OBSERVATIONS BY LIGHT MICROSCOPY AND TRANSMISSION ELECTRONMICROSCOPY ON INTESTINAL SPIROCHAETOSIS IN BABOONS (*PAPIO* SPP.)

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SUMMARY. Spirochaetes were recognised in the large bowel of 57 of 59 baboons as a basophilic fringe at the microvillous brush border of the epithelial cells. Both caecum and colon were usually affected, but seven animals had spirochaetes in the caecum alone. Examination of three animals by transmission electronmicroscopy revealed only one type of spirochaete; ring forms and cross-walls were present. Inflammatory changes were not seen in association with the infection, and the distribution of spirochaetes in 10 animals with soft or diarrhoeic faeces resembled that in normal animals.

INTRODUCTION

The attachment of spirochaetes to the surface epithelium of the large intestine has been reported in man (Harland and Lee, 1967; Lee *et al.*, 1971; Takeuchi *et al.*, 1974; Neutra, 1980; Douglas and Cruciani, 1981; Henrik-Nielsen *et al.*, 1982), rhesus monkeys (*Macaca mulatta*) (Takeuchi and Sprinz, 1970; Takeuchi and Zeller, 1972), guinea-pigs (McLeod *et al.*, 1977) and dogs (Turek and Meyer, 1978). The purpose of this paper is to report intestinal spirochaetosis in baboons (*Papio* spp.).

MATERIALS AND METHODS

Animals. Fifty-nine captured baboons (*Papio cynocephalus* and *Papio anubis*) of both sexes and weighing 3–7 kg were used as control animals in a series of drug-safety evaluations. They were individually caged and medically supervised in a controlled environment for 1 month before and 1–3 months after the beginning of each test. Each animal was given 250 g of Mazuri primate diet (Special Diets Services, Witham, Essex) and half an apple per day, 50 mg of ascorbic acid three times/week, and water *ad libitum*.

Necropsy techniques. All baboons were exsanguinated under pentobarbitone sodium anaesthesia and immediately necropsied. For histological examination, organs including stomach, duodenum, jejunum, ileum, caecum and colon were collected. In addition, samples of caecum were taken from three animals for transmission electronmicroscopy.

Histological studies. Portions of the gastrointestinal tract were stapled on to stiff card with

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the mucosal surface uppermost, placed immediately into Bouin's fixative and transferred to 70% alcohol after 24 h. The fixed tissues were processed by the paraffin method and 5- μ m sections were cut and stained with haematoxylin and eosin (HE). Additional selected sections were stained by the periodic acid-Schiff (PAS) method.

Electronmicroscopy. Samples of caecum (3 $\times$ 2 mm) were fixed in glutaraldehyde 2.5% in sodium cacodylate buffer, pH 7.2, for 4 h at 4°C, and post-fixed in osmium tetroxide 1% for 1 h at room temperature. They were dehydrated in a graded series of ethanols and embedded in Epon®; ultrathin sections were cut on an LKB Ultratome IV. After staining with uranyl acetate and lead citrate, they were examined in a Philips 301 transmission electronmicroscope.

RESULTS

During the period of observations, 10 of the 59 baboons had soft or diarrhoeic faeces for one or more days (table) but recovered spontaneously.

Histological examination revealed a basophilic fringe at the microvillous brush border of the large intestine (fig. 1). The basophilia was readily seen at low power and extended over the surface and into the mouths of the crypts of Lieberkuhn. The basophilic fringe stained positively with PAS. Fifty-seven of the 59 animals were affected and the distribution of the basophilic fringe in the large intestine is shown in the table. In most animals the basophilia in both caecum and colon was diffuse, but in others it was focal. In seven animals the caecum alone was affected. No inflammatory response was associated with the basophilic fringe, either on the epithelial surface or within the lamina propria, in any animal.

In two animals in which the basophilic fringe was diffuse, focal changes in the mucosa of the caecum were observed. In one there was a chronic ulcer comprising granulation tissue from which surface epithelium was absent. In the other there was focal epithelial hyperplasia, increased cytoplasmic basophilia and a few polymorph neutrophils within the epithelium (fig. 2). In both animals the basophilic fringe was absent from the altered mucosa although it was present on epithelial cells in adjacent areas. In all other instances in which the basophilic fringe was absent, the mucosa of the caecum, or colon, or both, revealed no abnormality. No basophilic fringe was seen at any other site in the gastrointestinal tract.

By electronmicroscopy, numerous spirochaetes were observed at the microvillous border of most columnar epithelial cells (fig. 3) and occasional goblet cells (fig. 4),

TABLE
Distribution of spirochaetes in the large intestine of normal baboons and those with soft or diarrhoeic faeces

Spirochaetal distribution in		Number of animals	
caecum	colon	without diarrhoea	with soft or diarrhoeic faeces (duration in days)
Diffuse	Diffuse	40	4 (1)
Focal	Diffuse	4	0
Focal	Focal	1	1 (1)
Diffuse	Absent	2	4 (1-14)
Focal	Absent	1	0
Absent	Absent	1	1 (7)

FIG. 1.—Caecal mucosa, baboon. The epithelial cell surface has a distinct basophilic fringe. HE. Bar = 50 μ m.

FIG. 2.—Caecal mucosa, baboon. The basophilic fringe is absent from an area of epithelium infiltrated by polymorph neutrophils (arrow). HE. Bar = 50 μ m.

where they indented the apical cytoplasm. These organisms corresponded to the basophilic fringe observed histologically. They measured 3–5 μ m in length by about 0.2 μ m in diameter and were tapered at both ends. At low magnification the cytoplasm was relatively dense and only one type of organism was seen. At higher magnification axial fibrils were observed; on cross-section these were seen to be situated between inner and outer membranes. Cross walls were occasionally observed (fig. 5) and in some areas the

FIG. 3.—Spirochaetes indent the apical cytoplasm of a caecal epithelial cell (E). EM. Bar = 1 μ m.

organisms appeared ring shaped (fig. 6). When the brush border was sectioned transversely, organisms appeared to be adherent to adjacent microvilli (fig. 7).

DISCUSSION

This study demonstrated a high prevalence of spirochaetosis of the large intestine in captured baboons (*Papio* spp.). To our knowledge this has not been reported previously. Spirochaetes were present in 57 of 59 animals, compared with 28% reported by Takeuchi *et al.* (1974) in rhesus monkeys and 3.7–9.8% in man (Lee *et al.*, 1971). There is uncertainty as to their possible pathogenicity, some workers believing them to be associated with clinical diarrhoea (Gad *et al.*, 1977; Douglas and Crucoli, 1981) and others regarding them as of little clinical significance (Lee *et al.*, 1971; Minio *et al.*, 1973; Neutra, 1980; Henrik-Nielsen *et al.*, 1983). It has been suggested (Gad, 1983) that the extent of the infection influences pathogenicity. In the present study, however, the organisms were widely distributed in most baboons, including four with soft faeces. Thus in our experience clinical signs were not associated with the presence of the organisms, despite a wide distribution in the large intestine.

FIG. 4.—Spirochaetes indent the apical mucus of a goblet cell (G) of the caecal epithelium. EM. Bar = 1 μ m.

FIG. 5.—A cross wall (arrow) divides the cytoplasm of a longitudinally sectioned spirochaete. EM. Bar = 0.5 μ m.

FIG. 6.—A ring-form of the spirochaete on the microvillous border of the caecum. EM. Bar = $0.5\ \mu\text{m}$.

FIG. 7.—Microvilli of caecal epithelial cells (small arrow) and adjacent spirochaetes (large arrow) are sectioned transversely at the epithelial surface. EM. Bar = $0.5\ \mu\text{m}$.

It was of interest that in a few animals the organisms were observed only in the caecum. In man, spirochaetosis was reported to occur more frequently in normal appendices than in those showing pathological changes (Henrik-Nielsen *et al.*, 1982), and in the baboon it is possible that the caecum is the predilection site. Furthermore, in two animals in which the organisms were widely distributed, infection was absent from areas of mucosal ulceration or inflammation. In man, Harland and Lee (1967) reported five cases in which organisms were absent from a rectal cancer but present on adjacent mucosa. It seems possible, therefore, that the presence of spirochaetes on the brush border in the large intestine of the baboon reflects the health of the large bowel.

Zeller and Takeuchi (1982) suggest that electronmicroscopy is not required to detect this infection in the rhesus monkey. In the baboon also, infection was evident by light microscopy and may in the past, as in man (Lee *et al.*, 1971), have been mistaken for the brush border. However, some features of these organisms were detectable only by ultrastructural investigation. First, only one type of organism was seen; in size and appearance it resembled the spirochaete previously described in the rhesus monkey and man (Takeuchi *et al.*, 1974; Neutra, 1980), but flagellates were not observed. Second, organisms with cross walls and ring-shaped organisms were recognised. These features have not been reported previously in intestinal spirochaetosis. It is possible that the cross walls are concerned with bacterial division (Hovind-Hougen *et al.*, 1982) and the ring forms represent transverse sections of tightly spiralled organisms.

Spirochaetes have also been associated with the microvilli (Takeuchi and Sprinz, 1970) and the peripheral cytoplasm (Antonakopoulos *et al.*, 1982) of goblet cells. However, in the present study the organisms appeared to indent the apical mucus of some goblet cells. Spirochaetes associated with the brush border of the large intestine of man have recently been cultured (Hovind-Hougen *et al.*, 1982).

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