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Biological effect of phytonadione administered orally as oily solution or solubilized with a non-ionic surfactant in rats

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It has been shown by Levy & Reuning (1964) and by Anello & Levy (1969) that the bioavailability of solubilized drugs may be determined by the concentrations of free drug molecules in the aqueous phase. On the other hand, Sobel (1952) and Engel & Riggi (1969) provided evidence for an increase of drug absorption when surface-active agents were used. In work from our laboratory (Thoma, Ullman & Fickel, 1970) the distribution of drug molecules between the aqueous and micellar phases was found to be critical for the efficiency of preserving agents; more recent studies have revealed that the changes in photostability observed in solubilized drugs like, e.g., menadione have to be ascribed to the micellar binding of the drug (Thoma & Pfaff, 1976). In our present experiments fatty acid esters of polyoxyethyleneglyceryl have been used.

So far there is limited knowledge about the effects of fatty acid esters of polyoxyethyleneglyceryl on the absorption of drugs with low solubility in aqueous media. In contrast to other lipid-soluble vitamins, only few data are available on the bioavailability of phytonadione (phytomenadione) (see Lowenthal & Taylor, 1959); obviously, studies on the effects of solubilizing compounds on the gastrointestinal absorption of this highly lipophilic vitamin have not yet been made. This led us to investigate the effect of polyoxyethylene-(20)glyceryloleate on the biological effect of phytonadione; neutral oil was used as reference formulation. As a measure of activity the antagonistic effect of phytonadione on the warfarin-produced increase in prothrombin time (Lowenthal & Taylor, 1959) was determined. In this experimental model (1/prothrombin time) *vs* (log phytonadione dose) is linear. Warfarin-pretreated male Wistar rats (150 to 180 g) were used. The substances used were: warfarin-sodium (Merell-Pharma, Gross-Gerau) and vitamin K_1 (phytonadione) (Hoffmann-La Roche, Grenzach); Miglyol 812 Neutralöl (Dynamit Nobel, Witten); calcium-thromboplastin (Behringwerke, Marburg); polyoxyethylene(20)-glyceryloleate (Th. Goldschmidt AG, Essen). Phytonadione was thoroughly mixed with the surfactant and the required amount of water added slowly.

Treatment schedule. The animals were treated with warfarin-sodium (10 mg kg^{-1}, i.p.) 24 h before the administration of phytonadione and fasted for the rest of the experimental period. An interval of 24 h is recommended since the warfarin effect reaches a plateau after 24 h and only slight changes are observed between 24 and 48 h (Niedner, Kayser & Meyer, 1974). After 24 h, phytonadione (30 mg kg^{-1}) was administered by stomach tube (1·0 ml/100 g body weight).

For the preparation of samples, the animals were anaesthetized with ether and blood was withdrawn by cardiac puncture into a plastic syringe containing 1/10 volume of citrate-citric acid buffer (0·1 M, pH 4·8). The samples were cooled to 4°, centrifuged at 2000 *g* and 4° for 15 min (plastic tubes) and kept at 4°.

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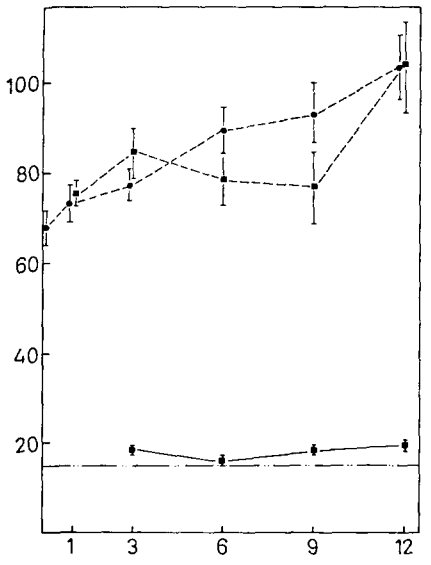

FIG. 1. Influence of the kind of solution on the effect of orally administered phytonadione (30 mg kg^{-1}) on the prothrombin time in warfarin-pretreated rats (10 mg warfarin kg^{-1}, i.p. 24 h before phytonadione administration). Time-effect curves (n=4–6). —·· — Normal prothrombin time (untreated controls). – – ● – – Pretreated rats (warfarin controls). – – ■ – – Dissolved in oil. —■— solubilized in 0·75% polyoxyethylene(20)glyceryloleate. Ordinate: prothrombin time (s). Abscissa: time (h).

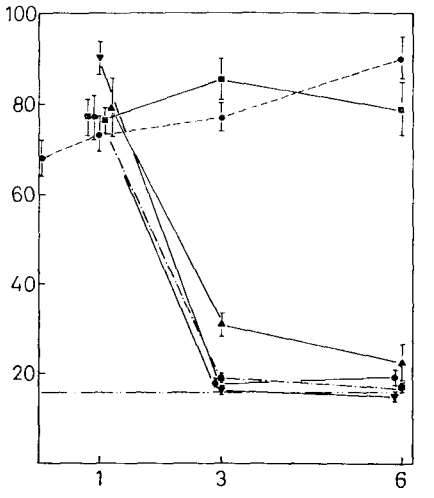

FIG. 2. Influence of the kind of solution and the concentration of the surfactant on the effect of orally administered phytonadione (30 mg kg^{-1}) on the prothrombin time in warfarin-pretreated rats (10 mg warfarin kg^{-1}, i.p. 24 h before phytonadione administration). Time-effect curves (n=4–6). —·· — Normal prothrombin time (untreated controls). – – ● – – Pretreated rats (warfarin controls). —■— Dissolved in oil. —▲— Emulsified with 0·04% polyoxyethylene(20)-glyceryloleate. —●— Solubilized in 0·2% polyoxyethylene(20)glyceryloleate. –·–■–·– Solubilized in 0·75% polyoxyethylene(20)glyceryloleate. —▼— Solubilized in 5% polyoxyethylene(20)glyceryloleate. Ordinate: prothrombin time (s). Abscissa: time (h).

Determination of prothrombin time. Determinations of the one stage-prothrombin time (Quick test) were performed in siliconized glass tubes within 3 h after the blood had been taken from the animals. To 0·1 ml plasma preincubated at 37° for 30 s, 0·2 ml of calcium-thromboplastin solution (37°) was added and the mixture carefully agitated by means of a platinum hooklet until clotting occurred. For each individual blood sample the clotting time was determined 5 times and the mean value taken as experimental result.

The experimental values given in text and figures are means from n experiments ±s.e.m. For statistical evaluations, Student's *t*-test was used. Under the conditions described, prothrombin time in untreated rats amounted to 16·9 s with a relative standard deviation of ±4·88%.

When phytonadione (30 mg kg^{-1}) dissolved in oil, was administered orally to warfarin-pretreated rats, the effect of the drug on prothrombin time was insignificant (Fig. 1). The same dose of phytonadione solubilized with polyoxyethylene(20)glyceryloleate, however, completely abolished the effect of warfarin in the pretreated animals: 3 h after administration of the solubilized vitamin, prothrombin time was in the normal range (Fig. 1). Control experiments were made to see whether the surfactant has any effect on blood clotting time. After administration of solutions containing the surfactant in concentrations that were used in the other experiments no changes in prothrombin time were measured. Such effects are unlikely as after oral administration of ester surfactants cleavage of the ester bond occurs (Oser & Oser, 1957); the polyoxyethylenes formed are poorly absorbed because of their relatively large molecular weight (Elworthy & Treon, 1967).

In an attempt to quantify the effect of polyoxyethylene(20)glyceryloleate on the absorption of phytonadione in rats, the vitamin was administered in increasing concentrations of surfactant and the clotting time was determined. Fig. 2, shows that phytonadione had no effect on prothrombin time 1 h after administration. After 3 h, however, clotting time in the warfarin-pretreated rats was normalized with all surfactant concentrations except the lowest. In this case, the polyoxyethylene(20)glyceryloleate concentration of 0·04% was not high enough to solubilize the vitamin but it was sufficient to form a water in oil emulsion with phytonadione as oily phase. This highly significant difference ($P < 0.001$) in the biological effect between emulsified and solubilized phytonadione was no longer seen 6 h after phytonadione administration (Fig. 2).

Our investigations in warfarin-pretreated Wistar rats have shown that the biological effect of orally administered solubilized phytonadione is much increased relative to phytonadione dissolved in oil. Even if the vitamin is only emulsified with very low concentrations of the surfactant polyoxyethylene(20)glyceryloleate it is still much more effective than its oily solution.

Similar effects of drug formulation on the pharmacokinetics of absorption have been shown for a variety of other vitamins and solubilizers (Sobel, 1952; Davis & Kreutler, 1971).

Generally, a marked increase in surface area is achieved when oily substances are transferred into o/w emulsions; the surface of phytonadione (particle size 5 μm) in 1 ml of the emulsion used in the above experiment can be estimated to be about 37 cm^2. However, the increase in the surface area does not provide a sufficient explanation for the improvement of phytonadione activity, since the difference in the anti-warfarin effects does not correspond to the ratio of the surface of emulsion and oily solution. That the absorption from an oily solution is slowed by the formation of a partition equilibrium between the oily solution emulsified in the gastrointestinal tract and the micellar phase of the natural surface-active compounds has to be considered. Moreover, lipophilic drugs will remain predominantly in the lipid phase until a larger portion of the triglycerides which constitute the emulsion droplet has been degraded and absorbed; only then, are the drug molecules dispersed in the micelles of the bile salts and adsorbed (Borgström, 1964). In contrast to oily solutions, the absorption of emulsified phytonadione depends neither on a partition equilibrium nor on an enzymic lipid degradation. Whether vitamin K_1 transfer through the membranes is accelerated by the effect of the surfactant itself on the membrane (Anello & Levy, 1969) cannot be judged from the experiment described here. The involvement of such processes cannot be ruled out on the basis of the present data.

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