## PROGRESS IN ACUTE LEUKAEMIA IN ADULTS

SIR,—The Toronto Leukaemia Study Group (April 5, p 786) has provided a much-needed baseline for population studies in adult acute myeloid leukaemia (AML). The figures show that we have hardly influenced the prognosis of AML in older patients. It remains to be seen whether the disease-free survivals in the age groups under 45 years are as good as those achieved with protocols using high-dose chemotherapy/radiotherapy and marrow rescue (allogeneic or autologous).

Acute leukaemia studies must define the population being studied and we think that this is especially important in the UK, where studies should have a regional or supraregional basis using the Toronto principle to create sufficient local interest to make them complete.

Your editorial on adult acute lymphoblastic leukaemia (ALL) provides a balanced view and a realistic appraisal of survival rates. Progress in adult ALL has now fallen behind that in AML in the younger age groups, and we[1] think that survival on conventional therapy in unselected populations is worse than that stated and that disease-free survival at four years is only about 20%. The much cited studies from the German National Co-operative Group[2] and the Memorial Hospital, New York,[3] are not population based. Dr D. Hoezler has indicated that the next German national study will ensure that investigators comment on cases arising during the study period, but excluded from the trial. However, the German study has proved that aggressive chemotherapy can now cure more people with T-cell ALL than was previously the case. Your editorial ends with few positive suggestions but it is not very helpful to suggest that transplantation should be used only in the "worst" group of adult ALL since 80% of adult ALL has a poor prognosis.

We have adopted an aggressive approach with the use of allogeneic and autologous transplantation in first remission, with the hope that this will provide a better way forward than waiting to transplant in second remission. 15 of 16 consecutive patients treated this way remain alive and disease-free 11 months post-transplant (median).

S. J. PROCTOR
P. J. HAMILTON
M. M. REID
A. G. HALL
P. CAREY

Department of Medicine (Haematology),
Royal Victoria Infirmary,
Newcastle upon Tyne NE1 4LP

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## ANGIOTENSIN CONVERTING ENZYME INHIBITORS AND LITHIUM TREATMENT

SIR,—Interaction between lithium and diuretics is well known.[1] We report here a case of lithium toxicity in a patient taking enalapril.

This 61-year-old woman had been taking lithium carbonate 750 mg for a manic-depressive psychosis diagnosed in 1977. Essential hypertension, found in 1975, had been successively treated with spironolactone, central antihypertensive agents, and beta-blocking agents (sotalol 320 mg daily). In November, 1985, persisting high blood pressure (180/100 mm Hg) led to hospital admission. Her ECG, renal function, and a digitalised intravenous arteriogram of renal arteries were normal. The plasma lithium was 0·88 mmol/l. Enalapril 20 mg daily by mouth was prescribed and the sotalol was stopped. Her blood pressure fell to 120/70 mm Hg on enalapril with a normal sodium diet.

5 weeks later, she was readmitted with a 2–3 weeks history of ataxia, dysarthria, tremor, and confusion. EEG revealed diffuse slowing and disorganisation of background rhythm. ECG revealed bradycardia with junctional rhythm and depression of the T wave. Her plasma lithium was 3·3 mmol/l. There was moderately altered renal function (serum creatinine 198 µmol/l, urea 20 mmol/l). Lithium and enalapril were stopped. Symptoms and EEG abnormalities disappeared and her plasma lithium fell to 0·42 mmol/l 7 days after drug withdrawal whereas renal function returned to normal in 48 h (table). 1 month later, lithium therapy was restarted, with nifedipine 40 mg daily by mouth without reappearance of lithium toxicity.

CREATININE AND LITHIUM LEVELS IN RELATION TO THERAPY

| Date | Therapy* | Serum creatinine (µmol/l) | Plasma lithium (mmol/l) |
|---|---|---|---|
| Sept 22, 1985 | Li | 108 | 0·88 |
| Dec 30 | Li + E | 198 | 3·30 |
| Dec 31 | " | 160 | 2·65 |
| Jan 2, 1986 | " | 127 | 1·57 |
| Jan 6 | " | 124 | 0·42 |
| Jan 19 | " | 97 | 0·01 |
| March 21 | Li + N | 93 | 0·66 |

*Li = lithium; E = enalapril; N = nifedipine.

In this patient plasma lithium levels rose when enalapril was added to lithium therapy. The mechanism for this interaction is unclear. An alteration in renal function induced by angiotensin-converting-enzyme (ACE) inhibition and the natriuretic effect of enalapril should be borne in mind. The increase in lithium levels cannot be totally explained by the moderate renal insufficiency. More probably, lithium retention is related to the increase in sodium excretion associated with decreased aldosterone secretion induced by enalapril.

This case points to the potential risk of lithium toxicity in patients receiving both an ACE inhibitor and lithium treatment.

Medical Service,
CHU Purpan,
35109 Toulouse, France;
Clinical Pharmacology Service,
CHU Toulouse;
Centre Midi-Pyrénées de Pharmacovigilance
et d'Informations sur le Médicament,
CHU Toulouse

PH. DOUSTE-BLAZY
M. ROSTIN
B. LIVAREK
E. TORDJMAN
J. L. MONTASTRUC
F. GALINIER

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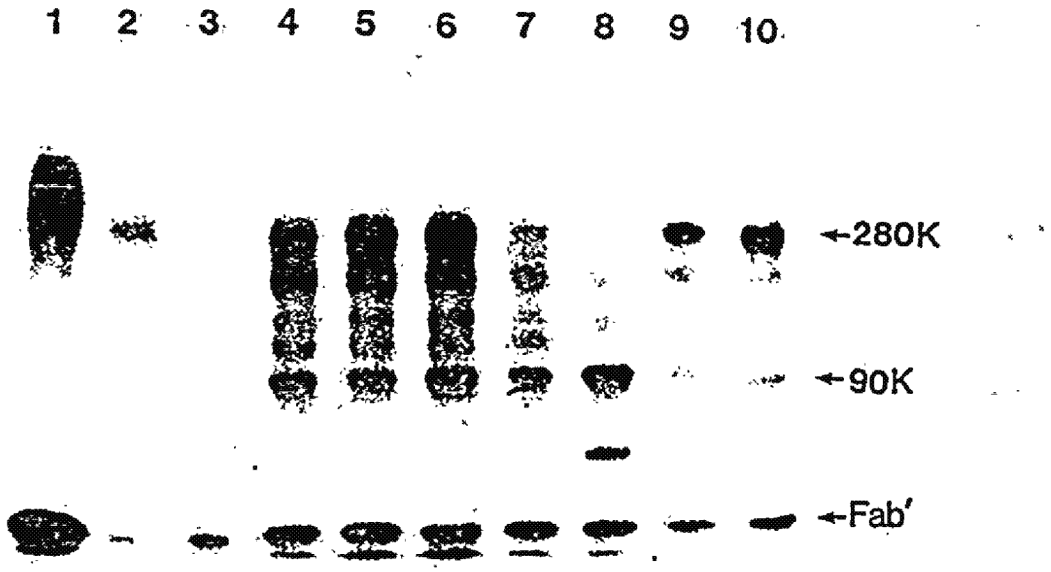
## FACTOR VIII DEGRADATION PRODUCTS IN HEATED CONCENTRATES

SIR,—To reduce the risk of infectivity with human immunodeficiency virus most manufacturers of factor VIII (FVIII) concentrates now heat their products in the freeze-dried state at 60–80°C for 10–72 h. Wet heating, in aqueous and organic solvents, is used by a few manufacturers. Whilst reduction in viral infectivity must remain the prime aim, concern has been expressed about the effects of heat on the integrity of the FVIII molecule. Initial studies in our laboratory revealed an increased VIII:Ag/VIII:C ratio in all heated products compared with the unheated products (unpublished); this presumably reflected loss of FVIII clotting activity during heat treatment.

We have now examined the FVIII polypeptide distribution in heated concentrates, using the method of Weinstein et al,[1,2] in which FVIII samples are incubated for 2 h with specific anti-FVIII $^{125}$I-Fab, prepared from a haemophiliac with a high-titre antibody, in the presence of polyethyleneglycol 4000 to aid complex formation. The complexes are subjected to electrophoresis in a 3–9% polyacrylamide gradient gel, in the presence of SDS under non-reducing conditions, and visualised by autoradiography. Although exact molecular weight assignments are uncertain, the method is useful for comparison, especially of impure samples such as plasma and concentrates which cannot be subjected to immunoblotting.

The figure is an autoradiogram of the seven brands of heated




**Peptide distribution of normal and haemophilic plasma and seven brands of FVIII concentrate.**

Molecular weights were assigned by comparison with protein markers run on same gel, after subtracting 50 000 for the Fab. Lane 1 = buffer; lane 2 = normal plasma; lane 3 = haemophilic plasma; lanes 4–10 = FVIII concentrates from seven manufacturers (diluted to 1 IU/ml of FVIII:C).

FVIII concentrate available in the UK, in comparison with normal and haemophilic plasma. Normal plasma has two high-molecular-weight bands, the major one being of an estimated molecular weight of 280 000, as observed by Weinstein et al.[1] Haemophilic plasma has no bands, confirming the specificity of the technique; the diffuse high-molecular-weight material strongly visible in lane 1 (and also faintly in lane 3 in the original autoradiogram) represents Fab aggregates, which are formed in the absence of FVIII binding material. In six concentrates there are five clear bands in the molecular weight range 90 000 to 280 000. The proportion of total FVIII antigen in the highest-molecular-weight band was estimated by densitometry to be 20–40% in these six concentrates, compared with 65% in plasma. In lane 8, less than 10% of the material appeared in the 280 000 region: the 90 000 band appeared strongest, and there was an additional band of apparent molecular weight 35 000 to 40 000 which was virtually undetectable in the other concentrates. Analysis of four different batches of each concentrate gave identical results and the patterns were not changed by inclusion of proteolytic inhibitors during incubation with Fab.

Although all concentrates show degradation of FVIII relative to plasma, the contribution of heat treatment to this is uncertain. Analysis of one of the dry heated products before and after heat treatment showed no major difference, but comparison of wet and dry heated products from the same manufacturers showed more extensive degradation after wet heat. Weinstein et al noted an increased preponderance of low-molecular-weight forms in FVIII concentrate, and especially in a highly purified VIII:C preparation.[2] Rotblat et al[3] showed that, even when FVIII was purified rapidly from fresh blood, with proteolytic inhibitors at each stage, extensive degradation was still observed. These results emphasise the extreme susceptibility of FVIII to proteolysis, and some degradation during large-scale fractionation is inevitable, regardless of heat treatment.

Proteolysis of FVIII can occur with a variety of cellular and plasma proteases.[4] Thrombin proteolysis is important for biological activity, leading to an increase in VIII:C activity by one-stage but not by two-stage assays.[5] This has been correlated with disappearance of the high-molecular-weight forms and intensification of the 90 000 band:[1-3,6] more prolonged thrombin treatment gives an inactive degradation product of 40 000.[3,6] Assays of most concentrates against a concentrate standard generally give similar results by one-stage and two-stage methods, but discrepancies between the methods sometimes occur.[7] Our current assays on the product in lane 8 show consistently higher values by one-stage than by two-stage assays, suggesting enhanced thrombin activation in this product. The ratio between the two methods ranged from 1·23 to 2·4, with an average over seven batches of 1·49. Thrombin treatment of one of the other products gave an additional band in the 35 000 to 40 000 region, in the same position as that observed in the untreated concentrate in lane 8, and this provides supporting evidence for more extensive thrombin proteolysis in this product.

Whilst there is no evidence that heated FVIII concentrates are any less effective haemostatically than their unheated predecessors, in some cases heat treatment may be accentuating proteolytic degradation. These results suggest the need for careful evaluation of in-vivo recovery and half-life of heat-treated concentrates.

T. W. BARROWCLIFFE
S. J. EDWARDS
G. KEMBALL-COOK
D. P. THOMAS

National Institute for Biological Standards and Control, London NW3 6RB

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