique à la figure 1	0,08	0,37
X	<chem>Nc1nc(N)c(O)c(N)n1</chem> + $\begin{array}{c} \text{CH}_2-\text{CO}_2\text{Et} \\ \\ \text{CO}-\text{CO}_2\text{Et} \end{array}$, hydrolysé par l'alcali	Identique à la figure 1	0,22	0,55
XI	Estérification de X	Identique à la figure 4	0,47	0,52
XII	<chem>Nc1nc(N)c(O)c(N)n1</chem> + $\begin{array}{c} \text{CH}_3-\text{CH}-\text{CO}_2\text{Et} \\ \\ \text{CO}-\text{CO}_2\text{Et} \end{array}$, hydrolysé par l'alcali	Identique à la figure 4	0,35	0,63
XIII	<chem>Nc1nc(N)c(O)c(N)n1</chem> + $\begin{array}{c} \text{C}_2\text{H}_5-\text{CH}-\text{CO}_2\text{Et} \\ \\ \text{CO}-\text{CO}_2\text{Et} \end{array}$, hydrolysé par l'alcali	Identique à la figure 4	0,73	0,68
XIV	<chem>Nc1nc(N)c(O)c(N)n1</chem> + $\begin{array}{c} \text{CH}_2-\text{CH}_2-\text{CO}_2\text{Et} \\ \\ \text{CO}-\text{CO}_2\text{Et} \end{array}$, hydrolysé par l'alcali ou la décarboxylation de XV	Identique à la figure 4	0,23	0,42
XV	<chem>Nc1nc(N)c(O)c(N)n1</chem> + $\begin{array}{c} \text{EtO}_2\text{C}-\text{CH}-\text{CH}_2-\text{CO}_2\text{Et} \\ \\ \text{CO}-\text{CO}_2\text{Et} \end{array}$, hydrolysé par l'alcali	Identique à la figure 4	0,24	0,75

a Chromatogramme sur papier avec le solvant: butanol, acide acétique, eau; b avec le solvant: solution de NH_4Cl à 3%. Et = C_2H_5 .

Summary

A pterin obtained from the larvae of *Bombyx mori* just after hatching is very similar to ichthyopterin, but not quite identical. It may be a derivative substituted at the position 6 of isoxanthopterin, but without carboxyl group.

Submicroscopic Structure of Reticular Tissue Fibres

There are few electron optical pictures of reticular tissue fibres in the recent literature. REED and RUDAL¹

¹ R. REED and K. M. RUDAL, *Bioch. Biophys. Acta* 2, 19 (1948).

have published pictures from sarcolemma, FERNANDEZ MORAN¹ from neurolemma, and GROSS² from subcutaneous tissue fibres of new-born mice.

This paper deals with some typical cases of reticular tissue of spleen stroma and lymphatic nodules. Before isolation of stroma, the spleen was thoroughly washed with Ringer's solution. Then the pieces were fixed in acetone and sectioned with the freezing microtome. The frozen sections underwent digestion in an alkaline (pH 7.8) pancreatine medium. Time-controls of the grids were made with the phase-contrast and polarized-

¹ H. FERNANDEZ-MORAN, *Exper. Cell. Res.* 1, 309 (1950).

² J. GROSS, *J. Gerontology* 5, 343 (1950).

light microscopes. These controls made it possible exactly to differentiate reticular fibres from collagen fibres which both invariably occur in spleen stroma and lymphatic nodules. Some of the isolated reticular fibres were fragmented by the usual mechanical means and by ultrasonic waves.

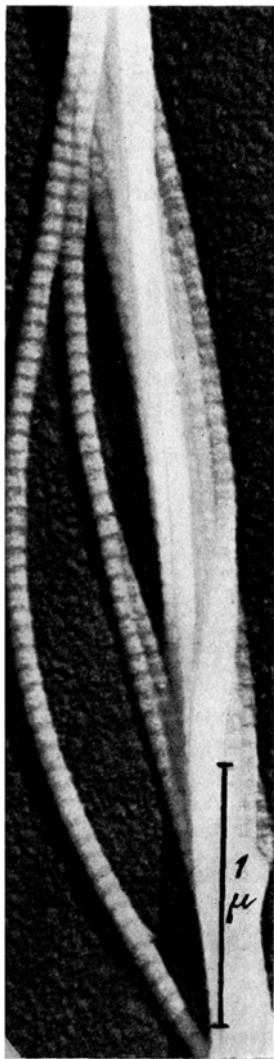


Fig. 1.

The Figures 1–3 give the most important and conclusive of our observations which will be described subsequently and commented upon.

Figure 1 shows a rather large reticular fibre from a lymphatic nodule network. In the electron microscope, these large fibres were shown to be made up of non-anastomosed spirally twisted fibrils: at least ten of these fibrils to a fibre.

In places where an agglomerated union of fibrils appeared, the electron optical picture was opaque. But, at those points where the fibrils were dissociated from each other, they were easily distinguishable. These isolated fibrils proved to have a diameter of about 800 Å and showed a clear periodicity. The range of their periodicity was inferior to 700 Å, i. e. between 600 Å and 690 Å. From this the authors draw the conclusion that this picture is clearly identical to those of the well-known typical collagen fibrils.

Figure 2 gives an electron optical picture of material which appeared under the light optical microscope to be like one of those anastomosed networks demonstrated by the ordinary histological technique of silver impregnation.

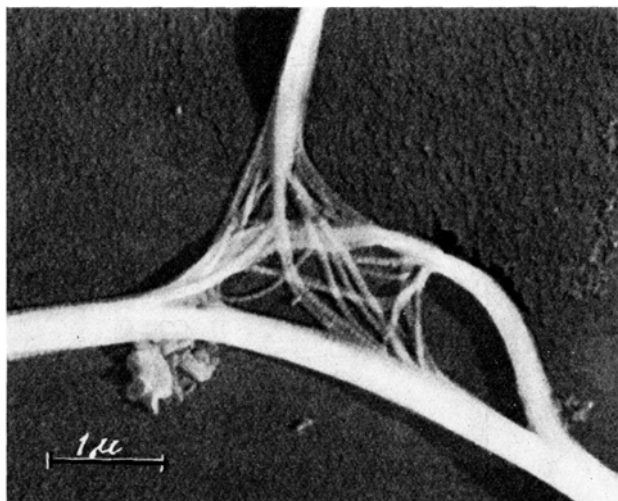


Fig. 2.

However, this figure reveals different converging fibres, which, at a certain point, spread out their respective fibrils passing from one fibre to another. The fibrils in regions of anastomosis have variable diameters; the smallest one, being inferior to 600 Å, clearly demonstrated periodicity.

Figure 3 shows the covering of a lymph nodule capillary vessel. The characteristics are an intricate plexiform network of fine fibrils with clear periodicity and with a variable diameter, ranging from 500 Å up to 800 Å.

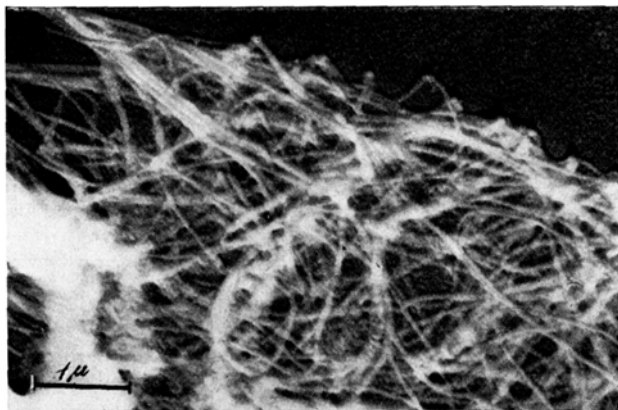


Fig. 3.

Furthermore we have observed another peculiarity which may be of some interest. By means of ultra-sonic waves and by mortar fragmentation, very thin and easily distinguishable isolated fibrils were obtained from adult spleen pulp. These well-isolated fibrils revealed extremely low width readings of about 300 Å and showed a striking periodicity.

Fibrils obtained adult organs with such small dimensions as stated here, have not been reported elsewhere as yet, so far as we know.

In the literature, there are many controversies about the typical collagen found in tendons, subcutaneous tissues, etc., and reticulin fibrils from stroma of parenchymal organs.

Up till now topographical distribution and affinity to silver salts were regarded conventionally as reliable criteria for differentiation of collagen and reticulin. But, bio-physical studies with polarized light and digestion tests have not, so far, given reliable data to prove actual differences between typical collagen and reticulin.

One of us, BAIRATI¹, has found, with the aid of dark-field observations, that the web-like network in reticular tissues demonstrated with the usual silver method is not formed by elementary anastomosed fibrils, but is actually made up of plexus of submicroscopic fibrils. The facts reported in this paper are also in favour of this conception.

The essential electron optical characteristics of the reticulin fibrils described here, reveal quite a clear identity with the characteristics of typical collagen fibrils. The only apparent difference seems to be their different diameter.

In addition to the bio-physical and digestion tests already mentioned as basic methods for differentiating and identifying reticulin and collagen fibrils, we consider the use of X-ray diagrams to be the ultimate deciding method to define the actual characteristics of pure reticulin fibrils. As ASTBURY² rightly points out, up till now it has been extremely difficult to obtain a reticulin specimen which is proved to be free from traces of other substances, especially from collagen fibrils, and which would be suitable for X-ray diagram research to give data upon pure reticulin.

Some time ago an attempt was made to present X-ray diagrams showing an example of pure reticulin fibrils: in this connection, HERINGA³ presented an amorphous X-ray diffraction pattern obtained from fibrils prepared from the subcutaneous tissue of new-born mice.

Later, however, GROSS⁴ controlled HERINGA's data and found that HERINGA's experimental material gave X-ray diffraction patterns similar to those of typical collagen. We should like, however, to add that we cannot accept subcutaneous tissue of new-born mice as a typical case of pure reticulin fibrils.

Judging by the new facts, we come to the conclusion that there is no essential sub-microscopic structural difference between collagen fibrils and reticulin fibrils.

The authors wish to thank Dr. STUDER, chief Assistant of the Electron Microscopy Laboratory of the University of Berne, Switzerland, for his helpful cooperation in their electron microscopic work.

A. BAIRATI, F. MASSARI, and G. MARSICO

Theodor Kocher Institute Berne, and Institute of Normal Human Anatomy, University of Bari, June 16, 1952.

Zusammenfassung

Die Verfasser haben die Gitterfasern von Milz und Lymphknoten mit verschiedenen Methoden präpariert und im Elektronenmikroskop untersucht. Die Gitterfasern sind zusammengesetzt aus voneinander unabhängigen Elementarfibrillen von periodischer Struktur. Zwischen dieser und der periodischen Struktur der typischen kollagenen Fibrillen besteht Übereinstimmung.

¹ A. BAIRATI, Z. Zellforsch. 30, 389 (1940). – A. BAIRATI, F. MASSARI, and G. MARSICO, Boll. Soc. Biol. Sper. 27, 1583 (1951).

² W. T. ASTBURY (personal communication).

³ G. HERINGA and A. WEIDINGER, Acta Neer. Morph. 4, 51 (1942).

⁴ J. GROSS, J. Gerontology 5, 343 (1950).

Faseranastomosen bestehen aus einem Geflecht von Elementarfibrillen. Es wird der Schluss gezogen, dass zwischen Retikulin- und kollagenen Fibrillen kein wesentlicher Unterschied in der submikroskopischen Struktur besteht.

Über eine Doppelbrechung in Kulturen von *Bacillus anthracis*

In einer früheren Mitteilung¹ wurde eine optische Anisotropie in Kulturen von *Mycobacterium tuberculosis* beschrieben. Virulente Stämme von Tuberkelbakterien bilden in Kolonien zopfartige Gebilde («cords»). Diese «Zöpfe» setzen sich aus zahlreichen, mehr oder minder exakt parallel geordneten Bakterien zusammen. «Cords» von genügender Dicke sind negativ doppelbrechend, bezogen auf ihre Länge.

Eine ähnliche Doppelbrechung sollte auch in Kulturen anderer Mikroorganismen zu finden sein. Mit derartigem war vor allem in Kolonien von *Bacillus anthracis* zu rechnen. Diese sind, wie man weiss, im allgemeinen durch eine charakteristische Struktur gekennzeichnet: Die länglichen Bazillen liegen orientiert in lockenartigen Strängen.

Methode. Prüfobjekt waren 24 Stunden alte, auf gewöhnlichem Nähragar gezüchtete Kulturen eines apathogenen Stammes von *Bacillus anthracis*. Klatschpräparate wurden auf einen Objektträger gebracht und durch zwei unterlegte Deckgläschen vor dem Zerdrücken geschützt. Um völlig undeformierte Kolonien untersuchen zu können, wurden auch Kulturen auf Cellophan angelegt und von dort vorsichtig auf ein Deckgläschen geschwemmt. Alle Messungen geschahen in 0,9prozentiger NaCl-Lösung.

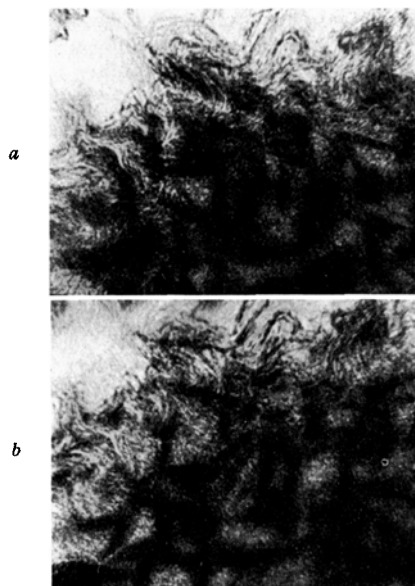


Abb. 1. Rand einer Kolonie von *Bacillus anthracis*. Vergrößerung ca. 45 $\times$. Gekreuzte Nikols und drehbarer Glimmerkompensator. Die Schwingungsrichtungen der Nikols entsprechen annähernd den Kanten der Bilder.

a Additionsstellung des Kompensators für Stränge, die von links unten nach rechts oben ziehen.

b Subtraktionsstellung des Kompensators für Stränge, die von links unten nach rechts oben ziehen.

Zahlreiche Stellen mit entsprechend gelagerten Strängen, die in a dunkel sind, sind in b hell, und umgekehrt.

¹ G. BOEHM, Exper. 5, 445 (1949).