MORPHOLOGY AND PATHOMORPHOLOGY

CERTAIN MORPHOLOGICAL ALTERATIONS IN ISOLATED PORTIONS OF THE INTESTINE AFTER THEIR DENERVATION

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When investigating the effects produced upon intestinal secretion by an interruption of the mesenteric nerves, one of the authors [2, 3] discovered that in dogs this "denervation" of portions of the intestine leads to pronounced alterations, both quantitatively in the volume of the secretion and in the qualitative composition of the enzymes present in the secretion.

In the present study our aim was to investigate the morphological alterations that take place in the mucosa and intramural nervous system of these portions and compare the results obtained with the known data on their secretory function.

The studies were conducted on dogs with two adjacent portions of the small intestine which had been isolated. One of the portions was operated following the method of Thiry, while the second was "denervated" by one of two methods: 1 to 5 months after having transplanted the portion under the abdominal skin of the animal, the nerve vascular bundle of this region was transected or else, while the isolated portion was being prepared, the visible mesenteric nerves were cut and the vessels were painted with a 5% solution of phenol. The intramural portion of the nervous system was maintained in these isolated bowel loops besides the fact that new nerves would grow into them so that the term, "denervated," really must be placed within the quotation marks as it is not true, strictly speaking.

Within 1-2 days following "denervation" by either method there took place a marked increase in the amount of the liquid volume of the secretion and also of the quantity of the solids in it so that "mucous clumps" were formed. However, the amount of enzymes produced per unit of time fell off markedly. This continued for 6-10 days. By the 12 to 20th day, the amount of the intestinal juice produced by the "denervated" portions fell to the level characteristic for Thiry fistulae. Then the secretion continued to diminish to levels 5-10 times lower and continued on this lower level all the time that these "denervated" transplanted subcutaneous intestinal segments continued to be observed. The segments which were allowed to stay within the abdominal cavity, after $2\frac{1}{2}$-3 months, would start to have an increase in the secretory levels although it never reached the levels characteristic for Thiry fistulae.

One to $1\frac{1}{2}$ years following the "denervation" 6 dogs were subjected to morphological studies. In 4 dogs biopsies were taken from the isolated portions, both Thiry and "denervated," while 2 dogs were sacrificed. Morphological studies were made on 4 dogs with isolated segments of the upper region of the small intestine while in 2 dogs the isolated segments were in the terminal portions of the ileum.

Snips from the isolated segments were placed in 12% solutions of neutral formalin. The mucous membrane stain was hematoxylin – eosin while the intramural nervous system was investigated by the use of the method of Bielschowsky-Gross.* Additionally, 3 dogs had two intestinal areas, one above and one below the operative site of the resection (as determined by the scar), subjected to microscopic studies.

EXPERIMENTAL RESULTS

Macroscopic investigation showed that the "denervated" intestinal segments, transplanted under the skin, atrophied markedly shrinking to half the original size. The diameter diminished in proportion. The "denervated" portions permitted to stay within the abdominal cavity macroscopically could not be distinguished from the usual Thiry fistula segments, the dimensions remaining unaltered.

Mucous membrane. Histological studies of the walls of the isolated segments operated by the Thiry method showed no pathological alterations. In these portions, as well as in the normally functioning intestine, the thickness of the mucosa, the frequency of the distribution of the villi and their dimensions were all the usual. The villous epithelium was high, prismatic; the cell nuclei were all on one level and the surface cupping was clearly outlined. The intestinal epithelium maintained the various stages of the secretory process [1]. The stroma of most of the villi was covered by an even layer of epithelium while on the sides of the villi the epithelium could be observed being gathered into folds or actually beginning to separate. The tops of some of the villi had some small tubular epithelial formations. In some areas between the villi much mucus, secreted by the goblet cells, could be observed gathered together. The mucosal epithelial layer itself was somewhat infiltrated by leucocytic elements. The intestinal crypts seemed to be built and distributed quite normally. The epithelium of the crypts evidenced mitotic figures (Fig. 1,a).

In the isolated portions, "denervated" by transections of the nervous twigs and allowed to remain within the abdomen, the mucous membrane looked hardly different from the mucous membrane lining the usual Thiry segments. Only in occasional places were there fewer villi and crypts. Within the epithelial layer there could be seen a greater number of goblet cells. The mucous membrane did not appear to exhibit other changes (Fig. 1,b).

In the isolated segments, "denervated" and transplanted subcutaneously, the epithelium evidenced severe atrophy. It was much thinner than the segments isolated by the Thiry method and permitted to remain within the abdomen. The shape of the villi altered markedly, the form becoming irregular and the number much less. The intestinal crypts became less frequent and more shallow at the same time. There was an increase in the quantity of interstitial connective tissue within the epithelial layer itself. The surface of the mucous membrane of the isolated segments of the lower portions of the small intestine became covered with cubical epithelium. In some areas of the epithelium the covering became uneven. In sections taken from the upper regions of the small intestine the epithelium, while remaining comparatively high, developed unequal cells of varying heights having uneven apices, the surface indentation being sometimes absent and the cell edges losing their sharp borders. There was no evidence of epithelial accumulation on the surfaces of the villi and only occasional villi had a little exfoliation of the epithelium (Fig. 1, c). The smooth muscle fibers within the epithelial layer and within the external muscular layer of the "denervated" sections which had been transplanted subcutaneously frequently displayed wrinkled, pycnotic nuclei.

Nerve plexi. In the isolated Thiry segments the intramural plexi, both the Meissner and the Auerbach, are difficult to distinguish from the intramural ganglia to be observed in the wall of a normally functioning intestine. Within the segments both the plexi have a larger number of ganglia having a variable number of nerve cells but their bodies appeared to have normal contours and the usual variation in the degree of impregnation from light to much darker. The nuclei were rounded, homogeneous, having nucleoli and the submucous plexi being darker than the cell bodies. The nerve cells within the ganglia were of various dimensions and varying stages of differentiation. The glial content of the nerve ganglia did not differ from the usual (Fig. 2,a).

Within the "denervated" intestinal segments left within the abdominal cavity, the intramural nerve system showed a few changes when compared with the nervous system observed within the isolated Thiry segments. Along with a large number of ganglia having a normal structure, there were encountered neurons manifesting a marked nuclear ectopia. Many nuclei and also the protoplasm had clumps of dark material heavily impregnated with silver as well as vacuoles. Within the Auerbach plexi there could be seen dying neurons with pycnotic nuclei and a vacuolated protoplasm. The nerve fibers seen in the plexi of segments, both "denervated" and

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left within the abdominal cavity as well as within the plexi of the Thiry preparations, could not be really differentiated from those seen in the walls of normally functioning intestine (Fig. 2,b).

Especially marked changes were to be observed within the intramural nervous system of the "denervated" segments which had been transplanted subcutaneously. Practically all the ganglia of the Auerbach plexus and the majority of those in the Meissner plexus had, along with the normal neurons, nerve cells showing degeneration and destruction. In the Meissner plexus of these segments the nerve cells were smaller than in the usually innervated intestinal portions, the neuronal nuclei were rounded, intensively impregnated or having angular shapes. Nucleoli could not be seen in all the nuclei. Around such nuclei could be seen cell debris of uncertain forms. In the Auerbach plexus there could be seen frequently neurons with nuclei lying at the cell periphery, the center and edges being taken up by large vacuoles. Cell shadows without nuclei could also be seen. Many cells were pycnotic and inordinately impregnated with silver. At times, remnants of such cells were encountered. In occasional ganglia of both plexi but especially Meissner, there would be seen just 3-5 sharply altered nerve cells or only satellite material (Fig. 2, c). Thus, when the physiological and morphological alterations in the isolated intestinal segments, whether "denervated" by various methods or left innervated, are compared, there is evident complete correlation between the morphological changes and the functional capabilities of these segments.

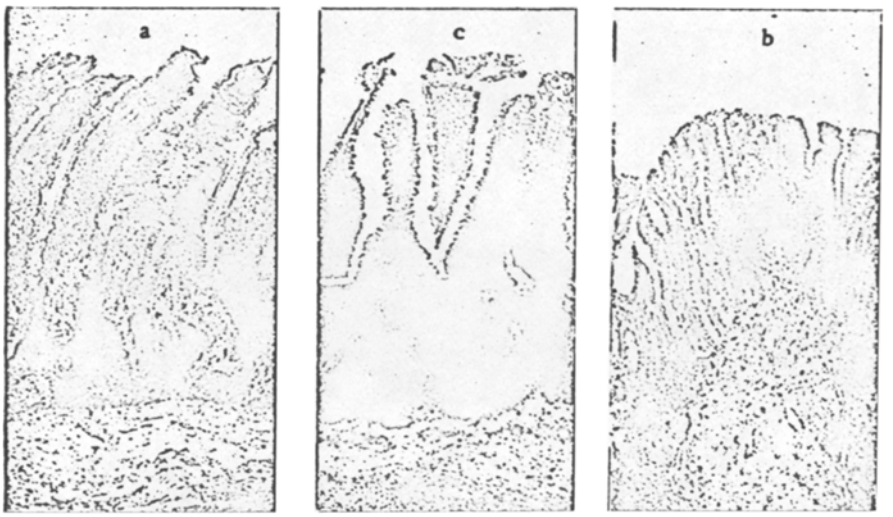

Fig. 1. Mucous membrane of isolated intestinal segments. Stained with hematoxylin-eosin. 60 fold magnification.
a) Isolated segment of an upper portion of the small intestine operated by the Thiry method, the nervous system remaining intact; b) same but "denervated" and allowed to remain within the abdominal cavity; c) isolated section of the terminal ileum, "denervated" and transplanted subcutaneously.

The morphology of the segments prepared by the Thiry method revealed no significant deviations from the structures seen in the normally functioning intestine of healthy animals. The secretory capacities of these segments were observed for $3\frac{1}{2}$-4 years and they remained well-expressed and constant. The "denervated" segments, after the sectioning of the mesenteric nerves, displayed a marked secretory disturbance. At first, "denervation" by both methods gave similar results. These, apparently, are the consequence of the removal of the trophic controls which had been arriving from the central nervous system. Following 1-2 months, the secretory capabilities within the segments tended somewhat to revert to normal but full restoration was observed only within the segments that had been permitted to remain within the abdominal cavity.

There are grounds for believing that new nerve fibers grow into the "denervated" intestinal segments, this leading to partial restoration of the trophic influences of the central nervous system. This restoration of

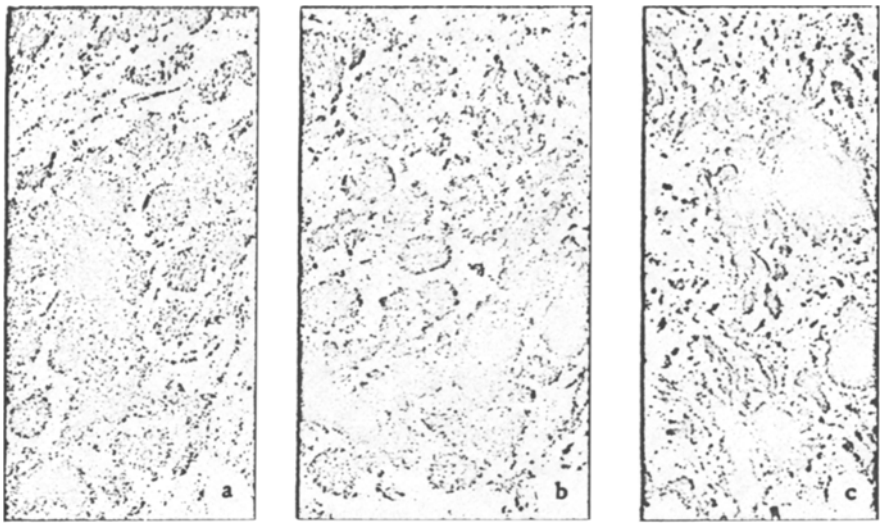

Fig. 2. Nerve ganglia of the submucous plexi within the isolated bowel segments.
Stained according to Bielschowsky-Gross. Magnification 550 x.
a) Isolated Thiry segment; b) "denervated" segment left within the abdominal cavity; c) "denervated" intestinal segment transplanted subcutaneously.

connections with the central nervous system is suggested by the fact that examination of the mesentery distal to the site of sectioning of the nerves reveals nerve fibers of normal structure and, apparently, quite functional. The extent of the return of the secretory function and the severity of the degenerative processes observed within the "denervated" intestinal segments appears to depend primarily on the character of the newly growing nerve fibers.

Into the intestinal segments transplanted to the subcutaneous region, the nerve fibers that grew in came from subcutaneous nerves which usually have no digestive associations. It is these segments that showed the heaviest morphological evidence of atrophy. Their capacity to secrete enzymes remained severely depressed.

Into the "denervated" segments which had been permitted to stay within the abdominal cavity there occurred an ingrowth of nerve fibers from nerve trunks innervating the digestive organs. These segments showed a much lesser degree of morphological evidence pointing to atrophy. Their secretory function tended to revert almost to normal, being much greater than in the transplanted segments even though less than what was seen in the segments prepared by the Thiry method only.

Our data all point to the powerful trophic effect exerted upon the intestine by the central nervous system by way of the mesenteric nerves. It would appear that the central nervous system regains control through the medium of the ingrowing nerve fibers and that its trophic influences are much more effectively displayed by way of nerve fibers normally innervating the contents of the abdominal cavity than by way of nerve fibers coming from the subcutaneous nerve net.

SUMMARY

Isolated intestinal segments were prepared in dogs. One segment was made by the usual Thiry method. The other was additionally "denervated" by one of two methods and then, either left within the abdominal cavity, or else transplanted subcutaneously.

The morphological and physiological alterations produced were observed over a period of several years.

The central nervous system exerts a powerful trophic effect upon the intestine. The "denervated"

segments are restored to central nervous system control by ingrowth of new nerve fibers. In the segment that had been left within the abdomen, these fibers come from nerves which normally innervate the digestive organs. The transplanted segment receives nerve fibers from the subcutaneous net. It is an experimental fact that the intra-abdominal segments tend to return to normal to a much greater extent than do the segments transplanted under the skin.

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