UNIDENTIFIED CURVED BACILLI ON GASTRIC EPITHELIUM IN ACTIVE CHRONIC GASTRITIS

SIR,—Gastric microbiology has been sadly neglected. Half the patients coming to gastroscopy and biopsy show bacterial colonisation of their stomachs, a colonisation remarkable for the constancy of both the bacteria involved and the associated histological changes. During the past three years I have observed small curved and S-shaped bacilli in 135 gastric biopsy specimens. The bacteria were closely associated with the surface epithelium, both within and between the gastric pits. Distribution was continuous, patchy, or focal. They were difficult to see with haematoxylin and eosin stain, but stained well by the Warthin-Starry silver method (figure).

I have classified gastric biopsy findings according to the type of inflammation, regardless of other features, as "no inflammation", "chronic gastritis" (CG), or "active chronic gastritis" (ACG). CG shows more small round cells than normal while ACG is characterised by an increase in polymorphonuclear neutrophil leucocytes, besides the features of CG. It was unusual to find no inflammation. CG usually showed superficial oedema of the mucosa. The leucocytes in ACG were usually focal and superficial, in and near the surface epithelium. In many cases they only infiltrated the necks of occasional gastric glands. The superficial epithelium was often irregular, with reduced mucinogenesis and a cobblestone surface.

When there was no inflammation bacteria were rare. Bacteria were often found in CG, but were rarely numerous. The curved bacilli were almost always present in ACG, often in large numbers and often growing between the cells of the surface epithelium (figure). The constant morphology of these bacteria and their intimate relationship with the mucosal architecture contrasted with the heterogeneous bacteria often seen in the surface debris. There was normally a layer of mucous secretion on the surface of the mucosa. When this layer was intact, the debris was spread over it, while the curved bacilli were on the epithelium beneath, closely spread over the surface (figure).

The curved bacilli and the associated histological changes may be present in any part of the stomach, but they were seen most consistently in the gastric antrum. Inflammation, with no bacteria, occurred in mucosa near focal lesions such as carcinoma or peptic ulcer. In such cases, the leucocytes were spread through the full thickness of the nearby mucosa, in contrast to the superficial infiltration associated with the bacteria. Both the bacteria and the typical histological changes were commonly found in mucosa unaffected by the focal lesion.

The extraordinary features of these bacteria are that they are almost unknown to clinicians and pathologists alike, that they are closely associated with granulocyte infiltration, and that they are present in about half of our routine gastric biopsy specimens in numbers large enough to see on routine histology. The only other organism I have found actively growing in the stomach is *Candida*, sometimes seen in the floor of peptic ulcers. These bacteria were not mentioned in two major studies of gastrointestinal microbiology[1,2] possibly because of their unusual atmospheric requirements and slow growth in culture (described by Dr B. Marshall in the accompanying letter). They were mentioned in passing by Fung et al.[3]

How the bacteria survive is uncertain. There is a pH gradient from acid in the gastric lumen to near neutral in the mucosal vessels. The bacteria grow in close contact with the epithelium, presumably near the neutral end of this gradient, and are protected by the overlying mucus.

The identification and clinical significance of this bacterium remain uncertain. By light microscopy it resembles *Campylobacter jejuni* but cannot be classified by reference to *Bergey's Manual of Determinative Bacteriology*. The stomach must not be viewed as a sterile organ with no permanent flora. Bacteria in numbers sufficient to see by light microscopy are closely associated with an active form of gastritis, a cause of considerable morbidity (dyspeptic disease). These organisms should be recognised and their significance investigated.

Curved bacilli on gastric epithelium.

Section is cut at acute angle to show bacteria on surface, forming network between epithelial cells. (Warthin-Starry silver stain; bar = 10 μm.)

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SIR,—The above description of S-shaped spiral bacteria in the gastric antrum, by my colleague Dr J. R. Warren, raises the following questions: why have they not been seen before; are they pathogens or merely commensals in a damaged mucosa; and are they campylobacters?

In 1938 Doenges[1] found "spirochaetes" in 43% of 242 stomachs at necropsy but drew no conclusions because autolysis had rendered most of the specimens unsuitable for pathological diagnosis. Freedburg and Barron[2] studied 35 partial gastrectomy specimens and found "spirochaetes" in 37%, after a long search. They concluded that the bacteria colonised the tissue near benign or malignant ulcers as non-pathogenic opportunists. When Palmer[3] examined 1140 gastric suction biopsy specimens he did not use silver stains, so, not surprisingly, he found "no structure which could reasonably be considered to be of a spirochaetal nature". He concluded that the gastric "spirochaetes" were oral contaminants which multiplied only in post mortem specimens or close to ulcers. Since that time, the spiral bacteria have rarely been mentioned, except as curiosities,[4] and the subject was not reopened with the

1. Gray JDA, Shiner M. Influence of gastric pH on gastric and jejunal flora. *Gut* 1967, **8**: 574–81.
2. Drasar BS, Shiner M, McLeod GM. Studies on the intestinal flora I: The bacterial flora of the gastrointestinal tract in healthy and achlorhydric persons. *Gastroenterology* 1969, **56**: 71–79.
3. Fung WP, Papadimitriou JM, Matz LR. Endoscopic, histological and ultrastructural correlations in chronic gastritis. *Am J Gastroenterol* 1979; **71**: 269–79.
1. Doenges JL. Spirochaetes in the gastric glands of *Macacus rhesus* and humans without definite history of related disease. *Proc Soc Exp Med Biol* 1938; **38**: 536–38.
2. Freedburg AS, Barron LE. The presence of spirochaetes in human gastric mucosa. *Am J Dig Dis* 1940; **7**: 443–45.
3. Palmer ED. Investigation of the gastric spirochaetes of the human? *Gastroenterology* 1954, **27**: 218–20.
4. Ito S. Anatomic structure of the gastric mucosa. In: Heidel US, Cody CF, eds. Handbook of physiology, section 6: Alimentary canal, vol II: Secretion. Washington, DC: American Physiological Society, 1967: 705–41.

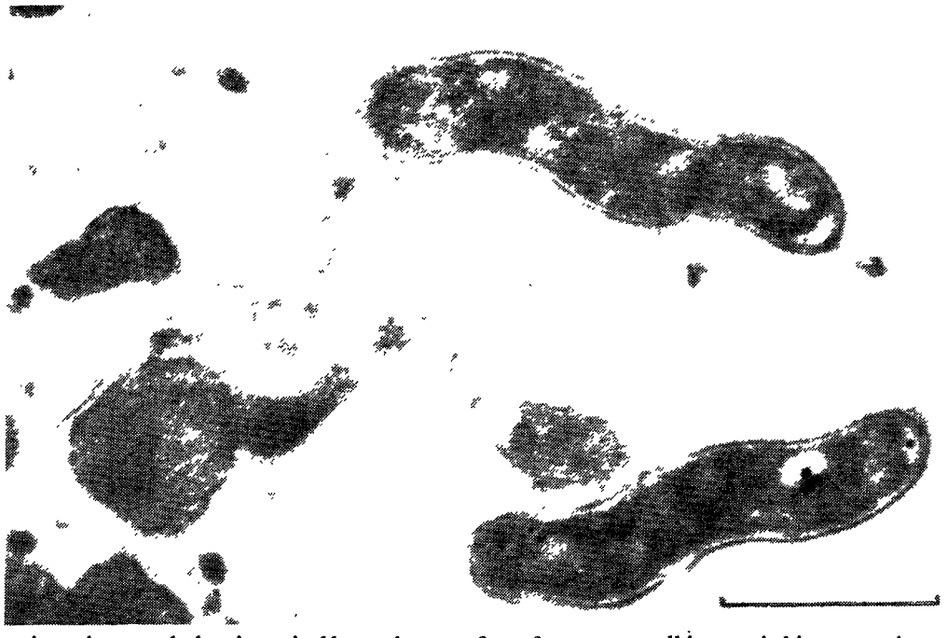

Fig 1—Thin-section micrograph showing spiral bacteria on surface of a mucous cell in gastric biopsy specimen. (Bar = 1 μm.)

advent of gastroscopic biopsy. Silver staining is not routine for mucosal biopsy specimens, and the bacteria have been overlooked.

In other mammals spiral gastric bacteria are well known and are thought to be commensals[5] (eg, Doenges[1] found them in all of forty-three monkeys). They usually have more than two spirals and inhabit the acid-secreting gastric fundus.[5] In cats they even occupy the canaliculi of the oxyntic cells, suggesting tolerance to acid.[6] The animal bacteria do not cause any inflammatory response, and no illness has ever been associated with them.

Investigation of gastric bacteria in man has been hampered by the false assumption that the bacteria were the same as those in animals and would therefore be acid-tolerant inhabitants of the fundus. Warren's bacteria are, however, shorter, with only one or two spirals and resemble campylobacters rather than spirochaetes. They live beneath the mucus of the gastric antrum well away from the acid-secreting cells.

We have cultured the bacteria from antral biopsy specimens, using *Campylobacter* isolation techniques. They are microaerophilic and grow on moist chocolate agar at 37°C, showing up in 3–4 days as a faint transparent layer. They are about 0·5 μm in diameter and 2·5 μm in length, appearing as short spirals with one or two wavelengths (fig 1). The bacteria have smooth coats with up to five sheathed flagellae arising from one end (fig 2). In some cells, including dividing forms, flagellae may be seen at both ends and in negative stain preparations they have bulbous tips, presumably an artefact.[7]

These bacteria do not fit any known species either morphologically or biochemically. Similar sheathed flagellae have been described in vibrios[7] but micro-aerophilic vibrios have now

5 Lockard VG, Boler RK. Ultrastructure of a spiraled micro-organism in the gastric mucosa of dogs. *Am J Vet Res* 1970; **31:** 1453–62.

6 Vial JD, Orrego H. Electron microscope observations on the fine structure of parietal cells. *J Biophys Biochem Cytol* 1960; **7:** 367–72.

7. Glauert AM, Kerridge D, Horne RW. The fine structure and mode of attachment of the sheathed flagellum of *Vibrio metchnikovii*. *J Cell Biol* 1963; **18:** 327–36.

8. Shewan JM, Veron M. Genus I vibrio. In: Buchanan RE, Gibbons NE, eds. Bergey's manual of determinative microbiology, 8th ed. Baltimore: Williams & Wilkins, 1974· 341.

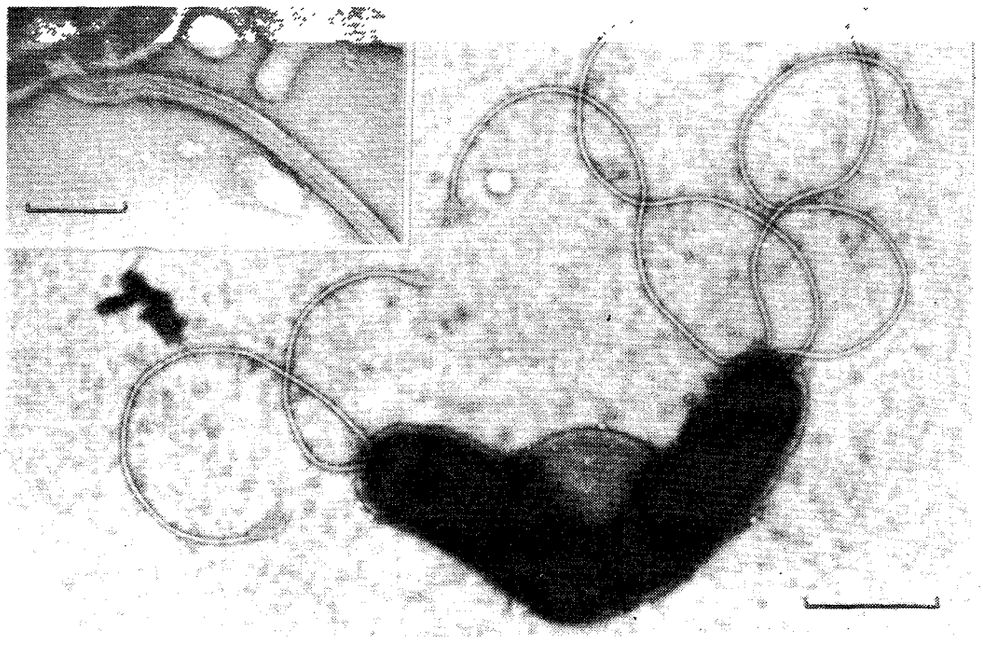

Fig 2—Negative stain micrograph of dividing bacterium from broth culture.

Multiple polar flagellae have terminal bulbs, (2% phosphotungstate, pH 6·8; bar = 1 μm.) Inset: detail showing sheathbed flagellum and basal disc associated with plasma membrane. (3% ammonium molybdate, pH 6·5; bar = 100 nm.)

been transferred to the family Spirillaceae genus *Campylobacter*.[8] Campylobacters however, have "a single polar flagellum at one or both ends of the cell" and the campylobacter flagellum is unsheathed.[9] Warren's bacteria may be of the genus *Spirillum*.

The pathogenicity of these bacteria remains unproven but their association with polymorphonuclear infiltration in the human antrum is highly suspicious. If these bacteria are truly associated with antral gastritis, as described by Warren, they may have a part to play in other poorly understood, gastritis associated diseases (ie, peptic ulcer and gastric cancer).

I thank Miss Helen Royce for microbiological assistance, Dr J. A. Armstrong for electronmicroscopy, and Dr Warren for permission to use fig 1.

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VASODILATOR PROSTANOIDS AND ACTH-DEPENDENT HYPERTENSION

SIR,—Dr Axelrod (April 23, p 904) proposes that the permissive effect of glucocorticoids on vascular tone is mediated via inhibition of prostacyclin production and that this may contribute to the hypertension of Cushing's syndrome. We became interested in this possibility following the suggestion by Rascher at al[1] that glucocorticoids may produce hypertension as a result of inhibition of phospholipase A_2 and a subsequent reduction in "vasodilator" prostaglandin synthesis. The demonstration by Weeks and Sutter[2] that prostacyclin (epoprostenol) infusion attenuated the development of DOCA (desoxycortone) induced hypertension in the rat was also relevant. We have reviewed the evidence for such a hypothesis in relation to steroid and corticotropin (ACTH) dependent hypertension.[3] Our own studies have been concerned with the mechanism of ACTH induced hypertension in sheep, a form of experimental hypertension and features of glucocorticoid and mineralocorticoid excess but in which these two classes of adrenocortical steroid activity do not appear to account for more than about half of the hypertension.[3] On the basis of detailed experiments in conscious sheep we concluded that although "vasodilator" prostanoids such as prostacyclin appear to modulate the ACTH induced rises in blood pressure they did not play a primary role in the development of the hypertension.

Although in sheep,[4] as in other species, indomethacin enhances vasoconstrictor responses to angiotensin II, ACTH treatment does not alter pressor responsiveness to either angiotensin II, noradrenaline, or arginine-vasopressin.[5-7] Also, indomethacin (3 mg/kg daily for 3 days) had no effect on blood pressure in normotensive sheep.[6] Further, pretreatment of sheep for 24 h with prostacyclin at a dose which lowered total peripheral resistance but not blood pressure did not alter the blood pressure response to ACTH.[6] This suggests to us that the proposal by Axelrod that ACTH-dependent hypertension is in any way caused by inhibition or prostaglandin synthesis is questionable.

Our evidence that prostaglandins may modulate the severity of ACTH dependent hypertension is based on three series of experiments. The first showed that although indomethacin infusion for 60 min, at a dose which blocks the vasodepressor effect of arachidonic acid, has no effect on blood pressure in normotensive sheep, it produced a further increase in mean arterial pressure of 26 mm Hg in sheep with ACTH-induced hypertension.[6] This rise in blood pressure was entirely due to a rise in total peripheral resistance. In the second series of experiments we showed that in animals pretreated with indomethacin for three days the rise in blood pressure in response to ACTH was significantly greater.[6] Finally we found that although graded doses of prostacyclin, infused for 10 min, produced similar falls in blood pressure in normotensive and ACTH hypertensive sheep, the fall in total peripheral resistance is much greater in the ACTH treated animals.[8] We speculated that plasma levels of vasodilator prostanoids such as prostacyclin may rise in response to ACTH administration. However, measurement of plasma 6-keto-$PGF_{1\alpha}$ (considered by some to reflect prostacyclin production) by Dr Murray Mitchell (Dallas, USA) showed a small but significant decrease with ACTH treatment.[3]

Our studies in sheep suggest a modulating rather than causal role for vasodilator prostanoids in ACTH-dependent hypertension.

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EPOPROSTENOL (PROSTACYCLIN) DECREASES PLATELET DEPOSITION ON VASCULAR PROSTHETIC GRAFTS

SIR,—Prostacyclin (PGI_2) is an important regulator of platelet deposition on vascular surfaces.[9] When a prosthetic vascular graft is inserted, a few weeks are required before the formation of PGI_2 by the pseudovascular wall cells reaches the same level of activity of tissue in the vicinity[10] because of the slow increase in prostacyclin synthetase[11] in the invading cells. Hence platelet deposition on the graft surface may be a significant factor in limiting graft survival[12] and causing early occlusion. PGI_2 can decrease platelet deposition on vascular surfaces,[13] so we wondered if platelet deposition on prosthetic grafts would be affected by a short term infusion of epoprostenol.

We examined nine male and two female patients aged 53–66 years between 48 and 72 h after surgery. Autologous platelet labelling was carried out with 100 μCi ^{111}In-oxine sulphate.[14] Platelet labelling efficiency amounted to 92±2%, and recovery 2 h after re-injection of autologous labelled platelets was 76±4%. 6 h after re-injection of autologous labelled platelets gamma-camera imaging studies were done. Epoprostenol (prostacyclin) 5 ng/kg/min was then infused for 24 h. Gamma-camera imaging was repeated (see figure) during and after prostacyclin infusion. Regions of interest (ROI) were

9 Pead PJ. Electron microscopy of *Campylobacter jejuni*. *J Med Microbiol* 1979; **12:** 383–85.

1 Rascher W, Dietz R, Schomig A, et al. Modulation of sympathetic vascular tone by prostaglandins in corticosterone-induced hypertension in rats. *Clin Sci* 1979; **57:** 235s–37s.

2. Weeks JR, Sutter DM. An antihypertensive effect of prostacyclin. New York: Raven Press, 1979 253–57.

3 Scoggins BA, Coghlan JP, Denton DA, Mason RT, Whitworth JA. A review of mechanisms involved in the production of steroid induced hypertension with particular reference to ACTH dependent hypertension. In: Mantero F, Biglieri EG, Edwards CRW, eds. Endocrinology of hypertension London: Academic Press, 1982: 41–67

4. Beilby DS, Coghlan JP, Denton DA, et al. In vivo-modification of angiotensin II pressor responsiveness in sheep by indomethacin. *Clin Exp Pharmacol Physiol* 1981; **8:** 33–37

5. McDougall JG, Barnes AM, Coghlan JP, et al. The effect of corticotrophin (ACTH) administration on the pressor action of angiotensin II, noradrenaline and tyramine in sheep. *Clin Exp Pharmacol Physiol* 1978; **5:** 449–55.

6. Mason RT, Coghlan JP, Denton DA. Do prostaglandins play a role in modulating the haemodynamic effects of ACTH administration? *Proc Endocrinol Soc Aust* 1981; **24:** 7.

7. Coghlan JP, Denton DA, Graham WF, et al. Effect of ACTH administration on the haemodynamic response to arginine-vasopressin in sheep. *Clin Expt Pharmacol Physiol* 1980; **7:** 559–62.

8. Mason RT, Allen KJF, Coghlan JP, Denton, et al. ACTH hypertension modifies the haemodynamic effects of prostacyclin infusions in sheep. *Clin Exp Pharmacol Physiol* 1980; **7:** 469–72.

9. Moncada S, Vane JR. Unstable metabolites of arachidonic acid and their role in haemostasis and thrombosis. *Br Med Bull* 1978; **34:** 129–36.

10. Sinzinger H, Silberbauer K, Winter M. Implanted vascular prostheses generate prostacyclin. *Lancet* 1978; ii: 840–41

11. Eldor A, Falcone D, Hajjar DP, Minick CR, Weksler BB. Recovery of prostacyclin production by deendothelialized rabbit aorta. *J Clin Invest* 1981; **67:** 735–41.

12. Harker LA, Slichter SJ, Sauvaage LR. Platelet consumption by arterial prostheses: The effect of endothelialization and pharmacological inhibition of platelet function *Ann Surg* 1977, **186:** 594–600.

13. Moncada S, Higgs EA, Vane JR. Human arterial and venous tissue generates prostacyclin (prostaglandin) a potent inhibitor of platelet aggregation. *Lancet* 1977; i: 18–21.

14. Sinzinger H, Schwarz M, Leithner Ch, Hofer R. Labelling of autologous human platelets with indium-^{111}-oxine sulphate for monitoring of human kidney transplants. *Nucl Med Biol (Paris)* 1982; 2752–55.