$$HO(COCHRNH)_nH + I \longrightarrow HO(COCHRNH)_nCONHCHRCOOH \quad (2)$$
II

tion of polymerization takes place when the amino group of a growing peptide chain reacts with carbon 2 of I, leading to an urea derivative (II), with the formation of a free carboxyl group. The presence of II (R = $CH_2C_6H_5$) in poly-DL-phenylalanine (prepared by bulk polymerization of I, R = $CH_2C_6H_5$, *cf.* substance 1 in the table) was demonstrated as follows: The polymer was hydrolyzed in acetic acid–hydrochloric acid and 5-benzylhydantoin-3-β-phenylpropionic acid[4] (III, R = $CH_2C_6H_5$) was separated from DL-phenylalanine by ether extraction. Both the racemic and *meso* forms of III were isolated (30 mg. from 1.5 g. of polymer) and identified by mixed melting points with authentic samples.

$$II \xrightarrow{H^+} (n-1)H_2NCHRCOOH + \text{(III)} \quad (3)$$

III

Since the polypeptides contain both terminated and unterminated chains, an excess of carboxyl groups is to be expected. The number average degree of polymerization should therefore be calculated from the total number of end groups (*i.e.*, both carboxyl and amino groups), present in the polymer.[5] We have found that the terminal amino and carboxyl groups of poly-α-amino acids can be determined by titration in anhydrous dimethylformamide with perchloric acid and sodium methoxide respectively, using thymol blue as an indicator. Results are summarized in the table.

TABLE I

Poly-α-amino acid[a]	Number of terminal groups per amino acid residue: COOH (titr.) (A)	NH_2 (titr.) (B)	NH_2 (Van Slyke) (C)	Calcd. degree of polymerization: 1/C	2/(A+B)
1[b]	0.033	0.011	0.010	100	45
2[c]	.048	.017	.014	72	31
3[d]	.118	.059	.059	17	11
4[d]	.091	.020	.019	53	18
5[d]	.072	.016	.016	63	23
6[d]	.056	.016	.012	83	28
7[e]	.143	.012	.010	100	7[f]

[a] Methods of preparation *cf.* E. Katchalski, *Advances in Protein Chemistry*, 6, 123 (1951.) [b] Poly-DL-phenylalanine. [c] Poly-δ,N-carbobenzoxy-DL-ornithine. [d] Poly-ε,Nc-carbobenzoxy-L-lysine (different samples). [e] Poly-β,N-carbobenzoxy-DL-α,β-diaminopropionic acid (polymerization in anhydrous dioxane initiated by diethylamine). [f] Calculated from 1/A + B.

There is fairly good agreement between the amino group titration and the Van Slyke analysis. The table clearly illustrates the considerable excess of carboxyl groups over amino groups in the polypeptides investigated. This fact, as well as the presence of carboxyl groups in amine-initiated polymers,[6] and the relatively low molecular weight of the polymerization products, are readily explained by the termination reaction.

(4) F. Wessely and M. John, *Z. physiol. Chem.*, **170**, 98, 167 (1927); F. Wessely, K. Schlögl and G. Korger, *Monatsh.*, **83**, 1156 (1952).

(5) The corrected degrees of polymerization appear in column 6 of the Table. Hitherto the values appearing in column 5 of the table were considered to represent the average degree of polymerization.

(6) J. H. Fessler and A. G. Ogston, *Trans. Faraday Soc.*, **47**, 667 (1951).

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DIRECT INTERACTION BETWEEN METAL ATOMS IN THE CRYSTALS OF BIS-(DIMETHYLGLYOXIME)-NICKEL(II) AND -PLATINUM(II)

Sir:

Previously the present writers have studied the dichroism of the crystals of Magnus green salt,[1,2] its related compounds,[2] and salts of tetracyanoplatinate(II),[3] and arrived at the conclusion that in the above crystals there exists a direct interaction between central platinum atoms of the planar complexes. Recently Godycki and Rundle[4] reported on the crystal structure of bis-(dimethylglyoxime)-nickel(II), suggesting the existence of a similar, though weak, interaction between nickel atoms. They have mentioned of the pleochroism of the nickel compound, but have not given any value for absorption coefficients. Moreover, their description on the pleochroism contradicts our results, though the conclusion reached agrees with ours. In this letter we wish to report on the results of the quantitative dichroism measurement, demonstrating the non-existence of the metal–metal interaction in the crystal of bis-(dimethylglyoxime)-copper and the possible existence of the metal–metal interaction in the crystals of bis-(dimethylglyoxime)-nickel(II) and -platinum(II).

Quantitative dichroism measurement by Tsuchida–Kobayashi's microscopic method[5] was performed with a microcrystal in the region from 2400 to 7000 Å. First, measurement was made with black prismatic crystals of bis-(dimethylglyoxime)-copper(II) (Fig. 1).[6] For the absorption band at the longest wave length region (Fig. 1), which is considered as due to transitions related to the metal–ligand linkages, a marked dichroism was observed. The following data were obtained: for ∥ absorption,[7] $\nu = 54 \times 10^{13}$/sec. and log $\alpha = 1.91$[8]; for ⊥ absorption, $\nu = 57.6 \times 10^{13}$/sec. and log $\alpha = 1.65$. The relation on the dichroism with this compound agrees with that induced for planar complexes of an ordinary type,[5b] indicating that there exists

(1) S. Yamada and R. Tsuchida, *J. Chem. Soc. Japan*, **70**, 44 (1949).

(2) S. Yamada, THIS JOURNAL, **73**, 1579 (1951).

(3) S. Yamada, *Bull. Chem. Soc. Japan*, **24**, 125 (1951).

(4) L. E. Godycki and R. E. Rundle, *Acta Cryst.*, **6**, 478 (1953).

(5) (a) R. Tsuchida and M. Kobayashi, "The Colours and the Structures of Metallic Compounds," Zoshindo, Osaka, Japan, 1944, p. 180. (b) See, for example, S. Yamada, THIS JOURNAL, **73**, 1182 (1951).

(6) About the crystal structure, see S. Bezzi, E. Bua and G. Schiavinato, *Gazz. chim. ital.*, **81**, 856 (1951).

(7) ∥ and ⊥ refer to results with polarized lights having their electric vectors parallel and perpendicular to the planes of the complexes, respectively.

(8) α denotes absorption coefficient per mm. of the crystal.

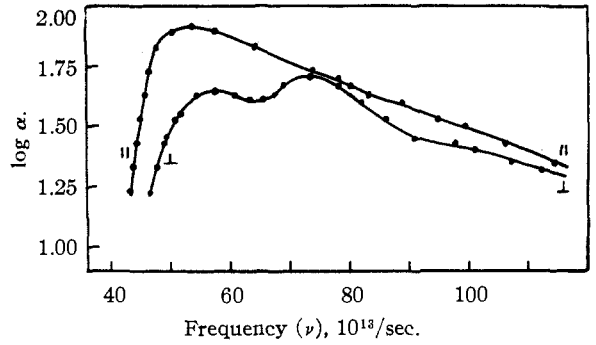

Fig. 1.—Absorption spectra of bis-(dimethyloxime)-copper.

no metal–metal interaction. Similar measurement (Fig. 2) revealed that the corresponding nickel compound exhibits a remarkable dichroism for the corresponding absorption band, maximum absorption being observed with electric vector along the *c*-axis.[9] The following data were

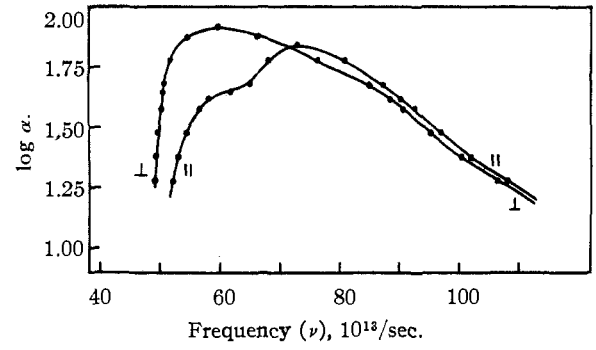

Fig. 2.—Absorption spectra of bis-(dimethylglyoxime)-nickel.

obtained: for ∥ absorption, $\nu = 60 \times 10^{13}$/sec. and log $\alpha = 1.62$; for ⊥ absorption, $\nu = 59.2 \times 10^{13}$/sec. and log $\alpha = 1.91$. These data indicate that the relation on the dichroism with this crystal is reverse to that in the ordinary case. A direct interaction between nickel atoms may be considered as responsible for the reversal of the effect. Similar dichroism measurement was made with chocolate-colored acicular crystals of the corresponding platinum complex. Although the crystal structure has not been determined, judging from the data of analogous compounds, planar complexes are supposed to be arranged parallel to each other to a greater or lesser extent, with their planes nearly perpendicular to the needle-axis. On the basis of the assumed structure, it is established that ⊥ absorption is bathochromic and hyperchromic to ∥ absorption. This relation is reverse to that for planar complexes of an ordinary type.[5b] The metal–metal interaction, as in the crystal of the corresponding nickel compound, may be expected in the platinum compound.

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(9) Godycki, *et al.*, reported (ref. 4) that maximum absorption was observed with the electric vector perpendicular to the *c*-axis.

BIOLOGICAL ACTIVITY OF A METABOLITE OF *p*-AMINOBENZOIC ACID (PABA) IN A HYDROXYLATING SYSTEM

Sir:

Sloane, Crane and Mayer[1] reported that resting cells of *Mycobacterium smegmatis* (101) hydroxylate aniline to *p*-aminophenol. Further investigations[2,3] indicated that the hydroxylation is an energy-coupled reaction. It was determined that chlortetracycline (Aureomycin[4]) and oxytetracycline (Terramycin[5]) compounds which uncouple oxidative phosphorylation[6,7] inhibit the hydroxylation reaction without affecting the oxygen uptake of mycobacterial cells[8] at concentrations ranging from 1.3 to 5.4 $\times 10^{-6}$ *M*.[9] Penicillin, chloramphenicol, dihydrostreptomycin, viomycin and neomycin do not inhibit the hydroxylation reaction by the mycobacteria at these concentrations.[9]

Isochlortetracycline, the biologically inactive rearrangement product of chlortetracycline[10] does not inhibit the hydroxylation, thus the antibiotic activity of chlortetracycline parallels its ability to inhibit the hydroxylation reaction.

It is the purpose of this communication to describe some biological and chemical properties of a metabolite of PABA, which non-competitively reverses the activity of chlortetracycline and oxytetracycline in this system. The data are shown in Table I. The metabolite appears to function as a

TABLE I

THE CHLORTETRACYCLINE–OXYTETRACYCLINE REVERSING ACTIVITY OF PABA-METABOLITE IN THE HYDROXYLATING SYSTEM

	M. Tuberculosis (#607 ≃ 600 mg. dried cells per 25 ml. of buffer–citrate–metals solution per 250-ml. flask (vigorous aeration (1))	μM. *p*-aminophenol, 16 hr.
1	Cells + buffer–citrate–metals + aniline (107 μM.)	4.62
2	1 + 0.136 μM. chlortetracycline	0
3	2 + 9.12 μM. PABA—metabolite[a]	1.54
4	2 + 4.56 μM. PABA—metabolite[a]	1.54
5	2 + 2.28 μM. PABA—metabolite[a]	0.93
6	2 + 1.14 μM. PABA—metabolite[a]	0.49
7	2 + 0.57 μM. PABA—metabolite[a]	0.13
8	4 without aniline	0
9	4 without aniline and chlortetracycline	0
10	Buffer–citrate–metals + 4.56 μM. PABA metabolite + 107 μM. aniline	0

[a] Maximum solubility of metabolite is 4.5 μM. per 25 ml.

cofactor or cosubstrate in the hydroxylation reaction. Amino acids, purines, pyrimidines and vita-

(1) N. H. Sloane, C. Crane and R. L. Mayer, *J. Biol. Chem.*, **193**, 453 (1951).

(2) N. H. Sloane, M. Samuels, C. Ritter, C. Crane and R. L. Mayer, *Federation Proc.*, **11**, 288 (1952).

(3) N. H. Sloane, M. Samuels and R. L. Mayer, *J. Biol. Chem.*, in process of publication.

(4) Aureomycin is the registered trade name of Lederle Laboratories Division, American Cyanamid Co.

(5) Terramycin is the registered trade name of Chas. Pfizer and Co.

(6) W. F. Loomis, *Science*, **111**, 474 (1950).

(7) Y. Miura, Y. Nakamura, H. Matsudaira and T. Komeiji, *Antibiotics and Chemotherapy*, **2**, 152 (1952).

(8) Non-pathogenic mycobacteria perform this hydroxylation.

(9) N. H. Sloane, unpublished data.

(10) C. W. Waller, B. L. Hutchings, C. F. Wolf, A. A. Goldman, R. W. Broschard and J. H. Williams, THIS JOURNAL, **74**, 4981 (1952).