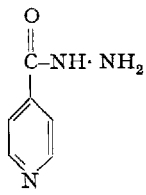
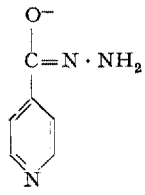
The Affinity of *Iso*nicotinic Hydrazide for Metals

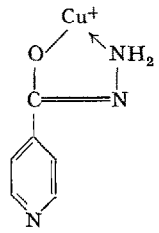
Is has been shown that *iso*nicotinic hydrazide (I) combines with cupric ions to give the 1:1-complex (III) and the 2:1-complex (IV)[1], and it has been claimed that the action of (I) against tuberculosis *in vitro* is increased when copper is supplied in excess of that normally present in the medium[2].



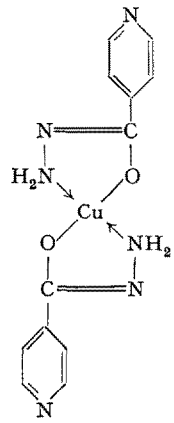
(I)



(II)



(III)



(IV)

A good deal of work has been done in this Department on the determination of the stability-constants governing the equilibria between biologically active substances and their complexes with the ions of heavy metals[3]. Moreover, in collaboration with S. D. RUBBO and Mrs. GIBSON of the University of Melbourne, we have been able to show that 8-hydroxyquinoline (and related substances) are toxic to bacteria only when traces of iron or copper are present in the medium[4]. In this case, the 1:1-complex has been shown to be the true toxic agent[5]. Thus the likelihood exists that a 1:1 metallic complex of (I) is the true curative agent in tuberculosis.

Accordingly it was interesting to determine the stability constants K' governing the reaction (I)$\rightleftarrows$(III)

$$K' = \frac{[\text{complex (III)}]}{[\text{free metallic ions}]\,[\text{free complex-forming species (II)}]}$$

This was done by potentiometric titration in 0·002 *M*-solution at 20°, by the method previously described[1]. First of all, the ionization constants of (I)[2] were determined and the pK_a values were found to be 10·77 ($\pm$ 0·05) for the acidic group, and 3·54 ($\pm$ 0·04) and 1·85 ($\pm$ 0·04) for the two basic groups. Thus, *iso*nicotinic hydrazide is an acid of approximately the same strength as phenol, and "free complex forming species" in the equation refers to the anion (II). The two basic functions are very weak, and so close together that they had to be separated by the method of NOYES[3]. The higher value probably belongs to the ring-nitrogen (cf. 5·2 for pyridine[4]) and the lower one to the more remote of the hydrazine nitrogens (cf. 2·4 for glycine hydrazide[5]). Of these three constants for (I), only the stronger basic one has previously been determined[6] and the result (3·52) is in excellent agreement.

The following values of $\log K'$ were obtained for the 1:1-complexes of type (III):

Cu^{++} (8·0); Ni^{++} (5·5); Zn^{++} (5·4); Co^{++} (4·8)

No values for Fe^{++} or Mn^{++} could be obtained because of the sparing solubility of the complexes. Fe^{+++} appeared to form no complex, in contrast to aureomycin and terramycin[7] which combine more avidly with this cation than with the common bivalent ions which give values of $\log K'$ very similar to those obtained for (I).

Values of $\log K_s$ were sought for (I). This is the constant which governs the equilibrium between (I) and the 2:1-complexes of type (IV)[8]. Because of solubility difficulties, $\log K_s$ was obtainable only for Ni^{++}, where it was found to be 9·8.

All these values for the metal complexes of (I) are low when compared with the corresponding values for the complexes of the amino-acids. This suggests that these complexes would be most stable in those cells where the concentration of free amino-acids was low. It would be interesting to determine whether the toxic action of *iso*nicotinic hydrazide on various strains of mycobacteria is in inverse proportion to their content of free amino-acids (or other intracellular metal-binding material). The abundance of such substances in the tissues of the host is commonly assumed and, if established, could explain the selectivity of (I).

Mr. E. P. SERJEANT is thanked for skilled technical assistance.

A. ALBERT

Department of Medical Chemistry, The Australian National University, London, N.W. 1., June 17, 1953.

Zusammenfassung

Die Konstanten, welche das Gleichgewicht zwischen Isonikotinsäurehydrazid (I) und den mit Schwermetallen gebildeten Komplex (III) beherrschen, werden ermittelt. Die Bedeutung dieser Konstanten für die Chemotherapie der Tuberkulose werden diskutiert.

[1] S. FALLAB and H. ERLENMEYER, Exper. *8*, 298 (1952). – E. SORKIN, W. ROTH, and H. ERLENMEYER, Helv. chim. Acta *35*, 1736, (1952).

[2] E. SORKIN, W. ROTH and H. ERLENMEYER, Helv. chim. Acta, *35*, 1736 (1952).

[3] A. ALBERT, Biochem. J. *47*, 531 (1950); *50*, 690 (1952); *54*, 646 (1953).

[4] S. D. RUBBO, Mrs. M. GIBSON, and A. ALBERT, Brit. J. experim. Path. *31*, 425 (1950).

[5] A. ALBERT, Mrs. M. GIBSON, and S. D. RUBBO, Brit. J. experim. Path. *34*, 119 (1953).

[1] A. ALBERT, Biochem. J. *47*, 531 (1950).

[2] Dried over P_2O_5 at 20°.

[3] H. BRITTON, *Hydrogen Ions* (Chapman and Hall, London, 1942), p. 198.

[4] A. ALBERT, R. GOLDACRE, and J. PHILLIPS, J. Chem. Soc. *1948*, 2240.

[5] C. LINDGREN and C. NIEMANN, J. Amer. chem. Soc. *71*, 1504 (1949).

[6] S. FALLAB, Helv. chim. Acta *36*, 3 (1953).

[7] A. ALBERT, Nature *172*, 201 (1953).

[8] S. FALLAB and H. ERLENMEYER, Helv. chim. Acta *36*, 6 (1953) have determined (spectrometrically) a value for $\frac{[\text{IV}]}{[\text{I}][\text{Cu}^{++}]}$. This value is not a constant but varies with the hydrogen ion concentration