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DISSOLUTION OF SLIGHTLY SOLUBLE DRUGS VIII. IN VIVO DRUG DISSOLUTION IN HUMAN SUBJECTS AND RABBITS

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SUMMARY

We used the deconvolution method to compare the in vivo dissolution of sulfisoxazole particles in human subjects and rabbits. In the former, the effect of particle size on the rate and extent of in vivo dissolution was appreciable. In both species, the in vivo dissolution rate correlated well with the absorption rate during the initial linear portions (2–4 h), and in the meantime, a good particle size-dependent correlation between in vitro and in vivo dissolution rates was observed in the human subjects.

INTRODUCTION

A study of the general relationship between drug solubility and bioavailability in rabbits revealed that particle size appreciably affected bioavailability when the solubility (in distilled water at 37°C) was less than 0.1% (Kaneniwa et al., 1978). This finding was based on the assumption that the absorption rate of highly lipid-soluble drugs, in solution form, is limited to the maximum value by physiological factors such as the gastric emptying rate, the presence of an unstirred layer at the absorbing membrane surface, and capillary blood-flow at the absorption site.

In rabbits fasted for 48 h, a good particle size-dependent correlation between in vitro and in vivo dissolution rates was observed for sulfadimethoxine and sulfadiazine that their solubilities were around or less than 0.01%; this correlation disappeared with increasing drug solubility (Watari et al., in press).

Although Chiou et al. (1969) had found the rabbit to be a poor model for oral absorption studies, we used the deconvolution method (Rescigno and Segre, 1966) to study the difference in drug dissolution in the GI tract of human subjects and rabbits. Then, we intended to elucidate further what degree of the difference of in vivo dissolution and absorption concretely exists between these two species, using sulfisoxazole particle size as an indicator for dissolution.

MATERIALS AND METHODS

Materials

Powder sulfisoxazole (Yamanouchi Seiyaku, Tokyo) was passed through varying-sized mesh (Japan Industrial Standard (JIS) sieves) to obtain an arithmetic mean diameter of 81, 230 and 545 μm for human, and 81, 163, 324 and 650 μm for animal experiments.

Study conditions and drug administration

Four healthy Japanese male volunteers ranging in age from 21 to 32 years and in weight from 57 to 67 kg were used. Each participant orally received 2.0 g sulfisoxazole after overnight fasting (week 1, sulfisoxazole in aqueous solution; week 2, 81 μm; week 3, 230 μm; week 4, 545 μm). Nelson and O'Reilly (1960), and Kaplan et al. (1972) have reported sulfisoxazole to be completely recovered in the urine during 48 or 72 h after oral drug administration and they found urinary excretion to be the sole route of elimination. Therefore, week-to-week differences due to the influence of any existing residual or cumulative effects of prior doses are negligible in the present study. Sample powders were administered just after vigorously mixing the suspension with 200 ml of water, and food was withheld for 5 h after each administration. The aqueous solution was prepared by dissolving 2.0 g sulfisoxazole in 200 ml water, converting the free drug to its sodium salt via the addition of 0.1 N NaOH.

Venous blood (0.5 ml) was collected immediately prior to and 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after drug administration and sulfisoxazole blood levels were determined as total sulfisoxazole after hydrolysis of 4-N-acetylsulfisoxazole by the method of Bratton-Marshall (1939). As the ratio of free sulfisoxazole to total in the blood ranged from 95 to 70% during the 12 h (Van Petten et al., 1971), the contribution of the metabolite may be less. In addition, about 20% of the dose is acetylated in man (Nelson and O'Reilly, 1960; Van Petten et al., 1971).

Animal studies and in vitro dissolution rate

Three adult male Japanese White rabbits, fasted for 48 h, orally received 4 particle sizes of 0.2 g sulfisoxazole in suspension form with 20 ml water and two administrations of aqueous solution orally and intravenously at 1-week intervals (Watari et al., in press).

In vitro dissolution rate was determined under sink conditions in distilled water using the same apparatus and procedure (Kaneniwa and Watari, 1974). The agitation speed used was 700 rpm so as to obtain thoroughly wetted and completely dispersed powder particles because we were interested in the relative value or ratio of dissolution rate.

Kinetic studies

If the body is regarded as a linear system, the blood drug concentration–time curve, X(t), obtained with an arbitrary but finite drug input into the body can be described by the convolution equation:

$$X(t) = \int_0^t F(\theta)G(t-\theta)d\theta$$

where G(t) is the blood drug concentration–time curve obtained with a unit drug impulse input and F(t) is the input function that, when integrated between limits of t = 0 and t, yields the cumulative amount of drug delivered to the impulse input point. When experimental data are available on X(t) and G(t), calculation of the function F(t) is usually carried out by "numerical deconvolution'. As in the present study, the input function is the released rate of drug in the gut, and the integration of the input function with time yields the cumulative amount of released drug to the unit dose.

The time (T_{50}) required for 50% ratio of released drug was determined from the integrated input function data for in vivo dissolution. Furthermore, to elucidate the dissolution kinetics of the drug in the gut, a plot of per cent undissolved versus time was made from the integrated input function data.

The computer programs used were CMNLR2 (OS7 Hitachi Statistical Analysis Program, 8700-7-004-02, Tokyo, Japan, 1972) for non-linear regression analysis and a deconvolution program in which the anterior phenomenon was approximated by an appropriate exponential polynomial, and the deconvolution operation was done from the posterior phenomenon by the point–area method (Kiwada et al., 1977). The absorption rate in the two-compartment model was calculated by the method of Loo-Riegelman (1968). The computation was carried out on a HITAC 8800/8700 digital computer at the University of Tokyo.

RESULTS AND DISCUSSION

Comparison of blood levels between human subjects and rabbits

The fastest blood peak occurred in the aqueous solution and there was a qualitative linear relation between particle size and peak time (Fig. 1). These findings indicate that the dissolution rate was rate-limiting and that sulfisoxazole dissolution is particle size-dependent.

In the human subjects, the aqueous solution produced a blood level peak within 0.5 h (Van Petten et al., 1971) and the observed characteristic 'nose' may be interpreted to suggest that the disposition of the drug follows the two-compartment model (Ronfeld and Benet, 1977).

Table 1 compares pharmacokinetic factors between the two species studies and shows the statistically significant difference between the aqueous solution and the 545-μm sample in the human subjects for AUC. In rabbits, the difference was minimal irrespective of particle size.

Comparison of the blood level peak time (T_{max}) revealed that T_{max} of the human subjects was faster than that of the rabbits, although in the two-compartment model, the elimination rate constant (k_{el}) for rabbits was 3 times that of the human subjects. These elimination rate constants from the central compartment in the two-compartment model were obtained from blood level data within 12 h after oral administration of aqueous solution for human subjects (Wagner, 1974), and from blood level data after i.v. injection of aqueous solution for rabbits, respectively. The elimination rate constant of the human subjects was in good agreement with the value ranging from 0.0912 to 0.131 h^{-1} for total rate constant of excretion and acetylation for sulfisoxazole, reported by Nelson and O'Reilly (1960). Furthermore, the peak blood level (C_{max}) in the human subjects

TABLE 1

COMPARISON OF PHARMACOKINETIC FACTORS BETWEEN HUMAN SUBJECTS AND RABBITS

Sample size (μm)	AUC [a] (μg/ml X h)	T_{max} [b] (h)	C_{max} [a] (μg/ml)	k_{el} [a] (h^{-1})
Human subjects				
soln.	1530 ± 189 (1.00)	0.5 (<0.5)	140.0 ± 2.1 (1.00)	
81	1540 ± 166 (1.01)	2.5 (2.0–3.0)	91.6 ± 3.5 * (0.88)	0.100 ± 0.009
230	1530 ± 157 (1.00)	6.0 (5.5–6.5)	74.6 ± 2.3 ** (0.72)	
545	1200 ± 129 * (0.78)	7.0 (6.0–8.0)	50.6 ± 1.7 ** (0.49)	
Rabbits				
soln.	613 ± 118 (1.00)	1.3 (0.5–2.0)	88.9 ± 9.0 (1.00)	
81	645 ± 113 (1.05)	3.7 (3.0–4.0)	73.3 ± 8.2 (0.82)	
163	685 ± 80 (1.12)	5.0 (4.5–5.5)	70.7 ± 3.2 (0.80)	0.309 ± 0.053
324	627 ± 113 (1.02)	7.0 (6.0–8.0)	66.0 ± 6.2 (0.74)	
650	644 ± 131 (1.05)	7.7 (7.0–8.0)	68.3 ± 8.7 (0.77)	

[a] Mean ± S.E. The ratio of the aqueous solution to each sample is shown in parentheses.

[b] Range of peak level time is given in parentheses. The significant difference between aqueous solution and each sample, using the Student's t-test is as follows: * $P < 0.05$ and ** $P < 0.01$.

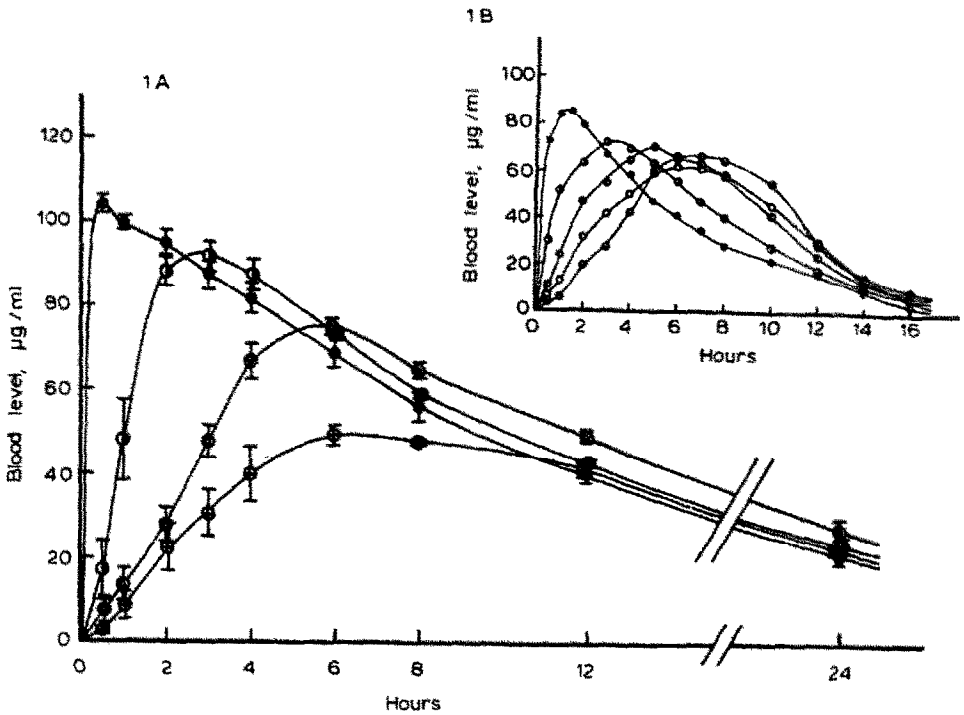

Fig. 1. Blood sulfisoxazole levels in human subjects and rabbits. Each point represents the mean ± S.E. for human subjects (Fig. 1A) and the mean of 3 rabbits (Fig. 1B). ●, soln.; particle size (μm): ●, 81; ●, 163; ●, 230; ○, 324; ●, 545; ●, 650.

decreased with increasing particle size while no marked effect of particle size was noted in the rabbits.

Relationships among in vitro and in vivo dissolution rates and absorption rate

In vivo sulfisoxazole dissolution was calculated from the deconvolution between blood levels after aqueous solution and each powder sample administration. The blood level after oral administration of aqueous solution was standardized as the anterior phenomenon. Fig. 2 shows the cumulative amount of released drug to the unit dose in the gut as a function of time. There was a statistically significant difference ($P < 0.05$, Student's t-test) between the 81- and 545-μm samples for the maximum value of cumulative amount of released drug in the human subjects. On the other hand, in the rabbits there was no significant difference among the different particle sizes; there is a remarkable difference between these two species for the dissolution of the drug in the gut. Furthermore, the results of the maximum amount of released drug were in good agreement with the data of AUC in both species, respectively.

To elucidate the in vivo dissolution kinetics, a semilogarithmic plot of per cent undissolved versus time was made from the integrated input function data and shown in Fig. 3A. The per cent unabsorbed–time plots calculated by the two-compartment model method are shown in Fig. 3B. The kinetic parameters used in the two-compartment model in calculating the absorption rate were obtained from the same data in calculating

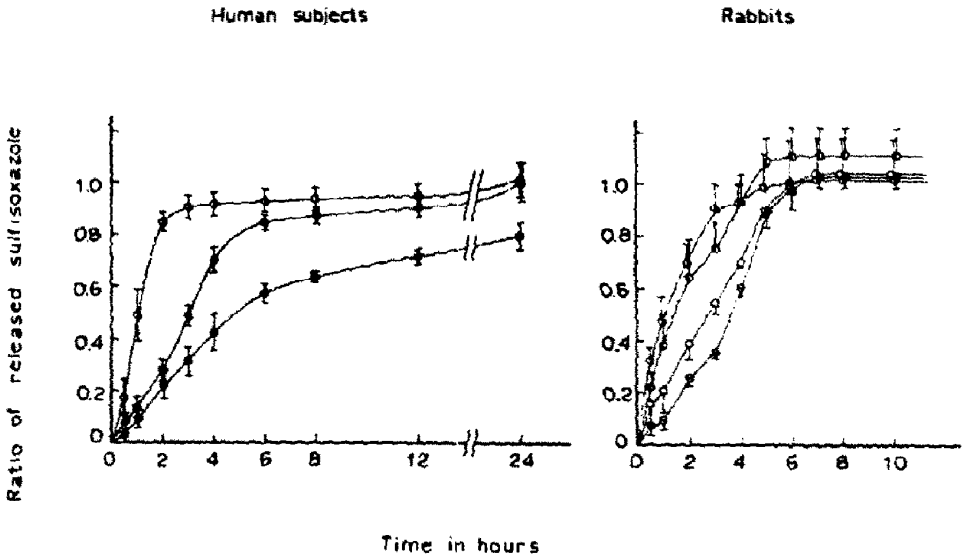

Fig. 2. Time course of the ratio of released sulfisoxazole in the gut. Each point represents the mean ± S.E. Particle size (μm): ⊕, 81; ●, 163; ●, 230; ○, 324; ●, 545; ⊖, 650.

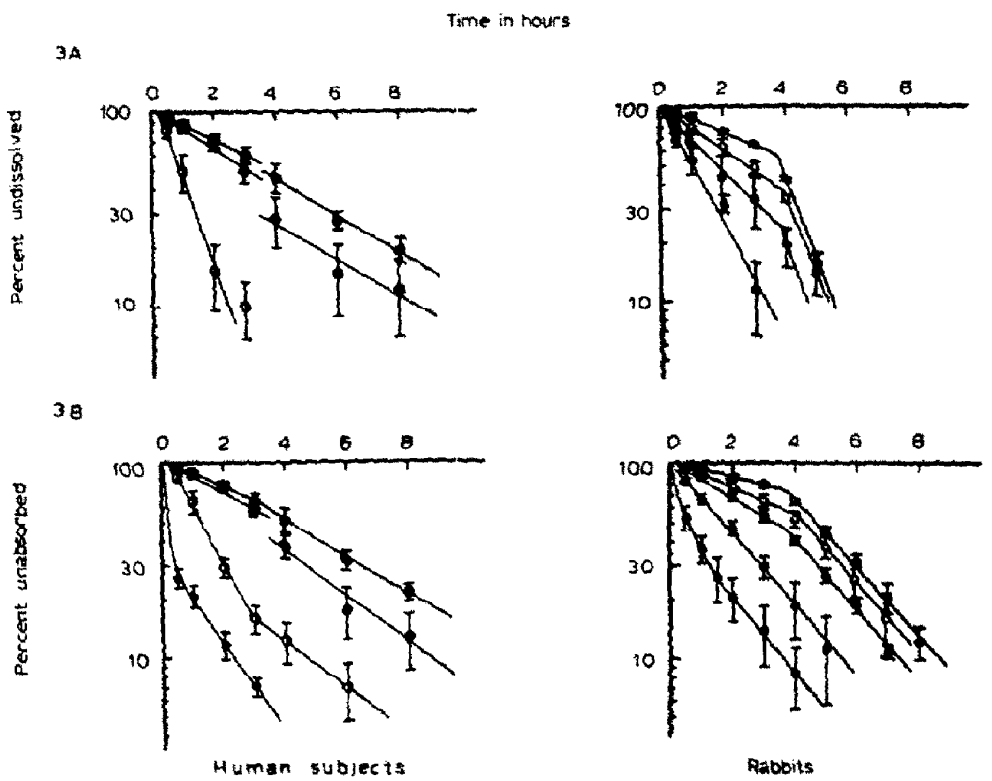

Fig. 3. Comparison of per cent undissolved or unabsorbed vs. time plot between human subjects and rabbits. Each point represents the mean ± S.E. The symbols are the same as in Fig. 1. The solid lines are calculated ones.

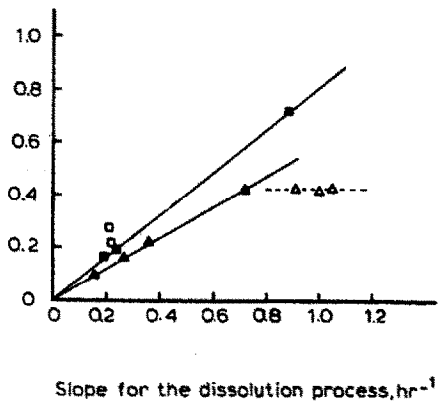

Fig. 4. Comparative plot of slopes from Fig. 3. ■, initial linear portion for human subjects; □, second linear portion for human subjects; ▲, initial linear portion for rabbits; △, second linear portion for rabbits.

the elimination rate constants in both species. Both undissolved–time and unabsorbed–time courses for powder samples were biphasic with convex-descending curves in both human subjects and rabbits except for the smallest sample size of 81 μm. These time courses of the mean value were fitted to two lines by the least-squares method. Fig. 4 shows the plot of the slope of the initial linear portion for the dissolution process (Fig. 3A) versus that of the absorption process (Fig. 3B). There was a good linear relationship in each species ($y = 0.802x + 0.009$, $r = 0.999$ for human subjects; $y = 0.576x + 0.007$, $r = 0.999$ for rabbits). However, in the second linear portion in rabbits, the slopes of the absorption process were almost constant, irrespective of the slopes of the dissolution process and approximately corresponded to that of the aqueous solution, indicating that absorption is rate-limiting (Watari et al., in press). In the human subjects, although the slopes of the absorption process for the 230- and 545-μm samples were similar to the slopes of the dissolution process, these relationships should be examined further.

The per cent unabsorbed–time courses of aqueous solution (Fig. 3B) were biphasic with concave-descending curves, in which the initial absorption rate compared with the second one is very large in both species. Crouthamel et al. (1971) and Koizumi et al. (1964) reported that the transfer of sulfa drug from the stomach to the body is one-tenth or less compared to that from the small intestine. Based on these considerations, we suggest that the aqueous solution did not remain in the stomach for long, but quickly moved to the small intestine and was transferred to the body. The slower second absorption rate may be explained by the fact that the aqueous solution moved quickly to the lower small intestine (e.g. ileum) or the large intestine and that the absorbability of the drug decreased (Watari et al., in press).

Fig. 3 illustrates that in vivo drug dissolution and absorption may follow the first-order process in both species. The biphasic convex-descending curves for large particle sizes may indicate that, as the drug moves down the GI tract, a change in the dissolution environment occurs, and an increase in pH results in an increase of the dissolution rate (Kaplan et al., 1972). The time taken for the initial linear portion for human subjects was smaller than that for rabbits. Furthermore, the larger slopes for the second linear portion of the

dissolution process in rabbits (Fig. 3A) were noted and this phenomenon may be explained by the difference in intestinal pH values between rabbits and humans (Crouthamel et al., 1975).

To facilitate correlations between in vitro and in vivo dissolution rates roughly, the in vivo parameters for the mean value of T_{50} were plotted against in vitro $T_{1/2}$ determined in distilled water (Fig. 5). Although as seen in Fig. 5, there were good linear relationships between in vitro and in vivo dissolution rates in both species, respectively, the slope of the line for human subjects was larger than that of rabbits, indicating that the in vivo dissolution in the human subjects was slow in large particle sizes.

To correlate in vitro dissolution with in vivo dissolution and absorption rate constants, the apparent rate constants for in vivo dissolution and absorption were adopted from the initial linear portions. In human experiments using the 545-μm sample, significantly reduced availability was noted, possibly due to the simultaneous occurrence of drug dissolution and movement down the GI tract. Hunt and Spurrell (1951) reported this movement to be according to a first-order process. Therefore, the 545-μm sample constant was obtained by multiplying the apparent rate constant by the fraction (F) of the orally administered dose absorbed or dissolved (Notari et al., 1972).

In both species, the rate constant of in vivo dissolution was larger than that of absorp-

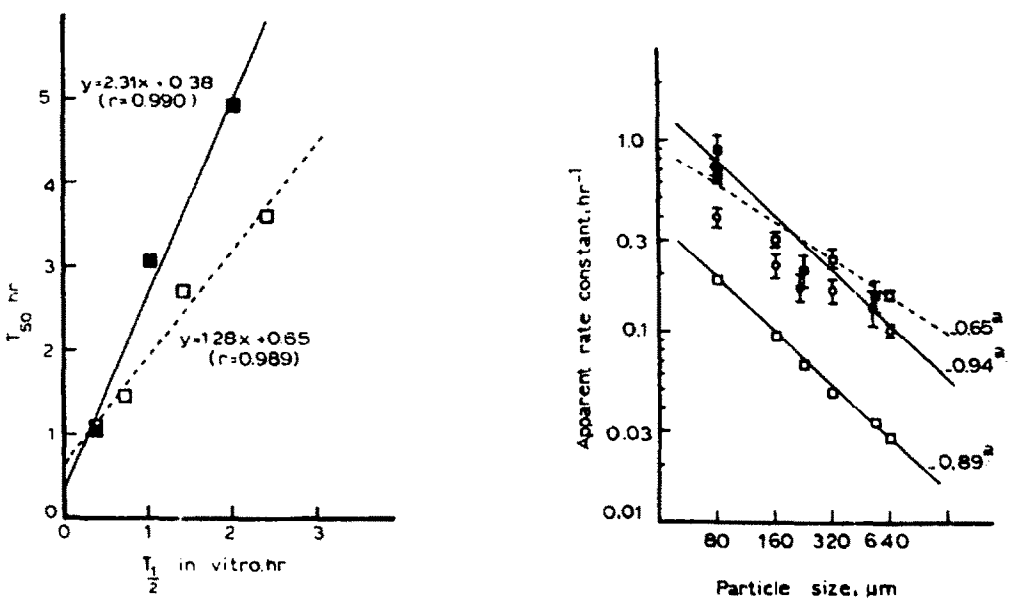

Fig. 5. Relationship between in vitro and in vivo dissolution rates. ■———■, human subjects; □– – –□, rabbits.

Fig. 6. Dependence of log rate constant on log particle size. Each point represents the mean and the vertical bars are the standard errors. [a] Effect of particle size on dissolution rate constants. The slope of the line is given. ■———■, dissolution rate constant for human subjects; □– – –□, dissolution rate constant for rabbits; □———□, in vitro dissolution rate constant ($mg^{1/3}$/min) by the Hixson–Crowell cube root law plot. ●, absorption rate constant for human subjects; ○, absorption rate constant for rabbits.

tion, and the ratio of rate constant of in vivo dissolution to absorption was similar for all samples in each species (1.23 ± 0.14, mean with S.D. for human subjects; 1.61 ± 0.19 for rabbits). The constant ratio between in vivo dissolution and absorption rates in both species indicates that it may be safe to utilize the ratio when correlating in vitro dissolution with in vivo dissolution rate.

We applied the Hixson–Crowell cube root law (Hixson and Crowell, 1931) to the data of cumulative amount of released drug for in vivo dissolution in the human subjects and this resulted in the plots becoming a straight line during the period of 2–4 h after drug administration. We previously reported the same result in rabbits (Watari et al., in press). This finding correlates well with the constant ratio of in vivo dissolution to absorption rate constants.

We calculated the effect of particle size on in vivo and in vitro dissolution rate constants in both species (Fig. 6). In the human subjects, a good particle size-dependent correlation was obtained between in vitro and in vivo dissolution rates.

Our results indicate that the faster human gastric emptying rate has little, if any, effect on drug dissolution. This is further supported by the inverse relationship between T_{50} and T_{max} in each species, i.e. T_{50} of the human subjects was slower than that of the rabbits but T_{max} in the human subjects was faster than that in the rabbits.

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