sults. The data obtained also showed that the kinetics of the hydrolysis of *o*-nitrophenyl-β-D-galactoside seemed fairly closely to follow the Michaelis-Menten behaviour under the restricted conditions employed.

The results obtained showed that the β-galactosidase briefly described in this communication resembles in many respects other microbial β-galactosidases. For example, one of the latest β-galactosidases studied, that of *Saccharomyces lactis*, acting on *o*-nitrophenyl-β-D-galactopyranoside, possesses a pH optimum at pH 7.2, and is activated by Mg^{2+} ions.[7] The organisms responsible for its production in the human oral cavity are unknown, although at least oral lactobacilli have been shown to be capable of forming this kind of enzyme. The best source of the enzyme studied seemed to be the soft bacterial covering of oral surfaces from which it can be obtained by extraction with buffers, or in greater amounts by disintegrating the microbial cells of the plaque with an ultrasonic disintegrator.

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Coordination Compounds of Phenylazothioformamides

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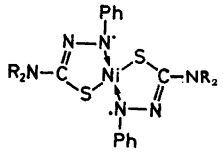
A characteristic difference between nickel(II) compounds of 2-alkylthiosemicarbazides and 1-alkylthiosemicarbazides is that the latter are easily oxidized in air, forming intensely coloured complexes. When sodium hydroxide is added to a solution of nickel(II) chloride and 1-methylthiosemicarbazide the usual brown innercomplex compound precipitates first but dissolves again in excess sodium hydroxide. This solution rapidly develops an intense blue colour, which originates at the phase boundary between air and solution and the colour development is accelerated by shaking the solution. The blue compounds formed from 1-methylthiosemicarbazide and other alkylthiosemicarbazides (except 1,4-di-*tert*-butylthiosemicarbazide) were too unstable for isolation. The 1-phenyl derivatives are more stable, however, but their stability is dependent upon the nature of the substituents in the 4-position. Very stable compounds were prepared from 1-phenyl-4-*tert*-butylthiosemicarbazide and from 1-phenyl-4,4-dialkylthiosemicarbazides (in the following discussion these ligands will be designated by LH_2). Oxidation of the innercomplex nickel(II) compounds, $Ni(LH)_2$, with iodine gave strongly coloured, high-melting crystalline compounds of the composition NiL_2 of high purity.

These compounds may be formulated as compounds with quadrivalent nickel — as was done for a corresponding derivative of thiobenzhydrazide [1] — but they may also be derived from the oxidized ligand, a phenylazothioformamide. It was found that phenylazothioformamides could be prepared in good yields by oxidation of thiosemicarbazides with benzoquinone (most other oxidants attack the sulfur atom). A few azothioformamides had been prepared earlier by essentially the same method [2] and after the completion of our work Pluijgers *et al.*[3] have prepared 1-

phenylazothioformamide by this method and shown that it possesses antifungal properties.

The phenylazothioformamides thus obtained react with nickel(0) compounds ($Ni(CO)_4$ or $Ni(P(OR)_3)_4$) with the formation of complex compounds which, according to appearance, analyses, and infrared spectra, are identical with those obtained by oxidation of the nickel(II) compounds. Considering the methods used for their preparation, the nickel complexes might be described as either nickel(IV) compounds, derived from the thiosemicarbazidate anion, or as nickel(0) compounds derived from the phenylazothioformamide. However, the properties of the compounds indicate that the most reasonable description of their constitution is as nickel(II) compounds derived from a thiosemicarbazidate anion radical:



For the nickel compounds extensive spin coupling of the unpaired electrons with the electrons of the unfilled *d*-shell of nickel(II) ion must take place, since the compounds are diamagnetic or only weakly paramagnetic. This is, of course, equivalent to saying that the formulation of the nickel compounds as nickel(IV) compounds, derived from the ligand ion L^{2-}, is not a bad approximation. However, the corresponding zinc compounds, even though resembling the nickel compounds very much in colour, are paramagnetic with a magnetic moment of approximately 2.4 B. M. In this the free electrons of the ligand cannot couple with unpaired electrons of the metal ion, accordingly, we actually are dealing with a zinc(II) complex of a radical ligand.

Nickel(II) compounds of thiopivaloylhydrazine and selenopivaloylhydrazine have been found to form similar strongly coloured complexes on oxidation.

The structure of the oxidized nickel compounds was confirmed by NMR spectroscopy. It has also been shown by polarography that they possess electron-transfer properties.

The details of this investigation will be published in a forthcoming paper in this journal.

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A Method for Determining Free Amino Groups in Polymers with Particular Reference to the Merrifield Synthesis

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For successful application of the solid-phase peptide synthesis method introduced by Merrifield [1] it is important to know the kinetics of the condensation reactions. The condensation reactions must go to completion, preferably at a high rate. Only one method has been described how to determine the free amino groups left, due to incomplete coupling.[2] We should like to publish another analytical procedure based on the condensation reaction of 2-hydroxy-1-naphthaldehyde with the free amino groups in the polymer. A quite stable aldimine (Schiff's base) is formed.[3] The chromophore is then displaced from the polymer by an amine, *e.g.* benzylamine, and the amount of the soluble aldimine thus formed is determined.

Procedure. 10 mg of polymer is allowed to react for 12 h with a great excess (50–100 times) of 2-hydroxy-1-naphthaldehyde at room temperature in absolute ethanol. After careful