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The number and distribution of lymphoid follicles in the human large intestine

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INTRODUCTION

The gut-associated lymphoid tissue has two main components, namely lymphoid follicles and lymphoid cells scattered diffusely through the lamina propria and epithelium. The follicles are composed of a ring of lymphocytes around a germinal centre and are mostly located astride the muscularis mucosae (Morson & Dawson, 1979; Shearman & Finlayson, 1982).

With respect to the follicles in the normal human large intestine, few quantitative studies have been performed and the results of these are open to question because of the techniques used. The density counts reported by Dukes & Bussey (1926) have been regarded as authoritative and have set the standard against which subsequent observations have been compared. However, recent radiographic and endoscopic evidence suggests that Dukes & Bussey's density counts are too low and appearances which in the past have been interpreted as lymphoid hyperplasia (Capitanio & Kirkpatrick, 1970; Robinson, Padron & Rywlin, 1973; Desmet, Tubergen & Martel, 1976) are now regarded as the normal follicular pattern of the intestine (Laufer & DeSa, 1978; Kelvin *et al.* 1979; Ell & Frank, 1981).

Using previously developed techniques (Langman, 1983), we have visualised the lymphoid follicles in the normal human large intestine and reassessed their number and distribution.

MATERIAL AND METHODS

Five specimens of large intestine, obtained at autopsy, were studied. Cases 1–4 were those of motor vehicle accident victims who suffered sudden death. Case 5 was a motor vehicle accident victim with head injuries who died after cranial surgery. Their ages, in years, were 19, 58, 21, 17 and 32 respectively. The interval between death and autopsy ranged between eight and 25 hours, with an average of 16 hours. No disease which would be likely to affect the lymphoid tissue of the intestine was revealed at autopsy. The specimens of intestine were opened and washed in 10% acetic acid until they were clean. After immersion in 10% acetic acid for at least 24 hours, lymphoid follicles became clearly visible as white patches (Fig. 1). The specimens were then laid out on a cork board and overlaid with clear plastic upon which their outlines were traced. The area of the total specimen or any section thereof could then be measured using a HP 9874A Digitizer interfaced with a HP 9830A programmable calculator.

The following preliminary test was performed to assess what percentage of the follicle population could be seen macroscopically in acetic acid-fixed intestine. Three segments taken from the large intestine of each of two other cases of sudden death

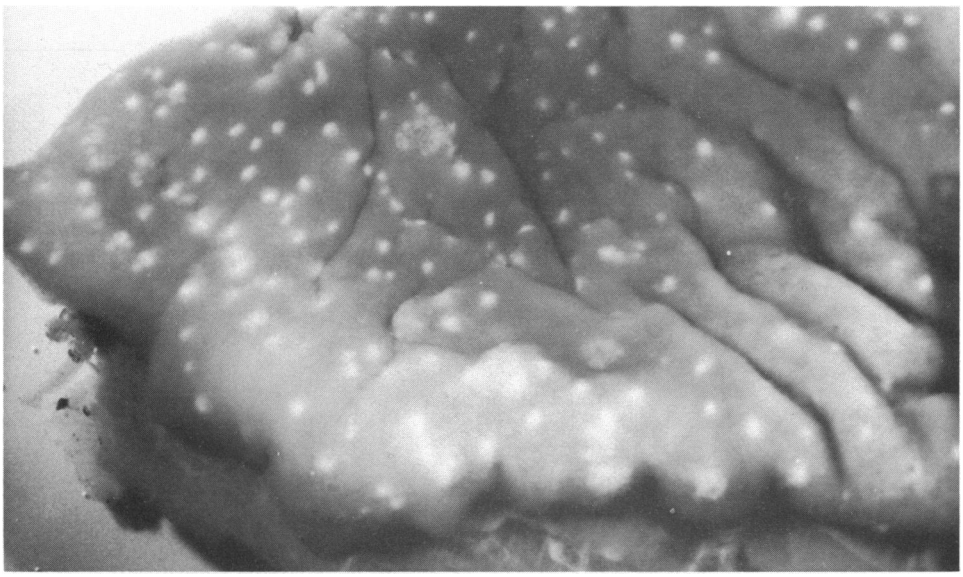

Fig. 1. Acetic acid-cleared large intestine. The lymphoid follicles stand out clearly as white spots in the mucosa. The mucosa is overhanging the cut edge of the main muscle coat. ×4.

were fixed in acetic acid and the numbers of follicles visible macroscopically were counted. The specimens were then blocked *in toto*, processed through alcohol and xylene into paraffin, serially sectioned and the follicles that were found microscopically were counted.

In the five test specimens, the following regions of the large intestine were selected for study because the large number of visible follicles made a total count impractical.

(i) Caecum.

(ii) Colon: starting from the ileocaecal valve, 4 cm lengths were marked off at 20 cm intervals.

(iii) Rectum: taken as the distal 10 cm of large intestine excluding the anorectal junction.

The density of follicles was measured by overlaying the intestine with a small piece of plastic on which a grid of 1 cm squares was drawn. Counts were made over 15 squares selected randomly from the caecum, from each 4 cm length of colon and from the rectum. Counting was easier if the intestine was placed under the lowest power of a dissecting microscope. A mean follicle density for the colon was obtained by averaging the density for each 4 cm length. Also, the total number of follicles in each complete specimen was estimated as follows: the area of each of the studied regions was multiplied by the follicle density of the region and the whole summed. Routine haematoxylin and eosin sections were prepared from each specimen.

RESULTS

Preliminary test result

A total of 80 follicles were counted macroscopically, 32 in the first specimen and 48 in the second. On examination of approximately 1280 serial sections from each specimen, 33 follicles were found in the first and 51 in the second, a total of 84. Thus

Table 1. *Anatomical data on specimens of large intestine used to quantitate lymphoid follicle density*

	Case 1	Case 2	Case 3	Case 4	Case 5
Sex	M	M	F	M	M
Age (years)	19	58	21	17	32
Length of intestine (cm)	131	175	112	126	147
Area of caecum*	109	82	69	75	91
Area of colon	823	1067	534	766	847
Area of rectum	87	103	67	124	98
Total area of intestine	1019	1252	670	965	1036

* All areas are in cm^2.

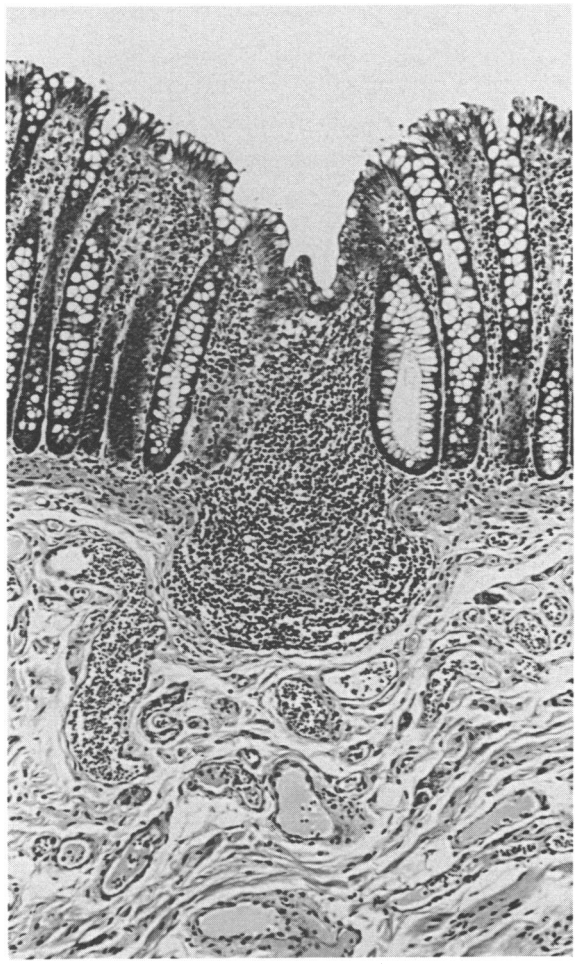

Fig. 2. A typical lymphoid follicle composed of a mass of lymphocytes lying astride the muscularis mucosae. × 100.

Table 2. *Density of lymphoid follicles in each region of the large intestine*

Area	Density of follicles averaged over 15 randomly selected 1 cm² areas					Mean follicle density for all cases
	Case 1	Case 2	Case 3	Case 4	Case 5	
Caecum	14·3±2·1	15·2±2·2	23·7±4·7	16·8±2·8	22·0±4·5	18·4±4·2
Colon 18–22 cm	10·2±2·0	11·9±1·9	17·6±2·8	11·4±2·3	15·3±2·2	—
38–42 cm	18·3±3·5	12·6±2·4	22·5±2·9	12·9±2·1	15·7±3·8	—
58–62 cm	20·5±4·3	17·0±2·2	17·2±2·8	11·5±1·8	16·5±3·6	—
78–82 cm	14·5±3·2	10·2±2·3	18·7±2·5	12·5±1·6	16·3±3·1	—
98–102 cm	16·8±3·6	9·1±1·8	—	—	14·7±1·6	—
118–122 cm	—	10·5±1·6	—	—	18·3±3·2	—
138–142 cm	—	11·6±2·3	—	—	—	—
Mean follicle density for all areas of colon	16·1±3·9	11·8±2·6	19·0±2·4	12·1±0·7	16·1±1·2	15·0±3·0
Rectum	25·6±7·7	24·3±3·9	30·8±5·2	18·0±2·9	28·5±9·3	25·4±4·9

Values are means±S.D. Gaps in the body of the Table result from differing lengths of each intestine.

Table 3. *Estimated total number of lymphoid follicles in each specimen*

	Case 1	Case 2	Case 3	Case 4	Case 5
Caecum	1559	1246	2256	1260	2002
Colon	13250	12591	10146	9269	13637
Rectum	2227	2503	2064	2232	2793
Total intestine	17036	16340	14466	12761	18432

95% of the follicles were identified macroscopically in the acetic acid-fixed material.

Test specimens

Each specimen was normal histologically (Fig. 2). Details of the quantitative data of the five cases are presented in Table 1. After acetic acid fixation, numerous circular or elliptical follicles were seen in all parts of each specimen. They nearly all occurred as single follicles between 0·5 mm and 2 mm in length. In three cases there were a few lymphoid aggregates comprised of a number of follicles, usually between two and six, which were mostly confined to the caecum and were counted as single follicles.

Follicle densities were consistently higher in the rectum than the caecum and they fluctuated down the length of the colon with no obvious pattern (Table 2). There was generally a small decrease in density from the caecum to the colon and a larger increase in density in the rectum compared with both the colon and caecum (Table 2). The estimated total numbers of follicles in the intestines range between 12761 and 18432 (Table 3).

DISCUSSION

The results of this study indicate that lymphoid follicles are distributed over the entire large intestine with a mean density of 18·4 per cm² in the caecum, 15·0 per cm² in the colon and 25·4 per cm² in the rectum. The estimated total number of follicles range between 12761 and 18432, the variation being due, at least to some extent, to

the differing total area of each specimen. The technique used here renders 91 % of the follicle population visible, which implies that our density figures must be very close to true values. The anorectal junction was excluded in this study but according to Morson & Dawson (1979) follicles are particularly noticeable in this area.

Dukes & Bussey's (1926) inappropriately low density figures can be accounted for at least, in part, by the technique used. They derived their figures by first scraping away the mucosa and then counting the follicles in the exposed submucosa. However, many follicles are mucosal and these would have been lost in the scraping. They derived a mean follicle density for normal large intestine of $3{\cdot}3 \pm 2{\cdot}5$ per cm^2. Robinson *et al.* (1973) observed lymphoid follicles with a mean diameter of 2 mm at a density of 7·0 per cm^2 in untreated large intestine in 26 out of 1000 consecutive autopsies. Taking Dukes & Bussey's (1926) figure as normal, they regarded their own figures as showing a pathological increase. We found that while more follicles are visible in fresh than in formalin-fixed specimens, only a small proportion of the follicle population can be seen (Langman, 1983).

The low figures obtained by Dukes and Bussey have led to confusion, because they were used as an anatomical baseline by those assessing follicles by other methods that were not comparable. Thus, the observation of numerous lymphoid follicles with an average diameter of around 2 mm has been called lymphoid hyperplasia (Capitanio & Kirkpatrick, 1970; Desmet *et al.* 1976; Burbige & Sobky, 1977; Kelvin *et al.* 1979; Ell & Frank, 1981), but is probably within normal limits and attempts consistently to associate such appearances with various diseases have been largely unsuccessful (Theander & Trägardh, 1976; Burbige & Sobky, 1977; Kelvin *et al.* 1979; Watanabe, Margulis & Harter, 1983). However, newer methods have also suggested that Dukes & Bussey's figures were incorrect because, as radiographic and endoscopic techniques have improved, lymphoid follicles are being seen more frequently (Theander & Trägardh, 1976) and it was suggested in 1978 (Laufer & DeSa) that the term 'lymphoid hyperplasia' may be a misnomer since it implies a pathological condition. While this has general acceptance in the literature (Kelvin *et al.* 1979; Ell & Frank, 1981), some doubt remains as to whether follicles are always visible in normal large intestine (Crooks & Brown, 1980; Watanabe *et al.* 1983) or, if present, are indicative of underlying pathology (Crooks & Brown, 1980; Bronen, Glick & Teplick, 1984). Also, some authors report a predominantly right-sided follicle distribution (Kelvin *et al.* 1979; Watanabe *et al.* 1983; Bronen *et al.* 1984) and others a predominantly left-sided distribution (Dukes & Bussey, 1926; Riddlesberger & Lebenthal, 1980). Diffuse follicles (involving more than 50 % of the colon) have been regarded as uncommon in the normal adult (Bronen *et al.* 1984). Some authors maintain that visible follicles are rare in patients past middle age (Watanabe *et al.* 1983; Bronen *et al.* 1984). Moreover, it has been stated that it is difficult or impossible to differentiate normal from hyperplastic lymphoid tissue (Ell & Frank, 1981; Watanabe *et al.* 1983).

This study has provided quantitative anatomical justification for the trend in the literature (which has been based largely on radiological evidence) to regard large numbers of small lymphoid follicles as normal. We have shown also that the follicles are distributed over the entire normal large intestine with a higher density than previously believed and that the follicle densities were similar at all ages in the cases studied.

The figures for the normal lymphoid follicle density in the caecum, colon and rectum presented in this study provide a more accurate basis from which to define and recognise abnormal states.

SUMMARY

The density of mucosal lymphoid follicles has been determined in the large intestines of five sudden death victims. The specimens were fixed in acetic acid which made the follicles visible macroscopically. The estimated total number of follicles in the large intestine ranged from 12761 to 18432. The average density of follicles was 18·4 per cm^2 in the caecum, 15·0 per cm^2 in the colon and 25·4 per cm^2 in the rectum. These results indicate that the density of lymphoid follicles has been grossly underestimated in the past where three to five follicles per cm^2 have been accepted as normal. The cause for this major discrepancy is discussed as is its bearing on the diagnosis of lymphoid hyperplasia.

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REFERENCES

BRONEN, R. A., GLICK, S. N. & TEPLICK, S. K. (1984). Diffuse lymphoid follicles of the colon associated with colonic carcinoma. *American Journal of Roentgenology* **142**, 105–109.

BURBIGE, E. J. & SOBKY, R. Z. F. (1977). Endoscopic appearance of colonic lymphoid nodules: a normal variant. *Gastroenterology* **72**, 524–526.

CAPITANIO, M. A. & KIRKPATRICK, J. A. (1970). Lymphoid hyperplasia of the colon in children. *Radiology* **94**, 323–327.

CROOKS, D. J. M. & BROWN, W. R. (1980). The distribution of intestinal nodular lymphoid hyperplasia in immuno-globulin deficiency. *Clinical Radiology* **31**, 701–706.

DESMET, A. A., TUBERGEN, D. G. & MARTEL, W. (1976). Nodular lymphoid hyperplasia of the colon associated with dysgammaglobulinemia. *American Journal of Roentgenology* **127**, 515–517.

DUKES, C. & BUSSEY, H. J. R. (1926). The number of lymphoid follicles of the human large intestine. *Journal of Pathology and Bacteriology* **29**, 111–116.

ELL, S. R. & FRANK, P. H. (1981). Spectrum of lymphoid hyperplasia: colonic manifestations of sarcoidosis, infectious mononucleosis and Crohn's disease. *Gastrointestinal Radiology* **6**, 329–332.

KELVIN, F. M., MAX, R. J., NORTON, G. A., ODDSON, T. A., RICE, R. P., THOMPSON, W. M. & GARBUTT, J. T. (1979). Lymphoid follicular pattern of the colon in adults. *American Journal of Roentgenology* **133**, 821–825.

LANGMAN, J. M. (1983). The quantitation of the lymphoid aggregates in the large bowel of the guinea pig. South Australian Institute of Technology, M.Sc. thesis, 4–7.

LAUFER, I. & DESA, D. (1978). Lymphoid follicular pattern: a normal feature of the pediatric colon. *American Journal of Roentgenology* **130**, 51–55.

MORSON, B. C. & DAWSON, I. M. P. (ed.) (1979). *Gastrointestinal Pathology*, 2nd ed., pp. 488–489. Oxford: Blackwell Scientific Publications.

RIDDLESBERGER, M. M. & LEBENTHAL, E. (1980). Nodular colonic mucosa of childhood: normal or pathologic? *Gastroenterology* **79**, 265–270.

ROBINSON, M. J., PADRON, S. & RYWLIN, A. (1973). Enterocolitis lymphofollicularis. *Archives of Pathology* **96**, 311–315.

SHEARMAN, D. J. C. & FINLAYSON, N. D. C. (1982). *Diseases of the Gastrointestinal Tract and Liver*, p. 807. Edinburgh: Churchill Livingstone.

THEANDER, G. & TRÄGARDH, B. (1976). Lymphoid hyperplasia of the colon in childhood. *Acta Radiologica Diagnosis* **17**, 631–640.

WATANABE, H., MARGULIS, A. R. & HARTER, L. (1983). The occurrence of lymphoid nodules in the colon of adults. *Journal of Clinical Gastroenterology* **5**, 535–539.