enzymatic pathway, and suggested that BUN levels of 100–300 mg/dl would be accompanied by cyanate concentrations of 0.75–1.40 mM.

Although our findings for warfarin binding to cyanate-derivatized BSA are consistent with the notion that cyanate is a mediator of the albumin binding defect for warfarin in uremia, more conclusive evidence remains to be reported. In that connection either an absolute or relative difference in carbamoyl substitutions on free amino groups of albumin isolated from uremic and normal serum would be compelling. Preliminary data recently reported by Erill *et al.* [20] suggest that carbamoylated human serum albumin binds salicylate and sulfadiazine less extensively than normal albumin, and describes a correlation between the magnitude of the free fraction of salicylate and the extent of carbamoylation. The synthesis of a qualitatively different type of albumin, binding inhibition effected by endogenous reversible binding inhibitors, and irreversible changes in albumin arising from covalent interactions with other uremic toxins, such as the guanidino compounds, may also contribute to the uremic binding defect.

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The dose-dependent effect of warfarin on vitamin K_1 metabolism and clotting factor synthesis in the rabbit

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It has been postulated that warfarin produces its anticoagulant effect by inhibiting the enzyme vitamin K_1 epoxide reductase which is responsible for the regeneration of vitamin K_1 from its biologically inactive metabolite vitamin K_1 epoxide [1]. In keeping with this hypothesis, we found that a series of 4-hydroxycoumarins, including warfarin, produced an accumulation of [^{3}H] vitamin K_1 epoxide in the plasma of rabbits dosed intravenously with [^{3}H] vitamin K_1 [2]. However, there was no correlation between the increase in [^{3}H] vitamin K_1 epoxide : [^{3}H] vitamin K_1 plasma concentration ratios and anticoagulant potency, in line with results obtained by other workers using the rat [3, 4]. One possible explanation for this lack of correlation is that the anticoagulants have different modes of action. We have investigated, therefore, the effect of several doses of one anticoagulant, warfarin, on the relationship between [^{3}H] vitamin K_1 metabolism and clotting factor activity in the rabbit.

Before each experiment the control prothrombin time was determined for each animal. Male New Zealand White rabbits (3.0–3.5 kg) were dosed with warfarin (0.16, 0.63, 2.5 and 10 mg/kg) intravenously into the marginal ear vein. One hour later, [^{3}H] vitamin K_1 (100 μCi; 2.1 μg) was injected intravenously into the same vein. Blood samples (4 ml) were taken from the other marginal ear vein 1, 2, 3, 4, 5 and 6 hr after administration of [^{3}H] vitamin K_1 for determination of [^{3}H] vitamin K_1 and [^{3}H] vitamin K_1 epoxide, as described previously [2]. The accuracy of the method was confirmed using reversed-phase chromatography on a partisil-10 ODS column using the solvent system acetonitrile–water (97:3, v/v). From 6 hr after injection of [^{3}H] vitamin K_1, blood samples (0.9 ml) were taken every four hours for determination of prothrombin complex activity (P.C.A.) by the method of Quick [5], as described previously [2].

The effect of warfarin on the [^{3}H] vitamin K_1 epoxide : [^{3}H] vitamin K_1 plasma concentration ratio is shown in Fig. 1. Figure 2 contains the corresponding prothrombin complex activity data.

Shearer *et al.* [5] have shown that there is a log-dose relationship between plasma warfarin concentrations and increases in plasma [^{3}H] vitamin K_1 2,3-epoxide concentrations in volunteers dosed with [^{3}H] vitamin K_1, but the corresponding changes in prothrombin complex activity were not measured. From Fig. 2 it can be seen that 0.63, 2.5 and 10 mg/kg of warfarin produced the same maximum rate of decay of P.C.A., suggesting that 0.63 mg/kg is sufficient to block clotting factor synthesis completely in

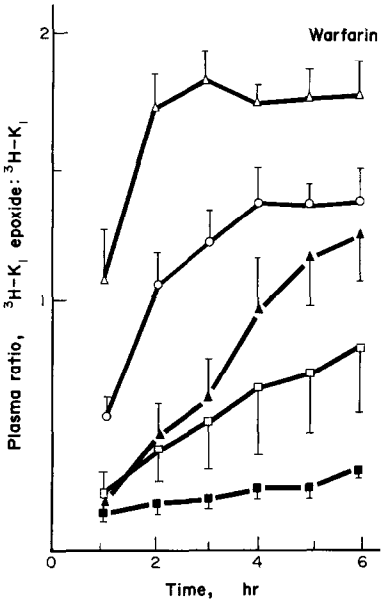

Fig. 1. The effect of warfarin 0.16 mg/kg (□), 0.63 mg/kg (▲), 2.5 mg/kg (○) and 10 mg/kg (△) on the [^{3}H] vitamin K$_1$ epoxide: [^{3}H] vitamin K$_1$ concentration ratio in rabbit plasma 1–6 hr after injection of [^{3}H] vitamin K$_1$ compared to controls (■). The results are expressed as the mean ± S.E.M.

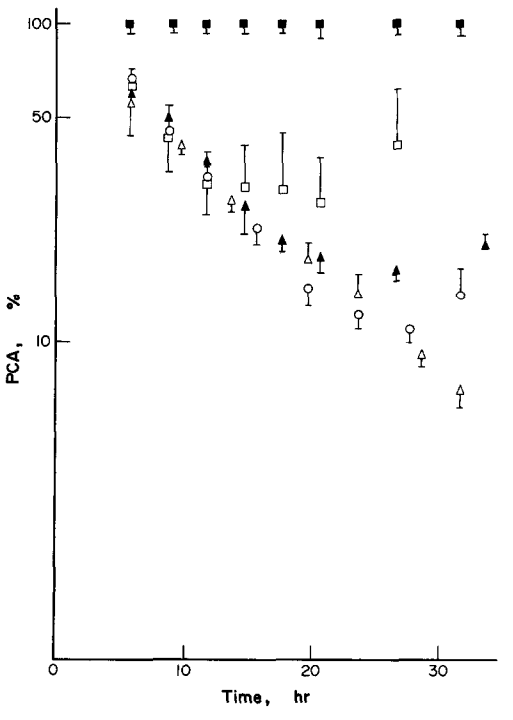

Fig. 2. The effect of warfarin 0.16 mg/kg (□), 0.63 mg/kg (▲), 2.5 mg/kg (○) and 10 mg/kg (△) on prothrombin complex activity in rabbit plasma compared to controls (■). The results are expressed as the mean ± S.E.M.

the rabbit for at least 16 hr. The minimum P.C.A. observed was dependent on dose, but this reflects the duration, as well as the degree, of inhibition of clotting factor synthesis. However, the 0.63 mg/kg dose did not produce the maximum change in vitamin K$_1$ metabolism, as the [^{3}H] vitamin K$_1$ epoxide: [^{3}H] vitamin K$_1$ plasma concentration ratios produced by the 10 mg/kg dose were significantly ($P < 0.01$, using Student's *t*-test) greater (Fig. 1). Thus the dose of warfarin required to produce maximum inhibition of clotting factor synthesis will not necessarily produce maximum pertubation of vitamin K$_1$ metabolism. This may explain why some workers have found a correlation between [^{3}H] vitamin K$_1$ epoxide: [^{3}H] vitamin K$_1$ concentration ratios [6] and others have not [3, 4].

It may be that only partial inhibition of the epoxide reductase is necessary to reduce endogenous vitamin K$_1$ to ineffectual levels at its side of action. However, there was no difference in the rate of onset of anticoagulation at the different doses, as the mean P.C.A. at 6 hr was similar for each group. An alternative explanation is that only partial inhibition of the vitamin K$_1$ dependent-carboxylation process is necessary to eliminate the *in vivo* biological activity of rabbit clotting factors. Esnouf and Prowse [7] have isolated bovine prothrombin with different degrees of carboxylation and found that appreciable biological activity, associated with the ability to bind calcium ions, is not apparent until more than seven of the ten glutamic acid residues have been γ-carboxylated.

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