

CORRESPONDENCE

Toxic effect of MDMA on brain serotonin neurons

Sir—U D McCann and colleagues (Oct 31, p 1433)¹ describe reduced [¹¹C]-McN-5652 binding, and by implication, serotonin transporter and serotonergic terminal loss, in users of (±)3,4-methylenedioxymethamphetamine (MDMA, ecstasy). This report provides some of the first biological evidence for the neurotoxic effects of MDMA in man, building up anatomical studies in non-human primates and complimenting indirect evidence from studies of neuropsychiatric symptoms in MDMA users.²

However, the results should be interpreted with caution because their meaning hinges on the accurate classification of participants in terms of cumulative use of MDMA. Unfortunately, this category depended on the participants' own estimates of their substance use, and, as the investigators acknowledge, the reliability of such estimates is questionable. The interpretation is also complicated by potentially confounding differences between the MDMA users and the controls. In our experience, heavy users of MDMA typically have quite different personalities from people who have never used the drug. Moreover, as Zuckerman and colleagues³ discuss, personality traits associated with substance misuse (such as impulsivity and novelty-seeking behaviour) may be linked to serotonergic function. Heavy users of MDMA commonly use several other psychoactive substances concurrently, and the effects of amphetamine, lysergic acid diethylamide (LSD), cocaine, and cannabis on the distribution of [¹¹C]-McN-5652 in man are largely unknown. It is thus difficult to be sure that the differences in [¹¹C]-McN-5652 binding are secondary to MDMA use itself.

More specifically, it is unclear whether [¹¹C]-McN-5652 binds to other the molecular targets in addition to the serotonin transporter, and whether the kinetic model of [¹¹C]-

McN-5652 distribution used by McCann and colleagues is the most appropriate for this ligand. The large range in [¹¹C]-McN-5652 binding among controls and the observation that most of the MDMA users fell within this range, questions the sensitivity and specificity of the technique. Furthermore, the investigators describe a negative correlation between cumulative MDMA dose and [¹¹C]-McN-5652 binding, but this correlation was evident only when the controls were included in the analysis: no relation is discernible when the MDMA users are considered.

Nevertheless, this study shows that neuroimaging has the potential to address the key question of whether MDMA use leads to neurotoxic effects in man. Our criticisms relate to the sensitivity and limitations of such ligand binding studies for an assessment of serotonin neuronal integrity and the functional consequences of impairments to this system. Neuronal systems may exhibit a wide range of adaptive and compensatory responses to cope with toxic effects or drug use. Such compensatory responses, perhaps resulting in increased serotonin transporter, might obscure the underlying toxic effects of MDMA. New imaging approaches that integrate cognitive function and neurotransmission,⁴ are required to tackle the problem of whether MDMA use leads to a functional impairment of the serotonin system. Thus, in addition to studies of serotonin transporters and receptors, we also need to examine the effects of experimentally manipulating serotonin transmission on brain activity in MDMA users, this is the approach we have adopted.

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1 McCann UD, Szabo Z, Scheffel U, Dannals RF, Ricuarte GA. Positron emission

tomographic evidence of toxic effect of MDMA ('Ecstasy') on brain serotonin neurons in human beings. *Lancet* 1998; **352**: 1433-37.

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Sir—The landmark study by U D McCann and colleagues provides convincing evidence of an association between decreased serotonin transporter binding sites and MDMA use. Nonetheless, it is important to note that this study does not allow comment on causal relations, and McCann and co-workers are over-eager to wring meaning from their findings.

The investigators briefly consider that MDMA users may pursue such use as a result of pre-existing serotonin function abnormalities, only to dismiss this on the grounds that "none of the MDMA users had a neuropsychiatric disorder in which serotonin has been implicated". Yet their exclusion of such disorders seems to have been based only on assessment of current axis-I disorder, as detected by the scheduled interview for diagnostic and statistical manual of mental disorders IV (SCID-IV). They did not exclude axis-I disorder before use of MDMA abuse, subclinical mood disorders, or previous or current axis-II diagnoses. With reference to the latter, abnormal serotonin binding sites may be particularly pertinent to personality disorders that involve impulsive or aggressive behaviour. Thus, some

people with mental disorders may self-medicate with MDMA.

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- 1 McCann UD, Szabo Z, Scheffel U, Dannals RF, Ricaurte GA. Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. *Lancet* 1998; **352**: 1433-37.

Sir—U D McCann and co-workers¹ show decreased brain serotonin transporter binding in MDMA users compared with age-matched and sex-matched controls who had never used MDMA. The investigators used a dual tracer approach: one radioligand [¹¹C](+)McN-5652 for imaging of composed specific binding, non-specific binding, and free ligand, whereas [¹¹C](-)McN-5652 imaging consists of only non-specific and free ligand. The latter technique is mainly affected by tissue perfusion.

Distribution volume of specific binding in the users of MDMA was found to be decreased in all regions except the thalamus. McCann used logarithmic transformations of distribution volumes corrected for non-specific binding to achieve a normal distribution in the control and MDMA groups. This approach raises the question: are the presented reductions based on the different kinetics of the non-specific radioligand [¹¹C](-)McN-5652 between controls and MDMA users? Figure 1 of the original article shows that the time-activity curve of this non-specific radioligand significantly differs between the study participants. An MDMA user had a more delayed inflow and outflow of this tracer into and from the brain than a control. We reanalysed the representative time-activity curves by means of Logan-Patlak plot.²

Results of the Logan-Patlak plots showed no difference in global distribution volume between an MDMA user (1.46 mL/mL) and a control (1.47 mL/mL) if corrected for different non-specific binding and free ligand.* If no such correction were applied, the figures are similar to those reported by McCann: 1.18 mL/mL and 1.47 mL/mL, respectively. The only difference was a completely different kinetics of non-specific binding and free ligand between an MDMA user and a control. The conclusion is that the main affective factor is different perfusion between the study participants, not the global serotonin specific binding. Regionally, it might be different.

A lesson of McCann and co-workers' report is that the biochemical fate of the tracers has to be known completely, and in this case multiple chemical species ([¹¹C](+)McN-5652 and [¹¹C](-)McN-5652) must be modelled simultaneously^{3,4} to analyse the effects of various tissue components on the specific binding. One cannot say whether the findings of McCann are based on the different kinetics of the non-specific binding and free ligand or on the true serotonin transporter binding in MDMA users.

*Full details are available from the authors.

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- 1 McCann UD, Szabo Z, Scheffel U, Dannals RF, Ricaurte GA. Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. *Lancet* 1998; **352**: 1433-37.
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Sir—The finding of U D McCann and colleagues¹ of reduced serotonin transporter binding in heavy users of MDMA lends support to evidence of the harmful effect of this drug on the serotonin system, which was previously reported in animals.² However, as the investigators acknowledge, little is known of the functional effects of MDMA-induced brain serotonin neurotoxic lesions. We report the effect of long-term consumption of MDMA on cognitive function.

We recruited participants who had a history of substantial and predominant MDMA use (mean estimated number of tablets consumed 235 [range 12-2600]) through popular magazines and the internet. The MDMA group (n=36; age 24.1 [SD 4.9] years; duration of MDMA use 4.3 [2.6] years) was compared with a control group (n=19; age 22.7 [2.3] years) who had never used illicit or prescribed psychoactive substances. The MDMA users had not taken the drug or any other psychoactive drug for some days before testing (79 days [2-400 days]). We took a detailed history of each participant's drug use together with current and past psychiatric state (Present State Examination). Four drug users met criteria for a diagnosis of neurotic depression, but there were

no group differences between groups on the Beck depression inventory (p=0.304), and cognitive impairment was unrelated to diagnosis and depression scores.

A multivariate analysis of variance of cognitive scores showed significant impairment of MDMA users on tests of learning, recognition, and recall.* Compared with controls, users of MDMA had worse immediate recall of words from a list, showed impairment in recognition memory for faces and in learning a repeatedly administered word list and a sequence of digits. Associative learning tasks revealed impairment in learning spatial information. By contrast, executive (frontal lobe) functions (measured by verbal fluency tests, and verbal and non-verbal working memory) were not impaired. Compared with normative data for the learning and memory tests, three MDMA users scored below the 2 SD limit on two tests, as did an additional eight users on one test, whereas only one control scored lower than the 1 SD limit on one test (p=0.021).

Most users of MDMA have also taken other illicit psychoactive drugs that may contribute to cognitive impairment and to serotonergic depletion. We selected individuals whose use of MDMA was more frequent and regular than of any other drug; we also recorded the frequency and use of other drugs. Other drugs did not correlate significantly with the deficits found with MDMA, although other deficits were implicated, such as frontal impairment with cannabis use. Our results do not represent a global impairment of cognitive function, instead we found discrete deficits in verbal and non-verbal memory and learning while other cognitive functions were intact. Accordingly, the deficits could not be explained as the result of impaired attention or poor motivation, which substantiates evidence of patterned cognitive impairment.^{3,4}

Chronic disruption of serotonergic transmission by MDMA is a possible explanation for the memory and learning deficits we found.

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- 1 McCann U, Szabo Z, Scheffel U, Dannals RF, Ricaurte GA. Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. *Lancet* 1998; **352**: 1433-37.

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Sir—U D McCann and colleagues equate self-reported use of ecstasy with the use of MDMA. Tablets described by users or dealers as ecstasy may contain one or more of various substituted amphetamines, including MDMA, amphetamine, ephedrine, ketamine, tiletamine, or other compounds.^{2,3} These drugs do not have identical neuropharmacological properties.⁴ Thus, what these investigators have shown is a difference in serotonin transporter activity between a group of individuals who thought they had taken MDMA in the past, compared with a group of people who said they had never taken MDMA before.

The MDMA users were tested for psychiatric disorders, such as anxiety and depression, and all proved normal. So, these differences in transporter activity relative to the control group existed without any anxiety and depression, as established by the investigators themselves.

Although MDMA may cause some brain changes, the evidence for chronic depression and anxiety disorders as a result of these changes is unconvincing. The midweek mood dip (which usually returns to normal by the end of the week) that follows weekend use of MDMA is due to an acute fall in serotonin, not to structural brain changes.

Were the MDMA users selected entirely at random from all the persons who replied to their advertisements, rather than from a highly selected subsample? None of the 14 cases reported includes people who described taking more than 400 pills. The manner in which the 14 cases seem to have been selected from a large initial sample raises questions about the statistics used. The investigators should clarify their selection procedure.

The statement that the absence of neuropsychiatric disorder means that the low concentrations of transporter could not have been pre-existing is unproven. People with lower concentrations of transporter and other neurochemical differences may experience drives to take drugs of this

nature, or to seek stimulation in other ways, without necessarily having a neuropsychiatric disorder. That the participants were tested and proved free from such disorders confirms the point that low serotonin transporter activity can co-exist with a normal mental state.

We believe that it would be valuable to compare serotonin transporter activity in heavy cocaine users with MDMA users. Cocaine is a stimulant that may be sought out by people who might have a pre-existing underactivity of some brain systems, such as parts of the dopamine and serotonin systems. Cocaine is not, however, a ring-substituted amphetamine and does not cause specific changes to serotonin fine terminals. So if heavy cocaine users proved to show similar changes in serotonin transporter mechanisms to MDMA users, we could conclude that these deficits, relative to non-drug use, are pre-existing and not caused by MDMA. We are not convinced that the differences reported by McCann were not pre-existing, because the putative use patterns and doses of MDMA were generally well below those that cause persistent changes in most animal studies. We agree with McCann and colleagues that some people take MDMA at levels equivalent to those used in some animal studies.⁵

Conclusive proof requires following a group of individuals who continue to use large amounts of MDMA alone, and a comparison of the progression of changes in their serotonin transporter activity with cocaine users and a group of people who do not use drugs.

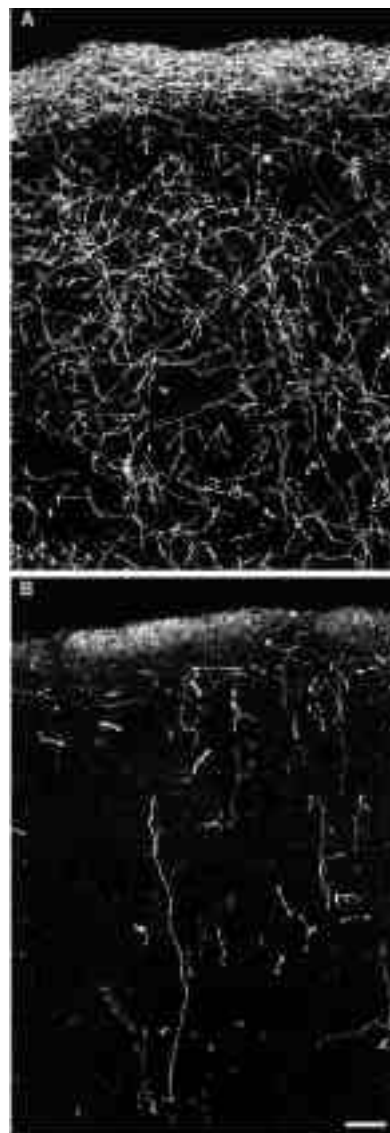
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Authors' reply

Sir—Laurence Reed and co-workers underscore the inherent difficulties of retrospective studies in individuals who have used illicit substances, and



Serotonin transporter immunoreactive fibres in prefrontal cortex of a control monkey (A) and a monkey treated with MDMA 2 weeks previously (B)

Scale bar 50 μ m. MDMA was given subcutaneously 5 mg/kg twice daily for 4 days.

question whether amphetamine, LSD, cocaine, and cannabis might also lead to reductions in the serotonin transporter. Neither cocaine nor amphetamine is toxic toward brain serotonin neurons and the structural requirements for substituted amphetamine neurotoxicity are quite strict. As to the selectivity of [¹²⁵I]McN5652 binding, neither dopamine nor norepinephrine transporter blockers inhibit specific [¹²⁵I]McN5652 binding, whereas serotonin reuptake inhibitors do.¹ Reed and colleagues are incorrect when they state that the negative correlation between extent of MDMA use and [¹²⁵I]McN5652 binding is lost when controls are dropped from the

analysis ($r = -0.51$, $p < 0.05$, without controls). It is not yet known if functional neuroimaging has the requisite sensitivity or specificity to show MDMA-induced serotonin neurotoxicity.

John Morgan and two of the other correspondents suggest that pre-existing low concentrations of serotonin transporters could be the cause of MDMA use, rather than the result of serotonin neurotoxicity. Although an intriguing possibility, it does not take into account animal data that provide compelling evidence for a longlasting neurotoxic effect of MDMA on brain serotonin neurons (figure).

Jyrki Kuikka and Aapo Ahonen question whether the observed reductions in specific [^{14}C]McN5652 binding might not be based on different kinetics of [^{14}C](–)McN-5652 in controls and MDMA users. Analysis of the data with only the active enantiomer, [^{14}C](+)-McN5652, shows that the differences are still significant (ANOVA, $p < 0.014$), mitigating against a confounding effect of non-specific binding. As to the analysis of our representative time-activity curves with a Logan-Patlak plot, it is inappropriate for several reasons. First, one should use the actual input function derived from metabolite-corrected arterial blood samples, as was done in our work. Second, we do not believe it is appropriate to derive general conclusions from time-activity curves of single individuals. Third, simple graphical analysis with [^{14}C](–)McN-5652 instead of the actual input function is inappropriate because the slow dissociation of [^{14}C](–)McN-5652 from non-specific binding sites leads to a gross underestimation of the actual distribution volume, as is evident from Kuikka and Ahonen's estimated values. These reservations notwithstanding, to assess the possibility of perfusion effects, we have calculated the K_1 indices (brain uptake) in MDMA users and controls and find that the two do not differ significantly (K_1 , control 0.312 [SD 0.032]; K_1 , MDMA 0.303 [0.053]; ANOVA, $p = 0.475$).

Karl Jansen and A R Forrest question whether MDMA tablets used by people in our study might have contained other illicit substances. While this is possible, it is noteworthy that among recreational drugs, only ring-substituted amphetamines like MDMA have been shown to cause serotonin neurotoxic injury.² Jansen and Forrest also suggest that, since none of our participants had anxiety or depression, our findings are clinically

irrelevant. However, we deliberately excluded such individuals from our study because of potential confounds on our outcome measures. The selection of MDMA users for PET studies was purely random and subject to participants meeting predetermined inclusion and exclusion criteria. All MDMA users who had undergone PET imaging by the time our paper was submitted are included in our report, including those kindly referred by Jansen. We expressed extent of previous exposure to MDMA in terms of the number of times or occasions participants reported having used MDMA, rather than the number of pills they recalled having taken, because the former approach is likely to provide a more accurate measure than the latter. Our finding that participants with lower degrees of MDMA exposure have evidence of reduced serotonin transporters should be viewed as further cause for concern, rather than as a reassuring observation.

We were interested in the findings of Anthony Klugmann and colleagues which accord with recent findings in other studies.³

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Does variant Creutzfeldt-Jakob disease have an Achilles heel?

Sir—A F Hill and co-workers' report (Jan 16, p 183)¹ of variant Creutzfeldt Jakob disease (CJD) in tonsillar lymphoid tissue, and an earlier similar finding in an appendix,² raise concerns about the safety of current procedures for sterilisation of surgical instruments. The outcome may be introduction of universal precautions to disinfect surgical instruments by means of six 3 min autoclave cycles at 134°C, or one 18 min cycle at 134°C. This method would require additional autoclaves, provision of extra space to accommodate them, and the purchase

of additional surgical instruments because of the longer turn-around time. A less costly way to inactivate the CJD agent is urgently needed.

A highly heat-resistant replicating agent (IFDO),³ may provide a model for the development of new strategies for sterilisation of CJD. Like CJD agent, IFDO can survive autoclaving at 134°C for 3 min, as well as exposure to formaldehyde and ionising radiation, and is of similar size to CJD.³ This agent occasionally survives autoclaving for 30 min at 134°C,⁴ but I found IFDO is inactivated by boiling in concentrated salt solutions that contain a weak acid or alkali. I used three different batches of IFDO with viable counts between 1.2×10^9 and 3×10^9 for these experiments. After autoclaving at 121°C with a holding time of 15 min the viable counts were reduced by less than 1 log. In 13 experiments in which 55 samples were tested for viability after boiling for 15 min in 2.0 mol/L sodium chloride, 0.02 mol/L disodium orthophosphate, the residual counts were: less than 0.5×10^2 in 35 samples, 0.5×10^2 in 16, 1.0×10^2 in three, and 1.5×10^2 in one.

The mode of action of sodium chloride in enhancing inactivation of IFDO is unknown. However, when IFDO is suspended in concentrated salt solutions it partly disaggregates, which may render acid or alkali susceptible targets more accessible, and raises the possibility that other chemicals and disinfectants might also be effective when combined with high concentrations of salts. The validity of IFDO as a model for sterilisation of CJD should be tested by observing the effect of the above procedure on the viability of CJD agent.

If confirmed as a valid model for sterilisation of CJD, IFDO would provide a simple and rapid tool for the development of new ways to inactivate CJD agent.

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Pentosan in transmissible spongiform encephalopathies

Sir—Christine Farquhar and colleagues (Jan 9, p 117)¹ proved the prophylactic potential of intraperitoneal pentosan polysulphate in transmissible spongiform encephalopathies in mice. Oral administration of the drug in the same doses was ineffective, which can be easily explained by its poor absorption rate (3%) from the gastrointestinal tract.² The efficacy of pentosan given weeks before or hours after the scrapie inoculation raises the question of where the action responsible for the long-term effect takes place.

Pentosan polysulphate is a highly polyanionic sulphated semisynthetic polysaccharide that is structurally related to the glycosaminoglycans synthesised by cells. Preclinical studies with parenterally administered radiolabelled pentosan polysulphate sodium showed distribution to the uroepithelium with a smaller amount found in the liver, spleen, lung, skin, periosteum, and bone marrow (www.alza.com/elmiron/elmironpi.htm).

About two-thirds of the dose undergoes partial desulphation in the liver and spleen within 1 h, partial depolymerisation also occurs in the kidney, and it is then eliminated in the urine over 24 h. One explanation is that during distribution the active pentosan polysulphate molecules attach to their target cell(s), and result in delayed structural or functional changes which thereby prevents the propagation of prion disease.

In scrapie-infected neuroblastoma cells, pentosan polysulphate in vitro stimulates the endocytosis of prion protein (PrP^C), rapidly and dramatically decreases the amount of PrP^C present on the cell surface, and prevents the accumulation of protease-resistant prion isoform.³ That this mechanism has a pivotal role in the central nervous system of infected mice is unlikely, because the blood-brain barrier is practically non-permeable to pentosan polysulphate.⁴ The potential cellular targets of the long-term effect of pentosan polysulphate can thus be splenocytes, lymphocytes, bone-marrow macrophages, and endothelial cells of blood-nerve and blood-brain barriers.

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Mortality and light to moderate alcohol consumption after myocardial infarction

Sir—Jorg Muntwyler and colleagues (Dec 12, p 1882)¹ showed that in male survivors of a myocardial infarction who consume two to six drinks of alcohol per week, 18 deaths per 1000 patient-years are prevented, compared with men who consume no alcohol. These findings, however, are flawed by the limitations of the study design.

In the multivariate analysis, Muntwyler and colleagues did not adjust for hypertension and hypercholesteraemia because “these risk factors may have affected alcohol-associated risk”. Furthermore, inclusion of these factors in a secondary multivariate model did not affect the results. The basis of the entire analysis is a single measurement of risk factors at baseline that may be subject to bias because of the diluting effects of random fluctuations in blood pressure and cholesterol concentrations. Not correcting for this “regression dilution bias” could lead to serious underestimation of the true effect of these factors on the outcome of interest.² The effects of variability and measurement errors for one individual on a one-time assessment of these risk factors may lead to an under assessment of their contribution as an intervening variable to explain the alcohol mortality relation. Thus, the independent association between alcohol intake and mortality could be the product of residual confounding. It would have been important to remeasure risk factors in this observational study in at least a representative proportion of survivors.

Muntwyler and co-workers provide no information on other prognostic co-factors and potential confounders, such as congestive heart failure or arrhythmia and treatment with β -blockers, inhibitors of angiotensin-converting enzyme, or antiarrhythmic

drugs. Insufficient control of these factors could have also affected the overall findings of the study.

Although the researchers' intention to illustrate the delightful merits of an epicurean lifestyle deserve our deep respect, one questions the value of a secondary post-hoc analysis of a cohort recruited 15 years ago and based on patients whose management of coronary heart disease probably does not reflect current practice.

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Sir—In the study by Jorg Muntwyler and co-workers¹ no data are provided in relation to the consumption of different alcohol beverages (wine, beer and spirits), and the effect of different drinks on mortality, especially cardiovascular diseases and cancer.^{2,3}

The composition of alcohol beverages differs, so their effect on health may also vary. It is important to find out whether the effect of alcohol on mortality depends only on the ethanol content or on the presence of other substances in its composition. The concentration of antioxidants is higher in wine than in beer or liquors. For example concentrations of resveratrol are much higher in wine than in beer, and this substance has in-vitro effects on the inhibition of the platelet aggregation⁴ and tumorigenesis.⁵

Despite the fact that there are contradictory epidemiological studies about the effect of alcoholic beverages on cardiovascular diseases and cancers, in-vitro data indicate that the protective effect of wine on cardiovascular or neoplastic diseases may be higher than that of other alcoholic beverages.

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Authors' reply

Sir—In our analyses we did not adjust for hyperlipidaemia and hypertension because these two factors are in the causal pathway of the alcohol effect. Thus, controlling for these factors might underestimate the effect of alcohol on risk of cardiovascular disease events. However, as we stated in our paper, we ran models including hyperlipidaemia and hypertension and the results were not substantially different from the models with these factors.

All measures of risk factors, including the alcohol variable, were based on a single measure. As Heiner Bucher points out, this measure may result in misclassification over time and yield an underestimation of the effect of any factor. Bucher is concerned about the effects of regression dilution bias on the potential confounders; however, this effect also applied to the alcohol measure. Since crude and adjusted risk reductions were not materially different, residual confounding is not likely to be an explanation of the observed association. On the contrary, the likely effect of having only one measure of alcohol exposure is an underestimation of the true alcohol effect. Finally, further adjustment for cardiac medications did not materially alter the associations.

Luis Bujanda and colleagues ask about the effects of different types of alcoholic beverages. Data were not available on beverage type in our cohort. Thus, we were unable to compare the effects of different beverages. Several investigators have postulated that the antioxidant substances in red wine render it more potent in reducing risk of cardiovascular disease. However, the evidence from available observational studies does not consistently show a greater benefit for wine compared with beer or distilled spirits.

In general, wine, beer, and spirits have been shown to reduce risks of coronary heart disease. Although some studies indicate greater benefit for wine consumption compared with other beverages, others have reported greatest benefit from beer or liquor.¹ The studies that shown a benefit for wine have not documented a more protective effect for red than white wine. In addition, since wine drinkers tend to be more educated and have a higher income, it has been speculated that apparent benefits of wine consumption may be due, at least partly, to confounding. Further, in some populations the pattern of alcohol consumption varies by beverage type, which could explain some observed differences. In a cross-cultural study, Criqui and Ringle² reported that the ethanol content rather than the total volume of type of wine were better predictors of the reduction in risk from coronary heart disease.

The evidence from both observational and experimental studies indicates that alcohol raises total HDL, and this moderate alcohol consumption seems to reduce the risk of coronary heart disease in large part by raising HDL cholesterol concentrations.^{3,4} Our research group found that all three beverage types, when consumed in a similar pattern, confer a comparable benefit, and that most of that benefit is derived from increasing HDL.⁵ This finding further indicates that alcohol is the major factor responsible for the protective association.

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Physical disorders in musicians

Sir—I wish to add my own experience of focal dystonia in response to the report by Victor Candia and colleagues (Jan 2, p 42).¹ For many years I have researched physical and psychological disorders in musicians—particularly physical injuries, including focal dystonia. As codirector of the ISSTIP performing arts clinic in the UK, I had the opportunity to work with several hundred musicians. Among them there were 29 keyboard players, 11 guitarists, and two violinists who had focal dystonia. Some of them had pains in the hands, arms, or shoulders, but most presented the usual incoordination—fingers curling under the palm, the player not able to bring them back in the initial position.

In my experience, all these musicians had misused their body and muscles, playing their instrument with excessive tension (stiffness) in the joints and muscles. The condition is caused, as in most cases of tendinitis, tenosynovitis, bursitis, ganglions, and other occupational ailments, by an exaggerated finger articulation (raising the finger as high as possible before striking the key), at the same time stiffening the wrists and forearms.

Since posture has a vital role in the playing of instruments, it is the responsibility of every teacher and therapist to address this issue. At our clinic, I work first and foremost on helping the musician to acquire a correct posture (perfect alignment of head, neck, and back), while learning how to liberate the body and muscles of any tensions, and especially, how to interact with the instrument and maintain the freedom of muscular coordination.

Once these skills have been acquired, we begin the work of retraining the motor-sensory processes, with the musician learning to use a different set of muscles to play the instrument. Instead of using finger articulation, I introduce a technique based on the downward and upward exertions of the wrist to produce the tone. When the wrist is dropped or raised, the fingers catch the keys in the hand's descent or when raising it. This technique is used in certain Russian and Hungarian schools of piano playing and was advocated and described by the great English pedagogue and theoretician, Tobias Matthay.²

I do not wish to present here detailed aspects of piano playing. Suffice it to emphasise that once the musician “thinks wrist movements, not finger movements”, he finds himself able to play with ease, without a recurrence of his condition.

The large body of publications by medical specialists in this field maintains

that "to date, no treatments have been found to be effective" but do admit that re-training by certain teachers has shown satisfactory results.^{3,4}

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A986S polymorphism of calcium-sensing receptor

Sir—David Cole and colleagues (Jan 9, p 112)¹ report that the A986S polymorphism of the calcium-sensing receptor (CASR) has a significant effect on extracellular calcium concentration in healthy Canadian women.¹ According to the previous reports of Heath² and Cole³ and their colleagues we assessed the same site of polymorphism and the relation to serum calcium concentration in healthy young women in Japan.

The participants were healthy students who attended a high school in Okayama Prefecture in Japan (aged 15–18 years). The distribution of genotypes AA, AS, and SS was 31, 1, and 0. We analysed the relation between these genotypes and serum concentration of calcium, but found no significant difference. This finding indicates that the genotypes AS and SS of codon 986 are rare in the Japanese population, and have little effect on serum calcium concentration.

The discrepancy between Cole and colleagues' findings and ours may be due to the difference in genetic background including ethnic origin, lifestyle, and calcium intake.

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Statins and C-reactive protein

Sir—Timo Strandberg and colleagues (Jan 9, p 118)¹ show that serum C-reactive protein (CRP) concentrations decrease in most cases after treatment with atorvastatin or simvastatin for 4 months in hyperlipidaemic patients with stable coronary artery disease. Since CRP is subject to seasonal fluctuations,² there is a risk that such fluctuations may account for the results of this non-controlled study.

We undertook studies on the effects of statins in different groups. First, we carried out a randomised study of simvastatin (20 mg daily) in 30 patients, versus acipimox (250 mg thrice daily) in 27 patients for 3 months, in hyperlipidaemic type II diabetic patients (mean age 61 years; 30 men, 27 women). We measured CRP with a sensitive in-house method.³ CRP concentrations at the start of treatment were similar in the two treatment groups and no significant changes were seen after 1.5 months. After 3 months of treatment, CRP concentrations were significantly reduced in the simvastatin group (median change -0.34 mg/L [interquartile range 1.21]; $p=0.013$), but not in the acipimox group (-0.06 mg/L [3.61]); in the simvastatin group, CRP concentrations fell in 23 patients and rose in seven patients; in the acipimox group, CRP increased in 14 and decreased in 13. However, the difference in change between the two groups was not significant. The change in CRP did not correlate with changes in total cholesterol, triglyceride, and HDL-cholesterol as a result of treatment. This finding confirms the short-term effect of statins not only for hyperlipidaemic patients with moderately raised CRP (median 1.55 mg/L),² but also for hyperlipidaemic type II diabetic patients known to have raised CRP (median 3.16 mg/L [7.19]),⁴ and the comparative design argues against seasonal effects.

Second, we carried out two studies on the effect of statins on CRP in groups with lower CRP. In a placebo-controlled study in 20 patients with familial hypercholesterolaemia, in which the treatment group received 40 mg pravastatin daily for 9 months, we found no change in CRP from baseline.⁵

In a study in 45 patients with familial hypercholesterolaemia (mean age 45 years; 21 men, 24 women) who received simvastatin (20 mg daily) for 1 year, median baseline CRP concentrations were 1.21 mg/L [2.47], and CRP concentrations were non-significantly reduced after 1 year of

treatment (0.97 mg/L [2.13]): CRP concentrations fell in 25 patients, remained the same in one patient, and increased in 19 patients.

Taken together, these data suggest that the effects on CRP are independent of lipid effects and occur mainly when the pretreatment value is increased. Further examination showed that the CRP decrements were predominantly present for values above the median in all studies. We propose that CRP reduction of statins is relevant for patients with high concentrations, which is a promising possibility for differential diagnosis and treatment, since it is known that patients with high cholesterol and high CRP have the highest risk of cardiovascular event.

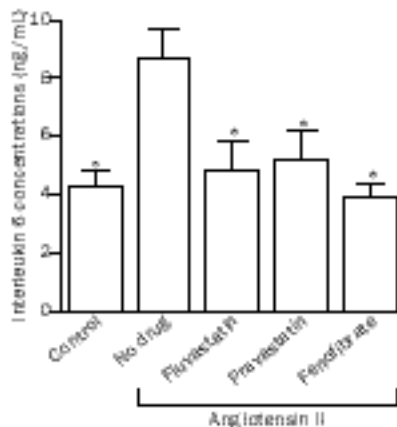
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Sir—Timo Strandberg and colleagues,¹ show that administration of statins reduced plasma concentrations of C-reactive protein (CRP), an established risk factor for coronary artery disease, in hyperlipidaemic coronary patients, although whether a relation exists between lipid lowering and CRP change is unknown.

Staels and colleagues² have reported that the hypolipidaemic fibrate drug fenofibrate prevented interleukin-1-induced secretion of interleukin-6, a marker for vascular smooth muscle cell (VSMC) activation, in cultured human VSMCs. They also investigated the effect of fenofibrate on plasma concentrations of acute-phase proteins in patients with hyperlipidaemia and coronary artery disease and found that administration of fenofibrate significantly lowered the plasma concentrations of interleukin-6 and CRP.



Effects of hypolipidaemic drugs (10^{-5} mol/L) on angiotensin II (10^{-7} mol/L)-induced interleukin-6 production by cultured human VSMCs

* $p < 0.05$ compared with angiotensin-II-stimulated cells.

We previously reported that plasma interleukin-6 concentrations are raised in patients with acute myocardial infarction³ and that interleukin-6 mRNA is expressed in human atherosclerotic lesions.⁴ Therefore, we speculate that the main source of plasma interleukin-6 in hyperlipidaemic coronary patients is vascular tissue. We also studied the effects of fluvastatin and pravastatin as well as fenofibrate on interleukin-6 production by cultured human VSMCs (figure). The addition of angiotensin II significantly increased interleukin-6 production, and fluvastatin, pravastatin, and fenofibrate significantly prevented angiotensin-II-induced secretion of interleukin-6. CRP is synthesised in the liver by stimulation of interleukin-6. Therefore, we speculated that the reduced plasma concentrations of CRP by the statins recorded by Strandberg and colleagues¹ were attributable to inhibition of interleukin-6 synthesis in the vascular tissue by drugs.

Hypolipidaemic drugs inhibit the progressive formation of atherosclerotic lesions, even in the absence of their lipid-lowering effect.⁵ Our findings and those of Strandberg and colleagues¹ suggest that in addition to their lipid-lowering effects, statins or fibrate drugs have direct anti-inflammatory effects on vascular tissue, leading to inhibition of atherosclerosis and postangioplasty restenosis.

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Rotavirus vaccine in Japan and Australia

Sir—According to Peter Wehrwein's Feb 6 news item (p 478),¹ the US Centers for Disease Control and Prevention (CDC) has made new vaccine recommendations that include an oral rotavirus vaccine, and the manufacturer of the first licensed rotavirus vaccine RRV-TV vaccine expects that as many as one million children will be vaccinated later this year.

Preliminary field trials of the RRV-TV vaccine showed remarkable efficacies for prevention of severe rotavirus diarrhoea in the USA and Finland.² Because the RRV-TV vaccine is recommended to be given at age 2, 4, and 6 months and the efficacy of fewer than three doses has not been established, a key factor for the vaccine to show good performance in a given country is the proportion of the children who become ill before the completion of the vaccination programme. In the USA, 22.2–29.9% of all paediatric cases of rotavirus diarrhoea were infants younger than 6 months.³ In the UK, a similarly large proportion (26.6%) of cases of rotavirus diarrhoea among infants aged 0–5 years occurred during the first 6 months of life.⁴ Such children would therefore be unprotected under the recommended vaccination schedule. By contrast, when cases of rotavirus diarrhoea among children younger than 5 years were considered, the percentage under age 6 months was only 7.4% in Japan (on the basis of the national surveillance data for 1990–94) and 9.1% in Australia⁵ (differences significant by χ^2 $p < 0.001$). Thus, the proportion that would not be prevented by the RRV-TV vaccine if administered according to the 2–6 month age regimen is substantially smaller in Japan and Australia; the RRV-TV vaccine may

be more effective in these countries than in the USA and Europe.

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Optimum doses of irinotecan

Sir—David Cunningham¹ and Philippe Rougier² and their colleagues (Oct 31, pp 1407, 1413), report that irinotecan was significantly better than best supportive care or infusion fluorouracil, respectively, in patients with metastatic colorectal cancer refractory to previous fluorouracil therapy.

In both studies, the starting dose of irinotecan was 350 mg/m² given every 3 weeks intravenously. However, it should be emphasised that in both studies patients aged 70 years or older and with a WHO performance status of 2 started at 300 mg/m². Patients with performance status of 3 or 4 were excluded from this study. Our experience in a phase I trial of irinotecan³ given every 3 weeks indicated that the recommended phase II dose is 320 mg/m² in patients who have not previously had radiation therapy. In those patients who previously had abdominal or pelvic irradiation, the phase II recommended dose was 290 mg/m².

This apparent difference in doses of irinotecan between our study and those of Cunningham and Rougier raises the question of what proportion of patients had dose reductions on the second course of therapy and what were the actual median doses delivered on cycle 1 and cycle 2 in the two studies. Taking our phase I experience together with additional information about dose reduction in the Cunningham and Rougier trials, it may be reasonable to start with irinotecan dose of 300 mg/m² on the first course with subsequent escalation if the drug is tolerated,

particularly among the elderly patients or those with poor performance status.

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- 3 Pitot HC, Erlichman C, Goldberg RM, et al. Phase I trial of irinotecan (CPT-11) given once every three weeks to patients with advanced solid tumors. *Proc Am Soc Clin Oncol* 1996; **51**: 494.

Author's reply

Sir—In response to our paper and that of Philippe Rougier and colleagues¹ Charles Erlichman and colleagues comment on the starting dose of irinotecan (350 mg/m²) with the 3-weekly schedule that might be too high in elderly patients or those with poor performance status. This proposal is supported by the data of a US phase I study with the 3-weekly schedule, in which the maximum tolerated dose was 320 mg/m² and, accordingly, the recommended dose of irinotecan was 290 mg/m².²

We agree with Erlichman and colleagues that the starting dose of irinotecan should be left to the physician, who should weight the benefit/risk ratio for each patient, with the opportunity to escalate the dose in patients who tolerate irinotecan. However, it must be reminded that in our studies, patients were treated with a starting dose of 350 mg/m², apart from those older than 70 years or a performance status of 2, who received a starting dose of 300 mg/m².

In our study, 15 of 183 patients treated at 350 mg/m² at cycle 1 were reduced to 300 mg/m² at cycle 2. The actual median dose (all patients) delivered at cycle 1 and 2 were 348.2 mg/m² and 345.1 mg/m² respectively. In the Rougier study,² seven of 94 treated at 350 mg/m² at cycle 1 were reduced to 300 mg/m² at cycle 2. The actual median dose (all patients) delivered at cycle 1 and 2 were 347.5 mg/m² and 344.1 mg/m², respectively. Of the patients who were treated at 300 mg/m² at cycle 1, because of age older than 70 or performance status of 2, two of the 21 patients in our study were reduced to 250 mg/m² at cycle 2, but none of the 20 patients in the Rougier study.

The small proportion of patients who had a dose reduction (7.5–8.2%), as

well as the actual median dose delivered at cycle 1 and cycle 2 in both trials, suggest that the starting dose of 350 mg/m² of irinotecan is appropriate, but this decision should be left to the physician's judgment.

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- 1 Rougier P, van Cutsen E, Bajetta E, et al. Phase III trial of irinotecan versus infusional fluorouracil in patients with metastatic colorectal cancer after fluorouracil failure. *Lancet* 1998; **352**: 1407–12.
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Missense mutations at *ATM* gene and cancer risk

Sir—Two studies published this year, one by Tatjana Stankovic and colleagues (Jan 2, p 26)¹, showed the presence of mutations in the *ATM* gene, which is defective in ataxia-telangiectasia (A-T), in B-cell chronic lymphocytic leukaemia (B-CLL). After the discovery of missense mutations in T-cell and B-cell malignant disease and our suggestion of a putative increase of A-T heterozygotes in patients with T-cell prolymphocytic leukaemia (T-PLL),³ both studies reported two missense mutations in the germ-line of patients with B-CLL, supporting the increased prevalence of the germ-line carriers of mutated *ATM* alleles among B-CLL patients. Stankovic and co-workers² argued that the transversion 3161C to G is a pathogenic *ATM* mutation since the change was not found in 68 A-T families and controls and the aminoacid substitution represents a non-conservative change.

Although we have shown this substitution in a breast tumour sample, we observed a loss of the rare allele in tumour cells,⁴ inconsistent with *ATM* being a tumour suppressor, as suggested for T-PLL.³ In addition, we found this variation five of 224 chromosomes in North European white people.⁴ Four guanosine-containing alleles were identified on 176 chromosomes in Swedish patients with familial breast cancer and the same number on 126 chromosomes in a control group of Swedish women.⁴ We also found 52 heterozygotes at this nucleotide position in 880 German patients with breast cancer, which did not differ from 22 heterozygotes in 335 controls. We identified five heterozygotes in 93 unrelated Iranians, although no heterozygotes were detected in 93 Gambians.

Since the overall frequency of this rare allele is in excess of the estimated population frequency of A-T heterozygotes and does not differ between controls and cancer patients in the same population, this particular change can hardly represent a genuine A-T or allele that predisposes to breast cancer. Can this substitution still confer a risk of developing B-CLL? Could such a risk be conferred by other changes found on the same haplotype?

At least a partial answer to these questions can be provided by the combination of mutation and large-scale allelic association/haplotype studies of B-CLL, which is more common than T-PLL, and so studies can yield sufficient cases. It will be crucial, however, to ensure a valid selection and sufficient number of controls, free of confounding effects of the population stratification or admixture. To distinguish rare variants in *ATM*¹ from genuine cancer-predisposing mutations, each rare allele identified in cancer patients should be analysed independently and in the context of a particular haplotype in cases versus controls. For example, we found that a subset of the guanosine-bearing alleles at position 3161 had a thymine to cytosine transition at position 2572. Similarly, A-T mutations such as the exon skipping 3576G to A substitution occur in *cis* on the 3161G haplotype.⁵ Such linkage disequilibria may contribute to disease associations and differential overall frequencies of missense changes in controls versus cancer patients.

We believe it is incorrect to regard the 3161G transversion as an A-T allele and premature at this stage to see it as a B-CLL-predisposing variant, in particular if a truncating *ATM* mutation was found in the same patient with B-CLL.³

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- 1 Bullrich F, Rasio D, Kitada S, et al. *ATM* mutations in B-cell chronic lymphocytic leukemia. *Cancer Res* 1999; **59**: 24–27.
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Biases in reporting of trials

Sir—As Iain Chalmers and Douglas Altman (Feb 6, p 490)¹ point out, a systematic approach is long overdue to prevent poor medical research not only during and at the end of realisation, but also during planning and designing. Clinical scenarios and outcome constructs in trials are increasingly complex, so a public place in the scientific community must be found where the formulation of rationale, hypothesis, and design of a clinical trial can be as vigorously discussed and peer reviewed.

A change in paradigms, however, is needed to improve the scientific culture of trial protocols which, at present, are too much dominated by formalism demanded by regulative agencies. The typical view, expressed in a clearing conference last year, was cited by J P Vandenbroucke: “progress in medicine is dependent upon young creative researchers who approach the problems with an abundance of novel ideas while the final demonstration of effectiveness by randomised trials might be left to their burnt-out superiors who are only good for administration and organization.”² The visionary future, however, was opened by the response of H R Wulff: “the planning of randomised trials presents a great intellectual challenge, and I disagree strongly with the former remark”.²

Electronic publishing offers new opportunities to handle trial protocols as original papers. More details can be made available for trial specialists and critical readers. Length of the article is not the problem for the publishing medium. But does it also take into account the information-exhausted reader?

Trial protocols have to be changed both in terms of content and format. At present they do not reveal why certain features of design or options have been chosen and just as importantly, why others have been considered but deliberately not used for a complex clinical scenario or methodological framework. Furthermore, trial protocols may require additional experiments to strengthen the necessity for a particular study condition. Such experiments may constitute small randomised trials in human beings³ or additional work in animals and cell cultures that are directly aimed to help decision making about the design of a particular trial feature.⁴ Such experiments were

coined as clinic modelling randomised trials (CMRTs) in basic biomedical research.⁴ Finally, trial protocols change the classic format of original papers since their result is methodology (materials and methods). The IMRAD format (introduction, methods, results, and discussion) is not appropriate. The structure of the CONSORT statement should be applied.

We have painfully learnt from the failure of the sepsis studies that multicentre trials include different clinical practice guidelines in various countries.⁵ Animal work has shown that this variability destroys the chance to show effectiveness of the intervention under investigation.⁴ A first attempt to overcome this fundamental drawback to the sepsis trials was consensus-assisted development of a study protocol on prevention of abdominal sepsis.⁵

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Sir—Iain Chalmers and Douglas Altman¹ show how important it is to reduce result-dependent biases in the reporting of randomised controlled trials and to publish reports of these studies in sufficient detail for quality to be assessed. Chalmers and Altman are correct to point out that electronic publishing can help to overcome these problems. The Current Science Group are endeavouring to respond to these opportunities and we started a new publishing initiative (which is under development) under the umbrella of Current Controlled Trials

Ltd (<http://controlled-trials.com>; accessed on Feb 25, 1999).

In response to an initiative taken in 1997 by a group with representation from the Medical Research Council, the NHS Executive, medical charities, pharmaceutical companies, the UK Cochrane Centre, and other publishers (the *BMJ* and *The Lancet*), we established an electronic metaregister of controlled trials (controlled-trials.com/search/actr.htm; accessed on Feb 25). We are collaborating with groups who are assembling and maintaining trials registers, and offering to make their registers available in the metaregister in the form of a minimum set of data, with links to other websites where users can find further information, if available. We also provide links to other trials registers, held separately, worldwide.

In areas where no registers exist, or where they are insufficiently complete, we will also endeavour to start registers ourselves if at all practical. The information in the metaregister is, and will continue to be, available free of charge through the Current Controlled Trials website, and we hope that the site will gradually make it much easier for everyone to obtain basic information about controlled trials that are underway.

We are also beginning to implement Chalmers and Altman's idea of a sequence of threaded electronic publications for controlled trials, ideally beginning with publication of protocols, continuing with reports of the resulting research that meet the CONSORT group's reporting standards, and going onto deposition of complete datasets when practical. This publishing project is being developed through various specialist publications that will combine print and internet versions.

We aim to collaborate with other publications and develop initiatives to improve and make more readily available information about trials. In conjunction with the two main initiatives outlined above, we will commission and publish reports, structured abstracts, commentaries, analyses and syntheses of information generated by controlled trials.

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Decriminalisation of opioid use

Sir—Jeff Ward and colleagues (Jan 16, p 221)¹ summarise the scientific evidence on the consequences of decriminalising non-medical opioid use. This evidence is scant but at least consistent. To allow opiate users access to legal opiates that have been subject to proper quality control improves both the health of users and of the communities they live in. The opiate itself does not seem to matter. Most evidence relates to methadone but results for other opiates have suggested similar effectiveness.² Needle exchange—the other main form of de facto decriminalisation in this area is also effective.³

Other therapeutic interventions for opiate users such as counselling do not involve decriminalisation of opiate use. The evidence for the effectiveness of this approach is less convincing,⁴ which is disappointing since, as with people who are problem users of legal drugs, the interventions seem to work.

The discrepancy may arise for various reasons. If a popular drug is illegal, then a black market is inevitable that will be augmented by diversion from legitimate sources. Problems associated with diversion are a proper concern of services that prescribe opiates to opiate users. As a result, a major activity of such services is the policing of prescriptions. Even in the situation when a more comprehensive approach is theoretically possible, prescription-related negotiations pervade everything. In this context, the lack of apparent added value from services that ostensibly provide more than legal drugs is not surprising. These services might have more success if drug provision was not their responsibility.

The medicalisation of the problem of addiction has undoubted benefits for individuals and was pragmatically sensible; however, such euphemisms as opioid maintenance may no longer be helpful. Scientists should not be constrained by the considerations that prevent politicians behaving rationally or speaking honestly in this area. Currently, decriminalisation is brought about through an elaborate, expensive system that is unpopular with many doctors. There are other approaches to decriminalisation. Legalisation, for example, would have the added social advantage of undermining the financial base of criminal entrepreneurs. Legalisation of opiates is unlikely to significantly reduce the number of opiate users, nor will it abolish the difficulties users experience (alcohol is legal). But the current evidence seems to be that adult opiate use should be

decriminalised and the most effective way to do this should be discussed.

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- 4 Mattick RP, Ward J, Hall W. The role of counselling and psychological therapy. In: Ward J, Mattick RP, Hall E, eds. *Methadone maintenance treatment and other opioid replacement therapies*. Amsterdam: Harwood Academic, 1998; 265–304.

Careless doctors are dangerous for their patients

Sir—Karen Birchard's Jan 16 (p 218)¹ news item gives an interesting summary of the Irish study about the bad self-care and treatment of ill doctors.² We want to point out the possible danger for the patients and for the public health as a whole, especially when doctors have contagious diseases.

We studied the rate of upper respiratory tract infections with fever ($\geq 38^{\circ}\text{C}$) in 177 Flemish general practitioners (GPs). Although 112 of the GPs believed it would be useful to stop work if they had upper respiratory tract infection with fever, only 12 did so. Some explanations can be given for this behaviour. 60% of GPs in Flanders work alone in private practices and have difficulties in finding another doctor to cover for them. Belgian GPs are paid fee for service and are without income when they do not work.

The vaccination status of each doctor shows the careless attitude and behaviour to the health of their patients. Half the GPs were vaccinated against influenza in winter 1996–97. Influenza vaccination for doctors is still controversial, although vaccination of health-care workers in long-term-care hospitals was shown to reduce the mortality of elderly patients.³ In 1991, only 56% of Belgian GPs were vaccinated against hepatitis B,^{4,5} notwithstanding that the vaccine had been available for more than 10 years at that time and GPs are judged at risk. The most frequently cited reasons for not being vaccinated were negligence and "I don't know why". Moreover, 12 GPs did not believe themselves at risk.

GPs in Belgium easily neglect their own protection against infection and their role in transmission of infections to

their patients, as a source themselves or as a vector between two patients.

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- 3 Potter J, Stott DJ, Roberts MA. Influenza vaccination of health care workers in long-term-care hospitals reduces the mortality of elderly patients. *J Infect Dis* 1997; **175**: 1–6.
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Near total sternal agenesis

Sir—Luigi Capasso and colleagues (Feb 6, p 504)¹ have radiographically and endoscopically studied the well preserved body of Santa Rosa da Viterbo (1233–52). Santa Rosa was apparently otherwise normally developed despite having total agenesis of the sternum. Capasso and colleagues suggest that this is the only case, historical or modern, of an individual surviving infancy with sternal agenesis, and without any other serious malformation such as anencephaly. However, there is at least one other such case.

Niculescu and colleagues² describe a 4-year-old boy who was otherwise normal, apart from a certain reticence in the eyes appreciated by the doctors and a certain excess tranquillity noticed by his parents. He had agenesis of the body, xiphoid process, and lower part of the manubrium of the sternum. The patient had successful surgical intervention. Niculescu and colleagues also mention that they are aware of at least two cases of otherwise normal individuals more than 20 years of age with sternal agenesis. Santa Rosa and the modern cases show that remarkable lives are possible, even in the presence of severe anomalies.

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- 2 Niculescu N, Papalicescu S, Olanescu A. Un cas d'agenésie sternale opérée. *J Chir (Paris)* 1967; **94**: 543–48.