LETTERS TO THE EDITOR

Methanogenesis in the human large intestine

EDITOR,—Methanogenesis is important in many anaerobic microbial environments. The human colon is such an environment. It is therefore a matter of curiosity that only some humans consistently produce significant amounts of methane. *Gut* published a paper concerning the apparent regulation of colonic methanogens by sulphate from the human diet (*Gut* 1992; **33**: 1234–8). Sulphate is a substrate for oxidative metabolism by sulphate reducing bacteria. A crucial argument of the authors of this paper is that sulphate reducing bacteria outcompete gut methanogens for hydrogen. They base this on their own in vitro work,[1] even though there are generally only small amounts of sulphate in faeces from humans on a high sulphate diet.[2]

Other investigators have reported the complete opposite. That is, human methanogens outcompete sulphate reducing bacteria in mixed faecal cultures, even with sulphate added[3 4]; that human methanogenic faeces consume hydrogen far more rapidly than non-methanogenic faeces;[4] and that faecal concentrations of sulphate and sulphide, the substrate and end products of sulphate reducing bacteria, are not even appreciably different between human methanogenic and non-methanogenic faeces.[2-5]

At least two of the three authors of the paper published in *Gut* knew of this other work as long ago as mid 1990. It is, therefore, disturbing and a little disappointing that this research work[3-5] was not referenced in their paper.

The control of human methanogenesis is not because of a competition between methanogens and sulphate reducing bacteria, so what does regulate human methanogenesis? There is good evidence that bile[6 7] and faecal stirring[8] inhibit hydrogen consumption by human methanogens. These are two of several possible factors, which could explain in a large part the epidemiology of human methanogenesis, including its rarity in patients with Crohn's disease of the terminal ileum. Ingested sulphate might act by augmenting gut motility and bile losses into the colon.[9]

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Reply

EDITOR,—The study of hydrogen metabolism in the human large intestine is providing new insights into colonic function and it is now being recognised that this may have implications for a number of diseases.[1-4] The control of methane production, which is one of a variety of ways for hydrogen disposal in man, has hitherto been incompletely understood. There is now substantial evidence, however, that competition for the growth substrate H_2 exists between methanogenic bacteria and sulphate reducing bacteria in some human populations.[5-8]

Since 1988[5] we have extended our studies of normal human faecal flora from the original 40 subjects to 127 and have found that 59 (46%) are methanogenic and 68 (53%) sulphate reducing. In 16 of the methanogenic subjects, low numbers of sulphate reducing bacteria were found and significant sulphate reducing activity occurred when methanogenesis was inhibited. In vitro, we have shown that competitive interactions occur between methanogenic bacteria and sulphate reducing bacteria, and that in the presence of sulphate from either organic (mucin) or inorganic sources, sulphate reducing bacteria are able to outcompete methanogenic bacteria for hydrogen.[7 8] In the paper,[9] sulphate feeding inhibited methanogenesis in half of the subjects when sulphate reducing bacteria were provided with an adequate amount of electron acceptor (sulphate). Such interactions between methanogenic bacteria and sulphate reducing bacteria also occur in marine sediments.[10] Methanogenic bacteria will always be outcompeted by sulphate reducing bacteria if sulphate is available unless hydrogen is present in great excess, the reason being that K_s for H_2 uptake favours sulphate reducing bacteria (1 μmol/l for *Desulfovibrio vulgaris*) at the expense of methanogenic bacteria (6 μmol/l for *Methanobrevibacter smithii*).[11] Also, the oxidation of H_2 by sulphate reducing bacteria is thermodynamically more favourable ($\triangle Go' = -152{\cdot}2$ kJ/mol) than by methanogenic bacteria ($\triangle Go' = -131$ kJ/mol).[12]

In this context, Dr Florin raises the interesting question of why there is no significant sulphate or sulphide in the faeces of methanogenic subjects.[13 14] As he will recall from the three years he spent with us in Cambridge, there are several possible explanations for this. The most important is that many species of facultatively anaerobic bacteria such as *Escherichia coli* can use sulphate as their sole source of elemental sulphur. In so doing, they reduce sulphate, a process known as assimilatory sulphate reduction.[15] By contrast, sulphate reducing bacteria conduct dissimilatory sulphate reduction in which sulphate acts as an electron acceptor during the breakdown (dissimilation) of organic matter. Both pathways occur in the colon and consume sulphate. Sulphide (SH^-) is of course rapidly absorbed and oxidised by the colonic mucosa.

Dr Florin suggests that other investigators have reported 'the complete opposite to be the case' namely that methanogenic bacteria outcompete sulphate reducing bacteria. His references do not support this argument. His own paper[16] which is essentially about measuring sulphide, contains no competition studies between methanogenic bacteria and sulphate reducing bacteria, or even data from mixed faecal slurries. The other paper[14] does include competition studies but no evidence that the non-methanogenic faeces contained viable sulphate reducing bacteria. No study, where sulphate was available in appreciable amounts, has shown that methanogenic bacteria outcompete sulphate reducing bacteria. It is worth noting that where an apparent absence of sulphate reducing bacteria is seen it is necessary to enumerate these bacteria after sulphate feeding, not just before, as such feeding may lead to growth of sulphate reducing bacteria which had not been dected before.[9]

So what of Dr Florin's suggestion that other factors control methanogenesis? We are happy to accept the findings of Levitt's group[17] that faecal stirring may be important. Stirring could increase the availability of hydrogen to methanogenic bacteria and thus allow bacteria to gain some advantage over organisms which might normally outcompete them in a more restricted environment. We note Strocchi and Levitt's comments about there being no quantitative data on stirring of colonic contents (in vivo).

We are not convinced of the role of bile as a controlling factor. Dr Florin may have misinterpreted his own experiments, which he reports in abstract form.[18] Every healthy colon contains bile acids, with which colonic bacteria coexist quite effectively. Miller and Wolin[19 20] have shown that methanogenic bacteria from the colon are not inhibited in their function by bile acids. These studies were conducted with *Methanobrevibacter smithii*[19] and *Methanosphaera stadtmaniae*,[20] the principal colonic methanogens. Of course, if you have enough bile acid then some inhibitory effects may be possible. Bile acid concentrations in ileal effluent, however, are less than 1 mM[21 22] while in the aqueous phase of stool they are even lower.[23 24] Dr Florin's studies, which actually failed to show an effect of bile acids on methanogenesis at concentrations of 0·05% (around 1 mM) were at concentrations of 0·1–1·0% bile acids (2·5–25·0 mM), which is unphysiological.

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Thyroid function and interferon treatment in chronic hepatitis C

EDITOR,—We read with much interest the article by Marcellin *et al* (*Gut* 1992; **33**: 855–6) about two cases of sustained hypothyroidism induced by recombinant α interferon in patients with chronic hepatitis C. The presence of antithyroid antibodies in both patients suggested an autoimmune cause of the hypothyroidism.[1,2]

We have recently seen a 51 year old woman with chronic active hepatitis C proved on biopsy examination randomised to receive 3 MU of recombinant interferon alfa 2-b (INTRON-A, Schering-Plough Corporation) subcutaneously three times a week for six months. The patient had no history of thyroid disease and had not received any drug known to be toxic to the thyroid. Serum triiodothyronine, thyroxine, free thyroxine, and thyroxine binding globulin were determined by RIA kits (Farmos Diagnostica Ltd, Turku, Finland); serum thyroid stimulating hormone, thyroid microsomal antigen autoantibodies, and thyroglobulin autoantibodies were measured by IRMA kits (Biocode, Switzerland). Serum samples were collected before treatment and every month for 12 months thereafter.

A transient reduction in triiodothyronine, thyroxine free thyroxine values started at month 4 and an increase in thyroid stimulating hormone values was recorded (Figure). The patient had no clinical signs of hypothyroidism; thyroid autoantibodies remained negative. On this basis, we suggest a multifactorial cause of thyroid function change induced by recombinant α interferon. In our case, a direct inhibition of thyroid hormone synthesis and secretion, or both by recombinant α interferon could have played a determinant role. This mechanism has been shown by in vitro experiments with γ interferon.[3,4]

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Reply

EDITOR,—We read with interest the comment by Picciotto *et al* on our paper reporting another case of hypothyroidism, probably induced by recombinant interferon alfa 2-b in a patient treated for chronic hepatitis C. No antithyroid antibodies were found and the authors suggest a multifactorial mechanism by which thyroid abnormalities are induced.

We agree that, in the absence of antithyroid antibodies, the role of a direct inhibition of thyroid hormone synthesis and secretion by α interferon, or both, might be considered. Indeed, among 22 patients developing thyroid abnormalities while receiving interferon, we found antithyroid antibodies (antithyroglobulin and anti-peroxydase) in only half of them (unpublished data). Ex vivo studies of patients' thyroid cells, if considered ethical, might provide pertinent information about the mechanisms of the cell damage induced by interferon in these patients.

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Biliary endoprosthesis and common bile duct stones

EDITOR,—The report from Peters *et al* (*Gut* 1992; **33**: 1412–5) of a group of patients with bile duct stones treated with a biliary endoprosthesis is of considerable interest. This technique has been used by many centres, provides excellent immediate drainage, and reasonable medium term results – but I wish to sound a note of caution. The justification for using stents as permanent treatment must depend on the results in the long term, indeed can only be assessed by lifetime follow up which no one has yet reported. Our own series with a follow up of 2–5 years was encouraging,[1] but more than half of the patients were still alive (despite being apparently at very high risk initially),[1] and many problems may have occurred subsequently.

The number of patients having stenting for bile duct stones in the King's series seems very high – no fewer than 40 of 146 of a consecutive series, including 27 (18%) as projected permanent treatment. The authors suggest that their low rate of duct clearance reflected their referral practice but referral centres should have special expertise. Of 343 patients with duct stones referred to this unit during the last two years, the clearance rate using standard techniques and mechanical lithotripsy was 94%. Stents were used as 'permanent' treat-

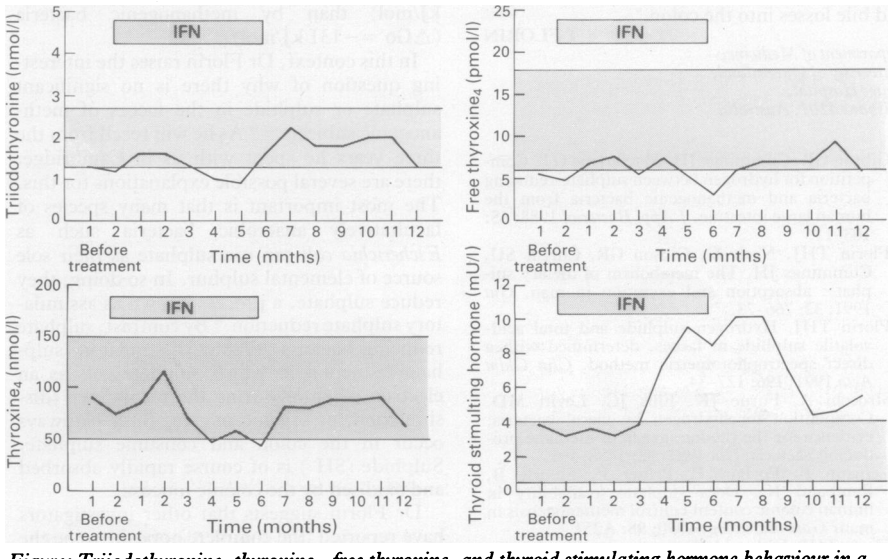

Figure: Triiodothyronine, thyroxine,, free thyroxine, and thyroid stimulating hormone behaviour in a patient with chronic hepatitis C treated with 3 MU of IFN alfa 2-b for six months.