change of enzyme structure. On testing formolized enzyme, it is found that the hydrolytic activity against salicin is specifically decreased. Moreover, the relative specificity is affected in the same way and to the same extent with formalin for two, three and six days respectively. These results indicate that chemical reaction is completed at least within two days under these conditions. From the point of view of species specificity of the enzyme, it should be noted that '*Aspergillus niger* β-glucosidase' is resistant to formalin, whereas almond emulsin is very sensitive[5,6]. The reaction of protein with formalin is more complicated than simply the formation of methylene compounds. In any event, free amino groups are blocked in addition to changes in other groups such as indole nuclei and imidazol rings. In the hydrolysis of *n*-alkyl-β-D-glucosides, nearly all the β-glucosidases from different sources exhibit an increase in the ease of hydrolysis with increasing chain-length except in the case of *Aspergillus niger* β-glucosidase[6,7]. In this respect it is significant that, after treatment with nitrous acid or formalin, *Aspergillus niger* β-glucosidase also shows a small increase with increasing chain-length.

There still remains, however, the question whether or not we are able to change greatly the enzymatic properties without losing its activity.

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Fate of Sodium 2,4-Dichloro-phenoxy-ethyl-sulphate in the Soil

SOME additional aspects of the "Fate of Sodium 2,4-Dichloro-phenoxy-ethyl-sulphate" which Prof. L. J. Audus has discussed[1] may possibly lend further understanding to this interesting problem.

Research on sodium 2-(2,4-dichloro-phenoxy)ethyl-sulphate for the past three years in this laboratory has indicated that the compound is readily hydrolysed under acid conditions to 2-(2,4-dichloro-phenoxy)-ethanol and sodium acid sulphate[2]. It has also been found that the soil organism, *Bacillus cereus* var. *mycoides* (Flügge) Smith, Gordon and Clark, possesses enzymes which can catalyse this hydrolysis[3,4]. The enzymatic conversion occurs in soil as well as *in vitro*[4].

An important point for consideration is the fact that 2-(2,4-dichloro-phenoxy)ethanol, which is quantitatively generated when sodium 2-(2,4-dichloro-phenoxy)ethyl-sulphate is added to soil[4], is a highly potent root growth inhibitor. It is extremely difficult to distinguish between 2,4-D and 2-(2,4-dichloro-phenoxy)ethanol on the basis of root growth suppression.

Although 2-(2,4-dichloro-phenoxy)ethanol can be oxidized to 2,4-D in soil[4], it is yet impossible to ascribe the herbicidal action of sodium 2-(2,4-dichloro-phenoxyl)ethyl-sulphate exclusively to one or the other of these compounds. Under field conditions it is most likely that when sodium 2-(2,4-dichloro-phenoxy)ethyl-sulphate is added to soil, the hydrolysis product 2-(2,4-dichloro-phenoxy)ethanol, as well as any 2,4-D which is generated, can suppress the root development of weed seedlings.

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THE possibility that phytotoxicity generated when sodium 2,4-dichloro-phenoxy-ethyl-sulphate is perfused through soil might be due to hydrolysis to a toxic 2,4-dichloro-phenoxy-ethanol has been seriously considered; but I have so far been unable to obtain any evidence that this compound accumulates in the perfusate. The toxicity seems to be due entirely to 2,4-D. Four independent reasons for this conclusion are given in the original paper[1]. The most convincing evidence comes from assays using paper chromatography to separate the toxic compounds in any possible mixture[2]. Only one compound has, as yet, been found in this very sensitive assay, which will detect 10^{-4} γ equivalents of 2,4-D, and this has an R_F value (0·7) identical with that of 2,4-D. A graph showing toxicity changes due to this compound in the perfusate is reproduced herewith. It is extremely unlikely that the ethanol has the same R_F value (solvent: butanol–ammonia) as the 2,4-D, but it has not been possible as yet to check this experimentally. The most rational explanation of the results to date is that the hydrolysis of sulphate to ethanol