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A Note on the Copper-retaining Power of a Humic Acid from Peat Soil

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Bremner, Mann, Heintze & Lees (1946) have suggested that trace elements may be retained in soil as metallo-organic complexes with the soil organic matter. Lees (1948) estimated, from results obtained with whole cacao soils, that the copper retention is about 1400 μ equiv. copper/g. soil organic matter. The copper-retaining power of 'humic acid' (the fraction of soil organic matter to which most of the trace element retention is presumably due) has now been measured directly.

EXPERIMENTAL

A peat soil from the San Juan Estate, Trinidad, was air-dried and a 100 g. sample repeatedly extracted with 500 ml. lots of 0.1 M-sodium oxalate at pH 7.0. Each lot of extractant was shaken with the soil intermittently over a period of 8 hr. after which the soil was allowed to settle for 16 hr.; the extract was then decanted and a fresh 500 ml. added to the residual soil. After five such extractions the combined extracts were acidified with conc. H_2SO_4 to a pH of approximately 2. The humic acid thus precipitated was centrifuged and then redissolved in 250 ml. water plus sufficient solid $NaHCO_3$ to bring the pH of the solution to 7.0. The humic acid solution was then filtered to remove clay and dialysed against repeatedly changed distilled water to remove salts. The humic acid was then reprecipitated by the addition of 4 vol. of 95% (v/v) ethanol and again filtered off. The precipitate was washed repeatedly with ethanol to remove as much water as possible and dried to a black cake in a desiccator over $CaCl_2$. This preparation of humic acid is based on the work of Bremner & Lees (1950).

A 1% aqueous solution of dried, powdered, humic acid was treated in 1 ml. lots with 1 ml. 0.5 M- $Ca(NO_3)_2$ solution (to precipitate the humic acid and to saturate its base-exchange groups) plus 1.0, 1.5, 2.0, 2.5, 3.0 or 3.5 ml. $CuSO_4$ solution containing 10 μ equiv. Cu/ml. solution,

Fig. 1. The distribution of copper (added as $CuSO_4$) between 10 mg. humic acid and 11 ml. of 4.5×10^{-2} M- $Ca(NO_3)_2$. (Each point is the mean of three determinations. Bracketed figures show total amount of copper (μ equiv.) present.)

with sufficient distilled water to bring the final volume to 11 ml. After standing for 2 hr. the suspended humic acid was centrifuged and the Cu in the supernatant liquid was determined by the method of Sherman & McHargue (1942). The Cu retained by the humic acid was calculated by difference. The results obtained by the method are given in Fig. 1.

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RESULTS AND DISCUSSION

The maximum copper retaining power of the humic acid tested, 1600 μ equiv. copper/g. humic acid, agrees reasonably well with the figure of 1400 μ equiv./g. obtained indirectly on whole soils (Lees, 1948). Humic acid contains about 56 % carbon (Page & duToit, 1932); therefore some 60 carbon atoms are associated with one copper atom in the fully saturated complex of copper and humic acid.

SUMMARY

1. The copper-retaining power of a humic acid from a Trinidad peat soil was 1600 μ equiv. copper/g. humic acid. This is in agreement with previous results.

2. The atomic ratio of carbon:copper in the complex so formed is about 60:1.

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Some Properties of Specific Cholinesterase with Particular Reference to the Mechanism of Inhibition by Diethyl *p*-Nitrophenyl Thiophosphate (E 605) and Analogues

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A full account of the properties of substances inhibiting cholinesterase has been given in a recent review by Koelle & Gilman (1949). In the following work the properties of certain organic esters of phosphoric acid which are powerful inhibitors of cholinesterase are described. In particular, the kinetics of the inhibitor-enzyme system have been worked out and certain conclusions have been drawn as to the mode of action of these inhibitors. The properties of a convenient preparation of cholinesterase which has been used for this work are given below.

METHODS

For the cholinesterase preparation blood was taken from a goat into a flask containing 2 ml. of 30 % (w/v) Na citrate per 60 ml. of blood, and was stored in the refrigerator at 4°. Washed cells lose their activity on storage. Prior to use in an experiment, the blood was centrifuged, the plasma removed and the cells washed three times with isotonic saline. They were then resuspended in buffer and diluted roughly to the original volume of blood taken.

Determination of cholinesterase activity was carried out using the method of Nachmansohn & Rothenberg (1945). The final concentrations in the Warburg flask, after tipping in the contents of the side arm, were NaCl, 0.15M; MgCl₂, 0.035M; NaHCO₃, 0.0312M and acetylcholine chloride 0.0138M. The pH of this buffer mixture, gassed with 5 % CO₂

in N₂, is 7.72 (calculated from Henderson-Hasselbach equation). Stock salt solutions of 20 times the strength used in the preparation of the buffer were stored in the refrigerator in paraffin-waxed flasks. The buffer was likewise stored after dilution from these stock solutions. The total volume of fluid in each flask was 4.0 ml. except where otherwise stated. All measurements of red-cell suspensions were made with a Krogh-Keys syringe pipette. The contents of the flasks were gassed in the bath at 37° with a slow stream of 5 % CO₂ + 95 % N₂ for 10 min., and allowed to equilibrate for 10 min. After the addition of the acetylcholine from the side-arm, readings were taken every 10 min., for 1 hr. After a visual inspection of the readings to see that a straight line relationship between time and output of CO₂ was sensibly satisfied, the slope of the line (output in μ l. of CO₂/min.) was determined by the method of least squares (Aldridge, Berry & Davies, 1949). The flasks were cleaned after each experiment by boiling in soap solution to remove all lanoline grease and protein, and once a day were further treated with ethanol and HNO₃, rinsed in distilled water and dried in a warm oven.

E 605 and its analogues, the formulae of which are given below, were dissolved initially in absolute ethanol (1 mg./ml.) and were diluted with buffer from that solution prior to the experiment. All the inhibitors were stored at 4° in the dark in bottles with stoppers sealed with paraffin wax.

Throughout this paper their code numbers (given in brackets) are used. They are all pale yellow liquids when pure, but both E 605 and Q 2 darken on standing, particularly when exposed to light. They all hydrolyse slowly in water to give a substituted phosphoric acid and *p*-nitrophenol or

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