and thiol-copper biochemical sites denaturation.[8] Another potential source of error in the use of NBT as a superoxide colorimetric detector may be related to the presence in some cell systems of a NBT reductase activity capable of directly reducing the dye without any superoxide participation.[1] It is clear that a 'true' superoxide mediated NBT reduction must be inhibited by superoxide dismutase, this being a fundamental criterion for assignment of a function of superoxide.[9] In such a context, studies dealing with oxygen radical production in cerebral vascular injury resulting from acute hypertension have shown that NBT reduction in brain microvasculature was inhibited by superoxide dismutase, thus showing superoxide participation in this pathophysiological event.[10] In Oshitani's study, superoxide dismutase failed to inhibit NBT reduction, though inhibition was seen with anaerobiosis and para-benzoquinone, which, however, are apparently not specific enough to discriminate superoxide as the species responsible for NBT reduction. Indeed, anaerobiosis may inhibit the mitochondria, and para-benzoquinone may react not only with superoxide but also with other reductants and radical species.

The authors have hypothesised that superoxide dismutase could have been ineffective in their model as a result of a lack of enzyme tissue permeability. Some much smaller molecules with a potential cell permeability, however, such as the cimetidine-copper complex, display a significant superoxide dismutase activity.[11] Moreover, superoxide dismutase conjugated to polyethylene glycol can enter cells, such as the endothelial, and maintain activity much longer than unconjugated superoxide dismutase.[12] We believe that these molecules should have been used by Oshitani *et al* to more precisely assign a role to superoxide in their investigation. Without similar experimental evidence, it does not seem correct to entitle the paper 'Location of superoxide anion generation in human colonic mucosa obtained by biopsy'.

D LAPENNA
F CUCCURULLO
Cattedra di Patologia Speciale Medica, Universita' GD'Annunzio; Presidenza Facolta' di Medicina a Chirugia, Via dei Vestini, 66100 Chieti, Italy

1 Oberley LW, Spitz DR. Assay of superoxide dismutase activity in tumor tissue. *Methods Enzymol* 1984; **105**: 457–64.
2 Spitz DR, Oberley LW. An assay for superoxide dismutase activity in mammalian tissue homogenates. *Anal Biochem* 1989; **179**: 8–18.
3 Jarasch E-D, Bruder G, Heid HW. Significance of xanthine oxidase in capillary endothelial cells. *Acta Physiol Scand* 1986; **548** (suppl): 39–46.
4 Topham RW, Jackson MR, Joslin SA, Walker MC. Studies of the ferroxidase activity of native and chemically modified xanthine oxidoreductase. *Biochem J* 1986; **235**: 39–44.
5 Bolann Bj, Ulvik RJ. Release of iron from ferritin by xanthine oxidase. *Biochem J* 1987; **243**: 55–9.
6 Fishbein MC, Meerbaum S, Rit J, Lando U, Kanmatsuse K, Mercier JC, *et al*. Early phase acute myocardial infarction: validation of the triphenyl tetrazolium chloride tissue enzyme staining technique. *Am Heart J* 1981; **101**: 593–600.
7 Biemond P, van Eijk HG, Swaak AJG, Koster JF. Iron mobilization from ferritin by superoxide derived from stimulated polymorphonuclear leukocytes. Possible mechanism in inflammation disease. *J Clin Invest* 1984; **73**: 1576–9.
8 Schechinger T, Hartmann H-J, Weser U. Copper transport from Cu(I)-thionein into apocaeuroplasmin mediated by activated neutrophils. *Biochem J* 1986; **240**: 281–3.
9 Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: *Clarendon Press*, 1989.
10 Kontos H. Oxygen radicals in cerebral vascular injury. *Circ Res* 1985; **57**: 508–16.
11 Goldstein S, Czapski G. Determination of the superoxide dismutase activity of cimetidine-Cu(II) complexes. *Free Rad Res Comms* 1991; **12–13**: 205–210.
12 Beckman JS, Minor RL, White CW, Repine JE, Rosen GM, Freeman BA. Superoxide dismutase and catalase conjugated to polyethilene glycol increases endothelial enzyme activity and oxidant resistance. *J Biol Chem* 1988; **263**: 6884–92.

Reply

EDITOR,—Thank you for giving us the opportunity to reply to the comments by Drs Lapenna and Cuccurullo. There is no specific probe to detect in situ generation of superoxide; nitroblue tetrazolium (NBT) is the most suitable probe available to detect generation of superoxide in in situ assay. Although inhibition of NBT reduction by superoxide dismutase is essential to prove the contribution of superoxide in the assay, it seems to be impossible to show an inhibitory effect of superoxide dismutase in organ culture, because it does not easily penetrate biopsy specimens.

In the organ culture system, specificity of the xanthine oxidase and interference of dehydrogenases or mitochondrial enzymes with the NBT reduction are important problems. Although a superoxide independent mechanism of releasing iron from ferritin by xanthine oxidase exists,[1–4] 70–95% of the aerobic reduction has been reported to be superoxide dependent,[2–4] hence direct reduction by the xanthine oxidase might not be an important pathway in NBT reduction. NBT reduction in the organ culture system is unique because of the permeability of the reagent. NBT probably does not penetrate the intact cells,[5,6] cytoplasmic enzymes and the mitochondrial respiratory chain might not interfere with the reduction under these conditions. The only interference found was the reduction of NBT by epithelial brush border enzymes, which was found to be non-specific to inflammation. Furthermore, the inability of mitochondrial respiratory chain inhibitor, KCN, to inhibit aerobic NBT reduction in the organ culture system and the inhibition of NBT reduction under anaerobic condition[7] might represent the participation of superoxide in this system. Based on these indirect findings, superoxide might participate in NBT reduction in the organ culture system.

In addition, we have already found that allopurinol combined with copper-cimetidine did inhibit NBT reduction by endothelium and infiltrating cells of the inflamed mucosa in this system.

N OSHITANI
Third Department of Internal Medicine, Osaka City University Medical School, 1-5-7, Asahi-machi, Abeno-ku, Osaka 545, Japan

1 Topham RW, Jackson MR, Joslin SA, Walker MC. Studies of the ferroxidase activity of native and modified xantine oxidoreductase. *Biochem J* 1986; **235**: 39–44.
2 Bolann BJ, Ulvik RJ. Release of iron from ferritin by xanthine oxidase. *Biochem J* 1987; **243**: 55–9.
3 Williams DM. Lee GR, Cartwright GE. The role of superoxide anion radical in the reduction of ferritin iron by xanthine oxidase. *J Clin Invest* 1974; **53**: 665–7.
4 Thomas CE, Morehouse LA, Aust SD. Ferritin and superoxide-dependent lipid peroxidation. *J Biol Chem* 1985; **260**: 3275–80.
5 Kontos HA. Oxygen radicals in cerebral vascular injury. *Circ Res* 1985; **57**: 508–16.
6 Peskin AV, Khramtsov AV, Morozov IA, Zemskov VM, Zbarsky IB. Visualization of reactive oxygen species formation by phagocytizing macrophages. *Exp Cell Res* 1984; **151**: 247–51.
7 Oshitani N, Kitano A, Okabe H, Nakamura S, Matsumoto T, Kobayashi K. Location of nitroblue tetrazolium-reducing activity in human colonic mucosa obtained by biopsy. *Dig Dis Sci* 1993; **38**: 546–50.

Selective affective biasing in recognition memory in the irritable bowel syndrome

EDITOR,—The account by Gomborone *et al* (*Gut* 1993; **34**: 1230–3) of selective perception, evaluation, and recall of emotionally loaded material by patients with irritable bowel syndrome (IBS) is fascinating. While their findings are resonant with personal clinical experience, they need to be seen and interpreted in a wider context before being judged representative of all patients with IBS.

The one year prevalence of IBS in the general population is about 20%; in other words, one in five adults selected at random from population registers meet the clinical criteria for a diagnosis of IBS when responses to a validated postal questionnaire are analysed.[1] A further substantial group of people have a complex of syndromes described as 'dysmotility like dyspepsia', which may represent a form of IBS predominantly affecting the proximal gut. Only about one third of these subjects, however, seek medical advice for their symptoms and the decision to do so is related to a number of factors.

In dyspepsia, symptom frequency and severity are poor predictors of the likelihood to consult, while concern about the significance of symptoms in terms of serious disease is much more strongly associated with consultation.[2] In IBS, consulting patients report more severe abdominal pain than those who do not consult; they are similarly concerned about the possibility of serious disease, particularly cancer, while those who do not consult are often positively dismissive of their symptoms.[3] In IBS the consulting patients are more often anxious or depressed, or both than the patients who do not consult.[3] This finding is consistent with other reports that conclude that psychological or frank psychiatric disorder is not a component of IBS in itself, but rather is an attribute of consulting patients and possibly a reason for consultation.[4]

The patients studied at St Bartholomew's were a selected group attending the hospital clinic, presumably because of severity or intractability of their symptoms. They also had a high level of psychiatric morbidity and cannot be regarded as representative of the much larger population of IBS patients without either of these characteristics. It would, therefore, be of great interest to know whether IBS patients in the community or consulting for the first time in primary care behave in the same way as this hospital based group. Other research questions arise from the findings of Gomborone *et al*. Do patients with other somatic symptoms for which a physical cause is difficult to find have similarly abnormal affective biases? Do these biases apply to perception, evaluation, and recall of physical symptoms as well as words? If they do, a key management question concerns the ways in which it is possible to investigate, diagnose, and treat irritable bowel syndrome without continually increasing anxiety, concern, and misinterpretation of symptoms.

R H JONES
UMDS Department of General Practice, 80 Kennington Road, London, SE11 6SP

1 Jones R, Lydeard S. Irritable bowel syndrome in the general population. *BMJ* 1992; **304**: 87–90.
2 Lydeard S, Jones R. Factors affecting the decision to consult with dyspepsia: comparison of consulters and non-consulters. *J R Coll Gen Pract* 1989; **39**: 495–8.
3 Kettell J, Jones R, Lydeard S. Reasons for consultation in irritable bowel syndrome: symptoms and patient characteristics. *Br J Gen Pract* 1992; **42**: 459–61.

4 Smith RC, Greenbaum DS, Jeffery B, Henry RC, Reinhart MA, Greenbaum RB, *et al.* Psychological factors are associated with health care seeking rather than diagnosis in irritable bowel syndrome. *Gastroenterology* 1990; **98:** 293–301.

Reply

EDITOR,—We welcome Professor Jones' comments on our recent paper. It is a criticism that can be levelled at any clinic based study of IBS that these patients are highly selected, not least because of high rates of psychiatric morbidity. These patients do, however, merit study in their own right as they are the very patients with whom gastroenterologists, and by inference physicians in primary care, most need help.[1] Furthermore, there are practical and ethical issues to be considered. Clinic patients are readily available and amenable to being studied. A positive result in these highly selected patients is possibly a necessary prerequisite to provide the impetus to extend an investigation into less selected groups.

Encouraged by our findings on selective affective biasing in clinic patients with IBS, we are now carrying out similar investigations in subjects with symptoms of IBS drawn from the community, both consulters and those who do not, and also in patients with other physical symptoms for which no sufficient organic pathology can be shown.

The word recognition memory test methodology that we use provides a direct but covert assessment of schema driven cognitive processing. The influence of such schemata is pervasive and we would therefore expect that these biases would apply to the preception and evaluation of physical sensations in terms of illness. IBS non-consulters have been reported to dismiss or normalise their bowel dysfunction.[2,3] The heightened receptiveness to negative material that we found in this study might be the mechanism that prevents IBS consulters from doing just this.

What we therefore expect to find in our study of selective affective biasing in IBS sufferers drawn from the community is that those who have consulted will show abnormalities similar to those that we have reported in our clinic patients. As yet these results are not available. We have found on a preliminary analysis of our data, however, that the IBS consulters drawn from the community resemble clinic patients with respect to other indices. Using DSM-III-R criteria, 64% of our community IBS consulters qualified for a diagnosis of an affective disorder, which is a similar prevalence to that found in clinic populations.[3] Also there was no difference between these community IBS consulters and our clinic patients in terms of dysfunctional illness attitudes.[4] Our clinic patients may therefore not be quite as highly selected as they seem.

J E GOMBORONE
P A DEWSNAP
G W LIBBY
M J G FARTHING
Department of Gastroenterology, St Bartholomew's Hospital, London EC1M 7BE

1 Kingham JGC, Dawson AM. Origin of chronic right upper quadrant pain. *Gut* 1985; **26:** 783–8.
2 Kettel J, Jones R, Lydeard S. Reasons for consultation in irritable bowel syndrome. *Br J Gen Pract* 1992; **42:** 459–61.
3 Gomborone JE, Dewsnap PA, Libby GW, Farthing MJG. Influence of psychiatric morbidity on self-appraisal of symptoms in irritable bowel syndrome. *Gut* 1993; **34** (suppl 1): S56.
4 Gomborone JE, Dewsnap PA, Libby GW, Farthing MJG. Community study reveals that dysfunctional illness attitudes are not wholly a reflection of patient status. *Gastroenterology* 1993; **104:** A513.

Left sided colon cancer

EDITOR,—The neurobiology of diverticular disease leading to left sided colon cancer in 7159 patients (2478 men and 4681 women (*Gut* 1993; **34:** 499–502) is suggested by reversed cerebral asymmetry in women with left sided breast cancer.[1] This hypothesis is supported by the association of specific frontal asymmetries with certain immune functions, and by compulsive ruminations occurring before oculogyric crises linked to inefficient cortical circuits and abnormalities of dopamine subserving gastrointestinal protection, immunocytes, and mood.[2–5] It is also supported by the association of severe psychiatric disorders with severe acute colitis[6] and by the protective role of dopamine in preferentially maintaining splanchnic blood flow.[7] These findings suggest screening patients with diverticular disease for increased risk of malignancy by monitoring dopaminergic neurotransmission.[8]

A possible strategy is suggested by the fact that delay-dependent speeding of reaction time, reflecting motor readiness, is abolished by depletion of dopamine.[3] Therefore, future studies may evaluate cognitive consequences of dopamine agonism and antagonism at intermediate dopamine tone in a medial-frontal-striatal 'activation' system underlying response organisation[9] by monitoring behavioural correlates of mood – that is, speech hesitation and switching pauses analysed on a time base by a microcomputer. This method is supported by participatory matching of pauses in dialogues at intermediate arousal, a joint, mutually responsive rhythm,[10] and by the concept of cellular tone.[11] Remote data acquisition[10] is an efficient, unambiguous strategy to evaluate the conveyance of ideas, a task that is possibly of sufficient complexity[12,13] to assess the role of dopaminergic neurotransmission in the development and progression of diverticular disease leading to left sided colon cancer.

E H FRIEDMAN
1831 Forest Hills Blvd, Cleveland, OH 44112, USA

1 Sandson TA, Wen PY, LeMay M. Reversed cerebral asymmetry in women with breast cancer. *Lancet* 1992; **339:** 523–4.
2 Friedman EH. Letter. *Soc Sci Med* 1993; **36:** 383.
3 Friedman EH. Read my lips revisited [Letter]. *J Clin Gastroenterol* 1993; **16:** 180–1.
4 Glavin GB. Central dopamine involvement in experimental gastrointestinal injury. *Prog Neuropsychopharmacol Biol Psychiatry* 1992; **16:** 217–21.
5 Mezey E, Palkovits M. Localization of targets for anti-ulcer drugs in cells of the immune system. *Science* 1992; **258:** 1662–5.
6 Labayle D, Delas N, Pariente A, Drouhin F, Naouri D, Bernard B, *et al.* Severe psychiatric illness and acute colitis. *Gastroenterol Clin Biol* 1992; **16:** 639–43.
7 Godkin A, Petros AJ, Bennett ED. The effect of dopamine on gastric intramucosal pH (pHi). *Gut* 1993; **34** (suppl): S13.
8 Rammsayer T, Netter P, Vogel WH. A neurochemical model underlying differences in reaction times between introverts and extraverts. *Personality and Individual Differences* 1993; **14:** 701–12.
9 Bilder RM, Lieberman JA, Kim Y, Alvir YM, Reiter G. Methylphenidate and neuroleptic effects on oral word production in schizophrenia. *Neuropsychiatry, Neuropsychology, and Behavioural Neurology* 1992; **5:** 262–71.
10 Friedman EH. Letter. *Soc Sci Med* 1993; **36:** 583.
11 Boswell CA, Joris I, Majno G. The concept of cellular tone: reflection on the endothelium, fibroblasts, and smooth muscle cells. *Perspect Biol Med* 1992; **36:** 79–86.
12 Friedman EH, Sanders GG. Speech timing of mood disorders. In: Miller MJ, ed. *Computer applications in mental health: education and evaluation.* New York: The Haworth Press, 1992: 121–42.
13 Jahanashahi M, Brown RG, Marsden CD. The effect of withdrawal of dopaminergic medication on simple and choice reaction time and the use of advance information in Parkinson's disease. *J Neurol Neurosurg Psychiatry* 1992; **55:** 1168–76.

Alcohol *v* epinephrine and polidocanol

EDITOR,—We have read with great interest the report by Rutgeerts *et al* (*Gut* 1993; **34:** 348–50). The authors state that 'absolute ethanol was superior to epinephrine-polidocanol, which was not significantly better than sham therapy'. As these results differ from other previous controlled studies even from the same group,[1] we would like to comment on some clinical, endoscopical, and methodological aspects that we consider of relevance.

It is worth noting that a high rebleeding rate (40%) and low haemostatic efficacy (68%) in the epinephrine-polidocanol injection group compared with the ethanol group (20% and 88% respectively) was seen, but these differences were not significant.

As noted by the authors, shock was more frequently seen in the epinephrine-polidocanol group (10 patients) than in the sham (five patients) and alcohol groups (seven patients). It is known that shock carries an increased risk of rebleeding,[2] and this could explain, at least partially, the high failure rate in the epinephrine-polidocanol group.

Apart from the type of injected substance, there are probably other factors influencing the efficacy of endoscopic injection, such as the site and size of the bleeding ulcer.[3,4] In the study by Rutgeerts *et al*, the authors consider the proportion of gastric and duodenal ulcers between groups but not their anatomical situation. Ulcers located high on the lesser gastric curvature or posterior in the duodenal wall are more difficult to reach and have a higher tendency to rebleed.[4–7] Furthermore, the size of the ulcer, probably one of the most important factors,[8] is not mentioned in the study. In this sense, it has been shown that endoscopic injection is significantly less effective in ulcers larger than 2 cm.[5]

Another remarkable aspect is that the study was designed specifically to compare both treatment groups (ethanol and epinephrine-polidocanol) with sham therapy (assuming a change in response of 75%). A much higher sample size would be necessary, however, to confirm differences between both treatment groups.

Thus, we believe that there is insufficient evidence in the study to conclude that absolute alcohol is superior to epinephrine-polidocanol. The efficacy of injection therapy is probably related less to the type or combination of substances used than to other factors, such as the size and site of bleeding ulcer. These variables should be considered in studies assessing the efficacy of endoscopic injection techniques.

R CAMPO
E BRULLET
X CALVET
C BROTONS
Endoscopy Unit, Hospital de Sabadell, Parc Tauli sn, 08208 Sabadell, Spain

1 Rutgeerts P, Broeckaert L, Janssens J, Vantrappen G, Coremans G, Hiele M. Comparison of endoscopic polidocanol injection and Yag-laser therapy for bleeding peptic ulcers. *Lancet* 1989; i: 1164–6.
2 Bornman PC, Theodorou NA, Shuttleworth RD, Essel HP, Marks IN. Importance of hypovolaemic shock and endoscopic signs in predicting recurrent haemorrhage from peptic ulceration: a prospective evaluation. *BMJ* 1985; **291:** 245–7.