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# Changes in D-glucaric acid excretion in relationship to alterations in the rate of antipyrine metabolism in man

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THE INDUCTION of hepatic microsomal enzymes may be an important factor affecting the rate of metabolism and hence the extent and duration of action of drugs in man. Enzyme induction may be produced not only by a large number of frequently used drugs,[1,2] but also by many chemicals, such as organochlorine pesticides and polycyclic hydrocarbons, which are encountered in man's environment.[3] Recently considerable evidence has been obtained that the urinary excretion of D-glucaric acid provides a quantitative, although indirect, index of microsomal enzyme activity in man.[4–6] We now report further studies of the relationship of changes in D-glucaric acid excretion to variations in the rate of metabolism of antipyrine, a drug which is metabolized in man by hepatic microsomal enzymes.

In an initial study, intersubject variations were eliminated by assessing changes in antipyrine half-life in relation to glucaric acid excretion in the same subjects before and after treatment with enzyme inducing drugs. Twelve normal volunteers (nine female, three male, aged 19–28) took part. An oral dose of antipyrine 18 mg/kg was administered after an overnight fast. Five blood samples were taken at intervals ranging from 3 to 15 hr after ingestion of the drug. Plasma levels of antipyrine were determined by the method of Welch *et al.*[7] At the same time, a complete 24 hr urine collection was made for glucaric acid, which was determined by the method of Marsh,[8] and expressed as $\mu$-moles of glucaro-1,4-lactone. Subjects then

received for 1 week varying doses of a barbiturate, phenobarbital, or glutethimide, after which measurements of antipyrine half-life and glucaric acid excretion were repeated.

In a further study, the absolute value of antipyrine half-life was related to glucaric acid excretion in thirty-three healthy volunteers and ambulant patients, aged 19–37, including seventeen women. Some were receiving no drugs; others were taking anticonvulsants, barbiturates, glutethimide or oral contraceptives. A 24 hr urine collection was made for glucaric acid excretion and blood samples were taken the following day for the determination of antipyrine half-life, as previously described.

Measurements of plasma antipyrine and urinary glucaric acid were performed at Huntingdon and Cambridge respectively and the results were not correlated until the study was complete.

In the initial study, the dose of barbiturate or glutethimide employed failed to induce hepatic enzymes in some subjects, as judged by glucaric acid excretion, and in these subjects there was no reduction in antipyrine half-life. In those subjects who showed an increase in glucaric acid excretion there was a definite reduction in antipyrine half-life. When the second antipyrine half-life was expressed as a percentage of the first and plotted against the degree of enzyme activity, as represented by the second urinary glucaric acid determination, there was a significant correlation ($r = -0{\cdot}59$, $n = 12$, $P < 0{\cdot}05$, see Fig. 1).

In the second study, glucaric acid excretion was within normal limits in most subjects, and only in those who were taking known enzyme-inducing drugs were increases found. Most subjects with increased glucaric acid excretion had relatively shorter antipyrine half-lives. However, the range of half-lives in those with normal glucaric acid excretion was wide, from 9 to 29 hr (Fig. 2), and many subjects with normal glucaric acid excretion had short antipyrine half-lives. Overall there was no significant correlation between antipyrine half-life and glucaric acid excretion ($r = -0{\cdot}28$, $n = 33$, $P > 0{\cdot}1$).

The correlation between changes in the rate of antipyrine metabolism and D-glucaric acid excretion found in our initial study provides further strong support for the value of glucaric acid excretion as an indirect index of hepatic microsomal enzyme activity in man. Variations in glucaric acid excretion show remarkable similarities to variations observed in microsomal enzyme activity in experimental animals. Both are low in the neonate[9,10] and rise with maturity. There is a diurnal variation in glucaric acid excretion, with a peak in the late afternoon and evening, which corresponds to that of microsomal enzymes.[11] Glucaric acid excretion is increased by a wide number of enzyme-inducing drugs, including barbiturates, glutethimide, organochlorine pesticides, rifampicin and phenylbutazone. Significant inverse correlation has been discovered between glucaric acid and the plasma levels of compounds which in man are metabolized by microsomal enzymes, such as bilirubin and pp′ DDE (the main metabolite of DDT). Furthermore, in guinea-pigs receiving phenobarbitone, there is a strong correlation between total liver content of the microsomal enzyme cytochrome P-450 and the urinary excretion of glucaric acid.[2,4–6,12]

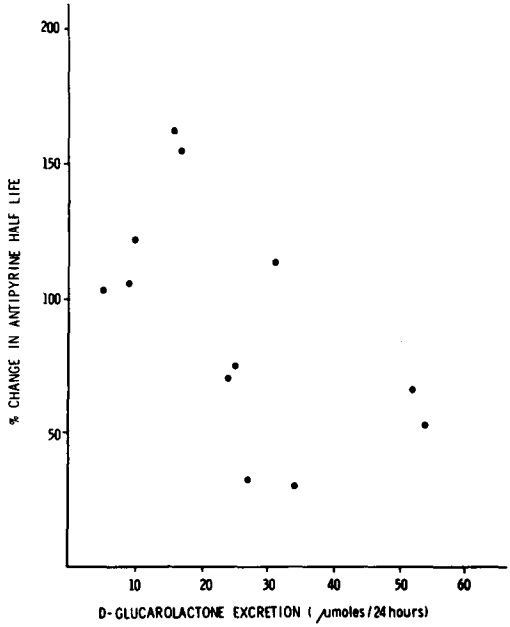


FIG. 1. Percentage change in antipyrine half-life correlated with urinary glucaric acid excretion in normal individuals.

Our second study, however, shows that, in relation to other factors, enzyme induction is comparatively unimportant in determining the absolute rate of drug metabolism in man. Although we found that subjects with enzyme induction tended to have short antipyrine half-lives, these were frequently little different from those of other subjects with normal glucaric acid excretion. There was no evidence of any environmental factor producing enzyme induction in our patients. It has been demonstrated recently that the absolute rate of drug metabolism is not closely related to the activity of cytochrome P-450 in specimens of human liver obtained by needle biopsy.[13] Although the half-life of antipyrine is very similar in monozygotic twins, it is frequently dissimilar in dizygotic twins.[14] This suggests that the most important factors governing the rate of drug metabolism are under genetic control. This appears to be true for drugs as widely different as ethanol,[15] halothane[16] and nortriptyline.[17] Only when genetic factors are kept constant by performing

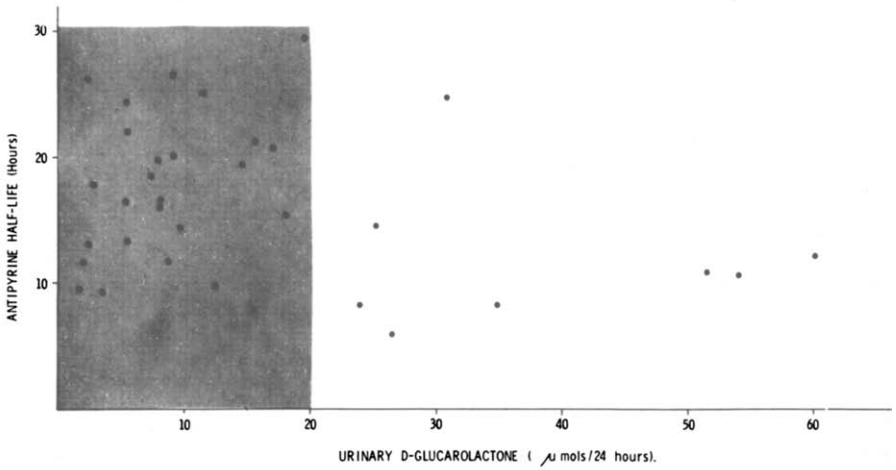


FIG. 2. The relationship between antipyrine half-life and urinary glucaric acid excretion in thirty-three normal subjects and ambulant patients. The normal range for glucaric acid excretion is shaded.

sequential studies, as in the first part of this investigation, does the activity of hepatic microsomal enzymes become a dominant influence on the rate of drug metabolism.

It may be concluded that, since genetic factors are usually more important than environmental ones in determining the rate of metabolism of drugs in man, little clinical benefit is likely to accrue from the routine determination of enzyme activity by such techniques as urinary excretion of D-glucaric acid. On the other hand, since the genetic factors which influence metabolism of one drug may also operate on another,[18] determination of the metabolism of a test substance may prove of value in predicting the rate of metabolism of other drugs, hence permitting the preparation of more accurate dosage regimes.

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