220 mg of butylated hydroxytoluene/kg or 100 mg of phenobarbitone/kg. Adrenalectomy diminished the initial effect of coumarin on microsomal amino acid incorporation \textit{in vitro}. However, the coumarin effect on microsomal protein synthesis could not be eliminated by the removal of either the pituitary or thyroid gland. Although the doses of coumarin used in these experiments are toxic and expected to impose stress on the animals, changes in the rate of amino acid incorporation \textit{in vitro} following single or repeated doses of coumarin treatment could not be entirely ascribed to stress-like phenomena. Stimulation of amino acid incorporation into protein \textit{in vitro}, or \textit{in vivo}, and the induced liver growth, which followed butylated hydroxytoluene and phenobarbitone treatment, were not influenced by the removal of adrenal, pituitary or thyroid glands.

These results suggest that although non-toxic inducers such as butylated hydroxytoluene increase the adrenal weight, steroid or thyroid hormones do not participate in the initial triggering effect of butylated hydroxytoluene or phenobarbitone on the onset of liver enlargement. The initiation of coumarin-induced 'toxic hepatomegaly' however, may be mediated by some effect of the adrenal hormones.


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Histochemical and Ultrastructural Analysis of the Effect of Electromagnetic Radiation on Rat Liver and Brain

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Electromagnetic radiation of various frequencies induces a variety of molecular and biochemical effects in normal and tumour tissues (Marha \textit{et al.}, 1971). However, it is difficult to compare most of the published data because in no previous studies were rigorous criteria adopted to establish a dose–response relationship and also various ranges and wave characteristics were used by individual authors. As part of a large research programme to study the 'non-thermal' effect of a wide frequency range of electromagnetic radiation pulses, we report our preliminary observation on the ultrastructural and cytochemical effect of pulsed electromagnetic radiation in rat liver.

White female Wistar rats (100 g body wt.) were used in these experiments. The animals were placed in restraining cages in such a way that the regions overlying the liver were freely exposed to electromagnetic radiation at a distance of 12.7 cm from the electromagnetic radiation generating probe. Groups of rats were irradiated for 10 min by a 'Diapulse' generator. The 27 MHz carrier frequency of electromagnetic radiation was amplitude-modulated into a series of pulses. In our initial studies we used 300 and 500 pulses/min. The duty cycle of the pulsed electromagnetic radiation was 2.4 and 4% at 300 or 500 pulses/min with a 'Diapulse' penetration setting of 3 and 5 respectively. The animals were killed and samples from the rapidly excised livers and brains were used for light- and electron-microscopic studies and for the assessment of various enzyme levels. The activities of succinate dehydrogenase, isocitrate dehydrogenase, lactate dehydrogenase, glucose 6-phosphate dehydrogenase, NADH-diaphorase, acid phosphatase, ATPase (adenosine triphosphatase), 5'-nucleotidase, \( \alpha \)-glycerophosphate dehydrogenase, alkaline phosphatase and monoamine oxidase, were determined histochemically.
In rats exposed to either 300 or 500 pulses/min no significant light-microscopic or electron-microscopic changes were observed. The activities of alkaline phosphatase, 5'-nucleotidase, isocitrate dehydrogenase, α-glycerophosphate dehydrogenase, lactate dehydrogenase, NADH-diaphorase and monoamine oxidase did not change significantly in brain or liver of animals exposed to either 300 or 500 pulses/min. Rats irradiated for 10 min with 500 pulses/min had, however, decreased glucose 6-phosphate dehydrogenase and succinate dehydrogenase activities around the central veins of the liver. ATPase activity was lower in the proximity of the bile canaliculi. Acid phosphatase was more diffuse in the cytoplasm of the irradiated liver than in that of the control. In the brain, however, no enzyme changes were observed in rats irradiated with either 300 or 500 pulses/min.


The Immobilization of Enzymes on Inorganic Supports

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The immobilization of lactoperoxidase on the ferromagnesium silicates, hornblende, biotite and muscovite, has been reported (Johnson et al., 1974). In our laboratories the suitability of these materials for the immobilization of other enzymes is under investigation. These investigations are along two broad lines. One line aims to establish the compatability of mineral surfaces, modified by various linkage techniques, with the successful binding, and long-term stability of enzymes. The other line of study aims to assess the suitability of mineral particles to the operation of enzyme reactors, with special reference to pressure decrease in plug-flow reactors, to possible problems of leaching of ions from the minerals, and to the long-term stability of the particles under process conditions. In the present paper studies on the immobilization of β-fructofuranosidase, creatinine hydrolase (creatininase) and β-galactosidases are reported.

Mineral samples were obtained and processed as previously reported (Johnson et al., 1974). Particles passing through nylon mesh having an average aperture of 125 μm, but retained by a mesh of average aperture 63 μm, were used in these studies. Porous glass (CPG-10, 200–400 mesh, average pore diameter 209 nm) and mineral particles were activated for enzyme attachment by the metal-link method (with TiCl₄) described by Emery et al. (1972), and by a process using a silane derivative and glutaraldehyde (Robinson et al., 1971). β-Fructofuranosidase and β-galactosidases were immobilized as described previously (Thornton et al., 1974; Byrne & Johnson, 1974). Creatininase was immobilized by stirring a solution of enzyme (1.0 mg in 10 ml of buffer; in the silane process at pH 6.5; in the metal-link process at pH 4.5 and 7.5) with 1 g of activated support.

Soluble and immobilized β-fructofuranosidase [Grade III, Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K.] were assayed as described by Thornton et al. (1974). The methods described by Byrne & Johnson (1974) were used to assay soluble and immobilized β-galactosidases. These were from Saccharomyces fragilis (type II, Sigma Chemical Co.) and from Aspergillus niger (Lactase LP, a gift from Wallerstein Laboratories, Mariner's Harbor, Stratien Island, N.Y., U.S.A.). Creatininase [Boehringer Corp. (London) Ltd., Ealing, London W.5, U.K.] was assayed by using alkaline picrate (Owen et al., 1954). Creatininase concentration in assay mixtures was 0.75 mM in phosphate buffer at pH 7.5. Immobilized creatininase was assayed in shaken vessels at 37°C at pH 7.5 in the case of the silane process, and at pH 6.5 and 7.5 in the case of the metal-link process. Units of activity were defined as follows: for β-fructofuranosidase that activity hydrolysing 1 μmol of sucrose/h under assay conditions; for creatininase, that activity hydrolysing 1 nmol of creatinine/min under assay conditions; for