Only the smile is left

SIR—The 30 June 1988 issue of *Nature* contains a "Scientific Paper" describing unusual results. In it, Davenas *et al.*[1] claim that images of molecules may be imposed on solvent such that when the original molecules are no longer present (for example, by diluting the solution sufficiently) their reactivity nevertheless persists. Specific antibodies, antigens, enzymes, ionophores and simple amines were claimed to exhibit such 'Cheshire cat'[2] phenomena. Because the assay used by Davenas *et al.* is closely analogous to one regularly used in our laboratory, it was simple for us to test the generalizability of their claims. We observed no results such as theirs. Evidently, whatever may have generated their own data is not readily reproduced. We therefore see no basis as yet for concluding that the chemical data accumulated over two centuries are in error, and that Davenas *et al.* have discovered a new chemical principle.

Rat basophilic leukaemia cells — a neoplastic line of mucosal mast cells of the 2H3(HR+) line[3] — were grown in stationary flasks and after harvesting were mixed with ^{3}H-labelled-5 hydroxytryptamine (5-HT) and mouse anti-dinitrophenyl immunoglobulin E (IgE). The mixture was distributed on 24-well polystyrene plates and incubated for 16 hours. After washing, the adherent cells were challenged with either purified rabbit anti-mouse IgE or dinitrophenylated bovine serum albumin[4]. In preparing sequential dilutions of the antibody and antigen, care was taken to vortex each dilution for approximately 12 seconds because Davenas *et al.* claimed this was important[1]. The dilutions were prepared and coded by one of us (H.M.); the assay performed and data analysed by the other (S.D.). Our results are shown in Fig. 1. No release of 5-HT was observed at high dilutions. It is apparent that the phenomenon described by Davenas *et al.* is not readily generalizable even to a closely related system.

It is reasonable to ask whether the observations of Davenas *et al.* should have been published by *Nature*. We think not. One of us (H.M.) reviewed their paper at the request of *Nature* in April 1987, and urged that the findings be checked by one or more laboratories chosen by the editor. Instead, Dr Benveniste made his own choice, and *Nature* decided to publish the report and then to despatch an international investigative team consisting of the editor, a magician and a scientist, none of whom has experience in the relevant field. Their report[5] provides no support for the published claims and will dismay serious scientists: it adds to the circus atmosphere engendered by the publication of the original paper. ("Homoeopathic enthusiasts are rejoicing while scientists scratch their heads..."[6]; "Homoeopathy finds scientific support"[7].) We believe that the approach chosen by *Nature* is regrettable. We feel that all ideas no matter how revolutionary, deserve to be heard. However, when new data are proffered that grossly conflict with vast amounts of earlier, well-documented and easily replicated data, a different editorial standard is required. Before the *imprimatur* inherent in publishing them in a leading scientific journal is granted, the new results must be reproducible by disinterested individuals familiar with the field. That is a fundamental principle of scientific objectivity. It's a shame really. It still takes a full teaspoon of sugar to sweeten our tea.

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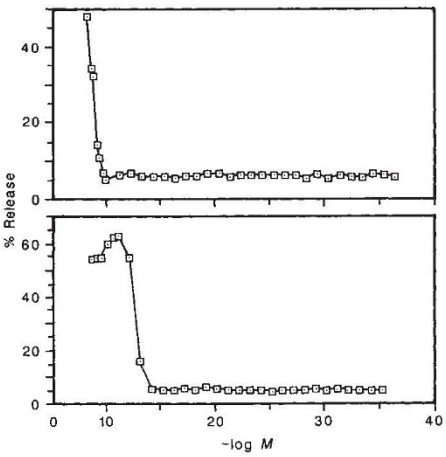

Release of incorporated ^{3}H-5HT from tumour basophils. Each specimen contained approximately 5×10^5 cells and 200 µl medium (100 mM NaCl, 5mM KCl, 1 mM $CaCl_2$, 0.4 mM $MgCl_2$, 5.6 mM glucose, 25 mM Na_2PIPES, 0.1% bovine serum albumin, *p*H 7.0). The appropriate (coded) dilution of antibody (*a*) or antigen (*b*) was added and incubation continued for 45 min. The supernatant was aspirated and counted; 250 µl of 1% Triton X-100 in phosphate buffered saline X3 was used to release the cells and counted. Percent release was calculated as previously described[4]. The solid squares on each graph show the average for three buffer controls performed in duplicate for each set. All data are the means. The ranges of duplicates were ±5% or less from the mean. Altogether there were 142 samples counted and all the data are shown.

SIR—In order that the controversial work of Davenas *et al.* should be considered credible, the reader must first be convinced that the data are genuine. Examination of the data represented in Table 1, however, convinces me that they are synthetic.

The results given are based on triplicate counts of approximately 80 cells and are expressed as the arithmetic mean and standard error. The standard errors vary between 0.5 and 4.8 with a median value of 1.5. This value is much less than that anticipated if normal chance variation in cell number were to have been observed. Even if no cell clumping occurred in the pipetted samples and the observers were wholly accurate, the value of the standard error is too small. In practice, this value will always be equal to or greater than the theoretical minimum standard error.

The most damning evidence is in their Fig. 1*b*. The number of basophils in the anti-IgG control wells, may be either greater or less than that observed in the untreated control wells, assuming that anti-IgG has no effect. It therefore follows that the "% basophil degranulation" should be less than zero in approximately 30 of the 60 dilutions used. The absence of any such 'negative' degranulation figures and the overall distribution of the results is disturbing.

As it is obvious that the data presented in Table 1 and Figure 1 are not strictly derived by experiment, the credibility of the remainder of the paper must remain in doubt.

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SIR—J. Benveniste and co-workers recently reported extraordinary observations on the effects of extremely diluted antibody solutions. They found that aqueous solutions of anti-immunoglobulin E (anti-IgE) still retain an ability to cause degranulation of human basophils (a type of white blood cell with cell surface antibodies to IgE) even when diluted to the point where there are no molecules of anti-IgE left in solution[1]. We suggest that these results are due to the production of reactive chemical species by cavitation during sample preparation.

As these authors state[1], the "importance of agitation in the transmission of information was explored by pipetting dilutions up and down ten times and comparing with the usual 10-s vortexing. Although the two processes resulted in the same dilution..., degranulation did not occur at high dilution after pipetting. Ten-second vortexing was the minimum time required.... So transmission of the information depended on vigorous agitation..."

The authors are apparently unaware of

the high energy chemistry associated with turbulent flow of liquids. These chemical effects result from cavitation: the creation, growth and implosive collapse of bubbles in liquids[2]. The implosive collapse of bubbles can generate intense, but transient, local heating[3] of the order of 5,000K. This is sufficient to induce the homolytic cleavage of O–H bonds of water[4] and C–C bonds of hydrocarbons[5]. Cavitation is usually associated with ultrasonic irradiation, but it also occurs during any turbulent flow. For example, Anbar demonstrated many years ago[6] the existence of cavitation and associated high energy chemistry during turbulent mixing of water. The effects of cavitation and associated shock waves on biological systems can be dramatic, leading to cell rupture and death as the limiting case[7].

We suggest that the degranulation observed by Benveniste and coworkers is an artefact of cell damage caused by reactions with small amounts of OH·, H·, H_2O_2, HO_2, etc., produced by their use of vortex turbulence. Our hypothesis is easily tested: do basophils degranulate upon addition of water or buffered solutions previously subjected to vortex mixing, to high speed propeller cavitation (from a turbine homogenizer), or to high intensity ultrasound (from a cell disruptor)? The treated water should not contain any protein, which could serve to scavenge reactive species. Alternatively, do atmospheres of helium or carbon dioxide suppress the observed effects? These gases dramatically diminish the temperatures reached during cavitational collapse and suppress most chemical effects.

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SIR—Davenas *et al.*[1] observed repetitive waves of degranulation of human basophils, which had been exposed to increasing dilutions of anti-immunoglobulin E (anti-IgE) antibody. Degranulation was reported at the highest dilutions, even in the calculated absence of anti-IgE antibody. The hypothesis was suggested that the molecular organization of water might have been responsible for transmission of biological information. There may be a simpler explanation, more readily accommodated by the existing scientific paradigms.

Davenas *et al.*[1] reported using Tyrode's solution to dilute antibody. As gleaned from their paper, this solution always appeared to contain heparin. Heparin has a number of interesting properties that are germane to what may be a novel role in mediating the activity of anti-IgE antibody. Heparin exists in a helical configuration, binding water molecules, as well as a number of monovalent and divalent cations (including Na and K, which are present in Tyrode's solution)[2]. It also binds to various proteins and synthetic polypeptides[2,3]. Like other glycosaminoglycans, heparin chains are further capable of interacting with one another, forming molecular aggregates[3].

I propose that anti-IgE antibody (or any of the other immunological stimuli noted in the paper[1], that were responsible for basophil degranulation) might have acted as a template for heparin, thereby inducing a specific conformation of the heparin molecule. This molecular conformation would then be stabilized, perhaps by interacting with another heparin molecule. Upon dilution with heparin-containing Tyrode's solution, the stabilized heparin conformation, although lacking biological activity, would itself serve as a template, effecting a new heparin conformation which would mimic the three-dimensional structure of the antigen-binding site of anti-IgE antibody (or other immunological stimulus). Presumably water molecules and perhaps the cations present in Tyrode's solution would stabilize this new, antigen-binding heparin conformation. Subsequent dilution with fresh heparin would, however, result in the antigen-binding conformation further acting as a template for formation of the biologically inactive conformation. Successive dilutions would generate the alternating heparin conformations.

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SIR—There may be a very simple explanation of Benveniste's conclusion (*Nature* **333,** 816; 1988). Imagine there is some degranulating active molecule, not necessarily an anti-IgE, that binds to one component of Hepes–Tyrode's solution, for example heparin or EDTA; then there will not be a real dilution of the active compound.

Imagine that the binding is or is not reversible depending on the degree of "vigorous shaking", you get an explanation for "rhythmic fluctuation" in the activity (not so rhythmic in fact). This explanation is more comfortable than 'water memory' or throwing away the Law of Mass Action or Avogadro's number.

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Creationism and evolution

SIR—Andrew P. Whipple (*Nature* **333,** 492; 1988) tries to take an even-handed approach towards science and creationism, but in my opinion fails in this attempt. First, he fails to give weight to the coherence between theory and observation that evolutionary theory is alone able to offer. It is this observationally multicentred coherence that gives scientific 'belief' a privileged character, not shared by other types of belief. Furthermore, Whipple gives a truncated account of the 'world views' in presence. He defines the naturalistic viewpoint as one of two basic positions, namely one that asserts that there is no reality beyond the physical and that denies the supernatural. This he opposes to the theistic viewpoint. In fact, there is a third position, not covered by your correspondent's analysis, which represents a different naturalistic view. This view also excludes the supernatural, but holds that the spiritual domain has a reality of its own as much as the world of phenomena does, a reality subject to the equivalent of natural laws, that is, engaged in obligatory, if unknown, relationships with the world of phenomena. These relationships need not be more arbitrary or inconstant than the relationships within the observable world itself and therefore are not in favour of a personalized and humanized god. The view I refer to holds that the mind, fully as real as, and distinct from, observable nature, is another side of nature. (By observable nature, I refer to the nature before us, not necessarily totally observable.) Our present ignorance of what this other side of nature *is* does not dispense us from acknowledging the existence of a philosophical position that is opposed by creationism, yet is not 'materialistic', but 'naturalistic' in a sense different from your correspondent's.

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