The distribution and induction of some drug-metabolizing enzymes in man

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The activities of certain drug-metabolizing enzymes were measured in visibly and histologically normal hepatic and extrahepatic surgical tissues. Fresh material was homogenized and sub-cellular fractions prepared using standard techniques. Two fractions, namely the supernatant of the 10,000 g×10 min fraction and the 105,000 g× 60 min (microsomal) fraction were used as sources of the various enzymes. Cytochrome P_{450}, hexobarbitone oxidase, ethylmorphine-N-demethylase, *p*-nitroreductase, UDP-glucuronyl transferase and the L-leucyl-β-naphthylamide splitting enzyme were chosen as representatives of the major classes of enzyme systems, namely oxidation, reduction, conjugation and hydrolysis, involved in the metabolism of foreign substances in the human body.

Enzyme activities were measured by standard methods in liver, kidney, placenta, lung, stomach, colon, spleen and prostate. For example, hexobarbitone oxidase was found in all tissues except prostate and had relative activities varying from 18·9% in the colon to 104% in the placenta (liver=100%). Ethylmorphine N-demethylase, however, was found only in the kidney and had a relative activity of 7·5% while cytochrome P_{450} was not detected in extrahepatic tissues. On the other hand, *p*-nitroreductase and UDP-glucuronyl transferase were found in most extrahepatic tissues but at levels less than 50% of the activities of the liver. The relative activities of L-leucyl-β-naphthylamide splitting enzyme which is considered to be involved in the metabolism of exogenous insulin varied from 35% in the lung to 352% in the kidney. Activities of the various hepatic drug-metabolizing enzymes as measured in the present work were higher than those obtained previously by Darby, Newnes & Price Evans (1970) from post-mortem specimens but were of the same order as those of Schoene, Fleischmann, Remmer & Oldershausen (1972) obtained in needle biopsy material. Comparison of the activities of hepatic microsomal enzymes between man and rat showed that they were higher in the latter species.

Preliminary results in 6 patients who received phenobarbitone before surgery suggests that daily divided doses of 90 mg for up to 10 days do not appear to induce drug-metabolizing enzymes in extrahepatic tissues.

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Dose-dependent enzyme induction

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Many drugs and other chemicals have been classified as inducers or non-inducers of liver microsomal drug metabolizing enzymes based on studies at one dose level. The purpose of the present study was to investigate if it is more meaningful to perform such studies at several dose levels. This has been done by administering varying doses of inducing agents to both man and rat and observing the degree of enzyme induction produced.

Administration of quinalbarbitone 100 mg nightly for 33 days caused a significant ($P<0·01$) fall in the steady state plasma warfarin concentration and rise in thrombotest

percentage in four of six patients. The percentage fall in steady state plasma warfarin concentration varied between 27 and 64%. In the other two patients, who showed no significant change in the plasma warfarin concentration, warfarin half-life or thrombotest percentage, the nightly dose of quinalbarbitone was increased on a subsequent occasion to 200 mg. This caused a significant fall in the steady state plasma warfarin concentration of 33·3% in one patient and 40·5% in the second patient. These changes were accompanied by significant increases in the thrombotest percentage. The plasma warfarin half-life decreased during the administration of quinalbarbitone 200 mg nightly in both, there being a fall of 59% in the first patient and of 28% in the second patient. On a further occasion the nightly dose of quinalbarbitone to the first patient was increased to 300 mg for a 33 day period. The changes produced were not significantly different from those produced by 200 mg of quinalbarbitone at night. Measurements of the plasma concentration of quinalbarbitone showed inter-individual differences with steady state concentrations varying from 0·38 to 1·21 μg/ml but the degree of induction produced by quinalbarbitone in the various subjects correlated poorly with both the plasma concentration of quinalbarbitone ($r=0·2$) and with the initial rate of warfarin metabolism ($r=0·18$).

The response to varying doses of four enzyme inducing agents—phenobarbitone, amylobarbitone, quinalbarbitone and antipyrine were studied in rats using the changes in pentobarbitone sleeping time and in the V_{max} for the N-demethylation of ethylmorphine by rat liver microsomes. The drugs were given by intraperitoneal injection to groups of rats at least 6 dose levels. Different dose response curves were obtained for the four inducing agents and phenobarbitone was shown to be at least twenty times more potent an inducing agent than the other drugs. This difference in potency is partly explained by a longer plasma half-life of phenobarbitone (241 min) than of amylobarbitone (34 min) and quinalbarbitone (45 min), and partly by a higher liver to plasma ratio for phenobarbitone (3·5 : 1) compared to amylobarbitone (1·6 : 1) and quinalbarbitone (2·0 : 1).

Effects of metformin on glucose uptake by isolated human skeletal muscle

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The hypoglycaemic effect of the biguanide antidiabetic drugs is seen only in diabetic patients (Madison & Unger, 1960). It has been suggested that these drugs act by increasing uptake of glucose into skeletal muscle (Butterfield, 1968). *In vitro* studies using the isolated rat diaphragm preparation have shown that metformin in a therapeutic concentration is without effect on glucose uptake by diaphragm muscle from normal rats, but increases uptake by muscle from alloxan-diabetic rats (Adnitt & Frayn, 1972). The effect is also seen in normal diaphragm muscle incubated in a medium containing n-butyrate (2·27 mM) which induces a metabolic pattern similar to that seen in diabetes. These results suggest that, in rat muscle, metformin increases intracellular utilization of glucose 6-phosphate rather than acting at the level of membrane transport (Frayn & Adnitt, 1972).

Confirmation of these effects in man is important in view of the between-species differences in response to the biguanides. Direct measurement of peripheral glucose uptake in man is, however, difficult. Studies were therefore carried out on the effects of metformin on glucose uptake by human skeletal muscle *in vitro*.

Pieces of human skeletal muscle removed at surgery (from the rectus abdominis or gluteus maximus muscles) were dissected along the fibres into pieces weighing from 100 to 250 mg. Subsequent treatment of the tissue and measurement of glucose uptake followed the method used with the isolated rat diaphragm (Vallance-Owen & Hurlock, 1954). Glucose uptake by such muscle pieces is stimulated by a physiological concentration of insulin (100 μU/ml), and impaired by incubation with n-butyrate (2·27 mM) (Frayn, Adnitt & Turner, 1972).