oestradiol concentration and the ratio of serum oestradiol concentration to serum testosterone concentration were raised in only nine of the 14 patients studied, and the serum testosterone concentration was low in only five. Scrotal ultrasonography therefore seems to be the most accurate investigation and its increased use in patients with gynaecomastia may reduce the large proportion of idiopathic cases.

The hypoechoic texture seen in the contralateral testis in the first patient can be explained by tubular atrophy, fibrosis, loss of Leydig cells, and hypospermatogenesis that commonly occurs as a result of an increased ratio of oestrogen to androgen concentrations.[2] Recognition of these textural changes may be valuable in diagnosis in patients without gynaecomastia but with otherwise unexplained loss of libido or potency.

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1 Nicholis GL, Modlinger RS, Gabrilove JL. A study of the histopathology of human gynecomastia. *J Clin Endocrinol Metab* 1971;32:173.
2 Gabrilove JL, Nicolis GL, Mitty HA, Sohval AR. Feminizing interstitial cell tumour of the testis: personal observations and a review of the literature. *Cancer* 1975;35:1184-202.
3 Hendry WS, Garvie WHH, Ah-see AK, Bayliss AP. Ultrasonic detection of occult testicular neoplasms in patients with gynaecomastia. *Br J Radiol* 1984;57:571-2.
4 Emory TH, Charboneau JW, Randall RV, Scheithauer BW, Grantham JG. Occult testicular interstitial-cell tumour in a patient with gynaecomastia: ultrasonic detection. *Radiology* 1984;151:474.
5 Kuhn JM, Mahoudeau JA, Billaud L, *et al*. Evaluation of diagnostic criteria for Leydig cell tumours in adult men revealed by gynaecomastia. *Clin Endocrinol* 1987;26:407-16.

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Intracerebral haemorrhage: incidence and use of computed tomography

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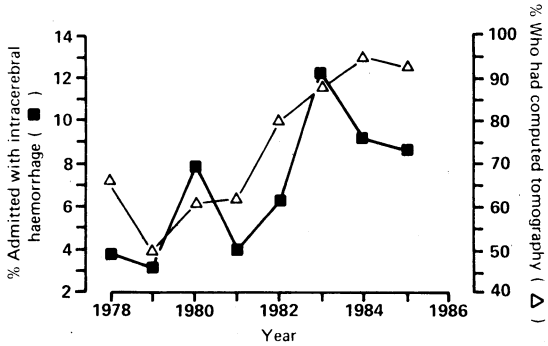
The incidence of primary intracerebral haemorrhage fell between 1950 and 1975.[1] We analysed admissions to this hospital's stroke unit from 1978 to 1985 to see whether the incidence of intracerebral haemorrhage bore any relation to the use of computed tomography.

Patients, methods, and results

The stroke unit was formed in 1977 to receive all patients presenting with suspected stroke or transient ischaemic attack. Computed tomography was performed whenever possible, and the proportion of patients scanned rose as access to facilities improved. Data accumulated prospectively on all patients (2537) were examined to determine the effect of computed tomography on the diagnosis of intracerebral haemorrhage and were analysed by the χ^2 test for trend and Pearson correlation coefficients. In addition the volumes of the haemorrhages were calculated by placing a grid over the scan, each square in the grid representing 1 cm^2 of brain. The number of squares that were more than half occupied were totalled, and the resulting number was multiplied by the thickness of the tomographic slice. This was repeated for all slices showing haemorrhage. A haemorrhage of 10 ml or less was classed as small. Many scans obtained in 1978-80 were not available, and the size of haemorrhage (small *v* medium or large) was then determined from the radiologist's report and the unit's records. A comparison of these methods showed that interpretation of the report tended to overestimate the number of small haemorrhages. Hence a bias in favour of more small haemorrhages for 1978-80 might be expected. Diagnosis of haemorrhages without computed tomography was based on findings at necropsy or the clinical features of a sudden rapid deterioration associated with headache, vomiting, and impairment of consciousness.

The figure shows that admissions for intracerebral haemorrhage rose significantly from 3·8% in 1978 to 8·6% in 1985 ($p<0·001$, χ^2 test for trend) with a peak of 12·3% in 1983 and that use of computed tomography increased from half of all admissions to 95% ($p<0·001$, χ^2 test for trend). A close correlation between these two events was found (Pearson correlation $r=0·77$, $p=0·027$). A greater correlation was found between



Percentage of patients admitted each year to stroke unit with intracerebral haemorrhage (total=186) and percentage who had computed tomography

use of computed tomography and incidence of small haemorrhage ($r=0·82$, $p=0·012$). At the same time mortality in hospital for patients with intracerebral haemorrhage fell significantly from 55% to 29% ($p<0·05$, χ^2 test for trend) and correlated inversely with increasing use of computed tomography ($r=-0·92$). In contrast, the annual incidence of haemorrhage among those not examined with computed tomography did not change significantly ($p<0·2$, χ^2 test for trend), and this group had a 94% mortality in hospital. Annual admissions to the stroke unit rose during the study, but no significant changes in the characteristics of the patients were found.

Comment

We found a strong correlation between the use of computed tomography and the incidence of intracerebral haemorrhage, particularly small haemorrhages. Garraway *et al* noted that the proportion of strokes due to haemorrhage in the population of Rochester, Minnesota, doubled after 1975 and suggested that this was due to the introduction of computed tomography.[2] We conclude that use of computed tomography also explains the apparent rise in the incidence of haemorrhage in our study and provide evidence to support this. The falling mortality reflects the fact that more small, benign haemorrhages were detected, and recent studies have shown that outcome, including survival, correlates closely with the size of haemorrhage.[3-5]

Two important conclusions may be drawn from our data. Firstly, an imaging procedure such as computed tomography is essential to establish the diagnosis of intracerebral haemorrhage in vivo. Secondly, any comparative or longitudinal study of the incidence of this and other types of stroke, based either in the community or in hospital, must use a reliable neuroimaging technique in a high and constant

proportion of patients if valid, durable results are to be obtained.

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1 Furlan AJ, Whisnant JP, Elveback LR. The decreasing incidence of primary intracerebral hemorrhage: a population study. *Ann Neurol* 1979;5:367-73.
2 Garraway WM, Whisnant JP, Drury I. The continuing decline in the incidence of stroke. *Mayo Clin Proc* 1983;58:520-3.
3 Steiner I, Gomori JM, Melamed E. The prognostic value of the CT scan in conservatively treated patients with intracerebral hematoma. *Stroke* 1984;15:279-82.
4 Garde A, Bohmer G, Selden B, Neiman J. 100 cases of spontaneous intracerebral haematoma. Diagnosis, treatment and prognosis. *Eur Neurol* 1983;22:161-72.
5 Helweg-Larsen S, Sommer W, Strange P, Lester J, Boysen G. Prognosis for patients treated conservatively for spontaneous intracerebral hematomas. *Stroke* 1984;15:1045-8.

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Filter paper sampling of blood infected with HIV: effect of heat on antibody activity and viral infectivity

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The filter paper technique for blood sampling has not gained wide acceptance, mainly owing to incomplete standardisation. It has several advantages over venepuncture: it is less distressing for patients; samples are more easily transported; and the risk of accidental transmission of infectious agents through broken glassware and needles, etc, is reduced. We have standardised the technique.[1] Antibodies to human immunodeficiency virus (HIV) and HIV antigens have been detected in extracts of dried blood on filter papers.[2,3] We assessed the effect of heating filter paper samples on antibody activity and infectivity of HIV.

Patients, methods, and results

We obtained venous serum samples and capillary blood collected on filter papers from 19 people attending an outpatient department for patients infected with HIV at Roslagstull Hospital for Infectious Diseases, Stockholm, and from one healthy laboratory worker. The blood samples on filter papers were dried at room temperature overnight and then either left at room temperature or heated at 56°C for one or 18 hours. A disc of 6·2 mm diameter was punched out of the filter paper and extracted in 0·5 ml phosphate saline buffer with 0·05% polysorbate 20 (Tween 20) for two hours at room temperature.[1] Serum samples were diluted 1:100 and eluates of filter paper discs used undiluted. HIV antibody activity was measured with an enzyme linked immunosorbent assay (ELISA) (Vironostika anti-HTLV-III micro-ELISA, Organon Teknika, Boxtel, Holland) and western blotting performed with antigen strips (Biotech, Rockville, Maryland).

HIV infectivity was measured by using HIV obtained from freshly infected HUT-78 cells when the culture had become antigen positive in an antigen capture enzyme immunoassay (Abbott Laboratories, North Chicago, Illinois). Discs of filter paper were soaked with 25 µl of the supernatant containing HIV and extracted in duplicate in 0·5 ml of the medium produced by Roswell Park Memorial Institute supplemented with 10% fetal calf serum. Samples were extracted immediately or after drying at room temperature or heating at 56°C for one hour. Each filter paper extract was then added to roughly 20×10^6 mononuclear cells from human peripheral blood stimulated with phytohaemagglutinin, and the supernatants were assayed for HIV antigens twice weekly for six weeks. A culture was regarded as positive when the supernatant showed a rise in absorbance value on two successive occasions. Declining absorbance values were regarded as indicating a lack of replication of HIV.

Ten patients had antibodies against HIV whereas nine patients and the healthy laboratory worker did not. Activity of the antibody in the filter paper extracts was found to be equal to that shown by the diluted serum samples. The western blots obtained with filter paper extracts and serum were indistinguishable.

Heat treatment of filter papers for one hour at 56°C did not alter the optical density of extracts. Incubation for 18 hours at 56°C reduced the antibody reactivity, but in all seropositive samples it was still high. Extracts from discs soaked with supernatants from the culture containing HIV gave rising or continuously high optical densities in the assay for HIV antigens, indicating the existence of replicating virus (table). When the filter paper discs were left to dry at room temperature or heated at 56°C for one hour before extraction no replication was found.

Mean optical density of culture supernatants of human mononuclear cells incubated with extracts from filter discs impregnated with HIV from freshly infected HUT-78 cells that were extracted in RPMI† medium immediately, after drying at room temperature for one hour, or after heating at 56°C for one hour. Values for discs heated at 56°C for one hour were all zero*

	Days of culture								
Treatment of discs	5-8	9-12	13-16	17-20	21-24	25-28	29-32	33-36	37-40
Immediate extraction	0·171	0·207	0·405	0·427	1·115	>2·000	>2·000	>2·000	>2·000
Dried at room temperature before extraction	0·134	0·091	0	0	0	0	0	0	

*Of two values.
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Comment

HIV antibodies in filter paper extracts resisted heating at 56°C for one hour, whereas no replicating virus could be shown when the filter paper was left to dry at room temperature or heated at 56°C for one hour. Martin *et al* reported that HIV diluted in human serum was inactivated when heated at 56°C for 10 minutes.[4] Resnick *et al*, however, reported that only after five hours at 56°C was the virus no longer detectable.[5] Our results show that heating capillary blood on filter paper for one hour at 56°C efficiently inactivates HIV but not HIV antibody. More exact evaluation of this is in progress.

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1 Evengård B, Lundbergh P, Linder E. Standardization of a filter paper technique for blood sampling. *Ann Trop Med Parasitol* 1988;82:295.
2 Farzadegan H, Quinn T, Polk BF. Detecting antibodies to human immunodeficiency virus in dried blood on filter papers. *J Infect Dis* 1987;155:1073-4.
3 Nielsen C, Bygbjerg IC, Vestergaard BF. Detection of HIV antigens in eluates from whole blood collected on filter paper. *Lancet* 1987;i:566-7.
4 Martin LS, Mcdougal JS, Loskoskki SL. Disinfection and inactivation of the human T lymphotropic virus type III/lymphadenopathy-associated virus. *J Infect Dis* 1985;152:400-3.
5 Resnick L, Veren K, Salahuddin SZ, Tondreau S, Markham PD. Stability and inactivation of HTLV-III/LAV under clinical and laboratory environments. *JAMA* 1986;255:1887-91.

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