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In vitro Response of Intestinal Segments of Mice with Hereditary Muscular Dystrophy to Various Pharmacologic Agents.* (29215)

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It has been observed in our laboratory that mice with hereditary muscular dystrophy (strain 129/J, genotype dy/dy) have a higher incidence of constipation and fecal impactions than do heterozygous (Dy/dy) or wild-type (Dy/Dy) animals of the same strain. Review of the clinical and experimental literature reveals little information regarding the status of the gastrointestinal tract both in patients with muscular dystrophy and in mice similarly affected. Patterson and Rios (1) reviewed the clinical literature and added a summary of their own observations on a group of patients with pseudohypertrophic dystrophy. In many of the cases in which gastrointestinal disturbances were present, diarrhea and vomiting are the predominant complaints, but constipation and intestinal atony were also frequently reported. To our knowledge, no systematic study of the autonomic nervous function of the gastrointestinal tract has been conducted, either in dystrophy patients or in affected experimental animals. In this communication are presented the results of an *in vitro* study of the responses to various autonomic drugs of selected segments of the gastrointestinal tract of mice with hereditary muscular dystrophy.

Materials and methods. The experimental animals were female mice of 3 genotypes: 1) homozygous dystrophic (dy/dy), 2) heterozygous (Dy/dy) and 3) wild-type (Dy/Dy) controls of the same strain. They had been weaned at 4 weeks of age and were subsequently maintained on Old Guilford Mouse Pellets and tap water *ad libitum*.

Between 8 and 12 animals of each genotype were sacrificed at 4, 12, and 20 weeks of age. At time of sacrifice mice were anesthetized with ether and the intestinal tract was rapidly dissected out and immersed in Tyrode's solution. Three centimeter sections of the proximal portions of the duodenum, jejunum, ileum, and large bowel were removed and were suspended in separate tissue baths containing 9.8 ml of Tyrode's and 0.2 ml of drug solution. Temperature of the baths was maintained at 35 ± 2°C and continual oxygenation was insured by bubbling air through the solution. The tissue was attached by fine sutures to a simple lever and kymograph system with a magnification of 20:1 and a resulting tension on the tissue of approximately 1 g.

All drugs were diluted in distilled water, with the exception of norepinephrine which was prepared in 0.01 N HCl. Drugs were injected into the tissue baths in volumes of 0.2 ml, bringing the total volume of the baths to 10.0 ml. Agents employed to assess the activity of the parasympathetic system included: 1) acetylcholine, in doses of 0.2, 1.0, and 5.0 μg (concentrations of 0.02, 0.10, and 0.50 μg/ml), 2) acetylcholine in the presence of 2.0 mg of hexamethonium, and 3) atropine sulfate in doses of 0.5 and 2.5 μg (concentrations of 0.05 and 0.25 μg/ml). The sympathetic system was tested with: 1) norepinephrine, 0.2 and 2.0 μg (0.02 and 0.20 μg/ml), and 2) guanethidine, 2.0 and 5.0 μg (0.2 and 0.5 μg/ml). In addition, the action of several substances on the muscle itself was determined: 1) barium chloride in doses

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of 2.0 and 4.0 mg (0.20 and 0.40 mg/ml), and 2) thyroxine sodium, 5.0 μg (0.50 μg/ml). The concentrations of acetylcholine, norepinephrine, and barium chloride were selected from the linear segments of previously determined dose-response curves.

Following suspension of the tissue in the baths, equilibration with Tyrode's solution was permitted for 5 minutes, and a 2-minute period of baseline contraction was then recorded. After testing with each of the drugs, the baths were flushed with 3-15 ml portions of Tyrode's, and no other drug was administered until original baseline conditions had been obtained for at least one minute.

The response of an intestinal segment to each agent was recorded as the distance (± 0.1 mm) from the baseline to the peak of contraction or relaxation. Data were calculated as mean response in millimeters ± standard error, and P values were determined by Student's t test.

Results. Only data obtained from the duodenal and jejunal segments are presented here, since the ileum often fatigued early in the experiment and the large bowel baseline recordings were frequently erratic. Valid assessment of the drug responses of the latter two segments was consequently not possible.

No differences in either rate or magnitude of spontaneous contractile activity could be detected among intestinal segments from dystrophic, heterozygous, or wild-type animals at any of the periods examined.

1. *Response to substances which influence the parasympathetic nervous system and the autonomic ganglia.* Since acetylcholine is the neurohumoral mediator of both pre-ganglionic and post-ganglionic parasympathetic impulses in the isolated preparation, the responses elicited following its addition to the tissue baths have been taken as an indication of the ability of intestinal segments to respond to parasympathetic nervous stimulation. When the addition of acetylcholine was preceded by that of the ganglion blocking agent hexamethonium, the observed effects were assumed to represent primarily those which result from stimulation of the postsynaptic myoneural junction directly. Atropine sulfate has been employed to inhibit the

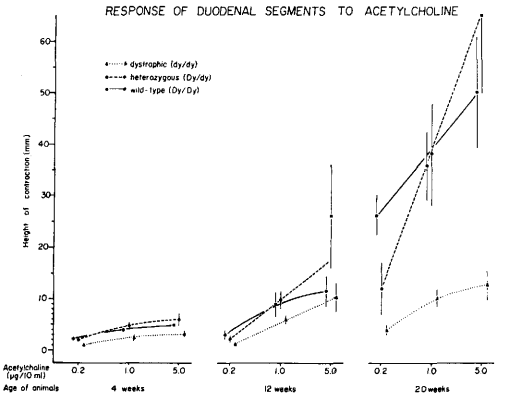

FIG. 1. Contractile response of duodenal segments to stimulation by acetylcholine.

myotrophic action of endogenous acetylcholine, and the degree of relaxation seen following atropine administration has been considered to reflect the contribution of the parasympathetic nervous system to the initial tone of the gut.

The responses to acetylcholine of duodenal segments from normal animals were significantly greater than were those of dystrophics at 4 and 20 weeks of age for all concentrations of the drug (Table I, Fig. 1). With progressive age the responses of the duodenum of wild-type mice to identical doses of acetylcholine increased markedly, so that by 20 weeks the differences between normal and dystrophic animals were striking (Fig. 2). The contractile responses of jejunal segments to acetylcholine (Table II) were, in general, similar to those obtained from duodenal segments. The activities recorded for normal tissue were greater than those recorded for dystrophic material, but the differences were significant only with higher concentrations of the drug.

When the addition of acetylcholine to the tissue baths was preceded by that of hexamethonium, the responses of individual specimens averaged 25-60% less than those which had been observed when acetylcholine alone was administered. There were, however, no differences in relative responses noted among specimens from the 3 genotypes.

Exposure of the various intestinal segments to atropine sulfate resulted in minimal relaxation which, nevertheless, tended to be greater among segments from normal and

TABLE I. Response of Duodenal Segments to Various Pharmacologic Agents.

Age (wks)	Geno-type	No.	Acetylcholine .2 μg	Acetylcholine 1.0 μg	Acetylcholine 5.0 μg	Hexamethonium 2.0 mg ff by acetylcholine .2 μg	Hexamethonium 2.0 mg ff by acetylcholine 1.0 μg	Hexamethonium 2.0 mg ff by acetylcholine 5.0 μg	Atropine .5 μg	Atropine 2.5 μg	Norepinephrine .2 μg	Norepinephrine 2.0 μg	Guanethidine 2.0 μg	Guanethidine 5.0 μg	Barium 2.0 mg	Barium 4.0 mg	Thyroxine 5.0 μg
4	Dy/Dy	10	2.2 ± .3	4.0 ± .3	4.8 ± .4	1.8 ± .2	3.6 ± .4	3.6 ± .5	− .5 ± .1	− .3 ±.1	− .1 ±.1	−1.0 ± .2	.4 ±.2	.6 ±.2	2.0 ± .3	2.1 ± .3	.4 ±.2
	Dy/dy	12	2.0 ± .4	4.7 ± .7	5.9 ± 1.2	2.1 ± .3	4.9 ± .7	5.0 ± .9	− .8 ± .2	− .4 ±.1	− .4 ±.1	−1.7 ± .3	.9 ±.2	.8 ±.2	3.1 ± .7	3.8* ± .7	.6 ±.2
	dy/dy	9	1.0‡ ± .2	2.4† ± .5	3.1‡ ± .4	1.3 ± .2	2.5* ± .3	2.8 ± .3	− .3 ± .1	− .1 ±.1	− .1 ±.1	− .7 ± .2	.3 ±.1	.4 ±.3	1.5 ± .2	1.9 ± .2	.1 ±.2
12	Dy/Dy	10	3.1 ± .9	8.9 ± 2.4	11.5 ± 2.9	3.3 ± .5	10.2 ±1.2	11.5 ± 2.6	−1.0 ± .2	− .6 ±.1	−1.0 ±.1	−2.5 ± .2	.3 ±.1	.3 ±.1	4.1 ±1.0	4.9 ±1.4	.3 ±.2
	Dy/dy	10	2.2 ± .5	9.8 ± 1.7	26.1 ±10.2	2.4 ± .3	9.1 ±1.6	17.4‡ ± 6.2	−1.2 ± .2	− .9 ±.3	−1.4 ±.2	−3.2 ± .3	1.1‡ ±.2	1.3 ±.2	4.8 ± .2	8.5 ±1.7	.5 ±.2
	dy/dy	11	1.3* ± .3	5.8 ± .8	10.3 ± 2.6	1.6† ± .3	5.2* ±1.5	7.4 ± 1.8	−1.1 ± .2	− .8 ±.2	− .5 ±.1	−1.4‡ ± .3	.4 ±.2	.1 ±.1	3.5 ± .7	3.4 ± .6	—
20	Dy/Dy	5	26.1 ±3.8	35.7 ± 6.6	49.4 ±10.7	8.6 ± .9	35.6 ±9.0	41.5 ± 8.4	−1.9 ± .3	−1.2 ±.2	−1.0 ±.2	−3.2 ± .4	1.4 ±.4	1.4 ±.3	12.4 ±2.7	16.7 ±5.8	.8 ±.5
	Dy/dy	7	12.0 ±5.1	38.2 ±10.1	65.2 ±15.1	5.5 ±1.3	33.1 ±9.9	59.7 ±17.3	−5.4* ±1.2	−3.2* ±.6	−2.7 ±.8	−7.4 ±1.6	1.8 ±.1	.8 ±.2	22.5 ±6.8	12.3 ±3.1	.8 ±.0
	dy/dy	6	4.0‡ ± .9	9.9‡ ± 1.8	12.6‡ ± 3.1	4.7† ± .8	10.3† ±2.1	12.1‡ ± 3.1	−1.2 ± .9	− .7† ±.1	− .5 ±.2	−2.1* ± .3	.3* ±.1	.9 ±.2	5.6* ± .6	7.0 ± .8	.2 ±.1

Data are expressed as mean ± standard error.
* $P < .05$ vs Dy/Dy animals.
† $P < .02$ vs Dy/Dy animals.
‡ $P < .01$ vs Dy/Dy animals.

TABLE II. Response of Jejunal Segments to Various Pharmacologic Agents.

Age (wks)	Genotype	No.	Acetylcholine .2 μg	Acetylcholine 1.0 μg	Acetylcholine 5.0 μg	Hexamethonium 2.0 mg ff by acetylcholine .2 μg	Hexamethonium 2.0 mg ff by acetylcholine 1.0 μg	Hexamethonium 2.0 mg ff by acetylcholine 5.0 μg	Atropine .5 μg	Atropine 2.5 μg	Norepinephrine .2 μg	Norepinephrine 2.0 μg	Guanethidine 2.0 μg	Guanethidine 5.0 μg	Barium 2.0 mg	Barium 2.0 mg	Thyroxine 5.0 μg
4	Dy/Dy	10	8.4 ± 2.2	39.8 ±15.5	59.3 ±17.0	3.9 ± .9	25.1 ± 1.6	43.7 ±14.8	−1.3 ± .4	− .8 ±.9	− .3 ±.1	−1.5 ± .5	.8 ± .2	1.1 ± .4	12.3 ± 3.3	11.7 ± 2.5	1.0 ±.1
	Dy/dy	12	5.5 ± 1.4	36.5 ±13.1	69.5 ±14.8	3.1 ± .7	34.3 ±12.0	63.9 ±13.5	−1.1 ± .4	− .5 ±.2	− .3 ±.1	−1.3 ± .4	.6 ± .3	.2* ± .1	41.2* ±12.9	31.7 ±12.3	.5 ±.3
	dy/dy	9	5.4 ± .8	10.9 ± 3.8	16.6* ± 5.7	1.7‡ ± .3	8.1‡ ± 1.6	11.4‡ ± 1.9	− .3* ± .1	.0 ±.1	− .3 ±.1	− .7 ± .2	.3 ± .1	.2* ± .1	7.2 ± 1.7	11.4 ± 3.3	.2 ±.1
12	Dy/Dy	10	24.3 ±12.3	57.2 ±13.1	89.0 ±12.1	6.9 ± 2.0	60.4 ±19.9	89.7 ±15.6	−1.9 ± .6	−1.1 ±.5	− .9 ±.4	−1.9 ± .6	.8 ± .4	.4 ± .3	39.8 ±11.5	47.9 ±13.9	.2 ±.3
	Dy/dy	10	8.1 ± 3.4	51.5 ±17.3	53.6 ±18.6	4.2 ± 1.3	42.8 ±16.2	68.4 ±20.6	−2.0 ± .8	−1.2 ±.3	−1.1 ±.6	−2.6 ± .8	.6 ± .2	1.5 ± .5	51.1 ±20.1	—	.8 ±.1
	dy/dy	11	4.0 ± 1.0	17.9† ± 3.0	32.4‡ ± 4.8	1.5‡ ± .3	10.3‡ ± 2.0	18.9‡ ± 2.7	− .7 ± .2	− .4 ±.2	.0* ±.1	- .7 ± .2	.3 ± .1	.4 ± .2	9.8* ± 1.2	14.0 ± 2.2	— —
20	Dy/Dy	5	71.8 ±22.2	100+	100+	68.0 ±21.6	100+	100 +	−6.5 ±2.8	−1.9 ±.8	−1.8 ±.8	−7.6 ±2.8	1.4 ± .7	.9 ± .5	90.0 ±20.2	100+	1.8 ±.8
	Dy/dy	7	50.8 ±18.4	100+	100+	26.8 ±12.9	100+	100+	−3.1 ± .9	−2.4 ±.4	−2.0 ±.3	−6.3 ±1.7	2.7 ±1.3	2.3 ±1.7	84.6 ±18.1	100+	1.9 ±.7
	dy/dy	6	31.9 ±10.7	82.0 ±16.6	97.3 ±11.7	9.5* ± 2.6	56.0 ±15.0	88.2 ±12.1	−1.2 ± .3	− .6 ±.2	− .3‡ ±.2	−1.7* ± .4	1.0 ± .4	2.0 ± .5	34.6‡ ±11.0	35.1 ± 9.7	.7 ±.2

Data are expressed as mean ± standard error.
* P <.05 vs Dy/Dy animals.
† P <.02 vs Dy/Dy animals.
‡ P <.01 vs Dy/Dy animals.

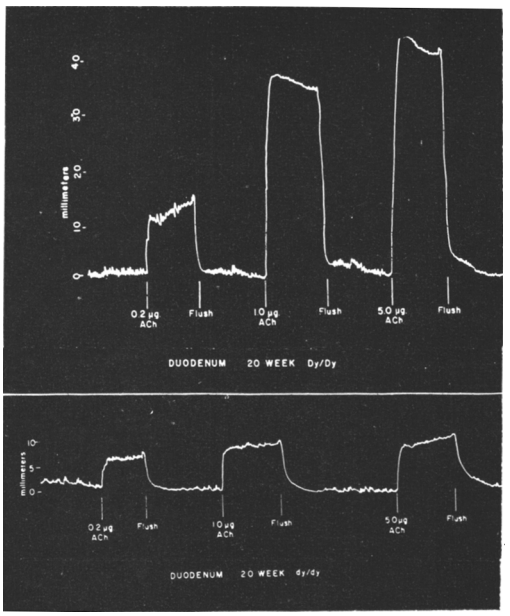

FIG. 2. Representative kymograph tracings of responses of duodenal segments of 20-wk-old wild-type (Dy/Dy) and dystrophic (dy/dy) animals to increasing concentrations of acetylcholine (ACh). A baseline period was recorded, drug introduced into tissue bath, contractile response recorded for 2 min. Bath was then flushed with Tyrode's solution and baseline contractions again recorded for a 2-min period before another concentration of the drug was added. Height of contraction in millimeters is indicated along the abscissa.

heterozygous than from dystrophic animals. Interestingly, the responses elicited with 2.5 µg of atropine were consistently less than those obtained with 0.5 µg of the drug.

2. *Response to substances which influence the sympathetic nervous system.* Since norepinephrine is the neurohumoral mediator of post-ganglionic sympathetic impulses, and since there are presumably no such impulses in the isolated preparation, the responses elicited following addition of norepinephrine to the tissue baths have been taken as an indication of the ability of intestinal segments to respond to sympathetic nervous stimulation. Guanethidine has been employed because of its ability to block the release of endogenous norepinephrine from post-ganglionic sympathetic axon terminals(2,3), and the magnitude of the contraction recorded following guanethidine administration has been considered to reflect the contribution of the sympathetic nervous system to the initial tone of the gut.

The responses of both duodenal (Table I, Fig. 3) and jejunal (Table II) segments of normal and heterozygous animals to norepinephrine were characterized by greater degrees of relaxation at 12 and 20 weeks than were those which were recorded for the dystrophics. There were no significant differences between the responses of tissue from normal and heterozygous mice at any of the periods examined.

The presence of guanethidine in the tissue baths permitted slight but insignificant contractions of both duodenal and jejunal segments. Although the magnitude of the contractile responses increased with the age of the animals, there were no consistent statistical differences between the groups.

3. *Response to substances which may influence the muscle directly.* Although the precise action of the divalent barium ion on neuromuscular activity is uncertain, it has been reported that barium stimulates smooth muscle both by a direct spasmogenic effect and by exciting the ganglion cells and myoneural junction(4).

The responses of the duodenum to barium (Table I) were such that, although there was a suggestion of greater contractile activity among segments from normal animals, only

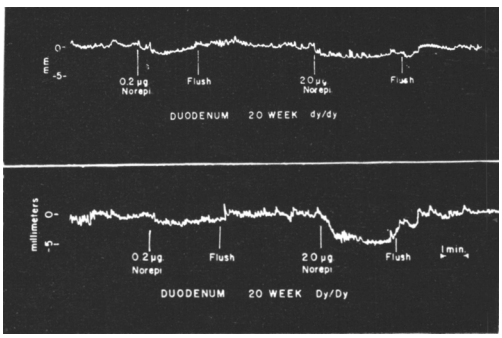

FIG. 3. Representative kymograph tracings of responses of duodenal segments of 20-wk-old wild-type (Dy/Dy) and dystrophic (dy/dy) animals to norepinephrine (Norepi.). A baseline period was recorded, drug introduced into tissue bath, and response recorded for 2 min. Bath was then flushed with Tyrode's solution and a 2-min period of baseline contractions again recorded before a greater concentration of the drug was added. Magnitude of relaxation in millimeters is indicated along abscissa.

one of the values approached the level of statistical significance. Differences in the responses to barium of the jejunum from normal and from dystrophic animals were more pronounced (Table II). In 20-week-old mice, the barium ion stimulated considerably greater contractile activity in jejunal segments from normal animals than it did in those from dystrophics.

The presence of thyroxine elicited slight and inconsistent increases in the tone of many of the segments, but there were no differences observed between segments from the 3 types of animals.

Discussion. By employing an *in vitro* tissue bath technique, it has been possible to examine the influence of several of the autonomic drugs on the motility of the gastrointestinal tract of mice with hereditary muscular dystrophy. In these animals there appears to be a non-specific impairment to the ability of intestinal smooth muscle to respond to stimulation by autonomic or myotrophic drugs. This impairment does not seem to be limited to the influence of any single drug and consequently it probably does not reflect autonomic imbalance.

Among normal and heterozygous mice there is a tendency for the magnitude of the responses to similar concentrations of the autonomic drugs to increase with the age of the animals. Although this tendency is likewise evident among the dystrophics it is less pronounced, and the differences between responses of normal and dystrophic tissue are consequently more marked in older animals. In addition, significant differences are more frequently noted with higher doses of a particular drug. This may be a reflection of the observation that dose-response curves obtained from 20-week-old normal and heterozygous animals tend to be steeper than those from dystrophics.

Although heterozygous animals do not display clinical or histopathologic evidences of the skeletal muscular disease, they have been shown to manifest varying degrees of the retardation in sexual development which characterize homozygous dystrophic mice(5,6). Since no consistent differences could be observed between the responses of intestinal segments from normal and heterozygous animals, there is no apparent evidence of smooth muscle disease in carriers of the gene.

The possibility is being considered that the non-specific impairment to contractile ability of intestinal smooth muscle from dystrophic animals may be the result of necrosis of the muscularis and subsequent infiltration by connective tissue. However, the observations reported here might also be attributable to a reduction in number or size of the muscle fibers.

Summary. The influence of several pharmacologic agents on the contractile activity of intestinal segments from dystrophic (dy/dy), heterozygous (Dy/dy) and wild-type (Dy/Dy) mice has been examined in an *in vitro* tissue bath system. There appears to be a non-specific impairment to the ability of intestinal smooth muscle from dystrophic animals to respond to stimulation by autonomic or myotrophic drugs. No consistent differences could be detected between the responses of segments from wild-type and from heterozygous animals.

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