Enzyme Induction and Medical Treatment

JOHN HUNTER, MB, MRCP, Senior Medical Registrar,
Addenbrooke's Hospital, Cambridge

Most fat-soluble drugs are metabolised in the body to more polar compounds which may be excreted more readily. By far the most important site for this metabolism is the endoplasmic reticulum of the hepatocyte, where a system of remarkably non-specific enzymes catalyses a considerable variety of reactions. It is now twenty years since it was first appreciated that certain foreign compounds had the property of increasing the activity of these enzymes (Brown *et al.*, 1954). This increase in activity was subsequently found to be accompanied by hypertrophy of the endoplasmic reticulum and an increase in the rate of metabolism of many drugs. It is now realised that a wide variety of drugs and foreign compounds may produce this phenomenon which is known as enzyme induction (Table 1).

Interactions between enzyme-inducing drugs and anticoagulants such as warfarin can produce spectacular results. Co-administration of a barbiturate increases the rate of metabolism of warfarin so that a larger dose is required to produce anticoagulation. If the barbiturate alone is then stopped, enzyme activity decreases and the metabolism of warfarin is slowed, leading to overdosage, with a risk of haemorrhage, if the warfarin dosage is not also reduced. Less well known, perhaps, but often no less dramatic, is the effect of enzyme induction on corticosteroid metabolism. A patient recently in Addenbrooke's Hospital, whom I studied in collaboration with Dr O. M. Edwards, provides a good example.

Case Report

A man, aged 22, who had suffered from pulmonary tuberculosis as a child, complained of vomiting, weakness and loss of weight. On examination, extensive buccal pigmentation was noted and the standing blood pressure was only 80/50. Plasma electrolytes were abnormal, with sodium 124 mEq/litre, potassium 5·7 mEq/litre, chloride 85 mEq/litre, and urea 72 mg/100 ml. The plasma cortisol at 9 a.m. was 6 μg/100 ml and was not increased by Synacthen 0·25 mg. Chest X-ray showed reactivation of the old pulmonary tuberculosis.

A diagnosis was made of tuberculous Addison's disease, and he was started on isoniazid 300 mg daily and rifampicin 600 mg daily with a

TABLE 1. Increased hepatic microsomal enzyme activity in man

Inducing agent	Substrate affected	Reference
Antipyrine	Cortisol Warfarin	Breckenridge *et al.* (1971).
Barbiturates	Anticoagulants	Cuccinell *et al.* (1965).
	Bilirubin	Crigler and Gold (1966).
	Chlorpromazine	Forrest *et al.* (1969).
	Cortisol	Kuntzman *et al.* (1968).
	DDT	Davies *et al.* (1969)
	Desmethylimipramine	Hammer *et al.* (1967).
	Dexamethasone	Brooks *et al.* (1972).
	Digitoxin Diphenylhydantoin	Jelliffe and Blankenhorn (1966).
	Phenylbutazone	Levi *et al.* (1968).
	Testosterone	Southren *et al.* (1969).
	Vitamin D	Hahn *et al.* (1972a)
Cigarette smoking	Nicotine	Beckett and Triggs (1967).
	Pentazocine	Keeri-Szanto and Pomeroy (1971).
	Phenacetin	Pantuck *et al.* (1972).
Carbamazepine	Diphenylhydantoin Warfarin	Hansen *et al.* (1971).
pp′-DDT	Bilirubin	Thompson *et al.* (1969).
	Cortisol Phenylbutazone	Poland *et al.* (1970).
op′-DDD	Cortisol	Bledsoe *et al.* (1964).
Diphenylhydantoin	Cortisol	Werk *et al.* (1964).
	DDT	Davies *et al.* (1969).
	Dexamethasone	Jubiz *et al.* (1970).
	Dieldrin	Davies *et al.* (1971).
	Digitoxin	Solomon *et al.* (1971).
	Metyrapone	Meikle *et al.* (1969).
	Thyroxine	Larsen *et al.* (1970).

course of streptomycin 0·75 g daily. He also received standard steroid replacement therapy of fludrocortisone 0·1 mg b.d. and cortisone acetate 25 mg b.d.

Despite this treatment the patient remained ill, with repeated episodes of abdominal pain, nausea, vomiting, and hypotension. The plasma sodium concentration remained persistently low and on one occasion he required intravenous saline and hydrocortisone. Therapy was therefore increased to cortisone acetate 25 mg t.d.s. and fludrocortisone 0·4 mg daily. Only on this high dose of steroids did he become free of symptoms.

Rifampicin is known to cause enzyme induction in man (Jezequel *et al.*, 1971) and the patient was investigated to see if this could be the reason for his high steroid requirements. The urinary excretion of D-glucaric acid is a

Table 1 *continued*

Inducing agent	Substrate affected	Reference
Endrin (or related compound)	DDT	Hunter *et al.* (1972).
	Cortisol	Jager (1970).
Ethanol	Antipyrine	Vesell *et al.* (1971b).
	Bilirubin	Waltman *et al.* (1969).
	Diphenylhydantoin, Tolbutamide, Warfarin	Kater *et al.* (1969).
	Ethanol, Meprobamate, Pentobarbital	Rubin and Lieber (1971).
Eucalyptol	Aminopyrine	Jori *et al.* (1970).
Glutethimide	Vitamin D	Greenwood *et al.* (1973).
	Warfarin	MacDonald *et al.* (1969).
Griseofulvin	Warfarin	Catalano and Cullen (1966).
Lindane	Antipyrine	Kolmodin *et al.* (1969).
	Phenylbutazone	Kolmodin-Hedman (1973).
Marihuana smoking	Delta-9-tetrahydrocannabinol	Lemberger *et al.* (1971).
Medroxyprogesterone	Testosterone	Gordon *et al.* (1971).
Nikethamide	Bilirubin	Sereni *et al.* (1967).
Phenylbutazone	Aminopyrine	Chen *et al.* (1962).
	Cortisol	Kuntzman *et al.* (1966).
	Digitoxin	Solomon *et al.* (1971).
Rifampicin	Increased smooth endoplasmic reticulum	Jezequel *et al.* (1971).
	Corticosteroids	This paper
Tolbutamide	Ethanol	Carulli *et al.* (1971).

useful indirect index of hepatic enzyme activity in man (Hunter *et al.*, 1971a), and in this patient D-glucaric acid excretion was raised to 35 μmoles/24 hours (normal: less than 20 μmoles), confirming the presence of definite enzyme induction. The half-life of injected cortisol was found to be reduced to 58 minutes (normal range: 90 to 120 minutes). We were later able to confirm that this increased cortisol breakdown was an effect of rifampicin, for the drug began to make him vomit, so it was stopped and ethambutol 1,450 mg daily was substituted. This drug is not an enzyme-inducing agent. At first the steroids were not changed, but the patient's blood pressure subsequently rose to 170/100. The half-life of injected cortisol was now found to be 184 minutes and D-glucaric acid excretion had fallen to 15 μmoles daily. So his steroid therapy was reduced and he is now well and normotensive, with normal electrolytes, on cortisone acetate 25 mg b.d. and fludrocortisone 0·1 mg daily.

Cortisol is normally excreted via the liver. The A-ring is reduced, producing tetrahydrocortisol and tetrahydrocortisone, which, after conjugation with glucuronic acid, are readily excreted in the urine, where they form a large percentage of the urinary 17-hydroxycorticosteroids. Only 2 to 3 per cent of the daily cortisol production is normally excreted in the urine as unconjugated steroids (Mattingly, 1968). After treatment with drugs such as phenobarbitone and phenytoin, cortisol metabolism is markedly changed. Urinary 17-hydroxycorticosteroids are much reduced and there is increased production of an unconjugated polar metabolite, 6-beta hydroxycortisol (Werk *et al.*, 1964). In guinea-pigs an increased excretion of this compound is associated with stimulation of a microsomal enzyme that hydroxylates cortisol in the 6-position (Kuntzman *et al.*, 1968). The excretion of cortisol by this route appears to be more rapid; so much so that a number of drugs known to increase 6-beta hydroxylation of cortisol in man have been discovered to produce clinical improvement in patients with Cushing's syndrome. These include op'-DDD (Bledsoe *et al.*, 1964), phenytoin (Werk *et al.*, 1966), and phetharbital (Southren *et al.*, 1969).

Cortisol metabolism is also increased in normal subjects by enzyme-inducing drugs. Two years ago, Dr C. Baum and I studied three epileptic patients, aged 18 to 24, who were receiving large doses of several anticonvulsant drugs, including phenobarbitone, phenytoin, and primidone, all of which are potent enzyme-inducing agents. Cortisol production rates, measured by the technique of Cope and Black (1958), were increased beyond the upper limit of normal in each case. A similar finding has been reported in patients taking phenytoin alone (Werk *et al.*, 1971). The rapid rate of corticosteroid metabolism is presumably compensated in normals by increased ACTH production, but clinical problems may arise, as in our patient taking rifampicin, when steroid availability is limited. For example, steroid-dependent asthmatics suffered a deterioration in their asthma when given phenobarbitone, and this was associated with an increased rate of clearance of steroids from the plasma (Brooks *et al.*, 1972).

Another steroid whose breakdown may be increased by enzyme induction is dexamethasone (Jubiz *et al.*, 1970). In normal subjects the morning level of plasma cortisol after 2 mg of dexamethasone given the previous day should be less than 4 μg per 100 ml (Mattingly, 1968). In six subjects taking anticonvulsant drugs, we found a range of 6 to 22 μg/100 ml (Fig. 1). It seems likely that this failure of suppression may have been a consequence of increased dexamethasone metabolism following hepatic enzyme induction. This is obviously of importance in the interpretation of dexamethasone suppression tests. It has been suggested by Carroll *et al.* (1968, 1970) that impairment of dexametha-

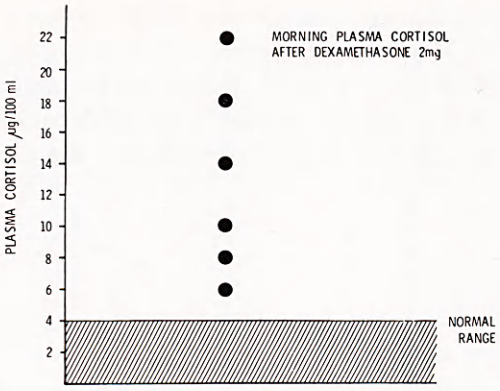

Fig. 1. Morning plasma cortisol levels in epileptic subjects taking anticonvulsant drugs who had been given dexamethasone 2 mg orally the previous day. The normal range is shaded.

sone suppression may be a consequence of psychiatric illness such as depression. However, many of the patients studied by these workers were receiving amylobarbitone, a potent enzyme-inducing agent which could quite conceivably have caused the abnormalities discovered.

VITAMIN D

The metabolism of the fat-soluble vitamin D may be modified by enzyme induction and osteomalacia may be a complication of treatment with anticonvulsant drugs (Kruse, 1968; Dent *et al.*, 1970); a low-plasma calcium concentration and a raised alkaline phosphatase are frequently found in epileptic patients (Richens and Rowe, 1970), even when their diet contains adequate amounts of vitamin D (Hunter *et al.*, 1971b). It is believed that increased breakdown of vitamin D using abnormal pathways in the liver as a consequence of enzyme induction may produce vitamin D deficiency and account for the low plasma concentrations of 25-hydroxycholecalciferol (the normal hepatic metabolite of vitamin D) which have been reported in these patients (Hahn *et al.*, 1972b). Stamp *et al.* (1972) reported that anticonvulsant osteomalacia could be corrected by administration of relatively small quantities of 25-hydroxycholecalciferol. We recently studied the effect of various vitamin D supplements on epileptic children who had abnormally high serum levels of alkaline phosphatase. The children were divided into three groups; one group acted as controls and received oral nut oil daily; a second group received oral vitamin D_2 (which is believed to be as active in man as the natural vitamin D_3)

1,000 IU daily; a third group was given weekly intramuscular injections of 8,000 IU vitamin D_2. Serum calcium and alkaline phosphatase levels were determined at intervals up to 12 weeks. The values for both were virtually unchanged throughout this period both in the controls and in those receiving intramuscular vitamin D (Fig. 2), as were those in the third group who re-

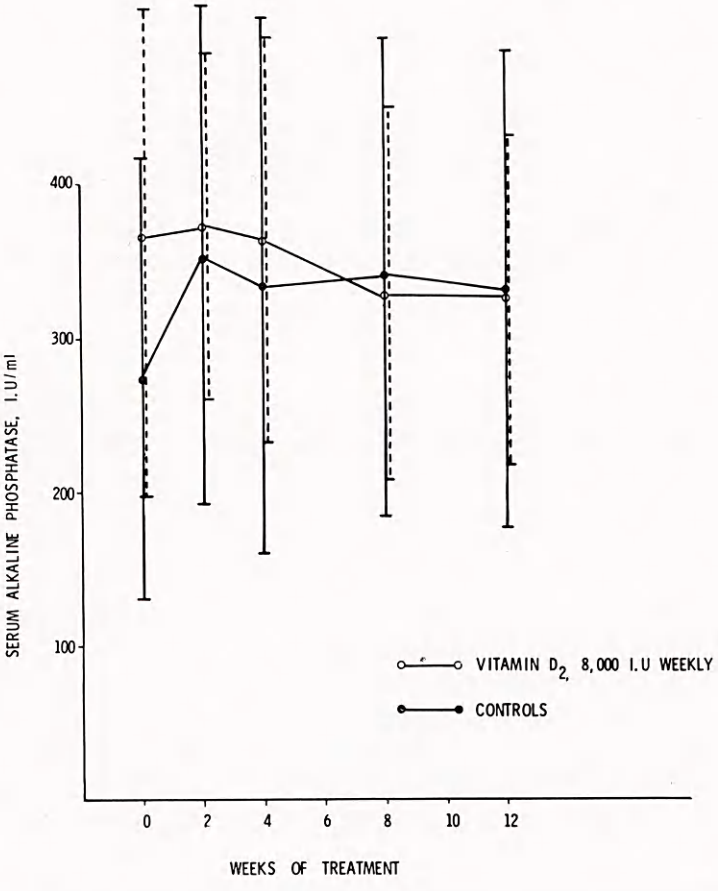

Fig. 2. Values for serum alkaline phosphatase in epileptic children taking anticonvulsant drugs who had been given intramuscular vitamin D compared with controls.

ceived oral vitamin D. Five children, however, were subsequently given a four-week course of 400 IU daily of oral 25-hydroxycholecalciferol. After two weeks of treatment the serum alkaline phosphatase had fallen in all five and the reduction was even more marked after four weeks (Fig. 3).

Thus, 25-hydroxycholecalciferol appears considerably more effective in the treatment of anticonvulsant osteomalacia than is vitamin D itself. We have also found that enzyme-inducing drugs may reduce the half-life of radioactive vitamin D to as little as six or seven hours, but that the half-life of 25-hydroxycholecalciferol is several days even in subjects taking large doses of anticonvulsant drugs (Hunter *et al.*, 1973a). It seems likely that this is because

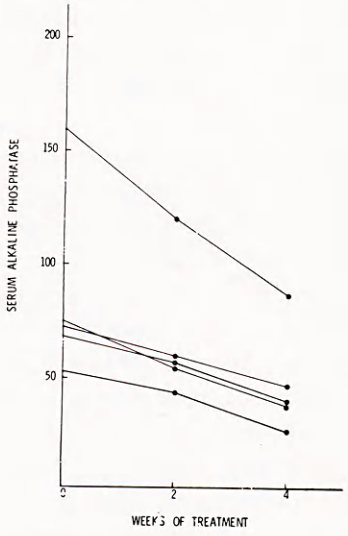

Fig. 3. Values for serum alkaline phosphatase in five epileptic children treated with 25-hydroxycholecalciferol 400 IU daily for four weeks.

25-hydroxycholecalciferol is more water-soluble than vitamin D. It is therefore a less suitable substitute for microsomal enzymes, and less affected by enzyme induction.

Many examples of enzyme induction affecting drug action in man are now known and some of the important ones are listed in Table 1. Although the common effect of enzyme induction is to reduce the effect of drugs, some substances are metabolised to more active compounds by hepatic microsomal enzymes and the effect of induction on these substances may be to lead to toxicity. An example is carbon tetrachloride in the rat (Garner and McLean, 1969). It has been suggested but not proven that the toxicity of anaesthetics such as halothane (Stenger and Johnson, 1972) and fluroxene (Reynolds *et al.*, 1972) may be increased by drugs such as phenobarbitone.

THE RELATIVE IMPORTANCE OF ENZYME INDUCTION IN DETERMINING THE RATE OF DRUG METABOLISM

Considerable differences exist between individual patients in their susceptibility to various drugs. Although enzyme induction is one factor that may alter the rate of drug metabolism, many others may be equally, if not more, important. Genetic differences in drug metabolism are well known (Vesell and Page, 1969) and there may also be differences in drug absorption, binding to plasma proteins and distribution within the body. Dr W. R. Burnham and I, in collaboration with Dr L. F. Chasseaud and Mr W. D. Down, have been using D-glucaric acid to study the relationship between enzyme induction and the rate of metabolism of antipyrine.

There is now considerable evidence that the urinary excretion of D-glucaric acid, a metabolite of the glucuronic acid pathway of the liver, is a reliable, although indirect, quantitative index of human microsomal enzyme activity. The similarities between urinary glucaric acid excretion and known facts of microsomal enzyme activity in experimental animals are remarkable. Both are low in the neonate (Mowat, 1968) and have a diurnal variation with a peak in the late afternoon and evening (Radzialowski and Bousquet, 1968). Both increase following administration of compounds as widely different as barbiturates, rifampicin and organochlorine pesticides, and there is a direct relation between the amount of glucaric acid and the dose of drugs taken. There is also a significant inverse correlation in man between glucaric acid excretion and the blood level of substances metabolised by microsomal enzyme systems such as bilirubin and pp′-DDE (the major metabolite of DDT) (Hunter *et al.*, 1971a, 1971c, 1972). Furthermore, in guinea-pigs given phenobarbitone 50 mg/kg intraperitoneally for 0 to 5 days there was a good correlation between the total liver content of the microsomal enzyme cytochrome P-450 and glucaric acid excretion in the 24 hours before death, a relationship that still obtained when actinomycin-D 1 mg/kg was given with the phenobarbitone to prevent enzyme induction (Hunter *et al.*, 1973b).

In the first experiment we attempted to eliminate other factors affecting drug metabolism by studying antipyrine clearance in the same subjects before and after a course of treatment with an enzyme-inducing drug. Twelve normal medical students and nurses took part, ages ranging from 19 to 28. Urinary D-glucaric acid excretion was measured at the beginning of the experiment and the antipyrine half-life was then determined using the technique described by Vesell *et al.* (1971a). Subjects then received a week's treatment with varying doses of either phetharbital or glutethimide, following which the 24-hour glucaric acid excretion and antipyrine half-life were repeated. Changes in antipyrine metabolism after induction were expressed by calculating the

second determination as a percentage of the first, and were plotted against the post-treatment glucaric acid excretion (Fig. 4).

The doses of drugs administered were not always sufficient to produce enzyme induction, and in some cases there was an increase in the antipyrine half-

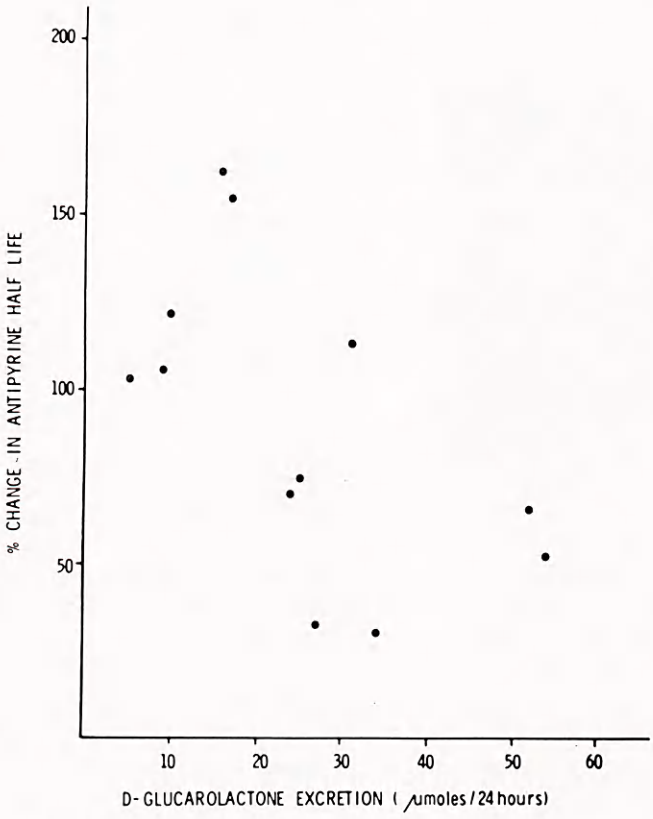

Fig. 4. Change in antipyrine half-life expressed as a percentage in relation to urinary glucaric acid excretion in normal subjects given various enzyme inducing drugs.

life. There was, however, a significant correlation ($r = -0{\cdot}59$, $p < 0{\cdot}05$) between glucaric acid and the change in the antipyrine half-life, confirming the importance of enzyme induction in determining the rate of drug metabolism when other factors are kept constant.

In a second experiment, however, the rate of antipyrine metabolism was compared directly with glucaric acid excretion in 33 normal volunteers and ambulant patients, some of whom were receiving no treatment whatsoever and some of whom were taking a variety of drugs including barbiturates,

glutethimide, anticonvulsants and oral contraceptives. A 24-hour urine save was made for glucaric acid. On the subsequent day the antipyrine half-life was determined as before.

The results are shown in Fig. 5. There is a wide range of antipyrine half-life, ranging from 6 to 29 hours. Although most of the subjects who had raised glucaric acid excretion had short half-lives, there was considerable variation

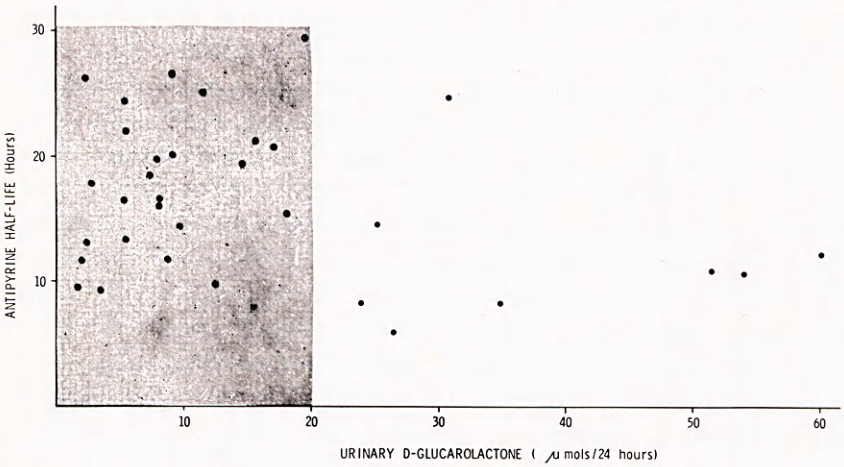

Fig. 5. The relationship between antipyrine half-life and glucaric acid excretion in normal subjects and patients receiving various drugs. The shaded area represents normal glucaric acid excretion.

in the antipyrine half-life in those subjects who had normal hepatic enzyme activity, and overall the correlation between drug half-life and the glucaric acid excretion was not significant ($r = -0{\cdot}28$, $p > 0{\cdot}1$).

This work indicates that, although enzyme induction may have dramatic effects in altering a patient's response to a drug, other factors are more important in determining the absolute rate of drug metabolism. Studies in monozygotic and dizygotic twins have suggested that genetic factors are the most important, controlling the rate of metabolism of drugs as widely different as nortriptyline (Alexanderson *et al.*, 1969), halothane (Cascorbi *et al.*, 1971) and ethanol (Vesell *et al.*, 1971b). It therefore seems unlikely that routine determination of hepatic enzyme activity by such techniques as glucaric acid excretion will increase the accuracy of drug dosage regimes or prevent the toxic side-effects that may result from enzyme induction. The best defence against the therapeutic difficulties caused by enzyme induction will remain the alertness of the physician and his awareness of the drugs likely to produce it.

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