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Pathological Changes in Abdominal Sympathetic Ganglia of Animals with Fulminating Experimental Diarrhea

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With 5 Figures

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Introduction

From time to time since the turn of the century the laboratory investigator, as well as the clinician, has hinted at a connection between the autonomic nervous system and various forms of diarrhea. Indeed, a review of the literature indicates that every level of the autonomic nervous system has been explored in search of a neurogenic mechanism of diarrhea or dysentery.

Penner and *Bernheim* (1960) presented convincing evidence for a central, perhaps hypothalamic, site of action of Shiga toxin with their cross-perfusion experiments in dogs. Gastrointestinal lesions reminiscent of those seen in bacillary dysentery suggested to them a disturbance of the central regulatory mechanisms, caused by the toxin — a known neurotoxin.

Tinel (1937) was impressed by the role of the local vasculature in the production of dysenteric pathophysiology. However, he considered the celiac plexus a more likely mediator of the vascular response, because of the extensive studies by *Reilly* and coll. (1935). The latter injected first live typhoid bacilli, later on various endotoxins, into the celiac ganglion of rabbits and rats. In this manner he was able to imitate lesions of dysentery to the point of intestinal ulceration. A mass of evidence has been quoted in support of such a role, direct or indirect, for the celiac plexus (*Popielski*, 1903; *Lium* and *Portsmouth*, 1941). With the fascinating report by *Palmerio* and coll. (1963) on the prevention of shock following "complete" denervation of the abdominal viscera, we can certainly no longer doubt the intimate association between abdominal sympathetics and vascular regulation.

On the other hand, the fact remains that direct injection of endotoxin into the superior mesenteric artery will reproduce most of the intestinal morphological changes described by *Reilly* and coll. (1935). In addition, (1965), we

have recently failed to reproduce *Reilly's* experimental results with various types of endotoxin, and find the intramural nervous system of the gut itself a more suitable site of action for bacterial toxins.

While the clinician has been able to apply the various experimentally derived, nervous theories of gastrointestinal disease to mucous colitis (*Jones* and *White*, 1938), ulcerative colitis (*Lium* and *Porter*, 1939), and Hirschsprung's disease (*Scott* and *Morten*, 1931), the pathologist has provided no clear clinicopathological correlation except for the well known deficit in congenital megacolon (*Bodian* and coll., 1949).

Even less neuropathological support has been furnished in the area of toxic-infectious diarrhea. Involvement of the abdominal sympathetic nervous system has been discussed by *Guizetti* (1898) in typhoid fever and *Mogilnitzki* (1923) in dysentery. Others (*Abrikossof*, 1923; *Leontyeff*, 1910) noted sympathetic changes in cholera. In general, most reviewers (*Graupner*, 1898; *Terplan*, 1926; *Herzog*, 1948) have either failed to mention sympathetic pathological alterations in gastroenteritis, or feel that the morphological picture in the regional abdominal ganglia and elsewhere in the autonomic system is unrelated to the clinical manifestations.

Recently *Kalas* (1961) furnished an important new model for the study of experimental diarrhea in guinea pigs. He challenged reserpinized animals with endotoxin, and observed a profuse propulsive diarrhea of truly astounding proportions. Because of the implied autonomic derangement here, we decided to study the abdominal vegetative nervous system in an effort to supply lacking neuropathological information about an area, which has been so demonstrably implicated by both the clinician and the physiologist.

Materials and Methods

The vegetative nervous system was studied in forty-four young adult Walter Reed strain guinea pigs of mixed sex, averaging 303 gm. body weight. Twenty-four animals were prepared with reserpine and endodoxin according to the method of *Kalas* (1961) and sacrificed with chloroform at intervals from 1 to 20 days. Ten guinea pigs were killed in a similar manner after receiving a comparable dose of intravenous endodoxin, while another 10 animals had control injections of reserpine alone. Five of these were given nultiple 0.3 mg./Kgm. body weight reserpine injections over a 2-week period.

The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

After fixation in 10% buffered formalin, 8—12 micron sections of diencephalon, medulla oblongata through tip of obex, thoracic spinal cord, abdominal sympathetic ganglia and mid-jejunum were prepared for H & E, Nissl stain, Bodian's silver, P. A. S. and the short silver diamine method. Duodenum and ileum were stained with methylene blue by immersion for the preparation of whole mounts. In some instances similar whole mount preparations were made from the mid-jejunum with formalin — 10% sucrose fixed tissue, which was stained with Cresyl Echt Violet.

Results

By themselves, intravenous endotoxin or reserpine have no morphological effect on the abdominal ganglia.

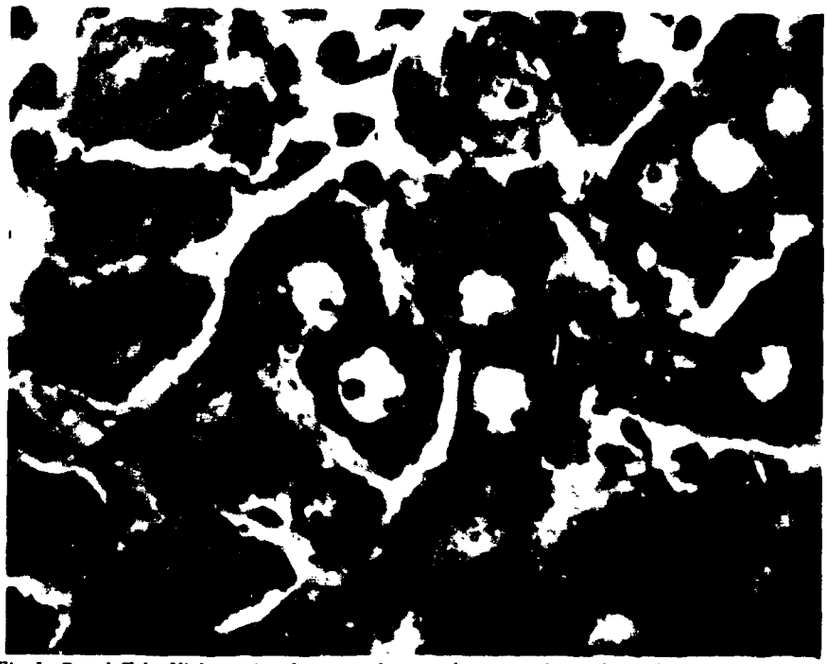

Fig. 1. Cresyl Echt Violet stain of a normal sympathetic ganglia such as the celiac. × 680. It demonstrates round nerve cells, the cytoplasm of which is filled with Nissl substance. Only a narrow clear rim is seen about the prominent, centrally located nucleus.

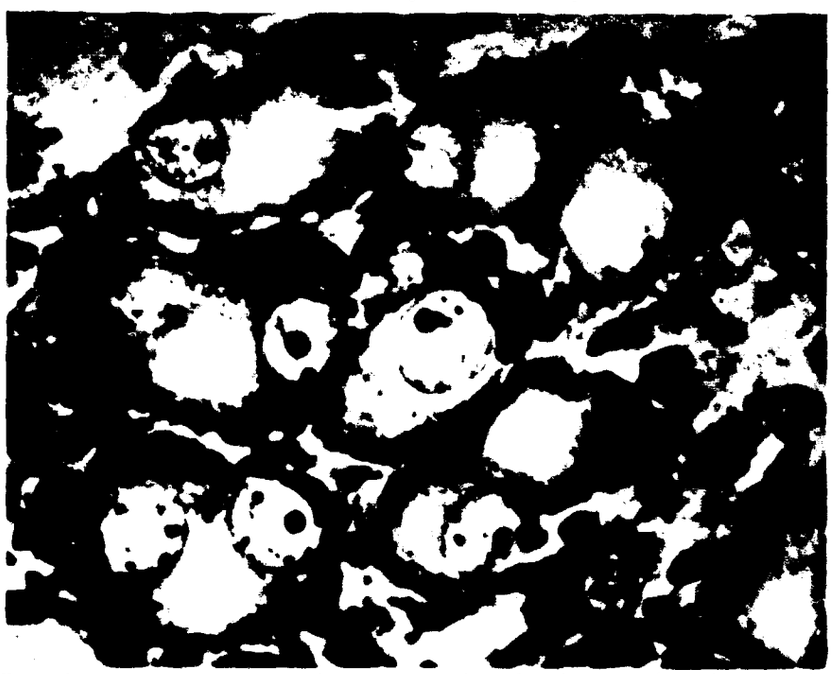

Fig. 2. Prominent changes in the celiac ganglion 8 days after the onset of diarrhea (C. E. V., × 680). Ganglion cells are swollen. The Nissl substance has formed a rim around the edge of the cy. plasm, which is now largely clear. Most nuclei are eccentric, more prominent than usual and the nucleoli at times appear increased in size or number.

During the first 48 h. after the administration of endotoxin to the reserpine primed guinea pig, the regional abdominal sympathetic ganglia are normal, save for a moderate degree of hyperemia. However, early morphological changes *can* be demonstrated in the intramural myenteric ganglia. It is the time of the most severe physiological derangement and many animals die in shock and dehydration.

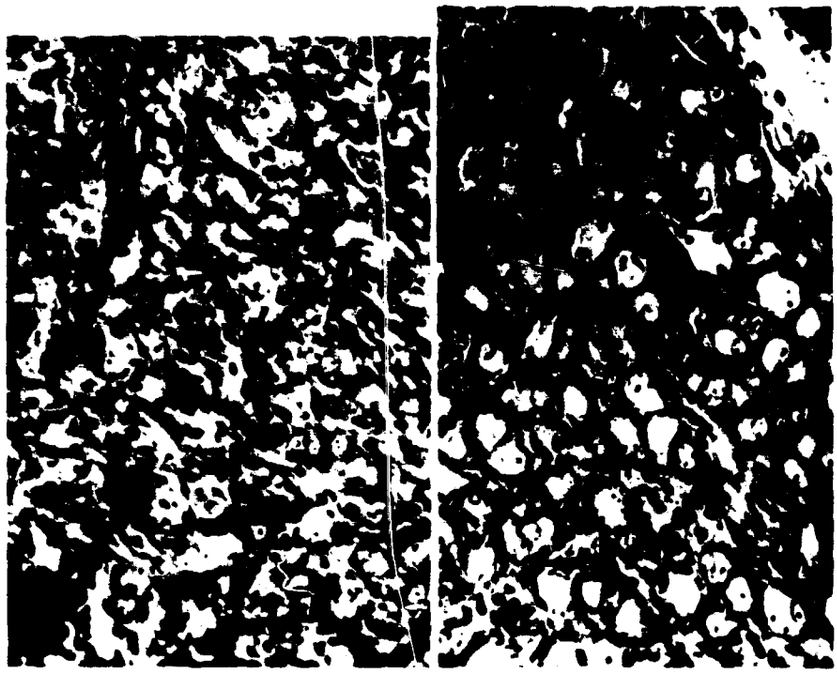

Fig. 3a Fig. 3b

Fig. 3. PAS stain of celiac ganglion ten days after Endotoxin-Reserpine (b). The cellular swelling is uniform throughout the ganglion, when compared to the control (a). The neuronal swelling, seems to shift the ratio of neurons to interstitial elements in favor of the former, whereas the nerve cells are much less evident in the control ganglion. No glycogen increase could be demonstrated in the cytoplasm of neurons in the experimental group, even with frozen section. (× 190.)

On the third day when survivors have begun to recuperate from their propulsive, profuse, watery diarrhea, microscopic alterations appear within the celiac and other regional sympathetic ganglia. Under low magnification the ganglia seem to be more lightly stained than normal, cytoplasmic borders stand out more clearly, and there is a greater ratio of neurons to supportive elements. Most neurons have become elliptical with a tendency for the nucleus to be eccentric (Fig. 1 and 2). Normally a narrow zone, free of Nissl substance, can be seen around the nucleus. Now the clear zone occupies most of the cytoplasm leaving only a slender rim of Nissl substance around the periphery of the cell. The nucleus occupies a marginal location and is perhaps

increased slightly in size — in addition the nucleoli appear more prominent and are seemingly larger and at times even increased in number. Alterations of this type take place in mono- as well as bi-nucleated neurons, and are found throughout each ganglion in a fairly uniform manner (Fig. 3 a and b). These nerve cells give negative reactions with P. A. S. for glycogen, and the short silver diamine stain for neuromelanin.

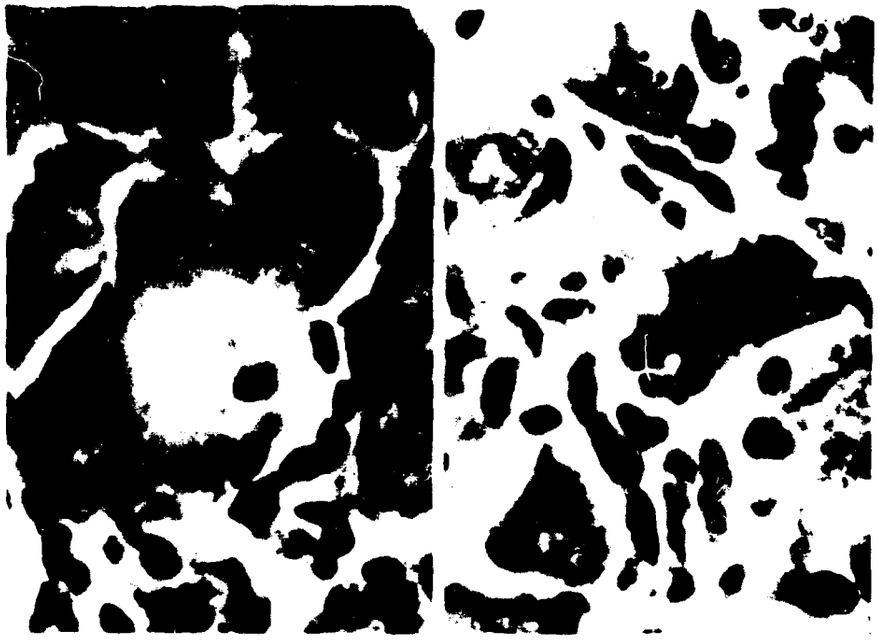

Fig. 4 a

Fig. 4 b

Fig. 4. a) A swollen neuron surrounded by normal ganglion cells in the celiac ganglion 21 days after the onset of diarrhea with reserpine-endotoxin (H & E, × 680). b) Another type of change noted three weeks after reserpine-endotoxin diarrhea in an otherwise normal ganglion was the presence of an occasional cell with basophilic irregular cytoplasm and a pyknotic nucleus (H & E, × 680). While such an appearance may be seen as a post mortem artefact when fixation is delayed more than 20 hr., we failed to note this change in the absence of diarrhea, and in immediately fixed tissue, and consider it compatible with the so-called "chronic cell change". This presumably is an irreversible condition.

Swollen neurons continue to dominate the histologic picture in the abdominal ganglia until about 2 weeks after the start of the experiment, when some cells present nuclear swelling and others have suffered a more severe cellular change, namely, increased cytoplasmic basophilia, irregular cellular outline, shrinkage of the cell and nuclear pyknosis (Fig. 4 a and b). At this point the earlier noted swelling of ganglion cells is less prominent, and by day 20 most of the sympathetic nerve cells have reverted to their normal size and appearance. An occasional neuron still retains its cytoplasmic corpulence and eccentric nucleus. In these ganglia 1 or 2 pyknotic neurons can be found per high power field on the 20th day.

In the acute phase general intestinal pathologic morphology of the reserpine-endotoxin group consisted of degenerative changes in the epithelium, blunting of the villi and hypercellularity of the lamina propria. In addition, capillary dilatation was significantly greater, and mucus cell depletion occurred earlier in this group than in control animals.

Morphological alterations have been alluded to in the intramural myenteric neurons during the initial 48 hr. (Fig. 5). Both endotoxin and endotoxin-reserpine animals demonstrated nuclear hyperchromasia cytoplasmic basophilia,

Fig. 5. Auerbach's ganglion in the small intestine of guinea pigs 7 hr. after the beginning of the experiment, in: a) normal; b) endotoxin; and, c) reserpine endotoxin animals. Only a few ganglion cells can be demonstrated in each section, however, the two groups of animals which received endotoxin, or the combination, demonstrated cytoplasmic basophilia, nuclear hyperchromasia, and some interstitial edema. This is compatible with some degree of early cell injury. (H & E, approximately × 100.)

and interstitial edema in Auerbach's ganglia. These changes are vague and difficult to confirm in cross-sectioned material, where only a limited sample of ganglion cells is available for comparison. However, this feature was consistent and striking enough to merit comment.

Discussion

The results obtained in this experiment indicate a definite relationship between the clinical manifestations of profuse watery diarrhea and subsequent anatomical microscopic changes in the regional sympathetic ganglia. Reserpine and endotoxin by themselves cause no apparent anatomic change in these structures. *Herzog* (1933) failed to detect pathological alterations in peripheral sympathetic ganglia of cats and rabbits with the administration of various poisons affecting the autonomic nervous system. There have been reports of neurotoxic effects in various forms of dysentery (*Kanai*, 1922; *Ecker* and *Wolpaw*, 1930). In most instances, however, the neurotoxic action has

been ascribed to the exotoxin of *S. Shiga* or *S. flexneri* (*Donald* and coll., 1956; *Blacklock* and *Guthrie*, 1937), and neurotropism as such does not appear to be an established feature of any endotoxin.

On the other hand, endotoxin does affect smooth muscle motility (*Ecker* and *Biskind*, 1929). Intestinal activity increases with small doses, but can be arrested with the administration of larger amounts. A similar effect has been reported for Shiga toxin by *Lucchini* (1923), with complete reversal of peristaltic movements in the antiperistaltic direction if large amounts were given. *Spiegel* and *Adolf* (1920) felt that changes in autonomic ganglia depended less on the type of toxin than it did on its intensity and duration of action. They were supported in this concept by *Laignel-Lavastine* (1903).

In *Kalas'* model the lesion in the regional abdominal sympathetic ganglia is characterized by cytoplasmic and nuclear swelling, nuclear eccentricity and peripheral Nissl chromatin, in the acute phase. We are unaware of previous lesions of this nature having been described in relation to acute gastroenteritis. *Loeper* (1919) noted intramural ganglionic lesions in autopsy material from 36 cases of dysentery, typhoid fever and colitis, which he considered important in the pathogenesis of the disease. There was thought to be an association between the tuberculous involvement of Auerbach's plexus and clinical diarrhea in *Leupold's* (1923) cases. *Nunez-Montiel* (1963), *Butterworth* (1958), *Lorentzen* (1923) and *Blaschko* (1883) have all made mention of pathological processes in the intramural nervous system of the gut in varying forms of enteritis, although without clear reference to the clinical symptomatology.

Two alternatives, therefore, present themselves in the analysis of our results: 1. the cell changes observed in the present experiment may be either the cause of diarrhea, or 2. a reaction to injury caused by the diarrhea. Excessive stimulation of nerve cells has been associated with considerable increase in cell volume acutely and, when stimulation continued, with nuclear and especially nucleolar enlargement (*Edström*, 1957). This is accompanied by a sharp increase in cellular glycogen (*Sulkin*, 1950). However, *Bertram* and *Barr* (1949) have pointed out that nuclear eccentricity is absent after stimulation and there is doubt that nervous stimulation is capable of producing real morphological changes in sympathetic nerve cells (*Eve*, 1896). In favor of our second alternative, i. e., that this is an axonal reaction, is the absence of glycogen, the nuclear eccentricity, as well as the magnitude of cellular swelling. Progression of changes also runs more or less according to *Nissl's* (1892) original description. Only a few cellular neurons in our animals seemingly proceeded to the chronic stage and cell death, which agrees with *Nissl's* (1896) concept that "restitutio ad integrum" is possible when the nucleus remains relatively unaltered.

It is our feeling that the violent contractions of the bowel musculature causes a local ischemia, with necrosis of the distal intramural nerve fibers. The suggestive evidence of early damage to the intramural ganglion cells, furthermore tends to support this hypothesis.

The Kalas model is an important one, because it demonstrates a definite clinico-pathological correlation within the autonomic nervous periphery. We

are also reminded to examine the regional ganglia in cases of protracted diarrhea, and made to wonder if repeated severe bouts of diarrhea may not eventually lead to a drop-out of enough inhibitory, sympathetic nerve cells, to establish the necessary foundation for an over-irritable bowel.

Summary

The abdominal vegetative nervous system has been studied in Walter Reed strain guinea pigs with experimental diarrhea produced by the method of *Kalas*.

During th diarrheal phase, the regional abdominal sympathetic ganglia are normal, but after approximately 72 hr. changes which we interpret as "central chromatolysis" take place in the ganglion cells. The latter remain uniformly and severely swollen for 10—18 days, when chronic changes and eventually cell death can be seen in occasional neurons. Most of the sympathetic nerve cells, however, have recovered by the 20th day, leaving a few pyknotic neurons behind in the ganglion.

We conclude that local ischemia within the bowel wall, during the severe propulsive diarrhea, is responsible for damage to intramural nerves and the subsequent ascending axonal reaction.

The significance of these findings is discussed.

Zusammenfassung

In dieser Studie wurde die Beziehung zwischen schwerem Durchfall und anatomischen Veränderungen in den Abdominalganglien des Sympathicus untersucht. Als experimentelles Modell dienten mit Reserpin vorbehandelte Meerschweinchen des Walter-Reed-Stammes, in denen, nach der Methode von *Kalas*, durch eine intravenöse Endotoxininjektion eine akute, schwere, choleraähnliche, wässerige Diarrhoe erzeugt wurde.

Anfänglich nach der für Stunden anhaltenden Phase des akuten Durchfalles bleiben die Abdominalganglien des sympathischen Grenzstranges intakt und zeigen keine Veränderungen. Jedoch nach ungefähr 72 Stunden beobachteten wir „zentrale Chromatolyse" der Ganglienzellen. Diese bleiben die ersten 10 bis 18 Tage durchweg stark geschwollen, ein Zeitraum in welchem wir in einigen Neuronen chronische Veränderungen einschließlich Zelltod feststellten. Nach Ablauf von ungefähr 20 Tagen ist die Mehrzahl der sympathischen Ganglienzellen zu ihrem Normalzustand zurückgekehrt und nur einige pyknotische Nervenzellen sind in den Ganglien erkennbar.

Wir glauben, daß während der schweren propulsiven Diarrhoe eine lokale Durchblutungsstörung zu einer ischemischen Schädigung des intramuralen vegetativen Plexus führt, der eine aufsteigende axonale Reaktion folgt.

Résumé

Le système nerveux végétatif abdominal a été étudié sur des cobayes de la souche Walter Reed atteints de diarrhée expérimentale provoquée par endotoxine et réserpine, par la méthode de *Kalas*.

Au cours de la phase diarrhéique, les ganglions sympathiques abdominaux sont normaux, mais après environ 72 heures ont lieu dans les cellules ganglionnaires des transformations que nous avons interprétées comme une chromatolyse centrale. Les cellules restent enflées uniformément et gravement pendant 10 à 18 jours, après quoi on peut observer dans quelques cellules nerveuses des altérations chroniques, et quelquefois la mort cellulaire. Toutefois, la plupart des neurones des

ganglions sympathiques abdominaux retournent à l'état normal vers le 20e jour, sauf quelques cellules nerveuses pyknotiques.

En conclusion, nous avons constaté une corrélation clinico-pathologique dans le système végétatif périphérique. Il est possible que l'ischémie locale dans la paroi intestinale, au cours de la diarrhée propulsive, puisse provoquer des lésions dans les nerfs intramuraux et la réaction rétrograde qui s'ensuit.

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