In order to investigate the pharmacokinetics of quinidine in the horse, an extraction-fluorescence method was developed for determination of quinidine in equine plasma. Plasma quinidine concentrations were determined by two fluorescence methods, which were the protein precipitate (PP) method and the extraction (EX) method. Although there was an excellent correlation of results between the two methods \( r=0.943, P<0.001 \), the value determined by the PP method was twice as large as that by the EX method. It was presumed that the higher concentration by the PP method might have been obtained by such contamination with quinidine metabolites as noticed in human plasma. In order to elucidate the true concentration of quinidine and to exclude its metabolites, the same plasma samples were assayed comparatively by very specific high-performance liquid chromatography (HPLC) and the EX method. There was an excellent correlation \( r=0.975, P<0.001 \) in results and a good consistency in value between the two methods. From these findings, it was concluded that the EX method was more accurate and specific than the PP method and might be available for a study of pharmacokinetics of quinidine in the horse.
Determination of Quinidine

mined between the PP and the EX method, because the PP method had been used in the horse. In it, preserved 51 plasma samples used were obtained from racehorses administered orally with quinidine sulfate in medical treatment. The PP method was carried out by the method of Oh-ishi et al.,7) which was a modification of that of Brodie and Udenfriend.1) The samples were assayed following deproteinization with 10% metaphosphoric acid. The EX method was carried out by the method of Cramér and Isaksson,2) in which samples were assayed following extraction with benzene and 0.1 mol/l sulfuric acid. The rate of recovery of quinidine in a concentration range of 0.5 to 5 mg/l from equine plasma was 86 to 92% by the PP method and 95 to 98% by the EX method. Comparison of quinidine concentrations determined between the two methods is shown in Fig. 1. There was an excellent correlation ($r=0.943, P<0.001$) of results between the two methods. The quinidine concentration estimated by the PP method was higher than that by the EX method. The regression line for the two methods was $y=2.5x + 0.04$. It indicated that the PP method yielded twice as large a value as the EX method. Similar results were obtained from human plasma by Huffman and Hignite8) and Kessler et al.9) On the other hand, Hartel et al.10) studied solvents for quinidine extraction and pointed out that the solubility of quinidine was not concerned with the polarity of the solvent, but that quinidine metabolites had a lower solubility in a less polar solvent. It is assumed that contamination with quinidine metabolites may be avoided more efficiently by the EX method with benzene than by the PP method, because benzene is a non-polar solvent and the metaphosphoric acid solution has a polarity. Consequently, it is shown that the EX method is more specific for the determination of quinidine than the PP method.

Next, to determine the quinidine specificity of the EX method, plasma quinidine concentration was compared between this method and high-performance liquid chromatography (HPLC) which is much more specific than this method. In it, 27 plasma samples used were obtained from 3 horses which had been administered orally with 40 mg/kg of quinidine sulfate. HPLC was done by the method of Guentert et al.5) In it, primaquine was...
added as internal standard to the plasma and extracted with ether-dichloromethane-isopropanol (6:4:1). The organic extract was evaporated. The residue was reconstituted into a mobile phase which consisted of hexane-ethanol-ethanolamine (91.5:8.47:0.03). An aliquot was injected into the column. The column (LiChrosorb Si 100, 15 μm, 4 mm I.D. × 25 cm) was kept at 30°C. The flow-rate of the mobile phase was 1.7 ml/min. The rate of recovery of quinidine from equine plasma by HPLC was 72 to 78% in a concentration range of 0.25 to 3 ml/l.

The quinidine sulfate preparations used in the study were proved to have contained dihydroquinidine by column chromatography and mass spectrometry. Guentert et al. reported that the results of the EX method had been affected by contamination with dihydroquinidine. Then, plasma samples were examined for the presence of dihydroquinidine by HPLC (Fig. 2, Peak 2). The rate of recovery of dihydroquinidine was 60 to 72% in a concentration range of 0.25 to 3 mg/l.

Although there was a tendency for HPLC to register a somewhat lower quinidine concentration than the EX method (Fig. 3), there was an excellent correlation of results between the two methods \( (r=0.975, P<0.001) \). Table 1 shows comparison of measurements in a horse between HPLC and the EX method. It indicates that the EX method detected dihydroquinidine in company with quinidine. The tendency for HPLC to give lower results may be due to both low quinidine recovery by HPLC and the measurements of quinidine plus dihydroquinidine by the EX method. This gap of results between the two methods, however, was small. Therefore, it was assumed that the results of the EX method might

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Fig. 2. Chromatogram obtained by HPLC for quinidine assay of plasma from a horse administered with 40 mg/kg of quinidine sulfate

Remarks. Peaks: 1; quinidine, 2; dihydroquinidine, 3, 5; metabolite, 4; internal standard (primaquine).

Fig. 3. Relationship of plasma quinidine concentration determined between EX method and HPLC

\[ n=27 \\
\[ r=0.975(P<0.001) \\
\[ y=0.9x+0.01 \\
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agree essentially with those of HPLC. From these findings, it was concluded that the EX method was more accurate and more specific than the PP method. It would be appropriate to use the EX method for studies on the pharmacokinetics of quinidine in the horse.

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馬の血中キニジンの定量法について——抽出-蛍光法の検討——

倉兼英二*・天田明男*

馬における抗不整脈薬キニジンの体内動態を研究するためには、キニジン特異性の高い定量法が必要である。最近、ヒトにおいてキニジン特異性の高い蛍光法として注目されている Cramér らの方法（抽出-蛍光法）が馬においても有用であるかどうかについて検討した。まず、従来から用いられてきた大石らの方法（沈殿-蛍光法）と抽出-蛍光法とによって同一試料の定量値の比較をした。その結果、両法による測定値の相関はきわめて高かった（r=0.943, P<0.001）が、沈殿法では抽出法の2倍以上の高い値が得られた。これはヒトでの報告にほぼ一致した成績であり、沈殿法ではキニジン代謝産物をも含めて測定することによるものと考えられた。すなわち、馬においても、抽出法の方が沈殿法よりキニジン特異性が高いものと推察された。そこで抽出法のキニジン特異性を検討するために、同一試料を抽出法と高速液体クロマトグラフ法で測定した。その結果、両法による測定値の相関はきわめて高く（r=0.975, P<0.001），しかも両法による測定値はほぼ1:1 の関係が認められた。以上の成績から、抽出-蛍光法は馬においてもキニジン特異性が高く、馬におけるキニジンの体内動態を検討するうえで有用な定量法であると考えられた。

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