skeleton. In combination with 2,4-dinitrophenol the frequency of insulin-induced defects of the maxilla was greatly raised, but the incidence of other abnormalities remained unaffected. When the administration of insulin was combined with that of *p*-hydroxymercuribenzoate, the results were the opposite to those just discussed, that is, the teratogenic effect of insulin on long bone development (micromelia) showed potentiation, but the incidence of abnormalities of the facial skeleton was not influenced. There was, finally, no quantitative alteration in the teratogenic effects of insulin by the added administration of carbonyl *m*-chlorophenylhydrazone.

It has been clearly shown "that chlorpromazine and dinitrophenol act at different points in the sequence of phosphorylation reactions"[7], and it seems likely that multiple loci of such interference exist[8]. This is in good agreement with our findings. There is also much evidence for similarities between insulin and uncoupling agents in their effects on metabolic events, and especially on dehydrogenase activity[9,10]. It is true that the uncoupling agents are not altogether specific for the enzymatic events of oxidative phosphorylation[11], but since many of our earlier observations had already pointed to interference with dehydrogenase activity as the most likely primary step by which insulin interferes with normal development[12], the present results may be taken as strong supporting evidence. The recent conclusion by Eisenhardt and Rosenthal[13] that "uncoupling agents operate by interfering with the synthesis of high-energy intermediates" may well apply to our material.

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Metabolic Fate of some Chlorinated Phenoxyacetic Acids in the Stem Tissue of *Avena sativa*

AN extension of the previous work[1] on the hydroxylation of phenoxyacetic acid by mesocotyl tissue of *Avena sativa* has demonstrated a broad relationship, at least along one pathway of metabolism, between the fate of certain phenoxyacetic acids and their pattern of ring substitution.

The techniques used are the same as those already reported. All metabolites were isolated in mg quantities by solvent extraction followed by chromatography. Characterization of the metabolites or their derivatives was by comparison with authentic compounds.

Phenoxyacetic acids with an unsubstituted 4 position were hydroxylated at that position, and the resulting phenolic acid was accumulated as the 4-O-β-D glucoside. Thus 2-chlorophenoxyacetic acid was converted into a more polar compound which could be hydrolysed with acid or β-glucosidase to glucose and a phenolic acid (m.p. 146° C). The acid showed selective light absorption in the ultra-violet region, λ_{max} 288 mμ at *p*H 2; λ_{max} 306 mμ at *p*H 12. This phenolic acid corresponds exactly, both in terms of these characteristics and the infra-red spectrum, with 2-chloro-4-hydroxyphenoxyacetic acid[2].

Similarly 2,6-dichlorophenoxyacetic acid was converted into a glucoside which yielded glucose and a phenolic acid on acid or enzymatic hydrolysis. Elementary analysis of the ammonium salt of this glucoside supports the formulation as a mono glucoside of a phenolic acid derived from 2,6-dichlorophenoxyacetic acid (found: C, 39·2; H, 4·77; Cl, 16·65; calc. for $C_{14}H_{19}NO_9Cl_2$: C, 40·5; H, 4·6; Cl, 16·87). This phenolic acid had λ_{max} 283, 287 mμ at *p*H 2 and λ_{max} 302 mμ at *p*H 12 and was identical in those respects with authentic 2,6-dichloro-4-hydroxyphenoxyacetic acid.

In contrast, phenoxyacetic acids with a chlorine atom at position 4 were not hydroxylated to any appreciable extent, but neutral products were obtained which proved to be substituted phenoxyacetylglucoses. Compounds of this type were obtained from 4-chlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid (2,4-D). The ultra-violet spectra of these metabolites were identical with their parent acids. The infra-red spectrum of the 2,4-D metabolite exhibited strong absorption at 3,300 cm^{-1} and 1,050–1,100 cm^{-1} characteristic of hydroxyl groups in the sugar moiety. Acid or enzymatic hydrolysis yielded equimolar quantities of glucose and the parent acid. The 2,4-D metabolite was obtained in the crystalline form, and was acetylated to give a material having an infra-red absorption spectrum identical with that of 1-O-2,4-dichlorophenoxyacetyl - 2,3,4,6 - tetraacetyl - β - D - glucose, which was obtained by condensing tetraacetylbromoglucose with the silver salt of 2,4-D.

The metabolic fate of 2,4,6-trichlorophenoxyacetic acid is exceptional: hydroxylation occurs at position 3 and the glucoside was isolated. Enzymatic hydrolysis yielded a phenolic acid having λ_{max} 291 mμ at *p*H 2 and λ_{max} 308 mμ at *p*H 12 identical with synthetic 3-hydroxy-2,4,6-trichlorophenoxyacetic acid.

In the case of 2,6-dichlorophenoxyacetic acid, in addition to the glucoside already described, a glucose ester of the unchanged acid was isolated.

Since the hydroxylation of auxins, benzoic acid, and cinnamic acids has been demonstrated by other workers[3–5], there can be little doubt that an enzyme system capable of effecting this transformation exists in plant tissues.

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Detection of 'Activated' Compounds on Paper

THE presence of acylating compounds on paper is frequently deduced from reaction with hydroxylamine and subsequent treatment with ferric chloride to reveal the hydroxamates so formed. This method has been used for the detection of nucleotide peptides and similar 'activated' complexes[1–8].