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"INTESTINAL SPIROCHAETOSIS" OF THE VERVET MONKEY

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ASTRACT

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Electron microscopy revealed that 80% of captured vervet monkeys, held in quarantine for experimental use, showed extensive proliferation of spiral-shaped bacteria on the mucosal epithelium of the large bowel. A consortium, consisting of a predominant spirillum together with a spirochaete, was usually seen as a lawn covering the colonic epithelium. Sparsely populated areas showed preferential colonization of the tubular glands. Pathological changes were minimal, being confined to the microvillus border, and affected animals showed no evidence of distress. These findings are compared with those of a similar condition known as "intestinal spirochaetosis" reported in other primates, including man.

INTRODUCTION

Spiral bacteria have been reported as members of the commensal flora of the gut of a wide range of monogastric vertebrates, including man (Harris & Kinyon, 1974; Leach, Lee & Stubbs, 1973). Although these organisms are generally refractive to culture in artificial media, their unique morphology makes them readily visible in microscopic preparations of the distal regions of the gastrointestinal tract, where they may be found in lumen contents, the mucous blanket or on epithelial cell surfaces.

There have been sporadic reports of a condition that has become known as "intestinal spirochaetosis" in which spiral bacteria, responding to some unknown stimulus, spread as a lawn over the colonic epithelium (Antonakopoulos, Newman & Wilkinson, 1982; Lee, Kraszewski, Gordon, Howie, McSeveney & Harland, 1971). In non-primates, the offending organisms are indeed spirochaetes (Turek & Meyer, 1978; 1979), but in primates the condition is considered to be a dual infestation with an unnamed flagellated spirillum together with a classical spirochaete (Neutra, 1980; Takeuchi & Zeller, 1972; Takeuchi, Jervis, Nakazawa & Robinson, 1974). Pathological changes are minimal, consisting mainly of damage to the brush border. There is no apparent debility to the host, which fails even to raise an inflammatory response to organisms that have rarely been observed to translocate across the epithelial basement membrane to the lamina propria. In rhesus monkeys, the reported incidence varies between 12 and 25 %, while in man the reported incidence is as low as 2 to 10 % (Takeuchi *et al.*, 1974).

We report here on an infestation by spiral bacteria of the large bowel of 80 % of vervet monkeys captured locally, which differs in a number of important aspects from previously described cases of intestinal spirochaetosis.

MATERIALS AND METHODS

Animals

Fourteen healthy vervet monkeys (*Cercopithecus aethiops*) were selected from a programme in which animals were captured from the wild from various regions in South Africa and held in quarantine for at least 6 weeks before surgical removal of kidneys for tissue culture cell lines. During quarantine, the monkeys were held initially in large communal cages and were then transferred to smaller individual cages prior to use. While in captivity, the animals were fed commercial dog pellets, fruit and water *ad lib*. Oral tetracycline was administered to animals which developed diarrhoea of undetermined aetiology.

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Electron microscopy

Ligated sections of duodenum, caecum and colon were removed post-mortem. Within an hour of collection, excised sections of gut wall were washed 3 times by 20 agitations in 50 mℓ volumes of 0,85 % NaCl and fixed for at least 24 h in 3 % phosphate buffered glutaraldehyde (pH 7,2). All tissues were post-fixed in 1 % osmium tetroxide, and dehydrated in alcohol. For scanning electron microscopy (SEM), blocks were critical point-dried, mounted, coated with 20 nm of gold and examined in a Cambridge Stereoscan. Examination of crypts was made after random fracture of critical point-dried blocks during preparation for SEM. For transmission electron microscopy, sections were cut from epon-araldite embedded blocks, stained with uranyl acetate-lead citrate sequence and examined on a JEOL 100S at 80 kV. Scrapings of washed, unfixed mucosal epithelium for negative staining were suspended in saline and stained with 3 % phosphotungstic acid on carbon-reinforced, formvar-coated grids.

RESULTS

In 3 of the 14 monkeys, the mucosal epithelium was free of attached bacteria, although a diverse mucous-associated flora was seen in fragments of mucous blanket that had resisted the washing process. In the other 11 monkeys, moderate to extensive colonization of the caecal and colonic gut wall by spiral bacteria was seen.

Two morphologically distinct spiral bacteria were found to be involved in the colonization of the mucosal epithelium. These bacteria were both within the same size range, 0,2–0,3 μm wide and 4,0–5,0 μm long. The predominant organism in all cases was a simple spiral organism with a single flagellum extending from each pole (Fig. 1), while the other organism had a typical spirochaete ultrastructure with a 4–8–4 or 3–6–3 periplasmic flagella configuration (Fig. 2). The spirochaete either co-colonized in low numbers or was absent altogether. Although in 8 animals the epithelia of the large bowel were completely covered by a lawn of bacteria, incomplete colonization in the remaining 3 monkeys revealed an unusual distribution, not reported in previous studies of intestinal spirochaetosis. In these monkeys, the flagellated organism showed a marked predilection for the epithelial cells lining the tubular glands of the colon (Fig. 3, 4 & 5). Goblet cells and extruded mucous were not colonized (Fig. 6). Both flagellated and spirochaete forms were found in a corona around gland openings.

Transmission electron microscopy revealed that both organisms were in intimate contact with the epithelial cells (Fig. 7 & 8). Disruption of microvillus continuity and loss of subsurface structure and organelles were also observed. Even in the most heavily infested monkeys no

FIG. 1 Flagellated spiral organism in scraping from monkey colonic epithelium. Negative staining. Bar = 0,5 μm

evidence was found of migration of spiral bacteria into the epithelial cells, nor of translocation through the basement membrane.

DISCUSSION

This study has demonstrated that the majority of captured vervet monkeys exhibited extensive colonization of the large bowel by spiral organisms. This infestation differs, however, from that described as "intestinal spirochaetosis", reported from both rhesus monkeys and man. In these hosts the predominant organism was a spirochaete, whereas in our study the numerically dominant organism was a flagellated spirillum. It is of interest to note, however, that, in spite of these differences, the 2 organisms involved appeared to be morphologically identical with those, organisms described in other primate intestinal spirochaetoses. Unfortunately, structural criteria are the only means of comparing non-cultivable organisms, but considering the diversity of spirochaetes found in the gastrointestinal tract of animals, the similarity between those involved in primate spirochaetosis is striking.

A further differentiating feature of the infestation seen in vervet monkeys was the preferential localization of these organisms in and around the tubular glands of the large bowel. Minio, Tonietti & Torsoli (1973) and Neutra (1980) have excluded tubular glands from the distribution pattern of bacteria involved in intestinal spirochaetosis both in man and in the rhesus monkey, although in these hosts the predominant organism was specifically identified as a spirochaete.

The limited pathology we observed in heavily infested monkeys was no more nor less than that reported by other workers in other hosts. The cellular degeneration described appears to be a non-specific response, while the severe loss of microvilli in areas of heavy infestation, even though widespread in some subjects, seems to cause little permanent debility to the host. There was no obvious association between diarrhoea, antibiotic therapy and spirochaetosis in the group of animals studied. Penetration by spiral bacteria into or through the epithelial layer has reportedly taken place under high epithelial challenge when the spirochaete member of the consortium appears able to traverse the epithelium. That we found no evidence of intracellular bacteria, even under very high challenge with the flagellate, would suggest that this organism is not capable of epithelial penetration.

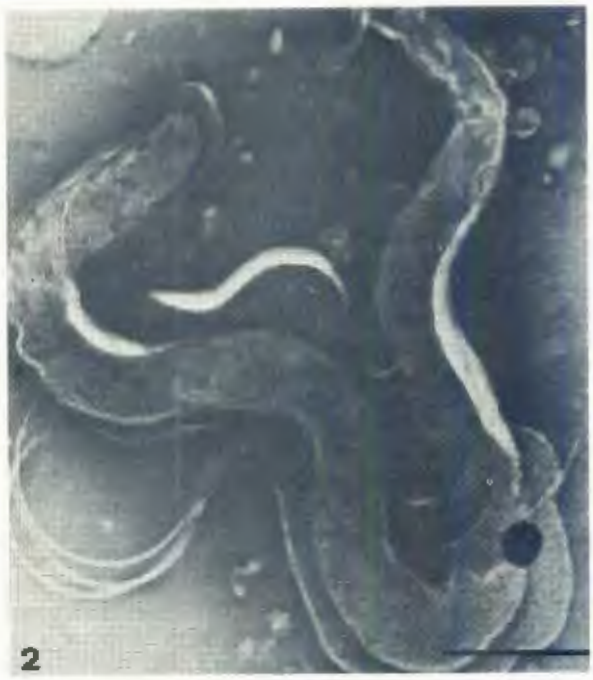

FIG. 2 Spirochaete in scraping from monkey colonic epithelium. Negative staining. Bar = 0,5 μm

Further conjecture on the significance of these results is not possible until it has been established whether the animals showing sparse colonization of the gut wall were in the declining phase of a heavy infestation or at the beginning of an expansion from the protected environment of the glands. Since intestinal spirochaetosis has been shown to be a chronic infection lasting up to 6 years (Lee *et al*, 1971), and since the animals in this study were examined within 3 months of capture, it is possible that this paper reports the early stages of infection. However, we cannot preclude the presence of this condition as endemic in wild monkeys, as no data are available from free-living animals. Although the presence of large numbers of spiral bacteria in the gut of captured vervet monkeys does not seem to cause patent debility nor overt pathology, we feel that the condition is not "normal" and advise caution in the interpretation of results of experiments performed on captured monkeys, particularly in nutritional and immunological studies.

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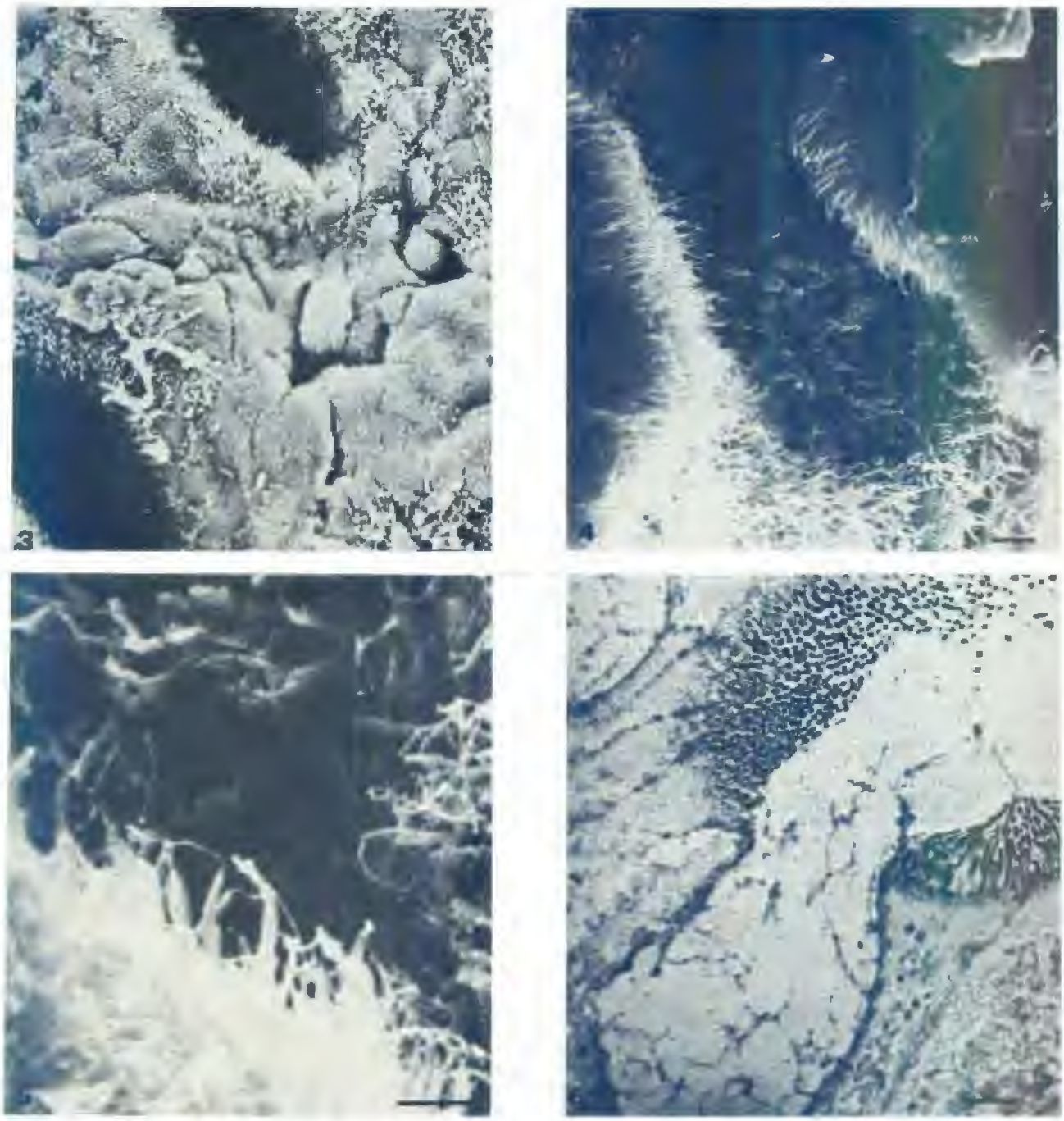
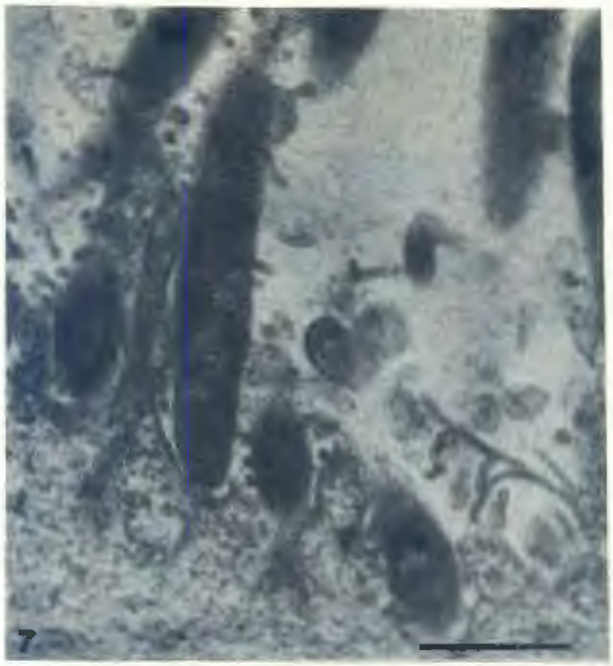


FIG. 3 SEM of spiral bacteria heavily colonizing the neck of monkey colonic tubular gland. Bar = 5 μm
FIG. 4 SEM of transverse section through crypt depicting colonization by spiral organisms. Bar = 2 μm
FIG. 5 Magnified view of Fig. 4 depicting polar flagellation. Bar = 1 μm
FIG. 6 Section of monkey colonic gland showing heavy infestation of epithelial cells but not of goblet cell mucin. Bar = 2 μm



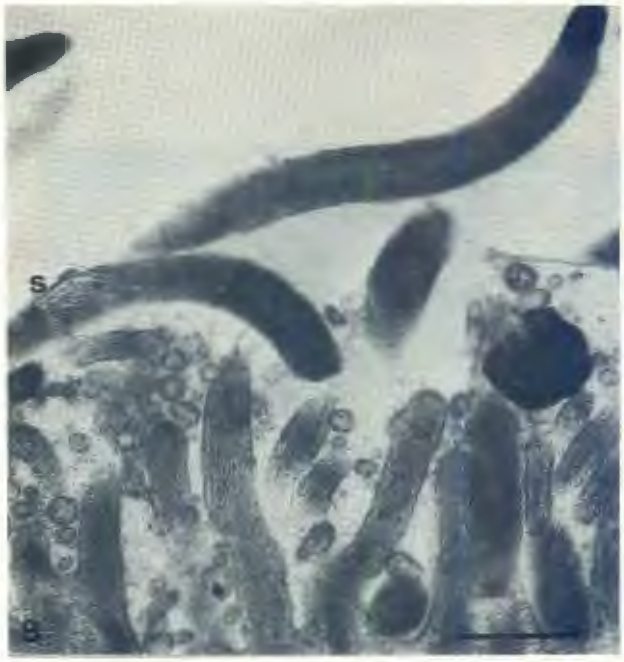


FIG. 7 Flagellated spiral bacteria attached to epithelium. Note regional loss of cellular organelles. Bar = 0,5 μm

FIG. 8 Spirochaetes (S) in close proximity to epithelial cells. Bar = 0,5 μm

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