acetoacetate and β-hydroxybutyrate concentrations, in the activities of enzymes concerned with hepatic acetoacetate formation and the activity of a key enzyme involved in ketone-body utilization by brain and heart.

Highest blood concentrations of acetoacetate are found at 5 days of age, after which time the concentration falls to reach the adult value by 30 days of age. Blood concentrations of β-hydroxybutyrate follow a developmental pattern similar to those of acetoacetate but increase to a greater extent during suckling. Both mitochondrial and cytoplasmic hydroxymethylglutaryl-CoA synthase (EC 4.1.3.5) activities were detected, with highest activities being found in the mitochondria at all stages of development. Activity of the mitochondrial enzyme increases rapidly immediately after birth, thereafter exhibiting a similar developmental pattern to blood ketone bodies. The cytoplasmic enzyme, on the other hand, increased steadily in activity after birth to reach a maximum at 40 days of age, after which time activity fell to adult values. Both mitochondrial and cytoplasmic acetoacetyl-CoA thiolase (EC 2.3.1.9) activities were detected, with the mitochondrial enzyme having considerably higher activities at all stages of development. The developmental patterns for both enzymes were very similar to those for the corresponding hydroxymethylglutaryl-CoA synthases. The activity of heart acetoacetyl-CoA transferase (3-oxo acid CoA-transferase, EC 2.8.3.5) remains constant from late foetal life until the end of the suckling period, after which time there is a gradual threefold increase in activity to reach the adult values. The activity of brain acetoacetyl-CoA transferase increases steadily after birth, reaching a maximum at 30 days of age, thereafter decreasing to adult values, which are similar to foetal activities.

Page, Krebs & Williamson (1971) have reported results for blood ketone bodies and brain and heart acetoacetyl-CoA transferase that are similar to those presented here.

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Significance of the Caffeine-Like Effect of Various Purines, Pyrimidines and Derivatives on Adipose-Tissue Phosphodiesterase

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Mammalian tissues are exposed to a variety of purines, pyrimidines and derivatives as endogenous metabolites, food constituents and drugs. The disturbances of metabolism brought about by one such group of substances, the methylxanthines, can largely be related to the elevation of tissue concentrations of cyclic AMP (adenosine 3′:5′-cyclic monophosphate) due to inhibition of the enzyme 3′:5′-cyclic nucleotide phosphodiesterase (Brodie, Davies, Hynie, Krishna & Weiss, 1966). We have compared the inhibitory effect of caffeine on this enzyme with that of other purines, pyrimidines and derivatives to which tissues may be exposed.

The phosphodiesterase of rat epididymal adipose tissue was shown to be probably heterogeneous, since it yielded K_m values that differed according to the range of substrate concentration used. Of the nucleosides and corresponding free bases tested only caffeine appreciably inhibited the fresh enzyme. However, after a 16h dialysis the enzyme was more susceptible to inhibition by inosine, guanosine or deoxyadenosine than by caffeine. This transformation was reversed by mercaptoethanol.

Isolated fat-cells derived from epididymal adipose tissue yielded a phosphodiesterase with K_m 1×10^{-5}M. Under circumstances where caffeine (1mM) caused 51% inhibition, this enzyme preparation was inhibited by various nucleosides and free bases, e.g. deoxyadenosine (1mM), 55%, adenosine (1mM), 30%, adenine (1mM), 29%, and guanosine (0.5mM), 24% (substrate concentration, 1μM). The actions of inosine and adenosine were shown to have competitive kinetics.

The inhibitory effect of caffeine on phosphodiesterase activity is well correlated with its effect on lipolysis in the intact fat-cell (Brodie *et al.* 1966). This correlation could be extended in some but not all instances to the selection of nucleosides and free bases tested (Davies, 1968).

Preliminary studies have been carried out to determine the extent to which the caffeine-like effects of these nucleosides and free bases could be manifested *in vivo*. Administration by stomach tube into rats (100mg/rat) led to responses in plasma non-esterified fatty acids and glucose that were generally small compared with those due to caffeine (50mg/rat). Work is in progress to determine the extent to which this is due to the detoxifying activity of the gut, liver etc.

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The Metabolism of 4-Chloro-2-Methylphenoxyacetic Acid in Plants

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The results described below were obtained from an investigation into the metabolism of the selective herbicide MCPA* in rape (*Brassica napus* var. *arvensis* var. Early Giant), a susceptible species, and in red campion (*Melandrium rubrum*) and peas (*Pisum sativum* var. Progress no. 9), two species that possess some resistance to the herbicide.

The presence of two ether-soluble metabolites, rigorously identified in peas as 4-chloro-2-hydroxymethylphenoxyacetic acid (hydroxy-MCPA) (Collins & Gaunt, 1970) and *N*-(4-chloro-2-methylphenoxyacetyl)-L-aspartic acid (MCPA-aspartic acid), was confirmed in rape and red campion by using radioactive-isotope-dilution analysis. A further ether-soluble metabolite, which was detected in peas, has not been identified.

A β-glycoside of hydroxy-MCPA was detected in all species and tentatively identified as 4-chloro-2-(β-D-glucopyranosidomethyl)phenoxyacetic acid. A β-linked sugar ester of MCPA was detected in rape and tentatively identified as 4-chloro-2-methylphenoxyacetyl-β-D-glucose. A further minor unidentified β-glycoside was detected in peas.

MCPA-aspartic acid, hydroxy-MCPA and the β-glucoside of the latter were synthesized and examined for auxin activity by using the *Avena* first-internode and pea third-internode bioassays. MCPA-aspartic acid showed growth-promoting effects similar to those of MCPA. Hydroxy-MCPA possessed some auxin activity at higher concentrations (10mg/l), but its β-glucoside was virtually inactive. After foliar applications to intact plants of each species hydroxy-MCPA was completely inactive. MCPA-aspartic acid, however, produced phytotoxic effects comparable with those produced by MCPA itself.

Foliar applications of ^{36}Cl- or *carboxy*-^{14}C-labelled MCPA were made to 21-day-old plants of each species and the distribution of MCPA and its metabolites was followed during the next 14 days. In rape about 50% of the label that entered the treated leaves was rapidly translocated to the rest of the plant. In peas and red campion translocation was markedly less extensive.

* Abbreviation: MCPA, 4-chloro-2-methylphenoxyacetic acid.

Differences were also evident in the patterns of metabolism in the three species. Rape showed conversion of MCPA, with MCPA-aspartic acid as the major product. In peas and red campion hydroxy-MCPA and the β-glycosides were predominant.

It seems probable that the differences in phytotoxicity of MCPA with these plants can be largely attributed to differential translocation and to a smaller extent to differential metabolism.

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The Subcellular Distribution of Tocopherols in the Green Leaves of *Pisum sativum*

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Previous studies on the subcellular distribution of tocopherols in plant tissues (Bucke, 1969; Dilley & Crane, 1963; Lichtenthaler, 1966; Peake, 1970) have been restricted by the limited ability of differential centrifugation to resolve cellular organelles and by the low capacity of density-gradient centrifugation in conventional rotors. Also, the recoveries of tocopherols after homogenization and centrifugation have often been very low. This could be attributable to the destructive action of tocopherol oxidase, an enzyme found in many plant tissues (Barlow & Gaunt, 1968).

In the present work the zonal rotor has been used to achieve large-capacity density gradients. Effective inhibitors of tocopherol oxidase have been incorporated in homogenization and centrifugation media. Green-leaf homogenates were fractionated into three fractions in the zonal rotor: a nuclear fraction, chloroplast fraction and a mixture of mitochondrial, microsomal and supernatant fractions. The last was then subjected to differential centrifugation at 20000*g* and 100000*g* to separate the three parts. Each fraction was analysed for tocopherols and related isoprenoid lipids.

The recoveries of tocopherols from the homogenization and centrifugation procedure were as follows: α-tocopherol, 65%; γ-tocopherol, 82%; δ-tocopherol, 87%; α-tocopherolquinone, 66%.

The purity of each subcellular fraction was assessed by analysis of marker compounds and by