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THE PRESENCE OF NERVES IN ORIGINAL AND REGENERATED SYNOVIAL TISSUE IN PATIENTS SYNOVECTOMISED FOR RHEUMATOID ARTHRITIS

By

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INTRODUCTION

Joint pain is one of the cardinal symptoms of rheumatoid arthritis. The exact origin of this has yet to be explained. Cartilage has despite the destruction it undergoes in this disease been ruled out as it does not contain neural elements (*Miller & Kasahara* 1963). In bone, however, nerves are frequently found. According to *Miller & Kasahara* (1963), they are intimately involved in the endosteum of the medullary trabeculae. They also occur in the Haversian Canals, as shown by *Milgram & Robinson* (1966) in a study on adult dogs. Since the osseous compartment of the joint may become part of the disease in rheumatoid arthritis it may well be a site for pain. Finally the soft tissues surrounding the joint, *i.e.* fibrous capsule and mesenchymal linings are richly supplied with nerve elements which may conduct pain (*Gardner* 1950, *Barnet et al.* 1954, *Polaczek* 1961, *Hirsch et al.* 1963). The fibrous capsule contains both capsulated and unencapsulated complex and free nerve endings, which are believed to be pressure sensitive and responsible for stereotactic and movements sensibility like *e.g.* the Ruffini, Vaer-Paccinian and Golzi-Mazzoni endings (*Boyd* 1954, *Skoglund* 1956, *Eklund et al.* 1960, *Lundberg et al.* 1960). The mesenchymal lining, which is the synovial tissue, contains nervous elements which are very scarce (*Gardner* 1950, *Barnett et al.* 1961) in contrast to the richly endowed fibrous capsule. There is some disagreement whether these

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synovial nerve fibres are afferent, but it is agreed that they are unmyelinated, probably autonomic associated with blood vessels (*Gardner* 1950, *Barnett et al.* 1961). *Kellgren & Samuels* (1950) nevertheless believe that synovial tissue contains afferents, a conclusion reached in a study on synovial sensitivity at arthrotomy in local anesthesia. Certain indications point to the synovial tissue carrying some of the responsibility for the articular pain in rheumatoid arthritis. First, it is a target tissue of the disease with an inflammatory infiltration of lymphocytes, plasma cells and phagocytes and fibrinous exsudate (*Norton & Ziff* 1966), the latter of which is believed to be pain-producing. Second, most often pain and other joint symptoms disappear for a variable length of time after synovectomy. By this procedure part of the target tissue of the disease is removed and thereby also nervous elements, whereby a partial denervation ensues. Certain indications suggest that decrease in pain may be due to desensitization. After synovectomy the regenerated tissue appears with the same pathomorphological elements as prior to surgery (*Goldie* 1967) within the same time limit as normal regeneration. Nerves do not appear within the same short time period. Also, some disease activity remains, as in a number of synovectomised cases the antiagglutination factor (AAF) is positive and elevated in joint fluid whereas it remains negative in serum (*Goldie* 1967). Nerve endings have not been encountered in these cases.

The regeneration of synovial tissue after synovectomy is a well established fact (*Key* 1923, *Lindström* 1963, *Marmor* 1966, *Whitefield & Stevens* 1966, *Goldie* 1967). So far, however, no reports have been encountered which describe the presence of nerve elements in regenerated synovial tissue. The object of this study has therefore been to demonstrate the presence of nerves in synovial tissue removed at synovectomy for rheumatoid arthritis and in regenerated synovial tissue at later arthrotomy or biopsy not less than one year and not more than three years following the first synovectomy.

MATERIAL AND METHODS

27 patients with well established signs of rheumatoid arthritis were selected for this study. Tissue samples were taken from three sources; first, from patients undergoing synovectomy for the first time in an affected joint; second, from patients who had undergone synovectomy from one to three years previously and who now volunteered for a second arthrotomy (total 12 of which 10 free of symptoms); third, from patients who had earlier undergone synovectomy and who now submitted themselves for biopsy with a Parker-Pearson Synovial Biopsy Needle (*Par-*

ker & Pearson 1963). Original tissue was studied in 15 specimens, 13 of which were obtained from knee-joints, one from elbow and one from wrist. Regenerated tissue was obtained in 12 patients of which 8 at arthrotomy of knee-joints and 4 by Parker-Pearson biopsy of knee-joints.

All tissue samples were submitted to the Pathology Department for pathomorphologic verification of the type of the tissue and diagnosis. The specimens were stained according to the intravital methylene blue technique and 6 according to the Gros-Bielschowsky silver impregnation method. The methylene blue technique was chosen because of its relative simplicity and its value in staining for nerve fibres and endings in a wide variety of tissues as shown by *Coers & Wolf* 1959, *Hirsch et al.* 1963, *Miller et al.* 1963 and *Goldie* 1964. One of the advantages with this technique is its employment of whole pieces of tissue which permits observation of nerve fibres in a three dimensional plane in translucent specimens.

Intravital Methylene Blue Staining

Fresh specimens are immersed in 0,005 per cent methylene blue in normal saline acidified to a pH of about 3,5 for 30–45 minutes (depending on size of sample) at room temperature (18–20° C). Then gentle rinsing in phys. saline for 10 minutes and oxidized for 10 minutes on a dampened piece of gauze in room air. The specimens are then fixed in 8 per cent ammonium molybdate for 8–12 hrs. at 8–10° C. Then rinsed in running tap water for 1½–2 hrs, and dehydrated in 96 per cent alcohol for 2 hrs. Before placing in the alcohol the specimens are flattened between two microscope slides held together with paper clips. Final dehydration in 100 per cent alcohol for 2 hrs. and then removed from slides and placed in Xylene for clearing. When cleared storing in benzyl benzoate in which medium the specimens are suspended during microscopic examination.

Gros–Bielschowsky Staining as Modified by Coers & Wolf 1959

The sections are placed in distilled water for an hour and are then transferred to 10 per cent silver nitrate for forty-five minutes. They are then placed without washing in 20 per cent formalin, filtered after being neutralized with magnesium carbonate. The solution is changed when it becomes cloudy. After fifteen minutes the sections are washed for a few seconds in two changes of distilled water and placed in a Petri dish containing the following ammoniacal silver solution. To 30 ml. of 20 per cent aqueous silver nitrate concentrated ammonia is added drop by drop until the resultant brown precipitate disappears after which a further 15 drops may be added if the sections darken too rapidly but this is not usually necessary. The sections are examined under the microscope whilst still in the solution and are left in the latter until impregnation of the terminal portion of the nerve fibres has occurred. If this is not achieved within a few hours a drop of the 20 per cent formalin solution may be added and diffused by blowing on the surface of the silver solution. This may be repeated at 15 minutes intervals. The sections must be removed before a precipitate forms or when impregnation is complete and placed successively in each of the following solutions: 20 per cent aqueous ammonia, distilled water, 1 per cent aqueous acetic acid, distilled water, 0.02 per cent

aqueous yellow gold chloride (half-one minute). The sections are then washed in distilled water dried on albuminized slides dehydrated, cleared and mounted in the usual way.

RESULTS

The methylene blue stain is not specific for nerve fibres, as reticulin and capillaries also take the stain. Moreover, this may accumulate in folds of the specimen which may make the interpretation deceptive. Nerve fibres appear as thin slender filaments of 1–3 microns with irregularly placed nodules along their course. Often they accompany a vessel or may be lodged in the wall of the same. The conclusive evidence is the termination into any of the types of nerve endings described below.

Capillaries are, as a rule, easily recognised as the endothelial cells take the stain and give the appearance of ghosted blue cells with a deeply stained nucleus. Reticulin fibres and folds generally appear as broken up, uneven and coiled strands the irregular course of which leave an impression of complete disorganisation.

The nerve endings encountered are of the free fibre ending type which terminate as single branches; complex unencapsulated endings which appear as complexly branched; and encapsulated endings which look like small bulbs.

In the original synovial tissue endings of the free fibre type and complex unencapsulated could be identified. The free fibre endings were seen as single branches tapering off either into a thin filament or into the appearance of a string of pearls (Figure 1). Branched filaments with tapered tips were observed (Figure 2). Verification of these observations was obtained in the Gros-Bielschowsky stain. Other methods, as *e.g.* the cholinesterase technique, were not utilized in this particular study.

In regenerated synovial tissue nerve fibres and endings identical to those found in the original tissue were observed (Figures 3, 4). The timelag of one to three years after synovectomy did not seem to have any influence on the presence of nerves.

COMMENT

Some limitations follow the use of the methylene blue staining technique which have become obvious in this investigation.

First, pieces of extirpated tissue were used which differs from the procedure of *Coers & Wolf* (1959) and *Miller & Kasahara* (1963) who

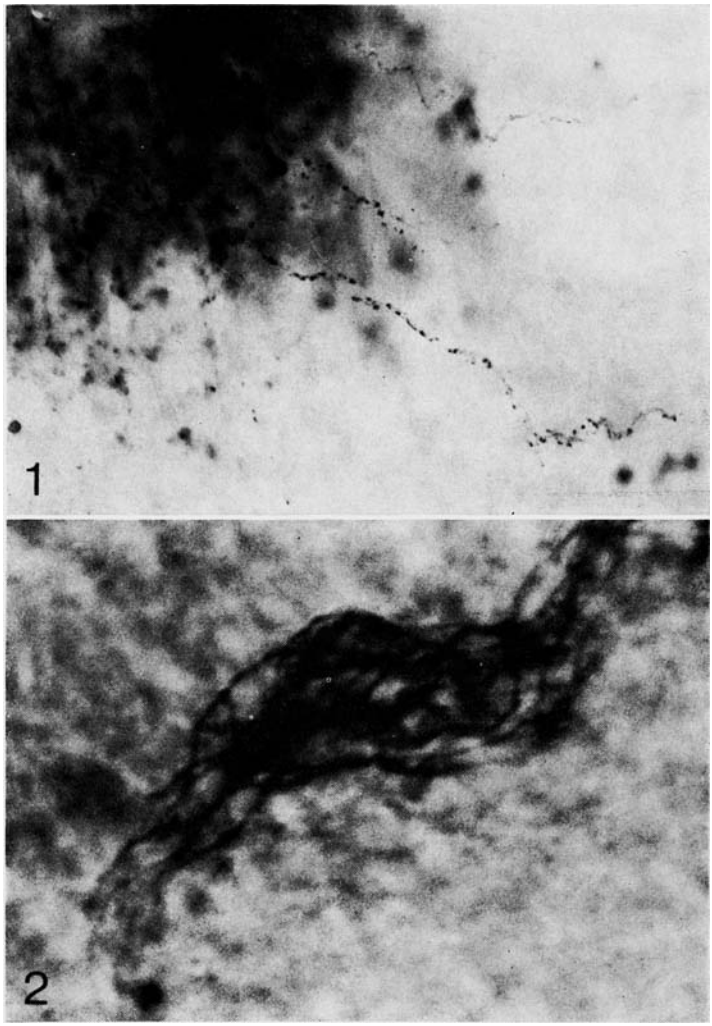

Figure 1. Free fibre ending with "varicosities". No terminal expansions. From original synovium. Methylene blue. × 200.

Figure 2. Complex unencapsulated nerve ending from original synovial tissue. Methylene blue. × 450.

injected the stain into intact tissue in situ. By not using the injection method an interference with the metabolic activity may arise and a disturbance of circulation in the extirpated tissue samples may ensue, which may, perhaps, make the uptake of stain less than optimal. In an immersion study by *Hirsch et al.* (1923) on connective tissues, especi-

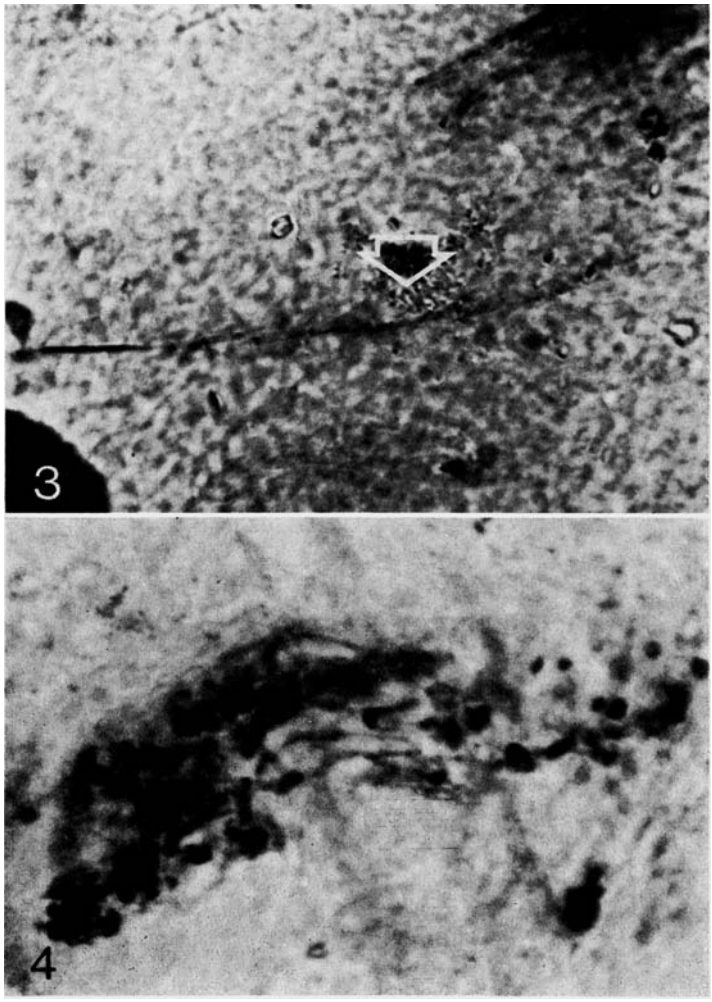

Figure 3. Arrow indicating free fibre ending running transversely in regenerated synovial tissue. Methylene blue. × *200.*

Figure 4. Complex unencapsulated nerve ending with some expanded terminal tips. Regenerated synovial tissue. Methylene blue. × *450.*

ally ligamentous, capsular and intervertebral disc structures, satisfactory results were achieved which no doubt justifies the employment of the method as described.

Second, in contrast to other investigators' techniques (*Hirsch et al.* 1963) the specimens in this study were not sectioned. The synovial tissue—both original and regenerated—often appears quite translucent

at excision and it was threfore thought not necessary to do any sectioning. As the method implies a specific pH (about 3.5) for sectioned material it is conceivable that a divergence from staining our specimens in thin sections might not yield the same results. In some samples which were rather large the deepest parts of these did not take up the stain satisfactorily. It was believed that by the thickness of specimens the low pH might in some way limit the uptake of the stain. Tentatively it was thought that the low pH denatures the proteins on the specimen surface producing a coagulum through which the stain could not penetrate and thus leaving the central interior unstained. Therefore different pH values were tested for thick specimens and it was found that staining in pH 7.0 gave a better penetration.

Third, because of poor penetration of the stain in some specimens, which has been discussed above, the course of nerve fibres was difficult at times to follow for any distance since they changed depth in the tissue which could reach 3–5 mm's thickness.

Fourth, because the specimens varied in size from about 5×2 mm to 5×7 mm the times in the staining procedure had to be adjusted empirically to get optimal staining.

These limitations make a statement on the density of innervation difficult, whereas the presence of neural elements can be proved without greater effort. In those cases where some doubt as to the presence of nerves arose verification with the Gros-Bielschowsky was obtained.

Our results confirm the findings of *Gardner* (1950) and *Barnett et al.* (1961) concerning the presence of nerves in original synovial tissues. As mentioned nerve endings of various types were encountered. It is, however, difficult to ascertain anything definite concerning the function of these nerves as their size, 1–3 μ, and difficulty in identification render neurophysiologic investigations difficult. These fibres, however, among other functions seem to play a role in the conduction of pain. It has been assumed (*Hirsch et al.* 1963) that free fibre endings are associated with pain, complex unencapsulated endings with tissue position and encapsulated with pressure perception. As, in this investigation, the different types of nerve endings were observed in the synovial tissues, it may be possible that part of the pain experienced in rheumatoid arthritis is transmitted through the free fibre endings. On the other hand, the surrounding fibrous structures are richly innervated and may either by distension of exsudate or exsudate or by inflammatory, oedematous infiltration create pain. At synovectomy it is impos-

sible not to injure these structures as well, and the absence of pain following synovectomy may be a combined result of the removal of synovial tissue and transection of nerve structures in perisynovial structures.

As regeneration commences the various morphologic elements seen in the original tissue reappear and in this investigation it has been possible to demonstrate the recurrence of nerve fibres and nerve endings of the same type as seen in the original tissue after a period of one year following synovectomy. Thus, free fibre endings and complex unencapsulated endings have been identified. Despite the existing similarities in histologic appearance of original and regenerated synovial tissues in rheumatoid arthritis there may be a variance in function which can serve as an explanation for the regenerated nerve endings not becoming involved in the transmission of painful stimuli.

In osteoarthritis of the hip joint *von Reis* (1945) has shown that denervation of the dorsal part of the 4th lumbar root gives satisfactory relief of pain.

Those joints which become painfree after synovectomy may therefore not necessarily owe their freedom of pain to a temporary denervation but more to a change of the disease process in which the role of synovectomy still remains obscure.

SUMMARY

The purpose of this investigation was to prove the presence of nerve fibres and nerve endings in original and regenerated synovial tissues in patients with rheumatoid arthritis. For this reason samples were obtained from 27 patients with established rheumatoid arthritis. Of these 12 consented to resampling one to three years after synovectomy. 10 were completely symptom-free.

With the methylene blue and Gros-Bielschowsky techniques it was possible to demonstrate nervous structures in original as well as in regenerated synovial tissues. The presence of nerve fibres in the regenerated tissue could be established one year after synovectomy. Free fibre endings and complex unencapsulated nerve endings were observed. Of these, the former are assumed to conduct pain.

RESUME

Le but de cette investigation a été de prouver la présence de fibres nerveuses et d'extrémités terminales de nerfs dans le tissu synovial

original et régénéré chez les malades souffrant d'arthrite rheumatoïde. A cette fin, il a été obtenu des prélèvements de 27 malades souffrant d'arthrite rheumatoïde constatée, Parmi ceux-ci 12 ont volontairement accepté la prise de prélèvements durant une période d'un à trois ans après la synovectomie. 10 d'entre eux n'ont présenté absolument aucun symptome.

Au moyen des techniques au bleu de méthylène et de Gros-Bielschowsky, il a été possible de démontrer des structures nerveuses aussi bien dans le tissu synovial original que régénéré. La présence d'extrémités terminales de fibres libres et des extrémités terminales de nerfs complexes sans gaine a été observée. Parmi celles-ci, on considère que les premières ont pour mission de conduire la douleur.

ZUSAMMENFASSUNG

Der Zweck dieser Untersuchung ist es gewesen, das Vorhandensein von Nervenfasern und Nervenendigungen in ursprünglichen und regeneriertem Synovialgewebe bei Patienten mit chronisch rheumatischer Polyarthritis nachzuweisen. Aus disem Grunde wurden Proben von 27 Patienten, alle mit sicherer Polyarthritis rheumatica, erhalten. Von diesen unterwarfen sich 12 der Probenentnahme ein bis drei Jahre nach der Synovektomie. 10 waren vollständig symptomfrei.

Mittels Methylenblau und Gros-Bielschowsky Technik war es möglich Nervengebilde sowohl im ursprünglichen als auch im regenerierten Synovialgewebe nachzuweisen. Das Vorhandensein von Nervenfasern im regenerierten Gewebe konnte ein Jahr nach der Synovektomie festgestellt werden. Freie Faserendungen und nichteingeschlossene Nervenendungen wurden beobachtet. Von diesen werden die ersteren als schmerzleitend angesehen.

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