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Elimination of Phenacetin and Phenazone by Man before and after Treatment with Phenobarbital*

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Summary. After the oral administration to volunteers of phenacetin (15 mg/kg) the highest concentration in blood was reached 2 h later. The decline appeared to follow first order kinetics with an apparent biological half-life (t'/2) of 0.8 h. The highest concentration in blood of the unconjugated metabolite, paracetamol, was twice that of phenacetin. About 70 per cent of a dose of phenacetin was excreted as conjugated paracetamol in the urine, and 70 per cent of this compound was eliminated during the first 12 h period. — After daily administration of 1.4, 2.5 and 3.6 mg/kg of Phenobarbital, each dose sequentially for 1 week, the apparent biological half-life of phenacetin and the elimination rates of its metabolites, unconjugated and conjugated paracetamol, remained unchanged. The elimination rate of phenazone (16 mg/kg) was increased by 40 per cent in the same subjects after treatment with phenobarbital. — The blood concentration of phenacetin varied more than 20 times between individuals, although no individual variation was found for the blood levels of unconjugated paracetamol.

Key-words: Enzyme-induction, man, phenacetin, phenazone.

Introduction

The enzyme system located in the endoplasmic reticulum of liver cells, which hydroxylates drugs, has been induced by many organic compounds in animals and in man [8].

Because of experimental limitations in human beings most investigators have studied the activity of this enzyme system by determining the biological half-life of a drug in blood, and/or by measuring the excretion rate of its hydroxylated products in urine.

However, most drugs become hydroxylated at several sites of the molecule leading to a variety of different products. In addition, in most instances we know of only a small fraction of the metabolic pathways followed by a drug.

Since the urinary excretion rate of even one hydroxylated product does not necessarily reflect the rate of a specific hydroxylation reaction, the pharmacokinetics of the formation and elimination of certain unconjugated products should also be followed. Only Che *et al.* [7] have reported simultaneous determination of a drug, aminopyrine, and its hydroxylation product, 4-aminoantipyrine, in man after treatment with an enzyme-inducing compound.

A search was made, therefore, for a substance almost completely hydroxylated by the microsomal enzyme system at a single important site in the molecule. The relatively safe drug phenacetin appeared to meet these requirements.

In man approximately 70 per cent of a dose of phenacetin is metabolized to paracetamol which is excreted in the urine in a conjugated form [3, 13]. The inducibility of the enzyme system that 0-dealkylates phenacetin has not yet been tested in man.

In rats an increase in the rate of phenacetin metabolism has been observed after administration of phenobarbital [6]. The doses of phenobarbital usually used in animal studies are known to be sufficient for enzyme induction; however, they exceed the therapeutic dose in man by more than 10 times. Therefore, it seemed of interest to investigate whether the blood concentration of phenacetin and its metabolite could be influenced by an inducing drug to an extent that might be of therapeutic significance.

Methods

Two female and 12 male volunteers, medical students between 24 and 26 years of age, participated in these studies. Their medical history did not indicate renal or hepatic disease. For six months before and during the experiment drugs, cigarettes or alcohol were not consumed in excess.

Test doses of phenacetin, 15 mg/kg, or phenazone, 16 mg/kg, were administered two hours preprandially at 8.30 a.m., with 2 cups of flavoured soda water and a slice of toast. Crystalline phenacetin (*Bayer*, DAB 6) had been ground in a mortar; the powder was placed on two starch discs, which formed the upper and lower parts of a flat starch capsule, 1.8 or 2.0 cm in diameter. Crystalline phenazone (*Bayer*, DAB 6) was administered in starch capsules.

For the determination of phenacetin and its metabolite paracetamol in blood, 8 samples were drawn for duplicate assays at varying intervals between 20 and 90 min, up to 8 h after the administration of phen-

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acetin. For determining conjugated paracetamol, urine was collected at 2 h intervals during the first 12 h, for the following 36 h it was collected in 12 h periods. Blood samples for quadruplicate analyses of phenazone were drawn 3, 5, 8 and 10 h after oral administration.

Chemical Analyses

Phenacetin and unconjugated paracetamol were determined according to Brodie and Axelrod [1, 3] in 5 or 3 ml of blood, respectively. For the determination of unconjugated paracetamol, the dye after coupling, was extracted into 1 ml of benzene. The extinction at 580 nm was read after 0.6 ml of the benzene solution had been mixed with 0.1 ml of 25% trichloroacetic acid. Amounts of 0.2 to 10 μg of phenacetin, or 1 to 20 μg of paracetamol per ml of preserved human blood, were recovered to 70 and 75 per cent, respectively.

For the determination of conjugated paracetamol 1 ml of urine, 0.5 ml of phosphate buffer (0.5 M, pH 6.6), and 0.1 ml of glucuronidase-arylsulfatase (Boehringer) were incubated for 5 hours at 37°C; 2 g of sodium chloride were added and the mixture was shaken with 20 ml of diethyl-ether for 20 min. 10 ml of the ether solution was evaporated on an Evapomix (Buchler). For deacetylation of paracetamol the residue was heated with 6 ml of 2.4 N hydrochloric acid for 1 h in a boiling water bath. With 5 ml the procedure was carried on according to Brodie and Axelrod [1]. The method of Welch and Conny [16], carried out concurrently in the first experiment, produced a greater variation in the results since the amount of p-aminophenol deteriorating in boiling urine correlated positively with the varying density of the urine samples.

Phenazone (antipyrine) was determined according to Brodie *et al.* [4]; 2 ml of water and 0.5 ml of 1 N sodium hydroxide were added to 3 ml of blood.

For the determination of phenobarbital, in duplicate, a mixture of 6 ml of blood, 2 ml of water, and 2 ml of phosphate buffer (0.5 M, pH 6.6) was extracted twice with 30 ml of chloroform. The chloroform was evaporated on an evapomix, and the residue was treated according to Büch *et al.* [5].

The method of Büch *et al.* [5] was followed for the determination of phenobarbital in urine.

For prevention of blood clotting a few crystals of sodium citrate were added to each sample.

All data presented have been corrected for loss on the basis of the recovery of phenacetin, paracetamol, and phenobarbital. Phenacetin and paracetamol in blood were determined immediately after blood was drawn. The other samples were kept at −25°C for up to 10 weeks; for the determination of the half-lives each set of samples was thawed in random order.

Statistical Methods

Biological half-lives were calculated from the data of at least 4 samples, taken at various time intervals, by applying the method of least squares analysis to the equation: $\log y = \log a - bx$; y is the concentration at time x. In this equation b equals $k_2 \cdot \log e$; $t/2$ was computed by equation $t/2 = ln2/k_2$. To compute b, the logarithms of the original data, corrected for loss, were handled by standard programme No. 201 for the Olivetti desk computer, Mod 101.

The area under the concentration curves of phenacetin and paracetamol was determined planimetrically.

The mean squares of analysis of variance were computed by programme No. 158 for the desk computer.

Results

Elimination of Phenacetin by Untreated Subjects

Test doses of phenacetin were given to 4 males and 2 females three times, each dose one week apart. The concentrations of phenacetin and unconjugated paracetamol in the blood and the urinary excretion of conjugated paracetamol were then determined.

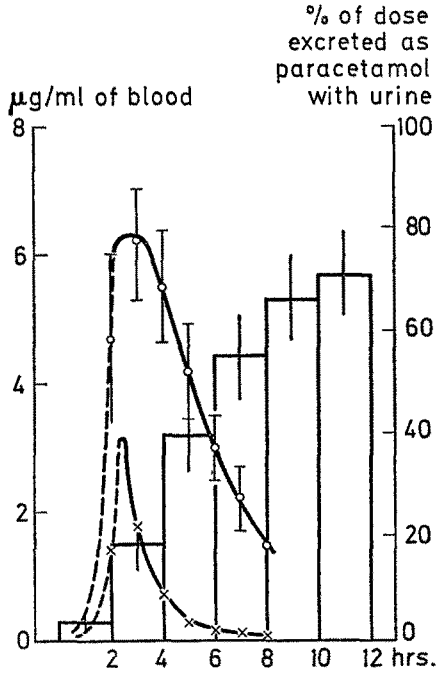

Fig. 1. Elimination of phenacetin by man after oral administration
Concentration of phenacetin (×) and unconjugated paracetamol (○) in the blood, and excretion of conjugated paracetamol in the urine (bars) after 15 mg of phenacetin per kg.
The experiments were carried out three times in each of 6 persons. The lines indicate the 95 per cent confidence interval of the mean.

The results are shown in Fig. 1. On average, the greatest concentration of phenacetin was reached about 2.5 hours after oral administration. The time at

which the highest concentration (t_{max}) was reached varied at random, and was found to be independent of the subject; never before the first hour, 7 times between the first and second hour, 8 times between the second and third hour, and once after the third hour of oral administration. The concentration of unconjugated paracetamol reached its maximum a little later than phenacetin and was approximately twice as high.

73 per cent of the dose of phenacetin was excreted after 48 h, 70 per cent of it being eliminated in the first 12 h.

After phenacetin administration, the falling blood concentrations of phenacetin and paracetamol were found to lie around a straight line on semilogarithmic paper after the third and fourth hour, respectively.

Elimination of Phenacetin and Phenazone after Treatment with Phenobarbital

Three of a group of 4 subjects were treated with single doses of 1.4, 2.5, and 3.6 mg phenobarbital per kg per day, each dose being given sequentially for 6 days. Test doses of phenacetin and phenazone were administered orally in random order 2 days apart. The tests were carried out five times in each individual, twice before treatment with phenobarbital, once after the second as well as the third period of treatment, and 8 days after the last dose of phenobarbital. Conjugated paracetamol in urine was not determined in this experiment.

Urine was collected in 24 h periods for the determination of phenobarbital excretion after the first and last oral dose of phenobarbital, as well as 8 days later.

Table 1. *Apparent biological half-life (t/2 [h]) of conjugated paracetamol (PU) in man, determined by urine analysis, following oral administration of 15 mg phenacetin per kg, and urine volume (UV) [ml] excreted during the first 12 h period after phenacetin administration*

	R.P.		T.V.		C.H.		K.H.		S.W.		J.B.	
	PU	UV	PU	UV	PU	UV	PU	UV	PU	UV	PU	UV
	3.2	866	4.5	450	2.7	674	4.2	696	6.7	253	2.3	1585
	6.5	714	3.4	629	5.4	722	2.7	1137	11.3	242	—	2524
	5.0	760	4.7	512	5.4	430	3.6	734	6.6	263	3.3	1151
Mean	4.9	780	4.2	530	4.5	609	3.5	856	8.2	253	2.8	1753

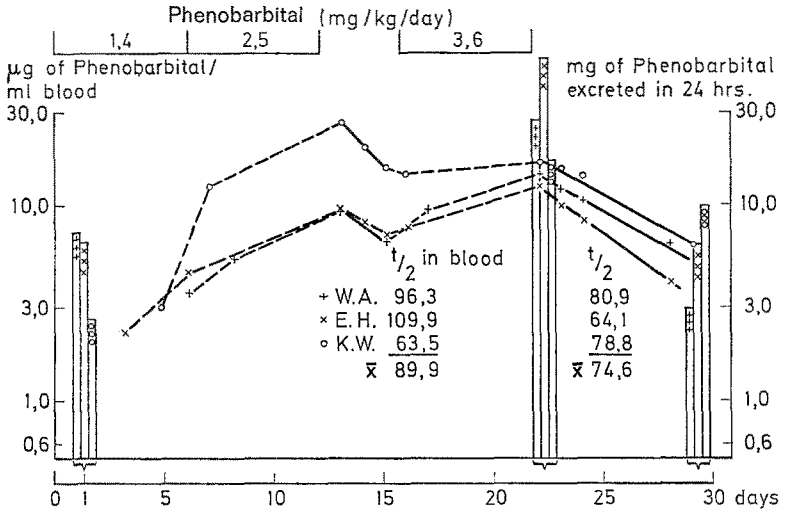

Fig. 2. Concentration of phenobarbital in blood (lines) and amounts excreted in urine (vertical bars) after daily doses of 1.4 mg, 2.5 mg, and 3.6 mg/kg/day, each dose for 6 days

Correlation coefficients of concentration vs time were $\geqq -0.930$.

Individual variations in the apparent biological half-lives of phenacetin and paracetamol were not found on analyzing the values for half-life, found in each experiment, by an analysis of variance.

The half-life of conjugated paracetamol was found to correlate inversely (p : 0.01) with the urine volume excreted during the first 12 h after phenacetin administration (Tab. 1). The mean differences between the subjects were not statistically significant.

In Fig. 2 the following parameters are plotted for each subject: time regime of phenobarbital administration, the concentration of phenobarbital in blood, the amount of phenobarbital excreted in 24 h periods, and the biological half-life of phenobarbital after the second and third period of treatment.

Phenobarbital accumulated when given daily for 6 days. After the third period of treatment the mean concentration of phenobarbital in the blood was 14.5

μg/ml, and 4.9 μg/ml 7 days later. The mean biological half-life dropped from 89.9 to 74.6 h during the third period of treatment, $p < 0.2$.

The apparent individual biological half-lives of phenacetin and phenazone before and after treatment with phenobarbital are listed in Table 2. The apparent biological half-life of phenacetin was not affected by the treatment with phenobarbital. In contrast, the apparent half-life of phenazone decreased by 40 per cent after the treatment with phenobarbital. If Duncan's multiple range test is applied to the data at each period of observation, the periods after treatment with phenobarbital exceed the pretreatment periods at the 5 per cent level. The values of the subject serving as the control indicate no enhancing effect of test doses of phenazone on its own rate of elimination.

The rate of disappearence of unconjugated paracetamol from the blood was also not affected by treatment with phenobarbital.

In subjects W.A., E.H., K.W., and D.R. the means of the five areas under the concentration curves of phenacetin and the means of t_{max} were 460, 2.1; 360, 1.9; 280, 1.7; 180, 1.6, respectively. The differences of the individual mean areas are significant at the 1 per cent level, whereas no individual variation in t_{max} and apparent biological half-life was found.

Individual variations in the concentration of unconjugated paracetamol, however, were much less, just exceeding the 5 per cent level. Between the concentration of phenacetin and the corresponding concentration of unconjugated paracetamol no correlation was found.

The results for phenacetin elimination before and after treatment with phenobarbital were confirmed in another set of 4 subjects at the same dose level of phenobarbital. In this experiment no test dose of phenazone was administered. The elimination of conjugated paracetamol in the urine as determined in this experiment, was also not affected by phenobarbital

Table 2. *Apparent biological half-life (h) of phenacetin (Pc) and phenazone (Pz) in 3 subjects before and after treatment with phenobarbital*

		Before Treatment with Phenobarbital				After Treatment with Phenobarbital mg phenobarbital/kg/day, each period for 6 days, sequentially 1.4 u. 2.5		3.6		8 days after last dose of	
weeks		0		1		3		4		5	
		Pc	Pz	Pc	Pz	Pc	Pz	Pc	Pz	Pc	Pz
Persons treated with phenobarbital	W.A.	0.70	17.2	0.81	14.0	0.64[a]	10.6	0.70	8.2	0.87[a]	10.8
	E.H.	0.74	14.4	0.80	18.4	0.73	12.1	0.96[a]	10.9	0.69	11.8
	K.W.	0.77	13.7	0.78	14.3	0.81	8.8	0.48[a]	7.0	0.85	6.5
Mean		0.74	15.1	0.79	15.6	0.73	10.5	0.71	8.7	0.80	9.7
Control	D.R.	0.61	13.8	0.66	10.9	0.63[a]	11.9	0.77	15.2	0.55[a]	12.0

[a] in these experiments the test dose of phenacetin was given 2 days prior to phenazone

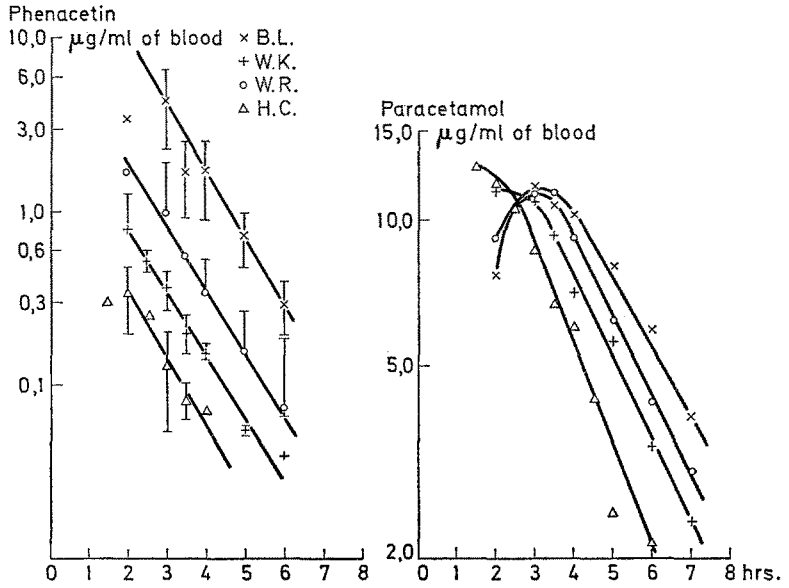

Fig. 3. Concentration of phenacetin and unconjugated paracetamol in the blood of 4 persons, following 15 mg of phenacetin per kg
Mean of 5 experiments in each subject. The bars indicate the 95 per cent confidence limits of the mean

treatment. However, individual variations in the phenacetin concentrations in blood were even more striking, as shown in Fig. 3. The concentrations differed more then 20 fold between the subjects with the highest and the lowest blood values, and yet, the apparent biological half-lives were identical as indicated by the slopes of the falling blood concentrations.

Statistical analysis of the apparent half-life of phenacetin after each test dose also did not reveal any significant individual variations.

In spite of the wide variation of the phenacetin level the maximal blood concentration of unconjugated paracetamol was approximately the same in the 4 persons.

In the presence of a falling blood concentration of phenacetin, the person with the lowest blood concentration showed the shortest apparent half-life of unconjugated paracetamol accompanied by the shortest t_{max}, and vice versa. The individual variation in the elimination of paracetamol from the blood ($t/2$ ranging between 1.54 and 2.13 h) did not exceed the 5 per cent level of significance.

Discussion

In normal volunteers, therapeutic doses of phenobarbital, administered for 3 weeks, did not affect the apparent biological half-life of phenacetin, and the elimination rates of its metabolic products, unconjugated and conjugated paracetamol.

In contrast, the half-life of phenazone was decreased by 40 per cent in the same subjects. The increased elimination rate of phenazone indicates increased activity of the hydroxylating enzyme system produced by the phenobarbital.

The failure to detect an increase in the apparent biological elimination rate of phenacetin after phenobarbital treatment might have been caused by a lesser degree of induction of the enzyme system that O-dealkylates phenacetin in man. According to statistical analysis of the data, a mean difference of at least 30 per cent would have been significant in the design chosen. Therefore, any decrease in the apparent biological half-life of phenacetin caused by the phenobarbital treatment, must have been less than 30 per cent. These results demonstrate that the apparent biological half-lives of drugs metabolized by the microsomal enzymes of liver cells, are not all equally affected by inducing agents.

In the case of phenacetin, however, the apparent biological half-life as measured, might not be identical with the true half-life as determined after intravenous injection of the drug.

After oral administration of phenacetin the pharmacokinetics in the blood follow Bateman's function [9]. The results of Priscott *et al.* [15] indicate that the rate of entry into the blood is dependent on the pharmaceutical formulation which can lead to a high blood concentration after rapid absorption. Following rapid oral absorption of phenacetin from small particles suspended in polysorbate (80), the apparent biological half-life was approximately 35 min [15], as compared to 45 min in the present experiments. If the half-life of 35 min approximates more closely to the true metabolic half-life, one can assume that the constants for invasion and elimination are almost identical. In such a pharmacokinetic situation the upper part of the falling concentration curve does not reflect the true rate of metabolic elimination since absorbtion has not been completed at that time [9]. After twice the time for t_{max}, the concentration of phenacetin was found to be approaching the lower margin of sensitivity of the analytical method. Moreover, in these experiments the pharmacokinetics have probably been complicated by a marked delay in absorbtion caused by the pharmaceutical formulation which kept together the bulk of water insoluble phenacetin for a certain time.

Unfortunately, the influence of the kinetics of absorption on the apparent biological half-life of phenacetin in man has not been investigated since a safe solvent for intravenous injection of phenacetin was not available.

For the same reason no experimental proofs can be provided to explain the striking individual variations of phenacetin concentration in the blood.

In individual subjects t_{max} and the height of the corresponding blood concentration of phenacetin varied in the same direction, i.e. the person with the lowest blood concentration of phenacetin showed the shortest time for t_{max}. This suggests no variation in individual absorption rates of phenacetin. It is likely, that incomplete absorption did not occur either, as the concentration of unconjugated paracetamol was equally high in all individuals.

However, the person showing a low blood concentration of phenacetin may actually metabolize phenacetin much faster than indicated by the slope of apparent elimination; in such a subject the slope at low concentrations of phenacetin is most affected by the phenacetin still being absorbed from the gut. To explain individual variations of phenacetin concentration on the basis of differences in metabolic rate in the presence of complete absorption, an individual rate difference of approximately 40 per cent would have to be assumed between persons showing the highest and the lowest blood concentrations of phenacetin.

Individual variations of phenacetin distribution in the body might afford an alternative explanation for the individual differences in blood concentration. However, no correlation was found between body weight (difference less than 11 kg) and individual phenacetin concentration. After intestinal absorption, individual differences in the capacity of the liver to store phenacetin also cannot be excluded.

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