Effect of Dicumarol on Rat Liver Slice Glycoprotein Synthesis

Although the coumarin anticoagulants uncouple oxidative phosphorylation in vitro and inhibit protein synthesis in liver slices, it has not been possible to demonstrate either action in vivo[1]. Nor has it been possible to correlate the coumarin anticoagulant in vitro effects to their therapeutic action of inhibiting prothrombin synthesis[2]. Since prothrombin is a glycoprotein, the therapeutic action of the coumarin anticoagulants might be associated with an inhibition of the incorporation of the polysaccharide side chain. In order to test the hypothesis that anticoagulants interfere with the glycosylation of proteins, we investigated the incorporation of glucosamine into glycoproteins of rat liver slices. Furthermore, we are reporting other findings on the effect of anticoagulants on glucosamine metabolites and glycolipids in rat liver slices.

Materials and methods. Male Wistar rats, fed ad libitum, were sacrificed by cervical dislocation after which their livers were excised and rinsed in cold saline. The livers were then sliced with McIlwain tissue chopper at a setting of 0.260 mm. The slices were rinsed in oxygenated Krebs-Ringer phosphate solution (pH 7.4) containing 50 mg/100 ml glucose blotted and weighed. Pooled slices (100–150 mg) were incubated in 3 ml of Krebs-Rinber phosphate solution gassed with 95% O_2–5% CO_2 at 37 °C in a Dubnoff metabolic incubator with shaking at 120 per min. The slices were incubated with D-glucosamine-1-^{14}C (10.1 mCi/mM, purchased from New England Nuclear) in the absence and presence of anticoagulant. The reactions were stopped by the addition of 0.5 ml of 2N perchloric acid (PCA)-10% phosphotungstic acid (PTA). The PCA-PTA precipitates were washed thoroughly and sequentially with PCA-PTA (5 times), hot ethanol, cold ethanol and acetone. The residues were dissolved in Nuclear Chicago Solubilizer, diluted with a toluene scintillation system and counted in a Packard liquid scintillation counter.

The incorporation of glucosamine into glycolipids was determined in experiments similar to those described above with the following modifications to recover the glycolipid fraction. The PCA-PTA washed precipitates were extracted twice with chloroform: methanol (2:1). The chloroform:methanol extracts were combined, concentrated under nitrogen, added to a liquid scintillation vial and further concentrated until all the chloroform and methanol had been removed. The resulting oil was counted in a 0.4% PPO-toluene scintillation system.

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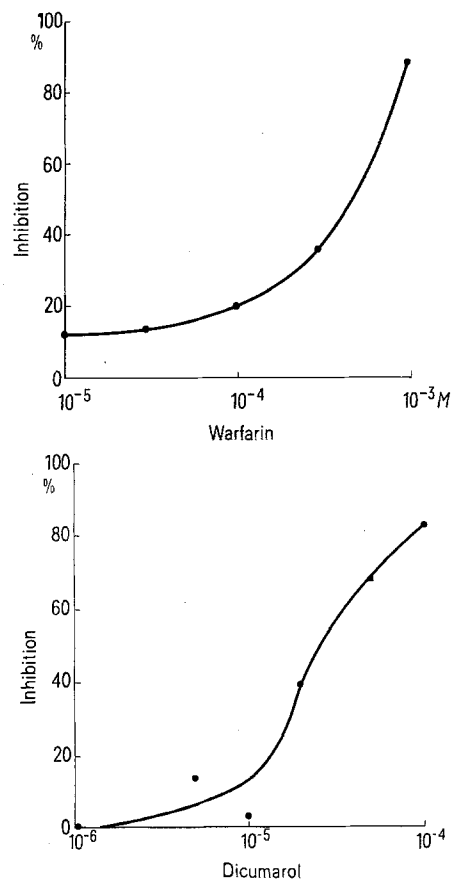

Fig. 1. Dose dependent inhibition of $6.7 \times 10^{-5}M$ D-glucosamine-^{14}C (0.2 μCi) incorporation into glycoproteins of rat liver slices by warfarin, top and dicumarol, bottom. Rat liver slices (ca. 100 mg) were incubated for $2^1/_2$ h at 37 °C in Krebs-Ringer phosphate solution (500 mg/100 ml glucose, pH 7.4). Results are expressed as per cent inhibition of solvent controls and are the averages of triplicate experiments.

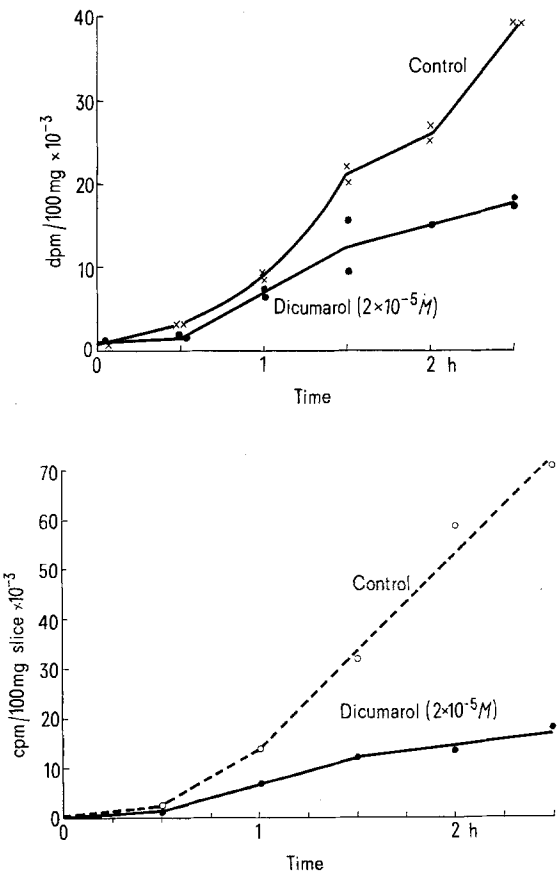

Fig. 2. Effect of $2 \times 10^{-5}M$ dicumarol on the incorporation of $6.7 \times 10^{-5}M$ D-glucosamine-^{14}C (0.2 μCi) into glycoproteins and glycolipids of rat liver slices as a function of incubation time. Top, glycoproteins. Bottom, glycolipids. Incubation conditions were as in Figure 1.

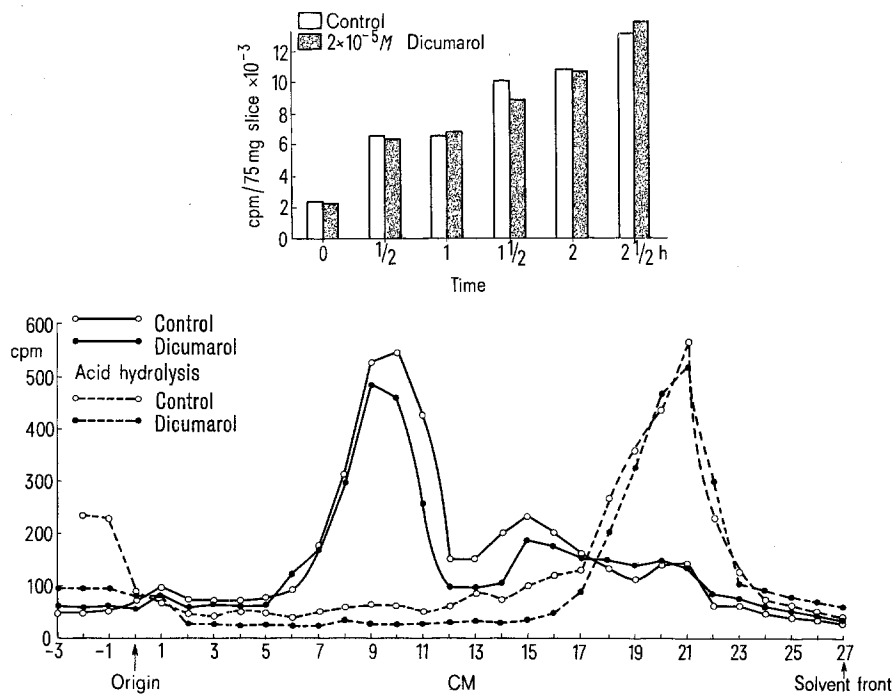

Fig. 3. Effect of $2 \times 10^{-5}M$ dicumarol on the incorporation of glucosamine-^{14}C into labeled metabolites. Top, time course of incorporation into total labeled metabolites. Bottom, chromatography of metabolites from $2^1/_2$ h incubation. Incubation conditions were as in Figure 1 and metabolites were handled as described in the text.

The sugar nucleotides of radiolabeled glucosamine present in the extracts from the slice experiments were adsorbed onto charcoal[3] (Norit A), washed with non-labeled glucosamine and eluted with 5% pyridine. Aliquots of 0.2 ml of the pyridine eluates were counted in Bray's solution[4].

Results and discussion. Dicumarol and warfarin exhibited concentration dependent inhibition of the incorporation of $6.7 \times 10^{-5}M$ glucosamine (0.2 μCi) into glycoproteins in rat liver slices (Figure 1). Dicumarol and warfarin caused a 50% inhibition at $2 \times 10^{-5}M$ and $5 \times 10^{-4}M$, respectively. The effect of dicumarol on the time course of $6.7 \times 10^{-5}M$ glucosamine (0.2 μCi) incorporation into glycoproteins was studied as shown in Figure 2, top. The incorporation of glucosamine into glycoproteins continued throughout the $2^1/_2$ h period studied, with $2 \times 10^{-5}M$ Dicumarol causing a marked diminution. The incorporation of $6.7 \times 10^{-5}M$ glucosamine (0.2 μCi) into glycolipids was similarly inhibited by $2 \times 10^{-5}M$ Dicumarol (Figure 2, bottom). The glycoprotein residues of the chloroform:methanol extractions exhibited the same rate of glucosamine incorporation as the ethanol-acetone residues depicted by Figure 2, top. Dicumarol ($2 \times 10^{-5}M$) caused a 66% decrease in the extent of glucosamine incorporated into the chloroform:methanol residues (glycoproteins) by $2^1/_2$ h.

Since dicumarol markedly inhibited the incorporation of glucosamine into glycoproteins and glycolipids in liver slices, we decided to determine whether this was secondary to a reduction of glucosamine uptake and metabolism to PCA-PTA soluble metabolites including carbohydrate nucleotides. Dicumarol ($2 \times 10^{-5}M$) had no effect on the amount of glucosamine-^{14}C metabolites present in the pyridine eluates (from charcoal), containing the carbohydrate nucleotides throughout the $2^1/_2$ h study (Figure 3, top). Chromatograms on Whatman No. 1 paper developed with 1 M ammonium acetate-ethanol (40:60 w/v, pH 7.5) of the pyridine eluates demonstrated that most of the radioactivity present in the pyridine eluates from control and dicumarol treated slices was contained in the same major metabolites (Figure 3, bottom). Upon acid hydrolysis there was a loss of the metabolite corresponding to sugar nucleotide migration with the appearance of an increase in radioactivity consistent with free glucosamine (Figure 3, bottom). Thus, the data of Figure 3 indicate that glucosamine uptake and metabolism is unaltered by Dicumarol when compared to controls.

In this report dicumarol has been shown to inhibit the incorporation of glucosamine into glycoproteins and glycolipids in rat liver slices. This inhibition has been shown to occur later than the uptake and metabolism of glucosamine. These findings are consistent with our earlier studies on the effect of dicumarol on carbohydrate incorporation into glycoproteins and prothrombin in vivo which demonstrated an inhibition of the rate of incorporation of glucosamine into prothrombin[5,6].

Résumé. Dans les tranches de foie, le dicumarol et la warfarine empêchent la glycosamine ^{14}C de s'incorporer aux glycoprotéines. Cette inhibition dépend de la dose employée. La glucosamine-^{14}C a été incorporée par des glycoprotéines et des glycolipides et elle se produisit au cours d'expériences d'une durée de $2^1/_2$ h. L'inhibition par le Dicumarol de l'incorporation de la glucosamine par les glycoprotéines et les glycolipides dans des tranches de foie n'a pas pu être expliquée par un effet de l'anticoagulant sur le métabolisme de la glucosamine en composés solubles dans les acides et adsorbés par le charbon de bois.

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[6] This research represents part of the work carried out by Michael A. Pereira in fulfillment of the requirements for the Ph. D. degree at The Ohio State University. Supported by Grant No. GM 01717 and Eastern Ohio Heart Association.