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COAGULATION, FIBRINOLYSIS AND PLATELET FUNCTION DURING COUMARIN THERAPY.

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The inactivation of factors VIII:C, V:C and fast acting TPA inhibitor by activated Protein C indicates that oral anticoagulation is more than simple reduction of prothrombin complex activity. To investigate these changes, six patients were studied after stopping oral anticoagulant treatment. Protein C activity and C antigen, Factors VIII:C, VIII:vWFAg, V:C, V:Ag, X:C, VII:C, fibrinogen and TPA activity were measured during long-term nicoumalone therapy (duration of therapy 8 - 96 months, mean 28 months), and after discontinuation on days 2, 4, 8, 10, 15, 30 and 42.

The INR on the last day of therapy ranged between 2.0 - 3.3, (mean 2.6). Protein C activity and antigen and factor X became normal by day 8; factor II by day 10. Factor VII activity peaked on day 8, falling to resting levels by day 30. Factor VIII parameters remained high throughout, whereas Factor V antigen showed no significant change. Factor V activity was not quantifiable until day 8 because of non-parallelism (? PIVKA effect), but was higher on day 8 than day 42 ($p < 0.002$ paired "t" test). The higher levels of factor V activity could be protein C dependent, but the high factor VIII appears unrelated. Fibrinogen levels were higher on coumarin treatment ($p < 0.05$ paired "t" test) and took 30 days to fall to resting level. The effect of Protein C on TPA inhibitor would be expected to increase the activity of TPA, but this activity remained unchanged. Raised fibrinogen levels did not, therefore, appear to be mediated by the effect of protein C on fibrinolysis. Fibrinogen levels in plasma influence ADP induced platelet aggregation which is known to be increased in patients receiving coumarin drugs. In conclusion, patients on coumarin treatment, in addition to showing a reduction in protein C activity, also have higher fibrinogen levels and increased platelet aggregability all of which may be undesirable.

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COMPUTER PREDICTION OF ANTICOAGULATION STATUS AND WARFARIN DOSE FOLLOWING CARDIAC SURGERY.

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Fluctuations are frequently seen in the anticoagulant status of patients in the immediate post operative period following prosthetic heart valve replacement. These patients are at high risk of haemorrhage or thromboembolism. We have used a pharmokinetic model of warfarin metabolism to develop a computer programme to predict the maintenance dose of warfarin from early prothrombin activity determinations. This will enable controlled anticoagulation to be achieved. The expression for warfarin kinetics employs 4 constants determined by the residual sum of the squares, which are used immediately to redefine dosage predictions. In a pilot study data obtained from 16 patients post operation 3, 5 and 7 days after commencing treatment, has been used to predict the required maintenance dose at 21 days. These predicted doses were then compared with the maintenance dose achieved by clinical practice. The programme was told to optimise its dose to achieve a PT ratio of 3 whereas clinically the ratio was allowed to vary in the therapeutic range of 2 to 4. Predicted doses at 21 days are shown in the table:

Dose after	3 days	5 days	7 days	Clinical
Pt activity	16.32+/-0.87	15.92+/-1.45	15.93+/-1.18	16.28+/-4.98
Dose	3.30+/-4.05	2.06+/-2.11	1.90+/-1.07	3.06+/-1.34
Correl. Coeff	0.07	0.28	0.71	-

Correlation between predicted and clinical maintenance doses after 3 and 5 days treatment was poor but had improved significantly by 7 days, despite similar levels of prothrombin activity. Predicted prothrombin activity never exceeded the upper limit of the therapeutic range, and the predicted dose can be uprated on addition of further data within 2 minutes.
After 7 days computer predicted warfarin dose has produced a good correlation with the clinical maintenance dose (the doses of only 3 patients varying by more than 1 mg/day). The significant fluctuations seen in the prothrombin ratio during clinical dosage were not observed with computer dosing and we now feel it is safe to use this programme to anticoagulate patients post operatively.

PROTHROMBIN - THROMBIN

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KINETICS OF HYDROLYSIS OF THE CHROMOGENIC SUBSTRATE S2238 BY ALPHA-THROMBIN : INFLUENCE OF THE pH AND THE IONIC STRENGTH.

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The knowledge of the pH and ionic strength dependence of k_{cat} and K_m for the hydrolysis of S2238 (HD-phe-pip-arg-pNA.2HCl) by alpha-thrombin is essential in determining optimal reaction conditions of residual enzyme in systems where also protease inhibitors and glycosaminoglycan catalysts play a role. We studied the kinetic behavior of S2238 in piperazine/glycylglycine/NaOH buffers from pH 6 to 11 and with a calculated ionic strength up to 0.7 M taking into account the pH-dependent concentration of the buffer species. The kinetic parameters of 60 Michaelis-Menten substrate functions were used for the setup of ionic strength and pH profiles. The k_{cat} values are dependent upon the ionic strength, increasing steeply up to about 0.3 M and decreasing again at high ionic strength. The K_m however, reflecting the affinity between enzyme and substrate, is nearly unaffected. The K_m values at very alkaline pH are markedly elevated, indicating a conformational form which does not readily bind substrate. The pH profiles for k_{cat} and k_{cat}/K_m are displaced towards the low pH side with increasing ionic strength. The ascending limb corresponds to the pK of the Asp-His charge relay system, decreasing with increasing ionic strength from 7.2 to 6.6 in the ES complex and from 6.8 to 6.6 in the free enzyme. Apparently substrate binding provokes a pK increase of the active His residue. The descending limb in the k_{cat} profile could be described by a hypothetical pK varying from 11.5 to 10.7 but the activity decrease is probably due to enzyme inactivation. The alkaline limb of the k_{cat}/K_m profile is governed by a pK of 9.4 which is rather independent of the ionic strength and could be attributed to the B-chain terminal isoleucine, forming a salt bridge with Asp 194 and stabilizing the active site conformation as proven for other serine proteases. Data analysis via a modified Debije function with appropriate estimates for the dielectric constant and the radius of the macro-ion can provide information on the charge density of the enzyme.

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MODEL STUDIES OF NEUTRALIZATION OF THROMBIN.

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A kinetic model, based on published studies of thrombin neutralization, is used to examine factors that limit spread of free thrombin in a simple plasma. It employs equations with presently available rate parameters which describe the courses of the major thrombin-binding reactions at 37°C in buffered saline solutions approximating plasma ultrafiltrate. Thrombin is bound reversibly by fibrinogen and fibrin-1 polymers as enzyme-substrate complexes (1) and by "fibrin" at a non-proteolytic site (2), and essentially irreversibly by antithrombins (3). These bindings reduce free thrombin levels and so limit spread of activity. The model equations with parameters from (1) and (3) show that thrombin neutralization by thrombin-substrate complexes is very brief and thrombin-antithrombin reactions are much too slow for early reduction of thrombin activity. However, parameters from (2) show that rapid reversible binding of thrombin by "fibrin" much reduces level of free thrombin and the level continues to fall as the thrombin is passed to the antithrombins. The model shows that a rapidly-acting antithrombin (e.g. heparin-ATIII) could reduce free thrombin fast enough to inhibit slower thrombin activations (e.g. of FXIII), and that a sufficient concentration of a reversible binder can govern the level of free thrombin. This suggests that a non-toxic reversible binder, with suitable K_d and half-life, would be valuable in treating thrombosis. Verification and extension of the model findings require better experimental definition of the parameters.

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