Bowel muscle in diverticular disease

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EDITORIAL COMMENT The thickened muscle found in diverticular disease of the colon could be due to a local increase in the number or size of the muscle cells or to sustained contraction. This study, by an ingenious chemical technique, does not resolve the controversy but provides strong evidence that the increase in muscle bulk is not due to an increase in the size of the muscle cells.

In diverticular disease of the colon the muscle wall is sometimes found to be thickened, and Arfwidsson and Lehmann (1964) consider that this increased thickness is due to hypertrophy of the muscle, which precedes the appearance of the diverticula. However, in a post-mortem series (Slack, 1962) it was shown that bowel muscle thickening was associated with diverticula in only two out of 26 specimens. In a clinical series described in the same paper it was noted that macroscopic thickening of the bowel wall occurred in each of 36 surgical specimens. No conclusions were reached as to the cause of the muscle thickening but it was considered that there was no good evidence to show that it was due to hypertrophy.

Hypertrophy has been defined as an increase in the amount of functional tissue of a cell, which is met by a synthesis of more cytoplasm. Although the increase in the mass of functional cytoplasm may continue until the cell is many times larger than normal, reduplication of desoxynucleic acid (D.N.A.) and mitosis do not occur (Florey, 1962). Hyperplasia may be defined as an increase in the number of cells per unit volume due to an increase in rate of normal cell division.

In an attempt to solve whether or not the thickened bowel muscle in diverticulitis is due to hypertrophy, a method was sought to establish the number of cells present per unit volume in normal and thickened muscle. Cell counting was attempted, but as the muscle fibres do not always lie in the same plane, it was realized that this method would give unreliable results. As the amount of D.N.A. is constant in the nucleus of a cell in any specific tissue, this can be used as an index of measurement for the number of nuclei present per unit volume (Davidson, Leslie and White, 1951; Hutchison and Munro, 1961). If the thickening is due to hypertrophy then

the amount of D.N.A. per unit volume would be less than normal.

MATERIALS AND METHODS

A series of 31 portions of bowel muscle were removed from macroscopically normal tissue on surgical specimens of carcinoma of the rectum and sigmoid colon. Fifteen of these were from circular muscle and 16 from taeniae coli. Thirty-three other portions of muscle were removed from various sites on surgical specimens of the colon that had diverticula. Six portions of carcinoma of the large intestine were also removed for analysis. Each strip of tissue was removed as soon as possible from the surgical specimen and kept at 1 to 4°C. until it could be stored at -10°C.

Strips of muscle weighing up to about 0.5 g. were dissected, freed of all adjacent tissues, and, after weighing, were homogenized by grinding in a pestle and mortar with distilled water; the homogenate was transferred to a graded test tube and made up to 10 ml. Two aliquot samples of 1 ml. were removed for estimation of their nitrogen content. The remaining 8 ml. of homogenate was centrifuged for 5 min., the supernatant being discarded. The resulting deposit was treated by the Schneider (1945) procedure and mixed with 10 ml. of 10% tri-chloroacetic acid (T.C.A.), which had been stored at 0°C., to remove the acid-soluble fractions. After centrifuging, the supernatant was discarded and a further 8 ml. of T.C.A. was added. This mixture was heated at 90°C. for 15 minutes, before filtering through no. 42 Whatman paper; the filtrate was then submitted to the Dische (1955) diphenylamine reaction, to convert the extracted D.N.A. to sugars. Aliquots, each of 2 ml. (with duplicates), were placed in test tubes to which were added 2 ml. of fresh diphenylamine reagent (0.2 g. diphenylamine, 2.0 ml. glacial acetic acid, and 0.4 ml. concentrated sulphuric acid), and heated at 90°C. for 10 minutes. Standard solutions of D.N.A. from thymus gland were made up and similarly treated with the diphenylamine reagent. The optical density was then determined in a spectrophotometer at 600 m μ and the amount of D.N.A. per gram of tissue was estimated.

TABLE I
COMPARISON OF MEAN D.N.A.:N. RATIOS

Muscle Specimen	No.	Mean	S.E.	t	P
Normal circular	15	0.03553	0.00332	0.64	Not significant
Thickened circular with diverticula	6	0.03765			
Normal taeniae	16	0.033675	0.00345	0.48	Not significant
Thickened taeniae with diverticula	6	0.035317			
Inflammation	14	0.048314	0.00374	3.67	< 0.01 > 0.001
Normal circular + normal taeniae	31	0.0345574			
Carcinoma	6	0.148			

The total nitrogen estimation was carried out using a micro-Kjeldahl method described by Ma and Zuazaga (1942). The amount of nitrogen per gram of tissue was established from the formula:—

$$\frac{\text{Amount of added N/100 hydrochloric acid} \times \text{factor 1.4}}{\text{Weight of original tissue}}$$

The amounts of D.N.A. and nitrogen per gram of tissue were expressed as a ratio D.N.A.:N.

From each strip of tissue that was submitted to analysis a portion was taken and fixed in 10% formol-saline; histological sections were taken to determine whether there was any infiltration of inflammatory cells, as the presence of these would invalidate the conclusion drawn from the readings for D.N.A. The sections were examined by an independent observer.

RESULTS

The results are grouped under eight separate headings and are shown in the figure: (a) Macroscopically normal circular muscle; (b) macroscopically normal taeniae coli; (c) macroscopically normal circular muscle from specimens with diverticula; (d)

taeniae coli from same specimens as (c); (e) macroscopically thickened circular muscle from specimens with diverticula; (f) taeniae coli from same specimens as (e); (g) specimens with diverticula which contained inflammatory cells; and (h) specimens of carcinoma of the colon or rectum.

In the figure it will be seen that there is little difference in mean D.N.A.:N. ratios in groups (a) to (f). The table shows that statistically the difference between normal and thickened circular muscle and normal and thickened taeniae is not significant. However, when inflammatory tissue is compared with normal circular muscle plus normal taeniae there is a significant difference in the mean levels ($p < 0.01 > 0.001$). The difference in the mean ratio between normal muscle and carcinoma specimens is even greater.

DISCUSSION

Some degree of thickening of the bowel wall is found

FIG. 1. Histograms of the D.N.A.:N. ratio in normal and pathological muscle.

in surgical specimens of diverticular disease. There is a variable degree of pericolic fibrosis, but the increase in thickness of the wall is mainly in the muscle layer. This increase in thickness is due either to hypertrophy of the muscle cells, or hyperplasia of the muscle cells, or a longitudinal contraction of a section of the bowel wall.

Arfwidsson and Lehmann (1964) believe that there is hypertrophy of the muscle coat and they have carried out careful microscopic studies in attempting to demonstrate this feature. However, the results of this investigation fail to support this theory and it seems likely that the increased thickness is due either to hyperplasia of the bowel muscle or a longitudinal contraction of the bowel wall.

CONCLUSION

The mean D.N.A.:N. ratios for specimens of normal colonic circular muscle and normal taeniae are the same, and the mean ratios for muscle from all specimens with diverticula are similar to those from normal muscle. The ratio for specimens with inflammatory cell infiltration is raised, as would be expected

with an increased number of nuclei per unit volume.

The result of this investigation suggests that the increased thickness of the bowel wall in diverticular disease is not due to hypertrophy of the muscle.

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