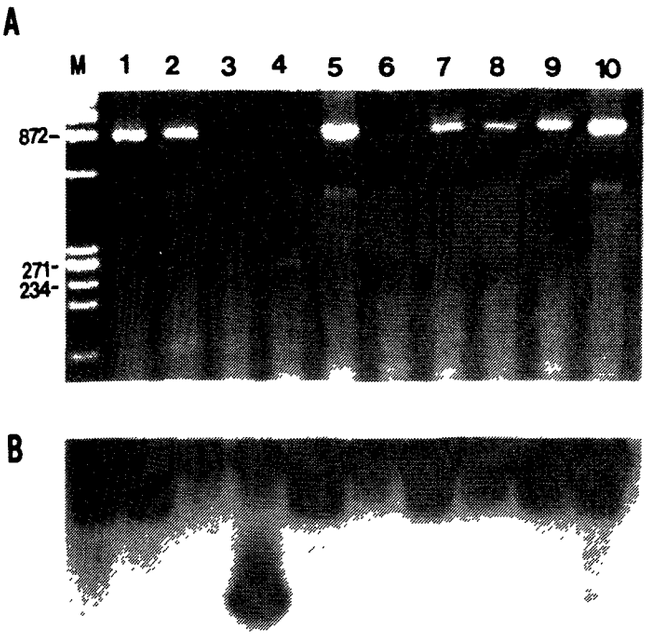

Electrophoretic (A) and Southern blot (B) analysis of RT-PCR products from AIDS-KS and normal skin tissues.

Lanes 1–5 and 10=AIDS-KS lesions, lanes 6–9=normal skin Samples for lanes 5, 6, 9, and 10 obtained from the same AIDS-KS patient. M=ϕX174 RF DNA/*Hae*III fragments

(5'-GAGGAGCACCCCGTGCTGCTGA-3' and 5'-CTAGAAGCA-TTTGCGGTGGACGATGGAGGGGCC-3')[7] and FGF6 (5'-CTCTAC-TGCAACGTGGGC-3' and 5'-TGACTCGTAGGCATTGTA-3')[8] in a PCR was done in buffer containing 10 mmol/l tris-HCl (pH 8·3), 50 mmol/l KCl, 1·5 mmol/l $MgCl_2$, 10 μg gelatin, 2 5 units of *TAQ* polymerase and 200 μmol of each dNTP Samples were subjected to 35 cycles of denaturation (94°C, 1 min), annealing (55°C, 1 min) and extension (72°C, 1 min).

Southern blot hybridisation was with both ^{32}PATP end-labelled probes for β-actin (5'-GAAATCGTGCGTGACATTAAGGAGAAG-3') and for FGF6 (5'-TTGGAAGCTGGGCGTTGCGTACAATCT-3'). Autoradiogram was obtained after exposure of Kodak X-ray film at −70°C

Primer pairs from separate exons were used to inhibit amplification of any contaminating genomic DNA sequences.

Six AIDS-associated KS tumour tissues and two normal appearing skin specimens taken from sites distant from KS lesions of these patients were studied. Two specimens of normal skin, obtained from patients undergoing plastic surgery, served as HIV-1 negative controls. RNA was extracted after specimens had been minced and ground. 1 μg RNA was treated with RNAse-free DNAse 1 (Promega, Wisconsin). After heat inactivation cDNA was made by using 200 units of Moloney murine leukaemia virus reverse transcriptase (BRL, Maryland), 200 ng random hexamer, 225 μmol of each dNTP, 50 mmol/l "tris" HCl (pH 8·3), 75 mmol/l KCl, 10 mmol/l dithiothreitol, 3 mmol $MgCl_2$, 2 μg nuclease-free bovine serum albumin at 37°C for 2 h. PCR was then performed by co-amplification using both primer pairs for β-actin (see figure legend). PCR products were analysed by gel electrophoresis and Southern blot hybridisation. As shown in the figure all the samples contained β-actin fragments (834 bp), showing that RNA had been amplified. An amplified fragment of 258 bp was found in one AIDS-KS sample. With the FGF6-specific probe and high stringency washes, a distinct band was clearly detectable on Southern blot hybridisation, which confirmed that FGF6 was strongly expressed in this sample. Weak expression of FGF6 was found in one other AIDS-KS specimen. There was no expression of FGF6 in the four other fresh AIDS-KS specimens or in any of the normal skin samples. The specimen with high expression of FGF6 had been obtained from a lesion on the tongue and histopathologically was a typical KS nodule with fascicles of spindle cells arranged in a herringbone pattern. The KS specimen with weak expression of FGF6 was from a biopsy-confirmed skin lesion in a patient with disseminated disease.

The FGF family includes oncogene-encoding growth factors which appear to have a similar broad mitogenic spectrum. They promote the proliferation in culture of a variety of cells of mesodermal and neuroectodermal origin and appear to have angiogenic properties too.[9] A role for one or more FGFs in the pathophysiology of KS has been suggested.[5] Histologically, KS represents a complex tumour composed of fasicles of spindle-shaped cells surrounding irregular shaped endothelial-lined vascular slits, extravasated red blood cells and a mononuclear cell inflammatory infiltrate. The FGF6 gene was identified by screening a cDNA library with probes to the HST/K-FGF gene.[8] The FGF6 protein is therefore closely related to HST/K-FGF. FGF6 seems to possess intrinsic oncogenic potential since it can transform cultured fibroblasts.[10] The finding that FGF6 is expressed in some fresh KS tumour tissue may be an important clue to a possible role for FGF6 in oncogenesis and/or angiogenesis in KS.

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Acute transverse myelitis after tetanus toxoid vaccination

SIR,—A case of optic neuritis and myelitis following a booster dose of tetanus toxoid was reported by Dr Topaloglu and colleagues (Jan 18, p 178). We recently managed a patient with acute transverse myelitis that also followed tetanus toxoid booster administration.

A 50-year-old man with a penetrating wound to the foot was given tetanus toxoid and immunoglobulin, single intravenous doses of benzylpenicillin and flucloxacillin, and a course of oral flucloxacillin. 6 days later he returned with a widespread erythematous, maculopapular eruption, which was attributed to flucloxacillin and which resolved on cessation of the drug. 10 days later he again returned complaining of generalised myalgia, lethargy, fatigue, and mild bifrontal headache. A viral illness was diagnosed and symptomatic treatment advised.

12 days after his initial presentation he was admitted after the onset, over several hours, of flaccid, areflexic paralysis of the legs, associated with sensory loss to T6, moderately severe midthoracic back pain, and urinary retention requiring an indwelling catheter. His temperature was 38·6°C but no focus of infection was evident. Cerebrospinal fluid (CSF) examination revealed neutrophil pleocytosis (white cells 1680×10^6/l, 90% polymorphs; red cells 885×10^6/l), raised protein (3900 mg/l), and a low glucose (1·6 mmol/l). Gram stain, bacterial antigen tests for *Streptococcus*

pneumoniae, Haemophilus influenzae, and *Neisseria meningitidis,* Ziehl-Neelsen stain, and india-ink stain were all negative. Peripheral blood leucocytosis ($18{\cdot}4 \times 10^9$/l, 80% polymorphs) was also noted. A myelogram imaged by computed tomography was normal. Acute transverse myelitis was diagnosed and treatment was started with intravenous dexamethasone 4 mg every 4 h and ceftriaxone 1 g daily (empirically in view of the fever and neutrophilia).

On the second hospital day a magnetic resonance imaging (MRI) scan revealed areas of increased signal intensity on T_2-weighted images at C7–T2 and T6–T7 vertebral levels. An MRI brain scan was normal. He subsequently received intravenous pulsed methylprednisolone 1 g daily for 5 days followed by oral prednisolone 100 mg daily (tapering over 2 weeks to zero). Repeat CSF examination on the fourth hospital day revealed white cells 65×10^6/l (75% mononuclear), red cells 40×10^6/l, protein 7200 mg/l, and glucose 5 mmol/l. CSF cultures for bacteria, fungi, and viruses were negative. Oligoclonal bands were not detected. Serology for syphilis, Epstein-Barr virus, herpex simplex virus type 2, and HIV was negative; positive IgG results for measles, varicella-zoster virus, and herpes simplex virus type 1 confirmed past contact. An autoantibody screen, anticardiolipin antibody titre, and serum vitamin B_{12} level were normal. After a month his neurological deficits remained unchanged and he was transferred to a rehabilitation unit.

Transverse myelitis has been reported after vaccination for smallpox, rabies, rubella, cholera, and typhoid and poliomyelitis, and for triple antigen[1] and diphtheria, tetanus, and poliomyelitis,[2] but Topaloglu et al were the first to report myelitis after tetanus toxoid vaccination alone. Reported neurological sequelae of tetanus toxoid include brachial plexus neuropathy,[3] polyradiculoneuritis,[4] and relapsing demyelinating polyneuropathy.[4] Although it is possible that the myelopathy in our patient occurred independently of vaccination, the timing and absence of an alternative explanation may implicate tetanus toxoid.

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Stroke

SIR,—In your recent Stroke Octet series, the use of anticoagulants after cardioembolic stroke was discussed. Dr Hart (March 7, p 589) advises that intravenous heparin should probably not be given until at least 48 h after stroke because the peak rise in haemorrhagic transformation (HT) takes place before this time. Dr Oppenheimer and Professor Hachinski (March 21, p 721) advise waiting 4 days from stroke onset because most HTs will have taken place by then. As these workers indicate, there is evidence that about a quarter of HT occur late.

Two studies of haemorrhagic infarction in which sequential computed tomographic (CT) scans were done and only low-dose heparin or aspirin was used[1,2] have shown a high frequency of HT after 3 days. Hornig et al[1] using sequential scans on days 3, 7, 14, and 21 after stroke found that 24 of 28 scans had HT after day 3 and the peak incidence was between day 7 and 14. Half of Laureno and co-workers' sequentially scanned cases had HT after day 3.[2] Okada et al[3] found that only 10 (15%) of 65 patients in whom HT developed within a month of cerebral embolism showed haemorrhage on the initial scan done 1–6 days after stroke.

Large cerebral infarcts are generally agreed to be a risk factor for HT,[2] and HT increases the risk of haemorrhage into the infarct in patients treated with conventional dose heparin.[4] The Cerebral Embolism Study Group[4] showed that HT was commonly associated with large infarcts, and less commonly with smaller infarcts. The only study that has established statistically that a specific size of infarct is associated with a low risk of HT is that of Okada et al.[3] These workers showed that infarcts of less than 10% of the ipsilateral hemisphere area had a very low frequency of HT (3%). Medium size infarcts had a high frequency of HT (50%), similar to that of large infarcts (51%).

The small size of an infarct on CT seems to be a better predictor of a low frequency of future HT than does the absence of haemorrhage on a 48 h or a 4 day scan. In deciding which patients to treat with anticoagulants after embolic stroke, the main purpose of an early CT scan should thus be to assess infarct size; the secondary aim should be to exclude patients in whom HT has already developed. Patients with infarcts larger than about 10% of the ipsilateral hemisphere area should be given anticoagulants only with caution because of the increased risk of future HT. A scan to assess infarct size should probably be done about 48 h after stroke since most infarcts are visible on CT by this time.

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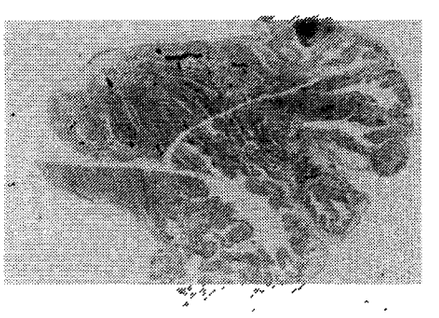
SIR,—Dr Hart's article (March 7, p 589) emphasising the importance of atrial fibrillation (AF) in cardiogenic embolism to the brain, affords the opportunity to address the regular absence of mural thrombus from the left atrium and appendage, at necropsy and on echocardiography, in non-valvular AF associated with embolism. In 1953, I briefly reported that in such cases special microscopic examination always disclosed a mural thrombus.[1] The details were not included and this information has not become generally known, although it forms the pathological rationale for prophylactic anticoagulation.

This study involved the post-mortem examination of the left atrial appendage in 20 cases of non-valvular AF associated with systemic embolism, in which the left atrium and appendage showed no grossly visible thrombus and no other source of an embolus. Each appendage was divided longitudinally after which the two halves were embedded in paraffin and sectioned serially at 8 μm, and every 50th section was stained with haematoxylin and eosin. Each appendage provided about 20 sections. In every case organising mural thrombus was found in the appendage. Often it was small and lay between the muscle trabeculae where it escaped the eye of the prosector, even when the appendage was opened longitudinally and examined with a loupe (figure). Likewise the thrombus was usually too small to be detected on echocardiography. Examination of two cases with AF but no embolism also disclosed microscopic mural thrombus. As a control measure the left atrial appendage was serially sectioned in 15 routine post-mortem cases without AF, and mural thrombus was absent.



Longitudinal section of left atrial appendage showing small mural thrombus (dark spot at top).

Haematoxylin and eosin stain (×1).