

Whale deaths caused by US Navy's sonar

Mark Schroppe

The US Navy has admitted that its use of a high-intensity sonar system caused a rash of whale strandings and deaths in March 2000.

Sixteen beaked and minke whales were found stranded on beaches in the Bahamas shortly after US Navy ships using the high-intensity sonar had passed by. Six are known to have died, and the rest were pushed back into the sea. But a fall in sightings of beaked whales has led researchers working in the area to believe that many more may have died. Autopsies on the animals revealed bleeding around the whales' inner ears and in one instance in the brain.

The Navy and the National Marine Fisheries Service (NMFS) responded to the incident by launching a series of investigations. An interim synopsis of the reports, released on 20 December 2001, concludes that the bleeding was caused by sound waves produced by the high-intensity sonar.

The synopsis was welcomed by environmental groups, which claim that high-intensity sonar may have caused other strandings. Autopsy evidence in previous



High and dry: the US Navy's use of high-intensity sonar left whales on the beach in the Bahamas.

cases could not identify the cause of the stranding. "This is the first time the Navy has really acknowledged responsibility for

anything like this," says Andrew Wetzler of the National Resources Defense Council, a New York-based environmental group.

But the report says that the high-intensity sonar may not pose a widespread threat to marine life. Such systems are commonly used, and the synopsis says the strandings were the result of unique local conditions. The sound waves were trapped in a layer of warm water, preventing their dissipation, and the whales could not escape because they were feeding in underwater canyons.

But according to Roger Gentry, coordinator of the NMFS acoustics team, the possibility that other conditions might cause similar problems cannot be ruled out, as it is not understood how the sound waves caused the bleeding. Several US research groups are looking into this issue.

Although he welcomes the report, Wetzler claims that the Navy is playing down the importance of its conclusions by focusing on unique characteristics of the event. "We really believe that this is an important piece of evidence that calls for all parties to re-examine all use of high-intensity sonar around the globe," he says.

The report comes at a sensitive time. The US Navy is seeking approval for a new high-intensity sonar system (see *Nature* 410, 505; 2001). The system, which operates at lower frequencies than that responsible for the Bahamas strandings, is needed to detect new, quieter submarines, the Navy says. The NMFS will have to approve use of the system under the Marine Mammal Protection Act, and Gentry says a decision should be made within a few months. But he says the report is unlikely to significantly affect the approval process. ■

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NIH faces action over HIV cat study

Erika Check, Washington

The US National Institutes of Health (NIH) is being sued over a research project that involves giving amphetamines to cats infected with the feline equivalent of HIV.

The Physicians Committee for Responsible Medicine (PCRM), a Washington-based pressure group that opposes animal experiments and advocates preventative health care, launched the action on 27 December. Using the Freedom of Information Act, it wants to force the NIH to release all the documents relating to the grant proposal made for the project.

The study is led by Michael Podell, a

veterinarian at Ohio State University in Columbus, who is investigating the interactions between drug abuse and AIDS.

Podell won a five-year grant worth \$1.7 million from the National Institute on Drug Abuse, part of the NIH, in autumn 2000 to study neurological changes in cats infected with feline immunodeficiency virus (FIV) and exposed to high doses of methamphetamine, the recreational drug more commonly known as speed.

The PCRM claims that the study is cruel and scientifically unnecessary, and wants the NIH to release more information about the project, including the outcome of Podell's pilot studies.

Podell, the NIH and Ohio State University have each declined to comment on the lawsuit. But in a previous statement, Podell said that his feline study will help to establish how HIV and drug abuse cause brain damage in human patients.

Murry Cohen, a psychiatrist affiliated with the PCRM, says that the feline model is too dissimilar from the human infection to yield clinically relevant information.

But Dennis Kolson, a neurologist at the University of Pennsylvania, says: "There are so few *in vivo* model systems for retroviral diseases like HIV that we have to utilize each one to its fullest extent, and the FIV system fits into that, as does the primate model." ■



Speed restrictions? Questions are being raised over a study that gives cats amphetamines.

Gas-bubble lesions in stranded cetaceans

Was sonar responsible for a spate of whale deaths after an Atlantic military exercise?

There are spatial and temporal links between some mass strandings of cetaceans — predominantly beaked whales — and the deployment of military sonar^{1–3}. Here we present evidence of acute and chronic tissue damage in stranded cetaceans that results from the formation *in vivo* of gas bubbles, challenging the view that these mammals do not suffer decompression sickness. The incidence of such cases during a naval sonar exercise indicates that acoustic factors could be important in the aetiology of bubble-related disease and may call for further environmental regulation of such activity.

Fourteen beaked whales were stranded in the Canary Islands close to the site of an international naval exercise (Neo Tapon, 2002) held on 24 September 2002. Strandings began about 4 hours after the onset of mid-frequency sonar activity. We necropsied eight Cuvier's beaked whales (*Ziphius cavirostris*), a Blainville's beaked whale (*Mesoplodon densirostris*) and a Gervais' beaked whale (*Mesoplodon europaeus*), six of which were very fresh. These animals showed severe, diffuse vascular congestion and marked, disseminated microvascular haemorrhages associated with widespread fat emboli within vital organs. Intravascular bubbles were present in several organs, although definitive evidence of gas embolism *in vivo* is difficult to determine after death⁴. No pathogenic bacteria were isolated from the carcasses.

These lesions are consistent with acute trauma due to *in vivo* bubble formation resulting from rapid decompression (as occurs in decompression sickness)^{4,5}. Bubble formation in response to sonar exposure might result from behavioural changes to normal dive profiles (such as accelerated ascent rate), causing excessive nitrogen supersaturation in the tissues (as occurs in decompression sickness); alternatively, bubble formation might result from a physical effect of sonar on *in vivo* bubble precursors (gas nuclei) in nitrogen-supersaturated tissues^{6,7}.

The beaked whales found in the Canary Islands are not the only stranded cetaceans to provide evidence of bubble-associated tissue injury. In strandings that occurred in Britain between October 1992 and January 2003, three out of 24 Risso's dolphins (*Grampus griseus*), three out of 342 common dolphins (*Delphinus delphis*) and one out of 1,035 harbour porpoises (*Phocoena phocoena*) necropsied, as well as the only Blainville's beaked whale studied, contained gas bubbles in their blood vessels and gas-filled cavities in their parenchymous organs.

The livers of these animals were the most consistently affected organ, with macroscopic gas-filled cavities (diameter, 0.2–6.0 cm) occupying 5–90% of the volume (Fig. 1) and having variable degrees of fibrotic encapsulation. Intrahepatic spherical non-staining cavities (gas bubbles) of diameter 50–750 μm were associated with compression of hepatic tissue, distension of portal blood vessels, and sometimes with haemorrhage, acute hepatocellular necrosis or fibrosis, indicating that this damage was inflicted before death. One of the *D. delphis* specimens also had bilateral acute renal infarcts associated with gas bubbles.

The cavitory lesions described here are new to marine-mammal pathology. Their presence in fresh carcasses in the absence of bacterial isolates, and the apparent progression through the stages of pericavitory fibrosis, are inconsistent with decompositional bubbles from bacterial activity. The coexistence of ante mortem gas bubbles and gas-filled fibrosed cavities suggests that *in vivo* bubble formation and expansion is the proximate aetiology of this disease process. Bubble formation may either have initiated in the liver and kidneys ('autochthonous' bubble formation) or in other tissues (fatty tissue, for example) before haematogenous transfer to the liver and kidneys as gas emboli.

Nitrogen bubbles and emboli can develop in decompression sickness in humans and

experimental animals as a result of expansion of pre-existing gas nuclei within nitrogen-supersaturated tissues⁵. Anatomical, physiological and behavioural adaptations may mitigate against *in vivo* formation of nitrogen bubbles in marine mammals^{8–12}, although there is empirical evidence of nitrogen supersaturation in cetaceans⁸. Some deep-diving species are predicted to undergo up to 300% nitrogen tissue supersaturation⁷. Static diffusion in nitrogen-supersaturated tissues is therefore a plausible mechanism for bubble development and is consistent with a greater prevalence of cases in deep-diving species such as Risso's dolphins and beaked whales.

Further investigation is needed into the physical and behavioural effects on cetaceans exposed to sonar, and the relation of these effects to bubble growth *in vivo* and to strandings. Necropsies should aim to compare fat and gas emboli in stranded cetaceans suspected of having been exposed to sonar with results from unexposed stranded controls. In a wider conservation sense, our findings need to be taken into account in considering the regulation and limitation of the adverse impact of anthropogenic sonar on cetaceans.

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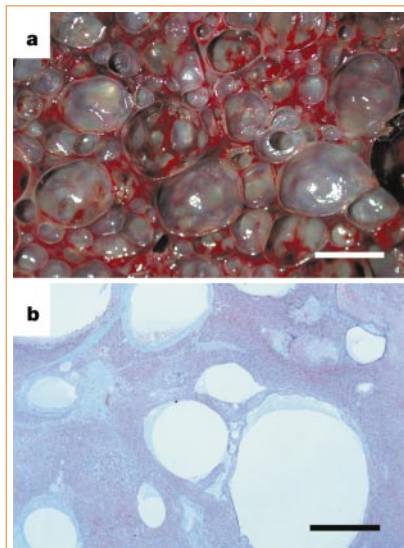


Figure 1 Gas-filled cavities in the liver of a stranded common dolphin (*Delphinus delphis*). **a**, Cut surface of the liver, showing that cavitory lesions have extensively replaced the normal tissue. Scale bar, 10 mm. **b**, Photomicrograph of liver section, showing multiple cavities (gas bubbles) within the portal tracts and hepatic parenchyma. Scale bar, 750 μm .

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Immunology

Hepatitis A virus link to atopic disease

Atopic diseases, including asthma, allergic rhinitis and atopic dermatitis, are caused by both environmental and genetic factors. Here we show that infection by hepatitis A virus (HAV) may protect individuals from atopy if they carry a particular variant of the gene that encodes TIM-1 (also known as HAVcr-1) — the cell-surface receptor used by HAV to infect human cells¹. Exposure to HAV is associated with poor hygiene, large family size and attendance at day-care centres, all factors that are also inversely associated with atopy^{2–6}. Our discovery indicates that interaction between HAV and TIM-1 genotype may contribute to the aetiology of atopic diseases, and provides a mechanism to account for the hygiene hypothesis.

Using a congenic positional cloning strategy, we identified *TIM-1* as a candidate gene for atopy and asthma in a region of mouse chromosome 11, which is homologous to a segment of human chromosome 5q31–33 that has been linked to atopy^{7,8}. TIM-1 is expressed by activated CD4⁺ T cells during the development of helper-T-cell (Th2) responses and regulates cytokine production⁷. We therefore investigated whether the interaction between HAV and TIM-1 on lymphocytes can modify T cells in a way that protects against atopy, and whether polymorphisms in TIM-1 can alter susceptibility to atopy⁷.

By sequencing complementary DNA from human lymphocytes, we identified a six-amino-acid insertion (ins) at residue 157, termed 157insMTTTVP (one-letter amino-acid notation), as well as two single-amino-acid changes, 195delT (where 'del' signifies a deletion) and A206T. The insertion 157insMTTTVP is located at the centre of an extracellular mucin-like region that is required for efficient HAV uncoating⁹ and, because 157insMTTTVP lengthens this critical region by 12–14%, this variation may affect the efficiency of viral entry (Fig. 1).

To determine the effect of the insertion 157insMTTTVP on the occurrence of atopy, we carried out a cross-sectional study of 375 individuals who were evaluated by history and tested serologically for atopy and prior HAV infection. To correct for potentially confounding effects of population admixture, we used stratified Mantel–Haenszel χ^2

tests to quantify the association between atopy and 157insMTTTVP in the total sample. We found that HAV seropositivity protects against atopy, but only in individuals with the 157insMTTTVP variant of TIM-1 ($P = 0.0005$; Table 1).

The protective effects of HAV therefore depend upon a common *TIM-1* allele that is carried by 63% of Caucasians, 46% of Asians and 64% of African Americans in this population (see supplementary information). As allelic variation in TIM-1 does not affect HAV-infection rates in our population ($\chi^2 = 1.567$, $P = 0.211$), we conclude that the interaction of HAV with TIM-1 genotype seen here is not due to variation in the rate of seroconversion following HAV exposure.

Before 1970, the seroprevalence of antibodies against HAV approached 100% in Western countries⁴, and infection with HAV may have protected many individuals against atopy³. However, modernization has led to a reduction in average family size and significant improvements in public health, causing anti-HAV seroprevalence to fall to 25–30%, while the prevalence of atopic disease has doubled⁴. Our finding that TIM-1 is associated with atopy in HAV-seropositive individuals indicates that exposure to a specific pathogen may influence the expression of atopy — so a declining prevalence of HAV infection could contribute to an increase in atopy by association with *TIM-1*. It will be necessary to determine whether HAV exposure must occur during childhood to have a protective effect, whether HAV can mitigate the severity of existing atopic disease, and whether HAV vaccination can reproduce the effects of natural HAV infection.

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Table 1 TIM-1 insert and protection against atopy

HAV status	157insMTTTVP genotype	Total	Atopic	Non-atopic	χ^2 (P)	Odds ratio (95% CI)
Seronegative (n = 198)	Insertion	120	83 (69%)	37 (31%)	0.463 (0.496)	1.285 (0.708–2.439)
	No insertion	78	50 (64%)	28 (36%)		
Seropositive (n = 123)	Insertion	65	31 (48%)	34 (52%)	11.978 (0.0005)	0.257 (0.116–0.570)
	No insertion	58	46 (79%)	12 (21%)		

Comparison of allele distributions across subjects using the Cochran–Mantel–Haenszel χ^2 test with racial stratification, two-sided tests of significance (P), and number of subjects within each genotype (see supplementary information). The six-amino-acid insertion 157insMTTTVP is associated with atopy in individuals seronegative for hepatitis A virus but not in seropositive individuals. HAV does not independently affect atopy ($\chi^2 = 0.513$, $P = 0.474$). Subgroup analysis of Caucasians and Asians confirms this association in both groups ($P = 0.024$ and $P = 0.036$, respectively), and Breslow–Day tests of the homogeneity of the odds ratios demonstrate no significant difference between racial strata (see supplementary information). This table excludes data from 54 individuals with an intermediate atopic phenotype (see supplementary information).

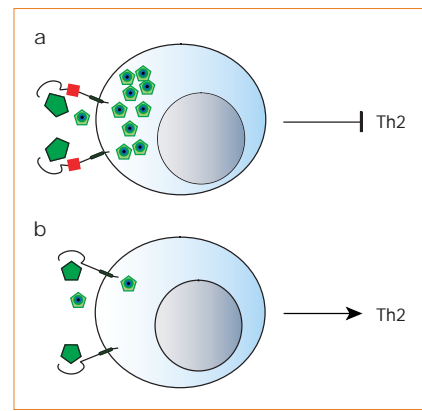


Figure 1 Possible mechanisms of interaction between the cell-surface TIM-1 receptor and hepatitis A virus (HAV), and the effect of HAV on cytokine production. **a**, A variant of TIM-1 ('hook') that carries a 6-amino-acid insert (red), 157insMTTTVP, may increase binding of HAV (large pentagons) to the receptor, thereby enhancing HAV viral uncoating (small pentagons) and infection of TIM-1-expressing T cells. This could lead to deletion of certain lymphocyte subsets, such as Th2 cells, or reduce Th2-cell differentiation, causing a reduction in atopy and asthma. **b**, Alternatively, HAV may bind less efficiently to the form of TIM-1 without the insertion, resulting in less HAV infection and hence more Th2-cell development and more atopy. The mechanism that underpins this interaction between TIM-1, its 157insMTTTVP region and HAV could relate to viral uncoating⁹, the extent and duration of HAV viraemia¹⁰, or to a direct effect on Th1/Th2-subset differentiation.

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