

Thesis
for
Degree of Doctor of Medicine

"The Regeneration of Tracheal Cartilages
after Division: an Experimental Research."

by

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I hereby certify that, since obtaining the Degrees of Bachelor of Medicine and Master in Surgery in August 1887, I have been engaged in the practice and study of my profession as follows:-
That, from September 1887 to September 1888, I held the post of Senior House Surgeon in the Cumberland Infirmary, Carlisle; That from October 1888 to January 1889 I practised in Charing Cross and other London hospitals, and that from March 1889 to March 1890 I have studied in the Universities of Vienna, Prague & Göttingen.

I also certify that the accompanying thesis for the Degree of Doctor of Medicine, entitled "An Experimental Inquiry concerning the Regeneration of Tracheal Cartilages after division" has been composed by myself.

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Edinburgh 30th April 1890.

1.

The manner in which cartilage behaves after traumatic injuries and under the action of irritants has been the subject of many researches, but the results obtained have proved, as will shortly be seen, to be of the most contradictory nature. Perhaps the weight of opinion however, inclines to ascribing a subordinate part to the cartilage in the process of healing. The object of the present experimental enquiry was to determine the changes which occur in divided tracheal rings under varying conditions.

Reviewing the literature on the subject chronologically we find that, as early as 1823, Béclard studied the behaviour of the costal cartilages after traumatic lesions, and their capability of regeneration. He bared the cartilages of their perichondrium and found that the cartilage itself produced no granulation tissue but that the loss of substance was repaired by the surrounding parts.

Donders and Meyer¹⁾ observed in the costal cartilages of old people, in addition to the fibrillar degeneration of the matrix, that the cartilage cells were transformed into large cell nests which were closely packed together. In subcutaneous wounds of the articular cartilages Redfern²⁾ found healing took place by the formation of fibrous tissue, consisting of white & yellow fibres,

¹⁾ Müller's Archiv: 1846

²⁾ Monthly Journal of Medical Science. March 1850

the former being produced by metamorphosis of the matrix, and the latter by the nuclei of proliferated cartilage cells becoming longer & changing into fibres.

E. A. Weber¹ made the same observation as Béclard, namely, that divided cartilages were reunited by means of the perichondrium.

According to Billroth², fractures of costal cartilages heal by means of ossifying callus, after vascularization and partial absorption of the fractured ends. And the same author³ observed changes first appear in inflamed cartilage after from 10 to 14 days.

Speaking of wounds of cartilage Rokitanisky⁴ stated: "They are no more united by new formed cartilage than loss of substance in cartilage is repaired"

Lyall⁵ and Thiersch⁶ give drawings of fractures implicating joints, which show the bone firmly united by callus but the articular cartilages still remaining ununited. The former author also says "Loss of substance in cartilage is at best replaced by a fibrous tissue, occasionally resembling cartilage but which is in reality never quite cartilaginous,"

¹ Hildebrandt's Anatomie I 305.

² Handbuch der Chirurgie III 2.

³ Beiträge zur patholog. Histologie 1858

⁴ Lehrbuch der patholog. Anatomie 1855

⁵ Handbuch der Lehre von den Knochenbrüchen 1862

⁶ Die feineren anatom. Vorgänge bei der Wundheilung 1867.

because no regeneration occurs in cartilage." In this opinion he is supported by Kölliker¹ who states "Cartilage does not possess the power of regeneration, nor do wounds of cartilage heal through formation of new cartilage" Up to this date therefore, we find that all investigators with the exception of Redfern, render prominent the fact that the cartilage itself plays a purely passive part in the process of healing.

Reitz² however, whose observations have an especial bearing on the present question from the fact that he examined wounds of the tracheal cartilages, described the cartilage cells as participating in the healing process. Thus, he saw those cells lying next the wound drawn out in their long axis and acquiring long fine processes which passed far out into the wound and united with similar processes from cells in the opposite cut end. He also speaks in a general manner of proliferation of the cartilage cells with simultaneous softening of the matrix, but gives no special description of the process, nor is it represented in his illustrations. From the consideration of the fact that he did not examine his specimens later than the 68th hour after the operation was performed, these rapid changes appear somewhat doubtful; and an examination of his drawings gives one the impression that he mistook the fine threads of coagulated fibrin

¹ Handbuch der Gewebelehre des Menschen 1867

² Sitzungsberichte d. Wiener k. k. Akad. 1867

for the new formed process of the cartilage cells which he describes. Thiersch¹⁾ indeed was of opinion that this so quickly originated tissue might quite well be a product of the surrounding soft parts, and this objection appears justified in so far that Redfern²⁾ observed similar changes occur after a much longer interval. Similar results had according to Meckel³⁾ been obtained by Dornier in 1798 in incised wounds of cartilage in cats.

Archangelzky⁴⁾ described connective tissue elements derived from the perichondrium passing into the wound of the cartilage and becoming gradually converted into true hyaline cartilage.

In suppuration of cartilage Kremiansky⁵⁾ + Brückner⁶⁾ agree in describing proliferation of the cartilage cells and their transformation into pus corpuscles.

Böhm⁷⁾ recognized the capability of the cartilage cells taking part to a slight extent in the formation of granulation tissue.

On the other hand through the experiments of Popp⁸⁾

¹⁾ loc. cit.

²⁾ loc. cit.

³⁾ Handbuch der patholog. Anatomie.

⁴⁾ Centralblatt 1868

⁵⁾ Wiener Med. Wochenschrift 1868

⁶⁾ Ueber Eiterbildung in Hyalinknorpel 1873

⁷⁾ Beitrag zur normal. u. patholog. Anat. d. Gelenke 1868

⁸⁾ Entzündung in Knorpelgewebe. Königsberg 1869.

on the ensiform cartilages of chickens, the opinion that the cartilage behaved passively was confirmed.

Barth¹⁾ agreed with Archangel'sky in stating that the wound edges of the cartilage play a slight or no roll at all in the healing process, but recognized the fact that they were excavated and absorbed by the newly formed connective tissue filling the wound and which subsequently became hyaline cartilage.

The researches of Hutob and Lang²⁾ which appeared about this time are relative to the present question in so far that the former author recognized the capability of cartilage cells proliferating under the action of irritants, while Lang made the same observation in the case of bone corpuscles. Both however observed only retrogressive metamorphoses of the young cells, i.e. the formation of pus from them.

In contradiction to these authors, and especially against Heitzmann's³⁾ views as to the nourishment of hyaline cartilage which were later confirmed by Henocque⁴⁾ and Petrone⁵⁾, we find Colomiatti⁶⁾ directing himself and being supported by various investigators who made observations on the reaction of hyaline cartilage under the influence of various irritants.

¹⁾ Ueber d. Regeneration d. hyalinen Knorpels. Med Centralblatt 1869

²⁾ Wiener Med. Jahresberichte 1871

³⁾ Wiener Med. Jahresberichte 1873

⁴⁾ Gaz. med. de Paris No: 46

⁵⁾ Giornale internat delle Scienze mediche 1879

⁶⁾ Rivista clinica di Bologna 1874.

Among these, Ewetzky¹⁾ described a zone of atrophy of the cartilage cells in the neighbourhood of the wound and a zone of proliferation further removed, the latter, however, not appearing till after the lapse of three weeks.

Genzmer²⁾ on the other hand, found neither proliferation of the cartilage cells nor the formation of pus cells from them, though he described an atrophic zone of cells with swelling and fibrillation of the matrix. Both authors agreed, however, in ascribing to the perichondrium the chief part in the healing process.

Schklarewsky³⁾ who experimented on the costal cartilages of dogs, came to the conclusion that the method of healing was different in 1st and 2nd intention. That, when suppuration occurred, the resulting cicatrix consisted of connective tissue which the cartilage cells assisted in producing. But, that when healing took place by first intention, the cicatrix after three months was composed of fibro-cartilage which in the course of a year became converted into hyaline cartilage; The production of this tissue being brought about by proliferation of the cartilage cells during the first days after the operation.

Tizzoni's⁴⁾ results from experiments on the articular cartilages were almost similar. He described both atrophy and proliferation of the cartilage cells. Where healing

¹⁾ Med. Centralblatt No 16. 1875

²⁾ Virchow's Archiv. 1876

³⁾ Hoffmann u. Schwalbe's Jahresberichte 1876

⁴⁾ Archivis per le scienze mediche 1877

took place by first intention it was by means of proliferation of the cartilage cells which formed either fibro-cartilage or connective tissue. Healing by second intention was brought about by granulations either from the synovial membrane or the marrow, and the connective tissue formed by them was gradually converted into hyaline cartilage by a direct transformation of the connective tissue cells into cartilage cells and the formation of an intercellular substance at first mucous in character but afterwards becoming hyaline. In the case of division of cartilages possessing perichondrium he found that union took place principally by means of the perichondrium, the cartilage cells taking only a very small part in the process.

Peyraud¹⁾ who made his experiments about the same time, found that regeneration of the cartilage took place provided the perichondrium remained intact but that when it was removed there was no regeneration.

In the case of fibro-cartilage Schwalbe²⁾ who operated on the ears of rabbits, found no change in the cartilage cells, the wound being healed by means of the perichondrium which formed new cartilage. And Gudden,³⁾ examining fractures of the ear cartilages, found that the cells were either unaltered or merely atrophied at the seat of the wound, but, that if the perichondrium

¹⁾ Compt. rend. No: 84. 1877.

²⁾ Jena Sitzungsberichte 1878

³⁾ Archiv f. patholog. Anat. u. Physiol. Bd. 51.

was implicated in the injury. new cartilage was formed from it.

Retzius¹⁾ described three changes which might occur in inflamed cartilage. Firstly, it might be transformed into connective tissue. secondly, simple hyperplasia might occur; and thirdly, granulation tissue might be formed by proliferation of the cells both of the cartilage and perichondrium, and this be later transformed into hyaline cartilage.

The results of Lyle's²⁾ experiments on the articular cartilage of dogs are of especial importance in relation to the question of regeneration of cartilage. inasmuch as he excluded all complications by operating aseptically, and by confining the wound strictly to the cartilage, taking precautions not to injure the subjacent tissue. In the neighbourhood of such wounds he found a zone of degenerated cartilage cells and fibrillation of the matrix, while further removed was a zone of proliferating cartilage cells, which first appeared after the lapse of a week. Up to the 150th day after the operation however he could not observe a trace of regeneration but merely a typical picture of arthritis deformans. He thus came to the conclusion that simple aseptic wounds of cartilage do not heal. Where the subchondral tissue was cut into or where the wound was not aseptic, a connective tissue cicatrix

¹⁾ Militärärztl. Journal 1880

²⁾ Deutsche Zeitschrift für Chirurgie 1883.

was formed which became later gradually converted into hyaline cartilage.

The process of healing in wounds of the tracheal cartilages has been carefully studied by Schottelius.¹ He performed tracheotomy on dogs which he made wear a cannula for 48 hours, the wound being subsequently allowed to heal. In his opinion the changes which occur in a divided tracheal ring depend mainly on the course of healing the wound follows. From one of his specimens he infers the possibility of the cartilage directly uniting but states that the usual course is for connective tissue elements to appear from the perichondrium, these sometimes becoming subsequently converted into hyaline cartilage. He describes both a zone of atrophy and proliferation of the cartilage cells, the latter being present by the 5th day; and also the formation of new cartilage both from the perichondrium and the cut surface of the cartilage.

Finally Schüller,² from his experiments on animals, confirmed by observations on the human subject, states that wounds of the tracheal cartilages are not closed cartilaginously, but by means of a more or less firm connective tissue cicatrix. In specimens examined several weeks after the operation he found the cartilages almost unaltered at the seat of the wound. Only the corners were somewhat rounded off and the cartilage

¹ Die Kehlkopf Knorpel Wiesbaden 1879.

² Deutsche Chirurgie Lief: 37.

cells there were pressed flat and arranged in rows across the cut end. He did not observe any proliferation of the cartilage cells.

Flesch⁹ examined a case in which tracheotomy had been performed 15 years previously, and found the cicatrix consisted entirely of connective tissue.

From the above retrospect of the literature on the subject, it will be seen that the cartilage has been described as playing widely different rolls in the healing process. Not only have we the two extremes, on the one hand of its remaining entirely passive and on the other of its reproducing new cartilage, but in addition almost every conceivable intermediate stage is described.

(Sitzungsber. d. Med. Phys. Gesellschft. Würzburg 1879)

In the series of experiments about to be described the animals employed were, with one exception, rabbits; and the method of operating was as follows. The animals being placed on their backs, the four limbs were secured by means of running nooses and the head was fixed in an extended position. The fur on the front of the neck was removed with scissors and then the skin was shaved & purified. An incision about an inch long was made through the skin in the middle line of the neck and the soft parts were divided till the trachea was exposed. The bleeding was minimal and the thyroid gland could be divided with impunity. The trachea was then opened in the middle line, from three to five of the tracheal

rings being divided, and the skin wound was then closed with sutures. In order to determine what influence the irritation produced by a metal cannula exerted on the cartilage, three series of experiments were made:-

1. Simple Tracheotomy; the skin wound being sutured.
2. Tracheotomy with insertion of a cannula for 48 hours, the wound being subsequently allowed to close.
3. Tracheotomy with the cannula worn till the end.

Those cases in which suppuration occurred are put by themselves in a separate division after each series.

The cannula employed was of nickelled metal.

The animals were killed at varying dates after the operation and the tracheas were immediately removed and placed in Flemming's solution for 24 hours, being subsequently hardened in alcohol. The seat of the wound in the trachea was not always easily recognized, being often marked merely by a fine white line, except where one cut end had been displaced in front of the other in which cases a slight elevation could be both seen and felt.

For the purpose of staining cells undergoing karyokinesis, saffranin, carbol-fuchsin and gentian violet according to Gram's method were used; picrocarmine, methylblue and haematoxylin being employed for simple nuclear staining. All the sections were cut in a plane parallel to that of the tracheal rings.

Series I

Cases in which simple tracheotomy was performed and the wound healed by first intention.

3rd Day.

In this case a cat was the animal operated upon. The two cut ends of the divided tracheal ring are separated from each other laterally to a small extent, and one end lies in a plane anterior to the other. In the interval between the two cut surfaces & applied closely to them is a mass of coagulated fibrin, which passes both into the lumen of the trachea and along its outer surface for some distance. Imbedded in its meshes are a number of leucocytes some of which show karyokinesis. The surrounding loose connective tissue has also passed to a certain extent into the wound, and its vessels are considerably dilated and show accumulations of round cells around them. Incidentally may be mentioned the presence of numerous "Mastzelle" in the submucous tissue. The cut surfaces of the cartilage are straight and show no appreciable alterations nor can any changes be seen in the cartilage cells. For a short distance from the wound the perichondrium appears somewhat swollen. The mucous membrane dips down towards the wound and some of its cells show karyokinesis.

5th Day.

The cut ends are separated in a similar manner to that described above, but the cut surfaces are no longer quite straight and show slight irregularities here & there. The intervening fibrin is rather less in amount but contains many more cells some of which are round but others already spindle-shaped. The perichondrium is seen to be considerably swollen for some distance from the wound and some of its cells are dividing. For a short distance from the cut edge the cartilage does not stain well and the cartilage cells there are seen to be shrunken and to have lost their nuclei. Sometimes the cartilage cells are absent altogether in the neighbourhood of the cut surface. The fat globules which are normally present in the cartilage cells have also disappeared in this area. These changes are especially seen in the cut end lying more externally.

6th Day.

The space between the two cut ends is here not quite so wide as previously and both ends lie nearly in the same plane. The intervening tissue has the form of two wedges applied apex to apex and consists mainly of spindle cells, some of which are seen to be dividing. It contains many capillaries and is continuous with the swollen and proliferating perichondrium. The cut surfaces of the cartilage are slightly concave and the free border is irregular in outline. Only the

central portion of the cartilage stains here but further away from the wound the portion that stains gradually increases in size till the whole of the cartilage is stained. So that on both inner & outer surfaces of the ring there is an unstained portion of cartilage which gradually diminishes in size as one passes away from the wound. The stained portion is consequently wedged shaped in appearance with the apex towards the wound. The cartilage cells near the cut edge show degenerative changes, some being shrunken, others having lost their nuclei and their protoplasm being very granular. Further away from the wound the cells present their normal appearance.

8th Day.

At this date the nucleus membrane is seen to be continuous over the seat of the wound which is marked in it merely by a furrow. The cut ends are displaced in the usual way but the tissue between them is now very vascular and consists entirely of cells mainly spindle shaped, there being no trace of the fibrin left. The nuclei of these cells show various shapes from round to spindle formed. The perichondrium is swollen & contains many more nuclei than normal and is seen to be continuous with the reformed cicatricial tissue. The corners of the cut ends are slightly rounded off and the cut surfaces are very irregular & much excavated. Passing into opened cartilage capsules and filling up the

excavations and irregularities of the cut border are seen the spindle shaped cells of the cicatrix. Here and there a small fragment of cartilage may be seen to be quite separated from the end of the ring by these cells. The cartilage cells near the cut border show the same atrophic and degenerative changes as described above. Nowhere is there any sign of their proliferating.

9th Day

Very similar changes to those just described are seen in specimens from this date. The cicatricial tissue is not quite so much developed nor are the cut surfaces of the cartilage so much excavated. Here and there where the ring has not been completely divided but simply notched a wedge shaped mass of young spindle cells is seen filling up the wound. The surrounding cartilage cells are atrophied and the matrix does not stain well.

11th Day

As before, merely a shallow furrow marks the site of the wound in the mucous membrane. The cicatricial tissue between the cut ends is not so vascular and towards the centre consists mainly of fibres. Next the cut surfaces, however, it is composed of cells and these pass into the excavations in the cartilage. These cells are irregular in shape and appear to be commencing to secrete a finely granular matrix around them. The cut ends of the cartilage still

show the same peculiarities as to staining and the matrix at the free border is ragged and finely striated in appearance. The cartilage cells in the neighbourhood of the cut border show marked atrophic changes, many of them being in an advanced state of fatty degeneration, as seen by the numerous small fat globules in their protoplasm.

The fat cells in the loose connective tissue outside the trachea are seen to show remarkable changes. The areolar tissue is infiltrated to a certain extent with round cells but the main changes are seen in the fat cells themselves. Starting with the normal fat cells at some distance from the wound, the first change observed is that the fat globule diminishes in size and the protoplasm of the cell is seen to distinctly surround it. The decrease in the size of the fat globule continues along with an apparent increase in the amount of protoplasm. At the same time there is seen to be a great increase in the number of the nuclei. That this takes place by karyokinesis appears probable from the fact that in a few cells in the first stage the typical figures of cell division were seen, though the number of such cells was certainly small. The process goes on till the fat globule has entirely disappeared and the fat cell is then represented by a large granular cell containing an enormous number of nuclei, as many as 20 or 30 being sometimes counted. It then has the appearance of an ordinary "giant cell". While the fat globule remains, the nuclei are often

arranged around it or at one side of the cell, but after its disappearance they appear to have no particular disposition in the cell, sometimes lying centrally, at others peripherally, or scattered throughout the whole cell. Some of these "giant cells" are seen to undergo fatty degeneration and so to disappear but in others the protoplasm seems to break up into a number of cells. In the connective tissue between the fat cells can be seen here & there rounded cells with transparent protoplasm and large rounded coarsely granular but highly refracting nuclei.

12th Day.

Here the two ends of the divided tracheal ring, owing to one being pushed behind the other, are applied to each other in such a way that the outer surface of the inner one is in contact with the inner surface of the outer. Otherwise very much the same appearances are seen as in the former case, namely, the cut ends being excavated and the cells of the cicatrix passing into and filling up the irregularities in the cartilage. There is also a similar atrophy of the cartilage cells in the neighbourhood of the wound. The excavation & absorption of the cartilage by the cells of the cicatrix is seen to take place to the greatest extent in that part of the cartilage which did not stain.

14th Day.

The divided ends are applied to each other in a similar manner to that just described. Though the cut surfaces are still excavated they are not so to such an extent as previously. The spindle and irregularly shaped cells of the cicatrix still pass into the opened cartilage cell capsules and the intervening matrix, but they appear to be commencing to secrete a matrix around them which is almost hyaline in character and which passes imperceptibly into the old matrix.

20th Day.

The cut ends are somewhat everted but are united by a new tissue cartilaginous in appearance. This has the form of a bridge between the two cut ends but extends only from the inner half of the one cut surface to the other. The outer half of the cut border on each side is covered by the external perichondrium which bends over the corner and then applies itself to the cut surface down as far as the middle. There it runs along the outer surface of the bridge of new tissue and joins with that of the opposite side. The inner perichondrium leaves the old cartilage at some distance from the wound and joins directly with that of the other cut end. So that the angle formed between the two ends internally is also filled with the new cartilaginous tissue. This new tissue consists of young cartilage cells closely packed together, and contains but little

intercellular substance. The latter is mainly finely fibrillar in appearance but here there seems to be almost hyaline. That this new tissue has been formed from the perichondrium there can be no doubt as the transition from the one to the other is very distinctly seen. Thus most externally are the densely arranged fibres of the outer perichondrium. Further in the structure is looser and more nuclei are seen. These have at first a spindle form but then become rod-shaped and finally round. At the same time the cell body becomes rounder and larger till finally typical young cartilage cells are seen. The line of separation between the old and new cartilage can still be easily recognized as the former stains more deeply and its cells are larger. There is no sign of proliferation in the old cartilage cells. In some preparations no union of the divided ends has taken place owing apparently to the presence of fat tissue between them. In these cases the perichondrium covers over the cut ends and a very small amount of new cartilage has been produced by it. But in other specimens the divided ends have been so closely applied to each other that there is merely a wedge shaped piece of new cartilaginous tissue passing in between them externally for some distance and internally a small amount of new cartilage has been formed from the inner perichondrium which fills up the angle formed by them internally. It is difficult to say whether the cartilage has

united directly in the middle or not. As a rule a small amount of new tissue can be seen passing in between the two ends there also. Where a ring has been merely notched the perichondrium is seen filling up the gap and forming new cartilage as already described. None of the old cartilage cells around are proliferating but there is a narrow zone of matrix around such notches which is devoid of cartilage cells. They having atrophied & disappeared.

24th Day.

Here the healing process is not quite so far advanced as in the previous case. The cut ends are everted as usual but their cut surfaces are almost in contact in their inner halves. The angle between them internally is seen to be filled with young cartilage tissue derived from the inner perichondrium as in the previous case. Externally, however, the wedged mass of new tissue passing between them still consists principally of spindle and irregularly shaped cells which are just commencing to secrete a matrix around them. There is no proliferation of the old cartilage cells to be seen.

30th Day.

The cut ends are separated by a considerable interval and the cicatricial tissue between them consists of spindle shaped cells. The perichondrium is seen to be as usual, continuous with this tissue.

The cut ends show the same staining changes as already described and are much excavated by the cells of the cicatricial tissue. Of these, those next to the excavated border of the cartilage are larger and rounder in shape than those further removed which are smaller and spindle shaped and gradually pass into ordinary connective tissue. The former appear to be secreting a finely fibrillar matrix around them. There is no sign of proliferation in the cartilage cells which appear almost unaltered up to the excavated border.

40th Day.

The cut ends are displaced in such a manner that the cut surface of the one is applied to the inner surface of the other. Filling up the small interval between them is young cartilaginous tissue similar to that already described, consisting of densely packed cells with little intercellular substance. That this has been derived from the perichondrium is quite evident, as it passes gradually into it, whereas there is a distinct line of separation between it and the old cartilage. The inner perichondrium has formed ^{new} cartilage for some distance on either side of the wound. The outer perichondrium runs without a break across the wound and passes gradually into the young cartilage between the cut ends. The outer corner of the externally lying end is rounded off, as is likewise the inner corner of the internally lying one. The perichondrium

is closely applied to these rounded surfaces. At these points the matrix does not stain & contains few cartilage cells. Those present are shrivelled and atrophied and have lost their contour. Occasionally only a globule of fat remains to show where a cartilage cell has been. Further away from this rounded border there is a zone of proliferating cartilage cells, that is to say, large capsules containing six, eight or ten cells. Still further away the cells are normal in appearance. These young cells in the capsules are granular in appearance and do not stain well. Some of them contain fat globules, others do not. The rounded border is as a rule smooth but here & there a spindle cell from the perichondrium is seen passing for some distance into the cartilage matrix, and occasionally a proliferating capsule lying nearer the edge than usual is seen to be opened into by these cells. None of these proliferating cells are seen in the cartilage except at the rounded off borders. Where the new cartilage joins the old, there is no sign of proliferation of the old cartilage cells, and the line of separation between them is quite distinct, on the one side there being the ordinary cartilage cells in the old matrix and on the other the young smaller cartilage cells closely packed together and with little intercellular substance. Karyokinesis was not observed in the proliferating cartilage cells.

50th Day.

Here the cut ends are united by a band of firm connective tissue derived from the perichondrium, which is seen to be thickened for some distance from the wound and to bend over rapidly itself closely to the cut surface. It then joins the perichondrium from the other side by a band of connective tissue stretching between the two cut ends. These themselves show very little alteration, the corners being occasionally slightly rounded off. The cartilage cells next the cut surface are flattened and seem to have been derived from the perichondrium.

60th Day.

In most of the preparations the wound is seen to be closed by a firm connective tissue cicatrix, continuous with the perichondrium and similar to that just described. Some of the divided rings however are united by a band of young cartilage similar to that previously described and also derived from the perichondrium. There are intermediate stages between these two extremes, in which the new cartilage formed from the perichondrium extends a certain distance across the wound and then joins that from the opposite side by means of a connective tissue band into which it gradually passes. The line of demarcation between old and new cartilage is still distinctly seen and the former is unaltered in appearance.

Cases in which Suppuration occurred.

4th Day.

There was a small abscess in the neck which communicated with the wound in the trachea. A mass of granular debris is in contact with the cut ends. These appear somewhat swollen and do not stain well for some distance; and the cut surface is slightly concave.

The cartilage cells nearest the wound are to some extent shrunken and have lost their nuclei and fat globules. Otherwise they exhibit no changes. Karyokinesis is seen in the cells of the mucous membrane near the wound.

6th Day.

There was a large abscess in the neck and the tracheal wound was gaping. Pneumonia was also present.

For a considerable distance from the wound the cartilage does not stain and in this area it is swollen, opaque and yellow in colour. The perichondrium shows the same appearances and its nuclei cannot be distinguished.

The cartilage cells in the unstained area are shrunken, faint in outline, and have lost their nuclei and fat globules. Further removed they are normal in appearance and show no sign of proliferation. The cut surface has a frayed and ragged appearance.

25th Day.

In this case there was an immense amount of subcutaneous emphysema two days after the operation.

and the wound had consequently to be reopened. When killed, an abscess was found in the neck but the tracheal wound appeared to be closed.

Between the cut ends are a number of round and spindle cells which, however, are in a state of advanced fatty degeneration. The cut surfaces of the cartilage are excavated, frayed and ragged; and the neighbouring cartilage cells show the usual signs of atrophy.

Series II

Cases in which a cannula was worn for 48 hours, after which the wound was allowed to close.

5th Day.

The cut ends are separated by a mass of coagulated fibrin containing many cells and passing both into the lumen of the trachea and into the loose connective tissue externally for some distance. The cartilage does not stain at the cut border nor for some distance from it and appears slightly swollen. The cut border is rough and irregular showing commencing excavation.

Karyokinesis in various stages is seen in many of the cells of the mucous membrane where it has been divided, and also in many of the cells entangled in the meshes of the fibrin, but no sign of it is seen in the cartilage cells. In the neighbourhood of the wound the latter show the usual atrophic changes, the nuclei having either disappeared or being shrunken and

the cell protoplasm being much vacuolated. The fat globules have also disappeared from the cells in the neighbourhood of the wound. The perichondrium is seen to be swollen but it does not extend further than the wound. The fat tissue lying externally is infiltrated to a small extent with small cells which are seen proliferating. Some of the fat cells contain two or three nuclei and occasionally karyokinesis can be observed in them. The round cells with large granular refractive nuclei as already described are also present. In the periphery of the lobule the fat cells present their normal appearance.

8th Day.

The cut ends are separated by a considerable interval and show different changes. Thus the inner cut end has spindle cells derived from the perichondrium in contact with it and they are excavating the wound surface of the cartilage to a considerable extent. There is also a marked atrophic zone of cartilage cells. The outer end, however, has a tissue in contact with it that is not so far developed and consists mainly of round cells in the remains of the fibrin. It is scarcely at all excavated. Both ends, however, exhibit the usual staining changes of the matrix. The mucous membrane has united and dips down into the wound for a short distance.

10th Day.

The cut ends are everted and one is displaced in front of the other. The newly formed vascular cicatricial tissue passes between and around them and consists of well formed spindle cells. The perichondrium is considerably thickened and passes directly into this tissue with which it is continuous. The cut ends show the usual changes with respect to staining and the cut surfaces are excavated in the usual manner by the spindle cells. Their corners are commencing to be a little rounded off. The cartilage cells have lost their fat globules, and show similar atrophic changes to those already described. The surrounding fat tissue also exhibits its peculiar atrophic process. These fat cells in the periphery of the lobule are stained with the osmic acid of the Flemming's solution and exhibit their normal appearance, but more towards the wound they are not stained. The increase in the amount of protoplasm and multiplication of the nuclei along with diminution in the size of the fat globule proceed until the "giant cells" are formed. Some of these are seen lying in the oval space previously occupied by the fat cell and are attached at intervals all round by processes of the cell protoplasm which pass to the border of the space. Only some of the numerous nuclei in these cells stain and possess sharply defined outlines, others being unstained and indistinct.

20th Day.

The cut ends are much closer together than usual and are only very slightly everted. The thickened outer perichondrium dips down slightly into the space between them where it joins with that from the other side. The inner perichondrium is thickened for some distance from the wound and is continuous with that from the other side. The tissue between the cut ends passes gradually into both outer and inner perichondrium, and consists of small rounded and irregularly shaped cells which are lying in a finely fibrillar matrix. The cut surfaces of the cartilage are still excavated to a slight extent but the staining changes are much less pronounced. The cartilage cells up to the excavated border show no change except that those in its immediate neighbourhood are devoid of fat globules.

30th Day.

Here the cut ends are also everted, but they are so close together that the cut surfaces are almost or quite in contact in their inner halves. The outer perichondrium is continuous over the wound and passes into it forming a wedged shaped mass which consists of spindle cells. The inner perichondrium has filled up the angle formed by the cut ends internally by a mass of young cartilage tissue, the matrix of which is either already hyaline or merely slightly granular in appearance. There is usually seen to be a junction of these two tissues formed from the

outer and inner perichondrium respectively, but sometimes the cut surfaces are so close, in contact that it is difficult to say whether there has been any intervening tissue between them or not. In the outer layers of the cut surfaces and corresponding to where the spindle cells are applied to them, the cartilage matrix does not stain for a short distance from the wound. The spindle cells are also seen to be passing into the matrix here for a varying distance. The cartilage cells in this unstained area of matrix have either completely disappeared or appear merely as shrunken granular masses of protoplasm with ill defined borders. Sometimes they are only represented by their fat globules. Some of the cells of the wedged mass derived from the outer perichondrium are rounded in shape and already possess a capsule around them.

Cases in which Suppuration occurred.

5th Day.

There was an abscess in the neck and the tracheal wound was open.

The cut ends of the cartilage do not stain for some distance from the wound and their cut surfaces are irregularly excavated and present a ragged appearance. The cartilage cells in the vicinity of the wound are shrunken and granular. The cut ends are surrounded by a mass of fibrin containing

pus cells and the latter are in contact with the cut surfaces and pass into the excavations.

7th Day.

In this case the wound was also open and suppurating, but it is of especial interest from the fact that the Ray Fungus (*Actinomyces*) was present. A case of actinomyces in an ox had been examined a week previous to the date of operating on this animal and in some manner the disease must have been inoculated.

Surrounding each cut end is a mass of fibrin, the meshes of which are full of round and spindle cells. In this tissue the Ray fungus is seen here & there and it is surrounded by a circumscribed mass of small cells. Its presence was best demonstrated by staining with saffranin, neither picrocarmine nor Gram's method giving satisfactory staining. Between the cut ends is a granular mass of debris. The cut surfaces are irregular and excavated to some extent and show the usual staining changes. The cartilage cells in the neighbourhood present the same degenerated & atrophic appearances as usual. Thus of some nothing is left but a mass of granules which stain deeply, while others are shrunken and have lost their nuclei. Nowhere is there any sign of them proliferating. A few of the fat cells at some distance from the wound show the changes already described; namely, the formation of "giant cells."

9th Day.

At this date after the operation the animal died.

The tracheal wound was gaping and there was suppurative pericarditis and pleurisy present.

Outside the cut ends is a mass of tissue consisting as before of round + spindle cells in a certain amount of fibrin. The cartilage does not stain for some distance from the wound but the cut surface is fairly straight exhibits only slight irregularities.

The matrix here is granular or faintly fibrillar in appearance. There is a mass of granular debris between the two cut surfaces. The cartilage cells in the neighbourhood of the wound have lost their fat globules and are, as a rule, atrophied, shrunken + granular, and the outlines of their capsules are no longer distinct. Sometimes they have disappeared altogether in the vicinity of the wound or only a mass of granules remains to show where they were.

In a number of the sections however, though by far the minority of those examined, there are to be seen near the wound capsules containing three or four cartilage cells. These capsules are larger than usual but the contained cells are small and possess a granular protoplasm. Karyokinesis could not be observed in these cells. Sometimes they are seen to be in a degenerated condition similar to the other cartilage cells.

15th Day.

In this case the animal also died and the same pathological condition of the organs was found as in the previous case.

There is a mass of tissue outside the trachea which consists as before of round & spindle cells. These are seen to gradually break down and form debris towards the cavity of the abscess which was present in the neck. The cut surfaces are irregular and much excavated and the cartilage does not stain for a considerable distance from the wound.

Applied to the cut surfaces is a layer of spindle and round cells which pass from the tissue outside the trachea and fill up the irregularities of the cut edge, into which they pass. The matrix here is finely fibrillar in appearance and the cartilage cells for some distance show the usual degenerative changes, most of them being represented by a mass of granules. There is no sign of proliferation.

Series III

Cases in which the cannula was worn till the animal was killed.

5th Day.

The cut ends show similar staining changes to those described in the preceding series. Their cut surfaces are fairly straight and only slightly

excavated, but the matrix has lost its hyaline appearance and is opaque and granular, and shows commencing fibrillation. The cartilage cells next the wound are in the same condition of degeneration as that already often described. The perichondrium is swollen and infiltrated with small cells for a short distance from the wound. The mucous membrane does not extend quite up to the wound but its cells show karyokinesis in various stages. There is no sign of the cartilage cells proliferating.

8th Day.

There is a large mass of tissue outside the cut ends consisting mainly of round & spindle cells & containing many capillaries. The perichondrium is more swollen & infiltrated than in the previous case. The cut ends show the same staining changes and the cut surfaces, though not much excavated, are ragged and the matrix there shows fibrillation. In the unexcavated area of matrix the cartilage cells show the usual degenerative appearances. Nowhere is there any sign of them proliferating. Karyokinesis can still be observed in the cells of the mucous membrane.

20th Day.

There is a large amount of inflammatory new tissue surrounding the cut ends both externally and submucosally. This consists of spindle cells & fibres and is rich in bloodvessels. The cut

ends are swollen and do not stain for a short distance from the wound, and then only stain in their central portions. Not only the cut surfaces but also the inner outer surfaces of the divided ring are excavated to a great extent by the surrounding tissue, the cells of which pass into the cartilage for varying distances.

That is to say, that portion of the cartilage which does not stain is being absorbed by these cells. Sometimes a small piece of cartilage has been separated from the cut end and it is seen to be burrowed through by the cells of the granulation tissue which open into the cartilage cell capsules & absorb the matrix.

The cartilage cells show only atrophy & fatty degeneration and exhibit no sign of proliferation. The cells of the granulation tissue which pass into the cartilage are not spindle shaped but three cornered or irregular in shape, and those furthest in are round.

Sometimes the perichondrium is separated for some distance from the cartilage by the granulation cells and does not stain. There is still to be seen a certain amount of karyokinesis in the cells of the mucous membrane.

The conclusions we can draw from the above results in regard to the method of healing of wounds of the tracheal cartilages are shortly as follows: -

1. That in simple tracheotomy, without insertion of a cannula and where healing occurs without suppuration the wound is closed by a new tissue of at first spindle shaped cells derived from the perichondrium which replace the fibrin that has previously filled the wound. That for a certain distance the cartilage cells and matrix atrophy & degenerate in consequence of the depression of vitality caused by the injury, and are absorbed by the spindle cells from the perichondrium. That the latter subsequently undergo a transformation into cartilage cells & secrete a hyaline matrix around them thus uniting the two cut ends by a mass of new cartilage. But that when the ends are too widely separated or there is any intervening tissue such as fat between them, union takes place by means of connective tissue only.

2. That, when a cannula was worn for 48 hours and the wound subsequently healed without suppuration, the healing process is practically the same but is slightly delayed and the atrophic processes in the cartilages are more pronounced from the depression of vitality being greater.

3. That, where a cannula was worn till the end, the atrophic changes are most marked and that the absorption of the cartilage probably goes on until a passage is formed for the cannula.

4. That, in cases where suppuration occurred, the atrophic & degenerative changes are also strongly pronounced sometimes, passing into actual necrosis both of the cartilage & perichondrium.

5. That proliferation of the cartilage cells occurs as the exception — only in two cases; and, that this proliferation is not regenerative but degenerative in nature. The reasons for this view are that, in the one case (I Series 40th Day) the proliferation was seen to be present in an area of matrix where absorption was going on by the perichondrium, that no new cartilage was produced by the proliferation and that some of the new cells were seen degenerating. In the other case (II Series 9th Day) suppuration occurred and the proliferation was next to the suppurating area. Some of the cells here were also seen to be degenerating.

This view is supported by various facts. Thus Gies¹⁾ in his experiments on the articular cartilages observed proliferation of the cartilage cells to occur but no new cartilage was formed from them, the new cells simply degenerating. Also, in Arthritis Deformans, proliferation of the cartilage cells occurs of a degenerative nature. And in the ribs cartilages of old people a senile degeneration occurs which is characterized by proliferation of the cartilage cells. The same occurs in the tracheal rings.

¹⁾ loc. cit.

6. Finally, that cartilage does not possess the power of regeneration after traumatic injuries and does not merely remain passive but actively degenerates.

The case in which actinomycosis was accidentally inoculated is interesting because "Eriksen" states that the disease has not been successfully inoculated on rabbits.

The changes that occurred in the fat cells are also of interest as they offer another example of a form of proliferative degeneration. The atrophy of fat cells in inflammation was first studied by Czajewicz², who came to the conclusion that an endogenous new formation of cells took place under its influence. Their behaviour both in inflammation and simple atrophy has been investigated by Flemming³ who distinguishes three different forms of atrophy, namely,

1. Simple atrophy, in which the fat cell as a whole becomes smaller, the protoplasm following the disappearance of the fat drop so that no space is formed between them in the cell.

¹ "Science and Art of Surgery."

² Mikroskop. Untersuch. üb. d. Textur, Entwickl. Rückbildung u. d. Fettzelle. Reichel + du Bois Reymonds Archiv. 1866

³ Ueber Bildung u. Rückbildung d. Fettzelle. Archiv f. Mikr. Anat. 7. 1870

Ueber Veränderungen d. Fettzelle bei Atrophie etc. Virchow Archiv 52. 1871

2. Serous atrophy, a space being formed in the cell which is filled with clear fluid. Later the cell shrinks.

3. Proliferative atrophy, in which there is an increase in the number of nuclei. This form he found occurred also in animals which were starved.

It was this last form, of proliferative atrophy, which was principally observed. Up to the present time karyokinesis has not been observed in this process.

It is interesting to note that merely the nuclei divide and not the cell protoplasm. Occasionally the protoplasm seemed to split up into a number of cells at a later date.

In conclusion I wish to take this opportunity of expressing my thanks to Prof. Orth in Göttingen, in whose laboratory the experiments were carried out, and to whom I am indebted for much kindly assistance and valuable advice.

W. Carr Sprague.