metallic ions[2]. We therefore examined this substance before and after it was mixed with cupric sulphate. Our results show a sharp contrast to those given by pterins. As Fig. 2 shows, the accelerating effect of riboflavin was much hindered by the addition of cupric ion which, if added independently, could accelerate the oxidation to a certain extent. A mixture of riboflavin and copper also showed less degree of acceleration than that of riboflavin alone. We believe that this may be due to the decrease of photodynamic activity of riboflavin, which was found previously to be proportional to the intensity of fluorescence[4], for we found that contact with the metal resulted in a considerable weakening of the fluorescence from riboflavin.

Another example of the effect of chelation may be mentioned. Zinc ion is the only metallic ion reported to accelerate the conversion of dopa-chrome (red substance) to leuco-substance—the second step in the formation of melanin[5]. This can be confirmed in Thunberg tubes. We prepared dopa-chrome by adding sodium hydroxide to dihydroxyphenylalanine. After neutralizing with hydrochloric acid, we put it into a Thunberg tube, de-aerated and mixed it with zinc sulphate at *p*H 6·3. The red colour disappeared gradually. Now, if xanthopterin was added to this system, the time of decolorization was prolonged somewhat. This again may be attributed to the capture of the metallic ion by xanthopterin, for the latter alone could have little effect upon this step.

Further details of our investigations will be published elsewhere.

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Stepwise Degradation of Polypeptides from the Carboxyl End

Many methods have already been described for the selective degradation of peptides from the carboxyl end[1]. However, the several chemical steps involved in those procedures do not allow their utilization on a micro-scale. Linstead, Shephard and Weedon[2] have recently published a study on the decarboxylation of α-N-acylamino-acids in alcoholic media by anodic oxidation into alkoxyalkylamides (acylaminoethers), with a 74–91 per cent yield.

We applied the same anodic oxidation on a micro-scale to N-acyl derivatives of polypeptides (1 cm.³ of 0·1 per cent solution in methanol; platinum electrodes of 1 cm.² total surface area, 3 mm. apart; 200–250 volts, D.C.; 50–150 m.amp.; 10–30 min.; 0° C.). The initial amino-acid of the acylated peptide (I) is decarboxylated into (II):

$$X\text{—NH.CH}R'\text{.CO—NH.CH}R\text{.CO}_2\text{H} \longrightarrow X\text{—NH.CH}R'\text{.CO—NH.CH}R\text{.OCH}_3 + \text{CO}_2$$

(I) (II)

$$\downarrow \text{HCl}$$

$$X\text{—NH.CH}R'\text{.OCH}_3 + \text{CO}_2 \longleftarrow X\text{—NH.CH}R'\text{.CO}_2\text{H} + \text{NH}_3 + R\text{.CHO} + \text{CH}_3\text{OH}$$

(IV) (III)

The initial amino-acid no longer appears on a paper chromatogram of the hydrolysis products of (II). If (II) is heated for a short time with dilute aqueous hydrochloric acid, the methoxyalkylamide is hydrolysed into (III). After evaporation to dryness *in vacuo* and re-dissolution in dry methanol, (III) is decarboxylated by a new anodic oxidation into (IV). The spot of the second amino-acid is no longer detectable on a chromatogram of the hydrolysis products of (IV).

By this simple method, which involves only manipulations easily carried out on a micro-scale, polypeptides were degraded stepwise from the carboxyl end, and the sequence of amino-acids was determined by their successive disappearance on chromatograms after hydrolysis.

We found that Sanger's dinitrophenyl derivatives of peptides may also be submitted to carboxyl anodic oxidation. Thus, time and material are saved by combining Sanger's method for the determination of the amino terminal amino-acid with our new method for the determination of the carboxyl terminal amino-acid.

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Metal Ion Catalysis of the Decarboxylation of Oxalo-Acetic Acid

In order to explain the catalysis of oxalo-acetic acid decarboxylation by metal ions, intermediate complex formation between the metal ion and the acid anion has been postulated. In an attempt to determine the nature of this complex, Steinberger and Westheimer[1] studied the kinetics of the decarboxylation of the dimethyl substituted acid. In this way they showed that the catalysis did not proceed through an enolic form of the acid. From a further consideration of the general characteristics of decarboxylation reactions, they suggested that the metal complex involved in the catalysis had the formula, MA, which was formed from MHA^+, by the loss of a proton.

$$MHA^+ \qquad \begin{matrix} CO_2^- \\ | \\ HO_2C.C(Me)_2.CO \end{matrix} \searrow \nearrow M^{++} \qquad MB \qquad \begin{matrix} CH_2.CO_2 \\ | \\ CO.CO_2 \end{matrix} \rangle M^{++}$$

Pedersen[2] examined the catalytic decarboxylation of the parent acid in greater detail. He obtained, from kinetic data, a stability constant of 10^4 for the formation of a cupric complex of the acid, and suggested that this complex was of the same kind as