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# THE RELATIONSHIP BETWEEN THE PARTICLE SIZE OF DICUMAROL AND ITS BIOAVAILABILITY IN DOGS PART I. CAPSULES

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## ABSTRACT

Three different lots of 50 mg. capsules of dicumarol produced markedly different effects on the blood clotting mechanism in dogs. Upon microscopic examination of the content of the capsules, a relationship between the particle size of dicumarol and the dissolution rate, inhibition of normal prothrombin activity and plasma concentration was found.

A general linear relationship was observed between the logarithm of drug concentration in plasma and the pharmacological effect of dicumarol produced by the three capsules. Also, as the area under the plasma concentration-time curve increased, so did the area under the percent inhibition of normal prothrombin

activity-time curve produced by the three dicumarol capsules.

## INTRODUCTION

In 1973, Akers et al (1) studied dicumarol (bishydroxycoumarin) tablets from three manufacturers and showed, in dogs, differences in the drug's bioavailability. Lozinski (2) in 1960, observed large differences in therapeutic response after administration of chemically equivalent dicumarol tablets to patients requiring anticoagulant therapy. He observed that the finely divided dicumarol was associated with increased solubility and a more pronounced clinical effect. The work of Reimer (3) in the late sixties, related the dissolution characteristics of dicumarol tablets to the "appearance of the crystals" of the drug.

The present study was undertaken to determine the relationship between the particle size of dicumarol and the drug's bioavailability and dissolution profile.

## EXPERIMENTAL

### Products Studied

Three lots of 50 mg. dicumarol capsules were prepared from three different lots of raw material supplied by two manufacturers. The lots of capsules were designated A, B and C.

## Particle Size Determination

A standard, photomicrographic technique (4) was used to visualize the contents of the three different dicumarol capsules. Two different magnifications were necessary to obtain satisfactory photographs.

## Dissolution

The U.S.P. XVIII - N.F. XIII* rotating basket assembly was modified by the addition of a stainless steel propeller, as Pernaroski et al.** One 50 mg. dicumarol capsule was placed into the stainless steel basket which was then immersed into 2000 ml. of pH 7.6 phosphate buffer*** with 0.1% (v/v) polysorbate 80, U.S.P. maintained at 37°C. in a 3000 ml. Griffin beaker. The basket was centered in the beaker about 2 cm. from the bottom and rotated at 100 r.p.m. Five ml. aliquots were withdrawn at 20, 60, and 120 minutes using a glass wool tipped pipet. The samples were read at 312 nm. on a spectrophotometer to determine the dicumarol concentration.

* U.S.P. 1970, p. 934; N.F. 1970, p. 802

** Pernarowski, M.; Woo, W.; and Searle, R. O., J. Pharm. Sci. 57:1419 (1968)

*** pH 7.6 phosphate buffer is 6.8 g. $KH_2PO_4$ and 1.52 g. NaOH in 1 liter of purified water

## Animals

Nine healthy female Beagle dogs (7.1 to 8.9 kg.) were collectively housed under controlled temperature and lighting.

## Protocol

The dogs were fasted approximately 16 hours before and 24 hours after drug administration. Water was available at all times during the study, and the dogs were randomly assigned to the three different capsules. One intact dicumarol capsule, equivalent to approximately 6 mg./kg., was administered to each dog. Following the capsule administration, a small amount of canned dog food was given to each animal. Blood samples were taken by venipuncture of the jugular vein and collected in 5 ml. citrated vacutainers. A sample was obtained just prior to dosing and 4, 8, 24, 48, 72, and 96 hours thereafter.

## Prothrombin Time Measurements

A plasma sample obtained at 0 time from each dog was serially diluted with sterile pyrogen free physiological saline to give plasma concentrations of 100, 50, 25, 12.5, and 6.25 percent. Normal prothrombin curves for individual dogs were established from the serial dilutions. Routine prothrombin times were performed in duplicate on each sample at the stated time intervals

using rabbit brain thromboplastin essentially as per the Quick method (5).

Assay for Dicumarol in Plasma

The spectrophotometric method as adapted by Nagashima et al (6) was used to assay for dicumarol in the dog plasma.

## RESULTS AND DISCUSSION

Figure 1 shows the photomicrographs of the contents of each different capsule. The granular background in each picture is the starch used in the formulation. The starch serves as a standard with which to judge the sizes of the crystals, but has the disadvantage of hiding the smallest crystals thus introducing a bias in estimating crystal sizes.

Dissolution of a particle can only take place from its surface; therefore, a surface area measurement is needed. However, the photographs give only a projection of the area onto the plane of the film and a true surface area is difficult to measure without complete knowledge of the interfacial angles of the crystals. While these angles could be calculated from the crystallographic data (7), we decided to use Martin's diameter of the particles (8) to approximate particle size. The data were then plotted as the percent of the particles smaller than a stated size against

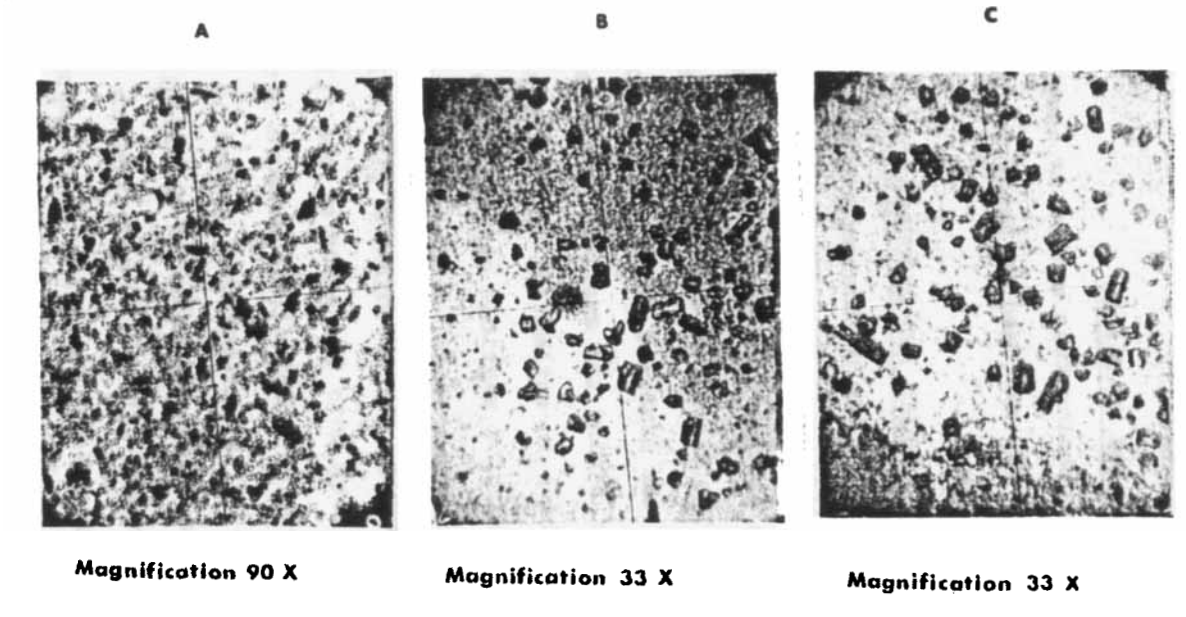


FIGURE 1. Photomicrographs of the contents of three different 50mg. Dicumarol capsules.

crystal size (Figure 2). The great disparity in crystal sizes between capsule A and capsules B and C is easily seen. The significant crystal size data are: capsule A, 50% of crystals are smaller than 25 μ and 94% smaller than 50 μ; capsule B, 50% of crystals are smaller than 105 μ and only 6% smaller than 50 μ; capsule C, 50% of crystals are smaller than 115 μ and only 1% smaller than 50 μ.

Figure 3[#] graphically presents the dissolution profiles of the three different capsules. Each of the individual capsules of product A showed more rapid dissolution than those of capsules B or C.

The mean percent inhibition of normal prothrombin activity after the administration of each of the 50 mg. dicumarol capsules is shown in Figure 4. These data show that capsule A has the most pronounced effect and capsule C the least effect on prothrombin activity. This was true for each animal as well as the mean of the three dogs.

Figure 5 depicts the mean plasma concentration-time curves. Capsule A gave higher plasma concentrations and the greatest area under the curve; whereas, capsule C was the lowest as measured by these parameters. This response was observed for each dog.

[#]The bars represent one standard deviation.

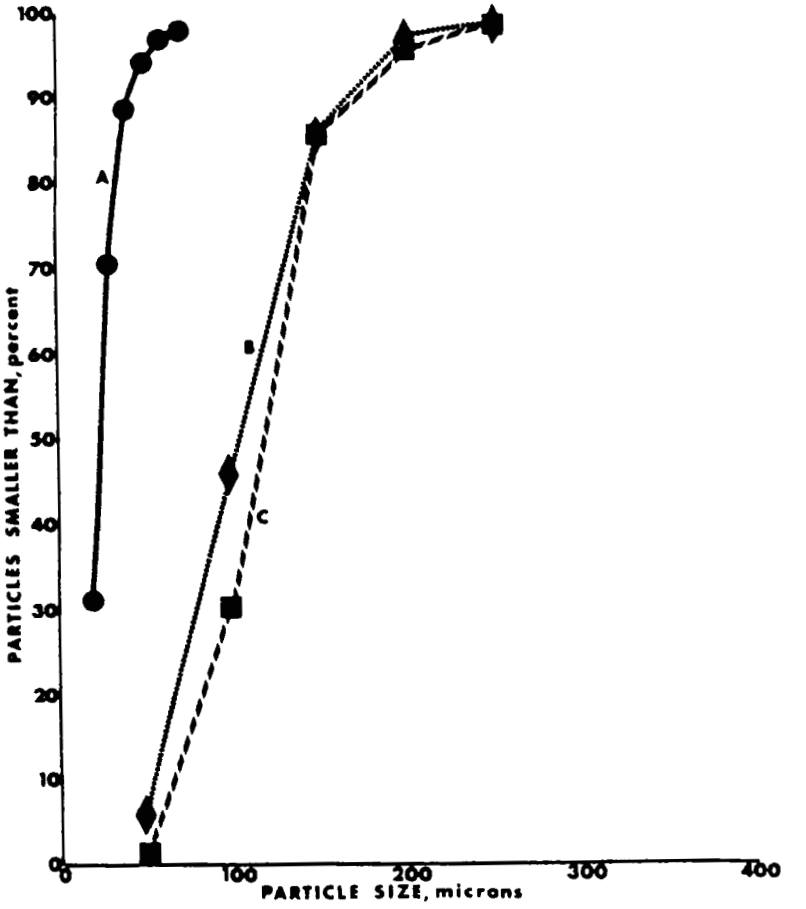


FIGURE 2. Particle size distribution of dicumarol crystals in three different 50mg. capsules.

O'Reilly et al (9) observed that the plasma concentration of dicumarol in man is directly related to the inhibition of "prothrombin complex activity synthesis rate." Our data in dogs show that as the plasma concentration of the drug in-

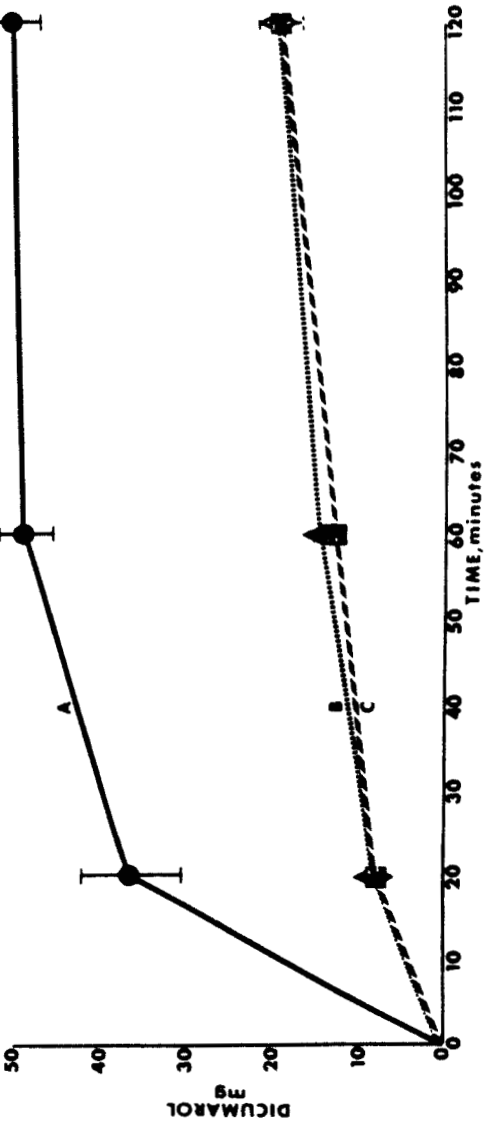


FIGURE 3. Mean dissolution of the dicumarol from three different 50mg. capsules.

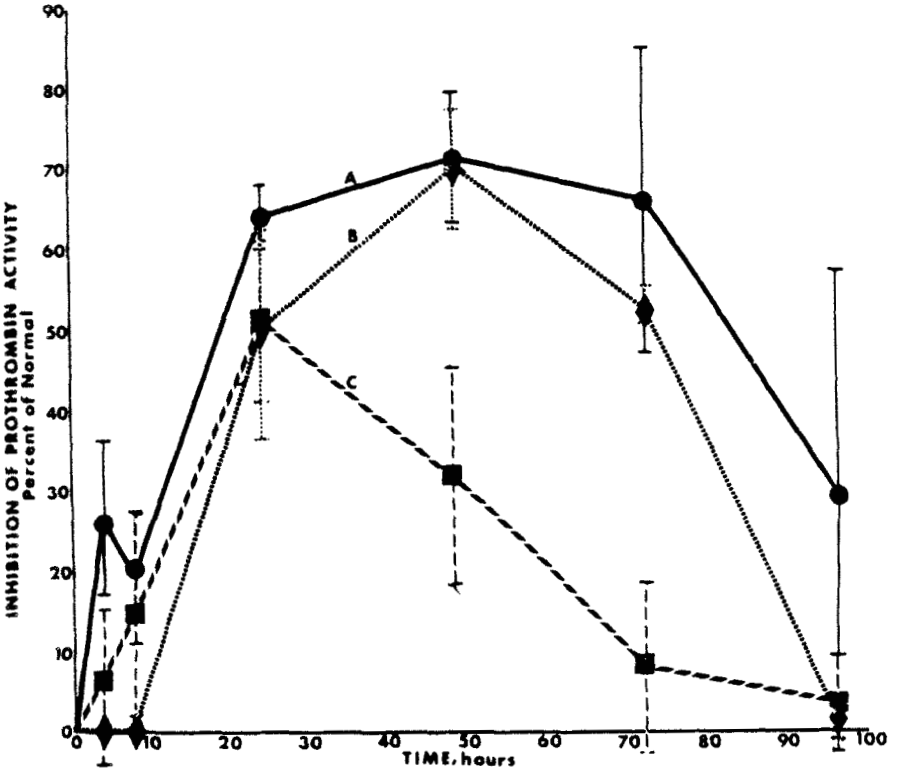


FIGURE 4. Mean percent inhibition of normal prothrombin activity in dogs after administration of three different 50mg. Dicumarol capsules

creased, the rate of synthesis of prothrombin complex activity was inhibited (Figure 6). The relationship is approximately log-linear for each different capsule and the scatter among the three different capsules compares well with the published data of O'Reilly in man.

Figure 7 is a plot of the area under the percent inhibition of normal prothrombin activity (PINPA) -

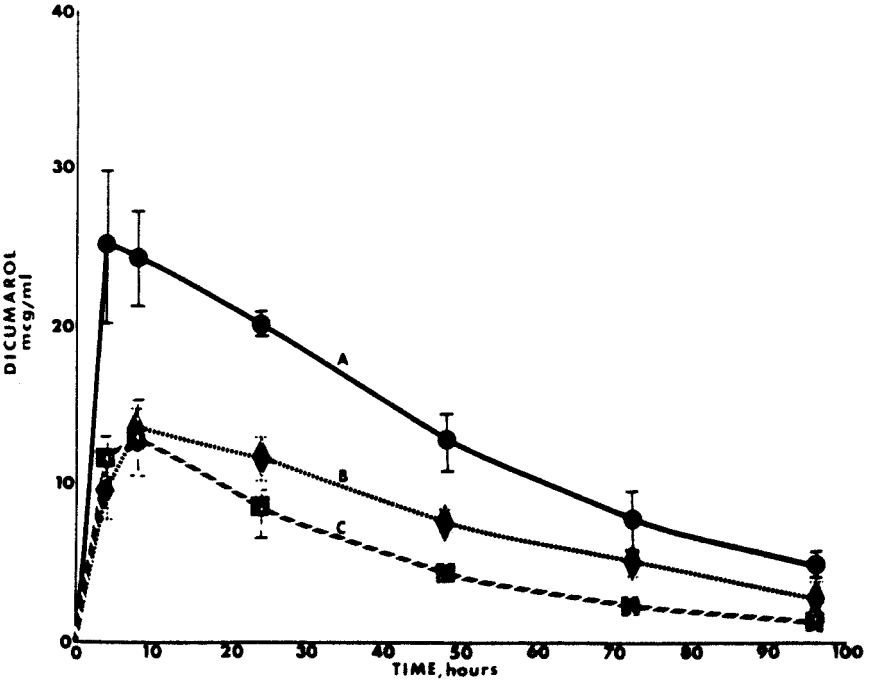


FIGURE 5. Mean plasma concentrations of dicumarol in dogs after administration of three different 50mg. capsules.

time curves versus the area under the plasma concentration-time curves for the three different capsules. The relationships were inconsistent within each lot, but when all the data were considered, a linear trend was found.

Apparently, within the characteristics of each lot, the relationship between plasma dicumarol-time area and PINPA-time area is minimal. When the large among lot differences are included in the model and a greater range is spanned, there is an overall increase in PINPA-

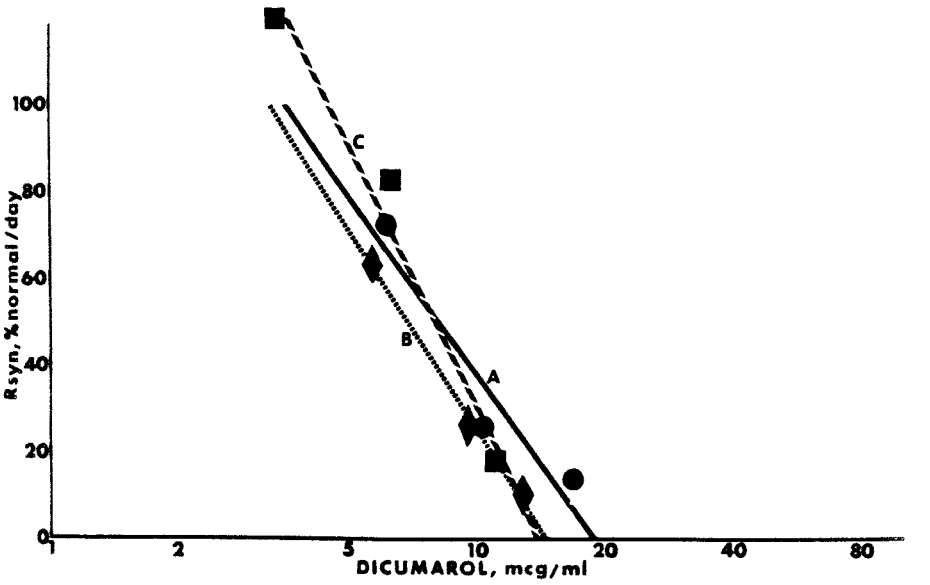


FIGURE 6. Relationship between synthesis rate of prothrombin complex activity and plasma dicumarol concentration after administration of three different 50mg. Dicumarol capsules.

time area with increasing plasma dicumarol-time area.

## SUMMARY

A study of the bioavailability of dicumarol from three different 50 mg. capsules shows a marked relationship between the particle size of drug and the dissolution rate of dicumarol. A strong relationship also exists between the particle size of the drug and the percent inhibition of normal prothrombin activity and plasma concentrations produced in dogs. Smaller particles of dicumarol are associated with a

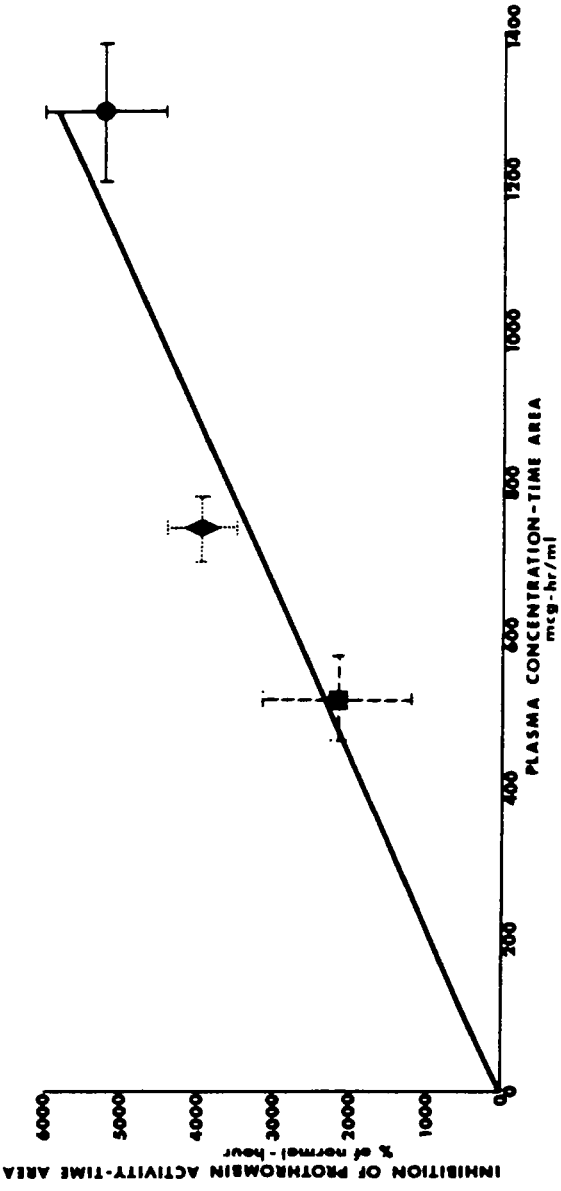


FIGURE 7. Relationship of percent inhibition of normal prothrombin activity-time area to the plasma concentration-time area of three different 50mg. Dicumarol capsules.

more rapid dissolution rate, a greater PINPA, and a higher plasma concentration of the drug.

A linear relationship was found between the synthesis rate of prothrombin complex activity and the logarithm plasma concentration of dicumarol. The areas under the plasma concentration-time curves are correlated with the areas under the PINPA-time curves.

Although no strong correlation was found between the dissolution rate of the drug in the three different capsules and their PINPA or plasma concentration, it is evident from the data that a relationship does exist.

ACKNOWLEDGMENT

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