REGENERATION OF AUERBACH'S PLEXUS IN THE SMALL INTESTINE.

By WALTER J. MEEK.

[From the Physiological Laboratory of the University of Wisconsin.]

In a previous communication on this subject from Dr. Carlson's laboratory, it was shown that after transection of the small intestine, physiological restoration, as determined by the passage of a peristaltic wave across the lesion, could be demonstrated as early as the eighth day. On closer analysis this peristaltic wave was found to depend for its transmission on a purely mechanical factor, the tug of the musculature at the line of transection. The passage of a wave of contraction was thus shown to be insufficient as a test for the regeneration of the nervous motor mechanism of the intestine. Recourse was then had to histological methods. By means of the gold chloride stain regeneration of certain fibres in Auerbach's plexus was shown in one of six dogs, one hundred and eighty days after transection. Unfortunately the origin of the fibres was not definitely known. Instead of being outgrowths of nerve cells in the intestinal plexus, they might have been postganglionic axones from extrinsic abdominal ganglia.

The problem has been taken up anew in hopes of being able to demonstrate conclusively a regeneration of the plexus of Auerbach accompanied by a return of complete physiological function. Regeneration has therefore been tested both histologically and physiologically. Histologically the gold chloride stain was again used and reduction brought about in arsenic acid. A more complete description of the technique will be found in the protocols.

Physiologically regeneration has been tested by the passage of a wave of inhibition through the transected areas. Bayliss and Starling in their well-known papers have shown that the contraction

1 MEEK: This journal, 1910, xxv, p. 367.
2 BAYLISS and STARLING: Journal of physiology, 1899, xxiv, p. 99.
wave in the intestine is a complicated phenomenon, the musculature being contracted above and inhibited for some distance below the point of stimulation. All investigators agree in assigning this complex reflex to the intrinsic nervous mechanism of the intestine. The reflex remains when the mesenteric nerves are degenerated.\(^3\) Pithing the cord does not eliminate it. It is inconceivable, as Bayliss and Starling have remarked, that muscular tissue could respond on one side of the stimulation by contraction and on the other by inhibition. Our own experiments show that the return of conduction for this wave of inhibition takes place at a time much later than the regeneration of the muscular coats. And finally Magnus\(^4\) found that the law of the intestine could not be demonstrated if the plexus of Auerbach were stripped off with the longitudinal muscular coat. It would seem therefore beyond question that the conduction of this wave of inhibition is taken care of by Auerbach's plexus, and the occurrence of this wave would therefore demonstrate the continuity of that nervous mechanism.

**METHODS.**

Cats and dogs were again used in the experiments. These animals had the small intestine transected and were then allowed to live from nineteen to two hundred and forty days, when they were used to test the passage of a wave of inhibition across the lesion. In our previous work the muscular coats had been cut down to the mucosa and an end to end anastomosis made. In this way the lumen of the intestine was unopened, an apt union of the layers was insured, and only a small amount of connective tissue developed in the scar. This method was again used on the cats.

In the experiments on dogs a different technique was employed. Dr. S. A. Matthews had noted in some intestinal work that a glass tube tied into a duct or loop of intestine in time passed out, the ligatures that held it having gradually cut through the tissues. This suggested that transection might be brought about by tying glass tubes in the intestine. A piece of glass tubing 5 cm. long and 1 cm. in diameter with the ends double flanged was slipped into the lumen of the intestine and then securely tied in place by means of two liga-

\(^2\) **LANGLEY** and **MAGNUS**: Journal of physiology, 1905, xxxiii, p. 34.

\(^4\) **MAGNUS**: Archiv für die gesammte Physiologie, 1904, cii, p. 123.
tures which encircled the gut exactly over the flanges. Silk twist, size C, was found convenient for the ligatures. Being tied tightly, the ligatures gradually transected the gut, and when they finally cut through the walls of the intestine the glass tube was of course freed and passed out with the feces. The tube was usually recovered at the end of seven days. The intestinal contents pass through a tube in such a position readily, and no obstruction was ever apparent. To make sure of an abundance of material two of the dogs were subjected to the procedure twice, making four transections in each.

This method of section is especially suitable for regeneration work. The layers are left exactly end to end and a minimum of scar tissue is produced. So little scar tissue is found and so scant are the adhesions that in one dog which had lived two hundred and forty days after the operation, the lines of transection could not be located until the intestine was opened and the mucosa scraped away. The line of section then showed as a white line around the intestine. In all other cases this white ring marking the lesion was apparent in the living animal, and by means of it and the recognition stitches absolute identification of the place was possible.

Records of the intestinal movements were made with a balloon and a Marey tambour. The balloon consisted of thin rubber fastened over the end of a small curved glass tube, all of which could be inserted into a coil of the gut so that the balloon rested a few centimetres below the line of section. The balloon was connected by rubber tubing to a recording tambour and to a small atomizer bulb which was used to raise the pressure. A manometer was not kept in connection, but the pressure was about 10 to 15 cm. of water. When the balloon was placed in the proper position, the lever of the tambour recorded the rhythmical contractions of the circular muscular coat. By stimulating a point above the balloon the rhythmical movements were at once abolished, which gave graphic evidence that a wave of inhibition had swept down the intestine.

**DESCRIPTION OF EXPERIMENTS.**

The first series consisted of five cats. These animals were used to find out whether complete physiological regeneration ever occurred, and if so at approximately what time after the transection. Cats
Regeneration of Auerbach's Plexus in the Small Intestine.

Nos. 1, 2, and 3 were operated on as described above, and kept for one hundred and eighty, two hundred and eleven, and two hundred and forty days respectively. One of the protocols will best illustrate the entire procedure.

Cat No. 2. — August 15. Intestine transected by cutting the muscular coats down to the mucosa. Rapid recovery.

March 18. Cat given 20 c.c. castor oil.

March 19. Two hundred and sixteen days later tested for passage of wave of inhibition across the line of transection.

9.15 A.M. Ether anesthesia. Tracheotomy.


9.35 A.M. Skin of abdomen opened along median line, reflected, and sewed to an iron ring. Cavity thus formed filled with warm saline. Abdomen opened and loop with transection drawn out.

9.50 A.M. Balloon placed in gut well below line of section. Circular muscles immediately began to show typical rhythmical movements.

9.55 A.M. Pinched intestine with forceps 5 cm. above lesion. Slight relaxation in parts surrounding the balloon.

10.11 A.M. Crystal of salt placed on intestine 2 cm. above section. No result except a marked local contraction.

10.19 A.M. Pinched lightly 2 cm. above section. Muscle around balloon inhibited ten to fifteen seconds, when rhythmical movements again gradually returned. (See Fig. 1.)

10.37 A.M. Salt crystal again applied above lesion. Marked constriction with inhibition over balloon.

10.42 A.M. Pinched above line of transection. Contraction at point stimulated and inhibition shown by balloon.

10.54 A.M. Cat used for injections not bearing on this problem.

From the protocol it will be seen that rhythmical contractions of the circular muscular coat below the line of transection were abolished by stimulation above. Fig. 1 illustrates this point. Cats Nos. 1 and 3 gave similar results. In each one a wave of inhibition was shown to pass the lesion. It is not thought necessary to give the protocols, since they are so nearly identical with the preceding. Intestinal movements are demonstrated with some difficulty in cats, but these rhythmical movements of the circular coat with inhibition below the point of stimulation were shown in every animal with ease. Giving castor oil to clear the intestines and eliminating extrinsic nervous
effects by pithing as suggested by Bayliss and Starling make the results almost certain. It will be noted that results were not so consistent when salt was used as a stimulus. In later experiments this method of stimulation was discarded, and pinching lightly with a blunt pair of forceps was used exclusively.

It would seem clear from the experiments just cited that a complete physiological regeneration occurs, and it may be looked for at least as early as the one hundred and eightieth day. The next point undertaken was to show that this complete return of function is associated with a tissue regenerating much more slowly than muscle, that is, the nerve plexus. Previous work has shown that the longitudinal coat of the cat's intestine may be almost perfectly renewed in nine days. Sections made from seventeen days on present a picture of complete muscular regeneration. Two cats, Nos. 4 and 5, were accordingly tested for passage of the inhibitory wave nineteen and forty days after transection. In each of these animals rhythmical contractions below the line of transection could not be inhibited by stimulation above. The protocols are unnecessary, since the technique was precisely the same as in the previous experiments. Fig. 2 is a tracing from Cat No. 5. The balloon was 5 cm. below the transection. At the signal marked 4 the gut was pinched 2 cm. above the lesion. No inhibition occurred. At the signal marked 5 a stimulus was applied as closely as possible below the line of section. As can be seen, a marked inhibition of the rhythmical contractions at once resulted. In both animals these results were obtained repeatedly, and not a single time did the wave of inhibition break through. We

\[ \text{Figure 1. — Showing the passage of a wave of inhibition across the line of transection in a cat two hundred and sixteen days after the transection was made. The arrow shows stimulation above the line of section.} \]
Regeneration of Auerbach's Plexus in the Small Intestine. 357

have seldom succeeded in staining the plexus of the cat intestine very successfully, and for that reason an histological examination was not made in the preceding experiments.

It would be interesting but relatively unimportant to know just what interval is required after transection to make regeneration of function complete, but no attempt was made to answer that question. The work seems to show conclusively that complete return of function is associated with some tissue regenerating after the fortieth day, presumably the nerve plexus.

The second series of experiments was carried out on four dogs. These animals had the intestines transected by means of ligatures tied around glass tubes as previously described, and they were then allowed to live two hundred and thirty, two hundred and twenty-five, one hundred and sixty-seven, and one hundred and twenty-two days respectively. The purpose of these experiments was to show the passage of the inhibitory wave across the line of transection in the dog, and in the same specimen demonstrate histologically the regeneration of plexus fibres. The plexus of the dog fortunately is usually stained

Figure 2. — Showing the failure of inhibition to pass the line of transection in a cat forty days after the transection was made. At 4 the intestine was stimulated above the transection. The rhythmical movements continue. At 5 a stimulus is applied just below the transection. The rhythmical contractions are at once abolished.

Figure 3. — Showing passage of the wave of inhibition across the line of transection in a dog two hundred and thirty days after the transection was made. At the signal the intestine was stimulated by pinching 4 cm. above the section.
with little difficulty. The protocol for Dog No. 1 will illustrate the entire procedure and present the results at the same time.

**Dog No. 1.**—August 19. Intestine transected by inserting a glass tube and ligating around the intestine.

April 6. Dog given 30 c.c. castor oil.

April 7. 1.45 P. M. Given 1 grain morphine.

2.30 P. M. Ether anesthesia. Tracheotomy. Skin of abdomen opened, reflected and sewed to an iron ring. Cavity thus formed filled with warm saline. Abdomen opened and loop of intestine with transection drawn out.

2.50 P. M. Balloon inserted in loop of intestine 6 cm. below transection. Rhythmical contractions of circular muscular coat at once recorded by tambour.

3.00 P. M. Stimulated by pinching 4 cm. above line of section. Contraction recorded by tambour at once inhibited.

3.05 P. M. Repeated above procedure with same results. (See Fig. 3.)

3.08 P. M. Repeated procedure with same results.

3.11 P. M. Repeated procedure with same results.

3.20 P. M. Cut longitudinal slit in intestine just above balloon to relieve air pressure.

3.22 P. M. Pinched 2 cm. above lesion. Contraction below transection at once inhibited.

3.30 P. M. Dog killed. Area with transection removed. Mucosa scraped off with scalpel. Placed in 0.5 per cent arsenic acid for thirty...
Regeneration of Auerbach's Plexus in the Small Intestine.

minutes, in gold chloride for forty-five minutes, and then reduced in 1 per cent arsenic acid over the water bath for fifteen minutes.

Fig. 3 shows the passage of a wave of inhibition across the transected area in the above experiment. In Fig. 4 is presented the appearance of a small portion of the transection after being treated with the gold chloride. Fibres may be seen crossing the scar tissue and entering the plexuses on either side. The findings just mentioned were exactly duplicated in two of the other dogs. No tracing was taken from Dog No. 2 because of some question as to the exact location of the transection, the recognition stitches for some reason having disappeared. The transected portions of all four dogs gave many examples of nerve fibres extending across the scars. The number of axones passing across amounts in itself almost to a demonstration of plexus regeneration. Fig. 5 is a tracing from Dog No. 4, one hundred and twenty-two days after transection. The balloon was well below the line of transection. At the signal marked 1 the intestine was pinched lightly 8 cm. above, and at the signal marked 2, 15 cm. above the transected area. The wave of inhibition

**Figure 5.** — Showing the passage of inhibition across lines of transection one hundred and twenty-two days after the transections were made. Stimulation at 1 was 8 cm. and at 2 15 cm. above line of section. In the case of 2 the wave of inhibition passes through two transections.
Walter J. Meek.

in this case passed down the intestine about 20 cm. and crossed two transections.

The above experiments seem to offer conclusive proof that in dogs as well as cats there is a complete return of physiological function after transection of the small intestine, and this return of function is accompanied by and no doubt depends on a regeneration of Auerbach's plexus.

SUMMARY.

The small intestine of cats and dogs was transected in order that regeneration of Auerbach's plexus might be tested at a later time.

Complete physiological regeneration as determined by the passage of a wave of inhibition across the line of transection has been demonstrated in three cats and three dogs. This return of function occurs in dogs as early as the one hundred and twenty-second day.

In four dogs this return of physiological function was shown to be accompanied by a growth of nerve fibres across the line of transection.

In cats it has been found that the wave of inhibition does not pass the line of transection until late in the course of recovery. Passage was impossible at the fortieth day, yet at this time muscular and epithelial regeneration is practically complete. Return of function is therefore associated with a slowly regenerating tissue, presumably the nerve plexus.

The above evidence we believe amounts to a demonstration of the regeneration of Auerbach's plexus in the cat and dog, accompanied by a return of complete physiological function.