

## METHODS

### VERIFICATION OF COMPLETENESS OF DIVISION AND REGENERATION OF EXTRAMURAL NERVE CONNECTIONS OF THE SMALL INTESTINE AFTER CENTRAL DENERVATION

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Experiments on dogs subjected to various operations (exteriorization of an innervated Thiry – Vella loop, total autografting of the small intestine into the pelvis, total severance of central connections of the small intestine, and surgical reinnervation after transplantation or severance of central connections of the intestine) show that the recto-enteral reflex and the duration of the inhibitory phase of action of methyldiazine on movements of the small intestine can be used as tests of the state of central innervation of the small intestine.

A lasting effect of abolition of central nervous influences on the small intestine can be obtained by simultaneous division of all extramural nerve fibers and transection of the intestine itself. However, even under these circumstances it is often impossible to be certain that all central connections of the organ have been severed. The validity of data obtained after surgical denervation can be assured only by the use of tests of the completeness of denervation immediately after the procedure has been carried out, and also tests to show the completeness of regeneration of connections of the central nervous system with the organ concerned, in this case the small intestine.

There are two possible methods of approach. The first [2-4, 5-7, 12] is to investigate the state of the entero-enteral and recto-enteral reflex, the arcs of which are connected in the central nervous system and the receptive field of which lies outside the denervated small intestine.

The second approach, which can be used to verify the degree of central denervation, is to investigate the sensitivity of the organ to humoral influences. It is based on the work of Cannon and Rosenbloom and of other investigators [1, 4, 8], who have shown that the sensitivity of denervated structures to humoral influences is increased after division of autonomic nerves. The degree of increase in sensitivity is dependent on the completeness of denervation and is reduced as regeneration of the nerve connections takes place.

When studying the effects of central denervation on the function of the small intestine the writers have used both methods to verify the completeness of division of the central nervous connections with the intestines and to study the rate of subsequent reinnervation.

The recto-enteral reflex was chosen as the first test; for the second method of verification the central cholinolytic methyldiazine was injected: in response to the injection of this drug the motor activity of the small intestine is inhibited in the clearest manner. To test the recto-enteral reflex, a pressure of up to 150-170 mm Hg was created in a balloon introduced into the rectum. The response was recorded by means of small balloons introduced into the Thiry – Vella loop of small intestine. Since, according to the

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TABLE 1. Changes in Recto-Enteral Reflex and Effects Produced by Methylthiazine after Central Denervation of the Small Intestine

Series of expts.	Recto-enteral reflex						Duration (in min) of action of methylthiazine ( $M \pm \sigma$ )				
	1-3	1-1½	2-3	4-6	6-9	10-24	½	5-7	8-10	11-12	13-24
	days	months					months				
Control (innervated intestine)	+	+	+	+	+	+	61 ± 16	82 ± 23	82 ± 12	80 ± 10	-
Autografting of small intestine)	-	-	-	-	±	±	161 ± 29	143 ± 64	156 ± 27	140 ± 39	127 ± 40
Central denervation of small intestine	-	-	-	-	±	±	143 ± 28	135 ± 33	140 ± 22	130 ± 42	127 ± 24
Autografting and surgical reinnervation	±	-	±	±	+	+	146-30	153 ± 42	105 ± 14	75 ± 17*	-
Central denervation + surgical reinnervation	±	-	±	+	+	+	124-43	68 ± 36*	82 ± 32*	46 ± 23*	-

Legend: + definite reflex inhibitory response to distension of rectum (Fig. 1a); - complete absence of inhibitory reflex response (Fig. 1b); ± weak recto-enteral reflex appearing after 10-15 sec (Fig. 1c); \* Results not significantly different from control.

literature [9-11], the inhibitory effect of humoral origin cannot take place sooner than 15-20 sec after stimulation, the appearance of a definite inhibition of movements of the intestinal loop during the first 15 min after stimulation was taken to imply a positive reflex response.

To investigate sensitivity to pharmacological agents a 0.5% solution of methylthiazine was injected intramuscularly in a dose of 150-200  $\mu$ g/kg body weight. The sensitivity of the intestine to the action of the drug was estimated from the duration of the inhibitory response of the tested loop. Motor activity of the Thiry - Vella loop was recorded by V. F. Mostun's modification of the balloon method. To make absolutely sure of the validity of the proposed tests, several types of experiments were performed. In the experiments of series I (control), a loop of small intestine was isolated by the usual Thiry - Vella method in 12 dogs. In these experiments, naturally, preservation of the extramural innervation of the loop of intestine was guaranteed. In the experiments of series II (9 experiments) total autografting of the small intestine into the pelvis was performed, for this operation ensures complete central denervation. In the 10 experiments of series III the model of central denervation of the small intestine which was used was division of all the tissues of the mesentery and treatment of the vessels with a neurolytic mixture (a saturated solution of phenol, etc.). After this procedure verification of the completeness of central denervation is essential. However, the series listed above did not allow for a study of the dynamics of complete regeneration of connections between the small intestine and the central nervous system, for natural reinnervation may develop late and may not be complete. For this reason, in the experiments of series IV and V (9 dogs in each series), after transplantation and central denervation of the small intestine a deliberate surgical reinnervation of the intestine was carried out by the writers' own technique: suture of the pelvic branches of the caudal mesenteric nerve plexus to the divided cranial mesenteric nerve plexus of the graft or suture of the divided ends of the cranial mesenteric plexus.

In all series of experiments the scheme used to investigate the animals was the same. The recto-enteral reflex was tested on the first 3 days after operation, and then monthly (218 observations altogether). The response to injection of methylthiazine was studied once every month (altogether 232 observations).

As the results given in Table 1 show, in the control series of experiments a well defined recto-enteral reflex was constantly observed at all times

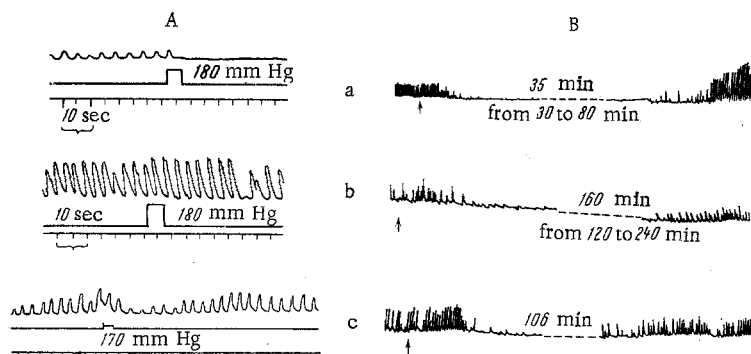


Fig. 1. Recto-enteral reflex (A) and response to injection of methyldiazine (B) for different degrees of preservation of innervation of small intestine. a) Innervated intestine; b) completely denervated intestine; c) intestine in period of reinnervation. A) (From top to bottom); contractions of small intestine, marker of stimulation, time marker (10 sec); B) contractions of small intestine, time of injection of methyldiazine indicated by arrow.

of observation, and the duration of the inhibitory response to injection of methyldiazine varied from 35 to 90 min (Fig. 1a). In the initial period after central denervation, by whatever method it was done, the recto-enteral reflex was absent and the inhibitory response to the intestine to injection of methyldiazine was considerably lengthened (Fig. 1b). The exception was a weak positive reflex observed in the first 2-3 days after surgical reinnervation. This phenomenon was evidently connected with the passage of nervous impulses across the zone of anastomosis, before the divided axons had degenerated. Six months after autografting or central denervation of the intestine, and 1.5-3 months after surgical reinnervation, the differences observed between the experimental and control series began to disappear.

The dynamics of normalization of the indices was especially clear after surgical reinnervation (in the case of autografting and denervation the indices did not return to normal during observations lasting 1.5-2 years after operation). The recto-enteral reflex recovered sooner than the response to methyldiazine.

It can be concluded from these investigations that the recto-enteral reflex can be accepted as a reliable criterion of the completeness of intestinal denervation. The appearance of a reflex indicates restoration of the reflex arc, and by its character it is possible to judge the rate and, to some extent, the degree of reinnervation. More complete evidence concerning the outcome of reinnervation is given by the results of the test with injection of methyldiazine, and also by the fact that the times of inhibition of motor activity in the control were always significantly different, not only from the results in the complete denervation experiments, but also from the times found in experiments in which the innervation was partly restored. Consequently, these two methods can be used to judge both the completeness of central denervation and the rates of restoration of the intestine at various stages after surgical division of the extramural nervous connections. In addition, the results of these experiments show that surgical denervation, like transplantation, is a good method of interrupting central nervous influences, for it ensures complete absence of nervous connections between the organ and the central nervous system for 6 months.

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