könnte, stets die gleichen sind, sondern starke strukturspezifische Verschiedenheiten zeigen. Die Werte zum Beispiel für $K_{Cu^{2+}}/K_{Fe^{2+}}$ zeigen starke Schwankungen, so dass je nach dem Konzentrationsverhältnis der Metallionen einerseits und der Liganden andererseits auch das «schwächere» Metallion Chelate ausbilden kann. Die Beobachtung von Rechenberger weist jedoch darauf hin, dass im Laufe der Zeit in zunehmendem Umfange eine Anreicherung der «starken» Metallionen eintreten kann.

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Anstalt für Anorganische Chemie der Universität Basel, den 12. März 1956.

Summary

The possible role of the metabolism of heavy metal ions in the process of ageing is discussed. It is suggested that, during this process, Cu^{2+} and Fe^{3+} as strong complexing ions, inhibit the activity of other metal enzymes by replacing their metal ion-activator. The relative stability of Cu^{2+}- and Fe^{3+}-complexes with various chelating compounds related to biological systems has been determined.

A New Concept of the Alkaline Oxidation of Uric Acid[1]

Scheme I shows the concept of the alkaline oxidation of uric acid (I), as based on the work of several German authors[2], and as generally accepted prior to 1953[3]. The key intermediate in this series of reactions is II, the so-called Behrend compound or hydroxy-acetylene-diureido-carboxylic acid (octahydro-6α-hydroxy-2, 5-dioxo-imidaz[*d*]imidazole-3α-carboxylic acid). This intermediate was postulated by Behrend[4], on the basis of work of Fischer and Ach[5], as the only possible symmetrical compound from uric acid able to undergo transformation into the following: uroxanic acid (III) by simple hydrolysis; allantoin (IV) by decarboxylation and hydrolysis with or without rearrangement (this reaction has been formulated in different ways); oxonic acid (allantoxanic acid) (V) and allantoxaidine (VI) by further oxidation, deamination and decarboxylation. Cyanuric acid (VII) was thought to result mainly from III by oxidative decarboxylation and ring closure with release of NH_3. However, it was stated that VII could also be formed from V or VI in a reaction believed to be an oxidative rearrangement[6].

In 1953 the present author called attention to the fact that oxonic acid and allantoxaidine cannot have structures V and VI, and he proposed *s*-triazine formulas for these compounds and their derivatives[3]. By degrading 2-, 4-, 6- and 8-labelled ^{14}C-uric acids and 7-labelled ^{15}N-uric acid, it could be shown that the uric acid carbon atoms 2, 4 and 8, and the nitrogen atoms 3 and 9 are incorporated into the ring of oxonic acid and into allantoxaidine. The third nitrogen in the two degradation products is contributed 50% each from former uric acid positions 1 and 7, and the carboxyl carbon of oxonic

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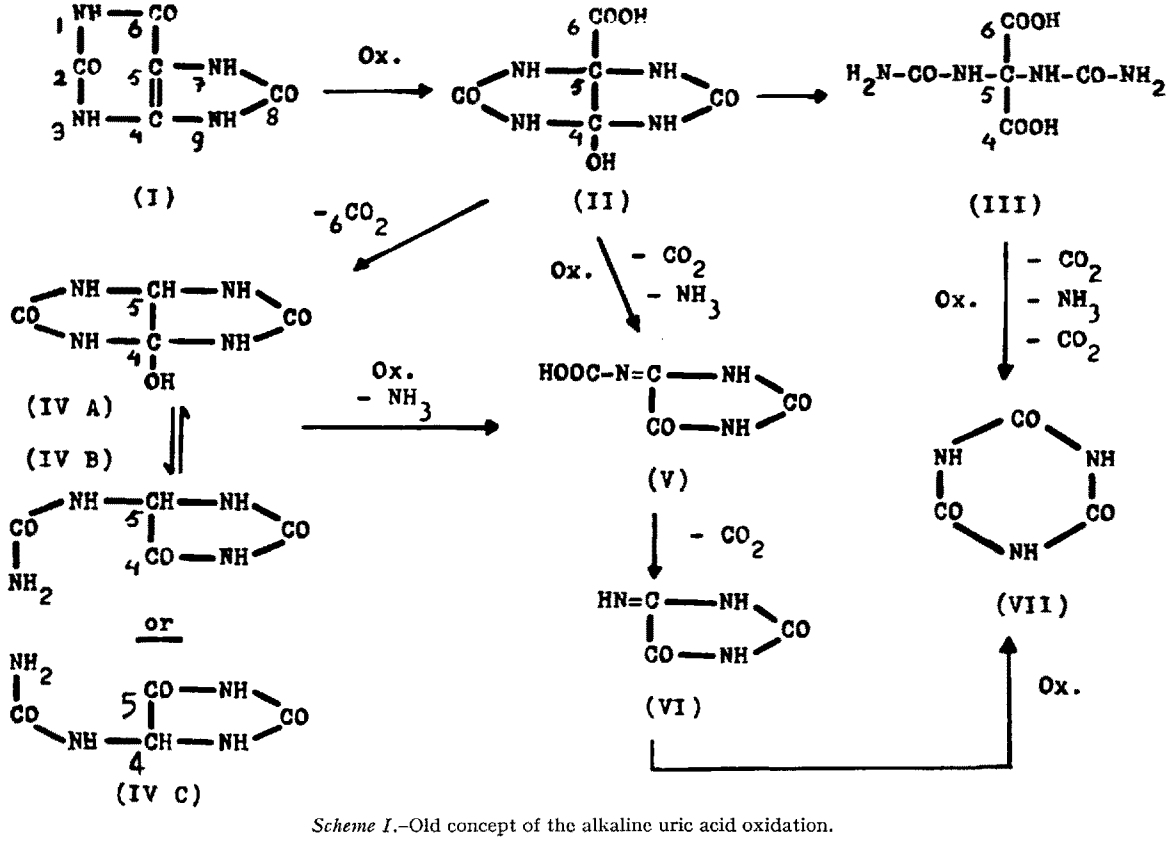

Scheme I.–Old concept of the alkaline uric acid oxidation.

acid from the former uric acid position 5[7]. This tracer work could be confirmed by degradation of 5-^{14}C-uric acid[8], the earlier proposed structures proved by ultraviolet and infrared spectrophotometry[9]. Oxonic acid, therefore, must be formulated as 2,4-dihydroxy-6-carboxy-1,3,5-triazine (VIII), allantoxaidine as 2,4-dihydroxy-1,3,5-triazine (IX).

Further, it has been shown together with R. H. BRANDENBERGER[9] that for the formulation of allantoin alternative IV *C* is the correct one, because the tertiary carbon atom originates from the former uric acid position 4 and not from 5. No evidence could be found for structure IV *A*, with which IV *B* has often been thought to be in equilibrium[10]. Allantoic acid (X) is the real precursor of allantoin, as well as an intermediate in its oxidation to the triazines VIII and IX. This oxidation may involve hydroxy-allantoic acid (XI) and/or dehydro-allantoic acid (XII) as further intermediates[9].

The work has also been extended to the formation of cyanuric acid (VII), which may be considered the end-product in this oxidation series. If III were its precursor, VII should contain the former uric acid carbon 5. However, by degrading the different labelled radioactive uric acids to cyanuric acid, it was possible to establish only the presence of the uric carbon atoms 2, 4 and 8 in VII[11]. The intermediates in the oxidation to cyanuric acid are, therefore, the dihydroxy-triazines VIII and IX, and not compound III.

To the experiments already reported, a reinvestigation of the structure of uroxanic acid and a study of the nature of the intermediates in the first oxidation step have now been added. They lead to a completely new conception of the mechanism of the alkaline uric acid oxidation which is summarized in Schema II. The additional facts in support of this new concept are:

(1) During the course of the oxidation and decarboxylation reactions in the alkaline degradation of radioactive labelled uric acids, the carbon atom number 4 never could be split off as CO_2.

(2) Uroxanic acid is not 2,2-diureido-malonic acid (III), as has been believed so far, but is 4,5-dihydroxy-4-carboxy-5-ureido-imidazolidone-2 (XIII). This is indicated by the facts that this compound contains only one free ureido amido group (VAN SLYKE determination, as modified by G. KAINZ[12]), and that the carbon monoxide split off on heating with concentrated phosphoric acid reveals the presence of an α-hydroxy acid. The insolubility of uroxanic acid in water or acid solutions agrees more with the properties of a compound having formula XIII than III.

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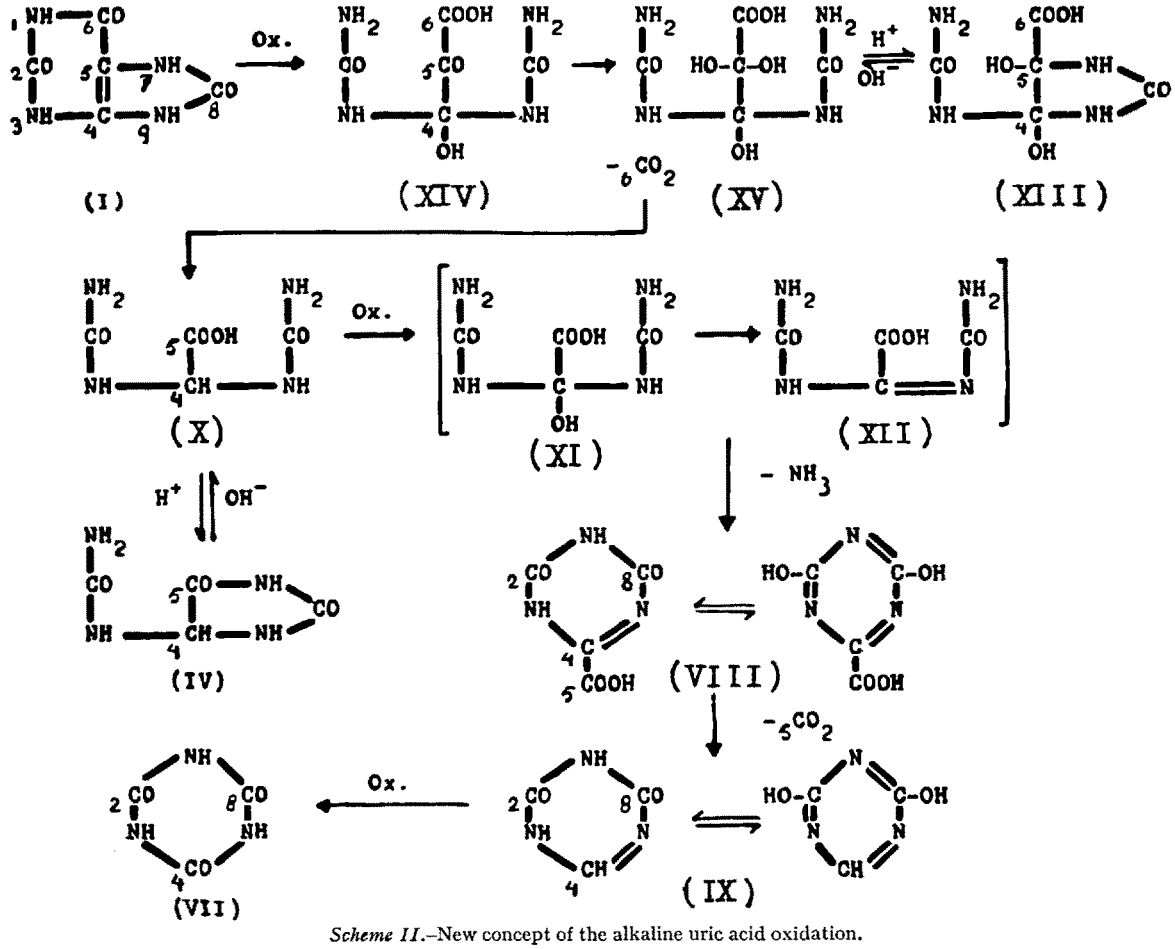

Scheme II.–New concept of the alkaline uric acid oxidation.

(3) The imidazolidone ring of XIII is a secondary formation, occuring on acidification as in the case of allantoin. Salts, isolated from the alkaline solution after permanganate oxidation of uric acid, contain half of the total nitrogen as primary ureido amido groups. On dissolving XIII in alkaline solutions, the imidazolidone ring is cleaved, and the compound is reconverted into salts of the same open-chain product from which it has been prepared. The infrared spectra of corresponding silver salts, prepared before and after conversion to XIII, could be superimposed.

(4) During alkaline hydrogen peroxide oxidation of uric acid, the intermediary formation of a compound with an absorption maximum between 320 and 350 mμ could be followed spectrophotometrically. This compound is very unstable, and we have not been able to isolate it; but the ultraviolet maximum and the previously reported tracer experiments suggest strongly the α-keto acid formula XIV (β-diureido-β-hydroxy-pyruvic acid).

(5) Microanalytical results of the silver salts mentioned in paragraph 3 are consistent with the structure of a monohydrate of XIV, probably XV (α, α, β-trihydroxy-β-diureido-propionic acid). Analysis and properties of SCHULER and REINDEL's silver salts[13], believed by these authors to be salts of the BEHREND intermediate II[4], agree with structure XV as well as II. Their infrared spectra show all the bands of the silver salt spectra mentioned before; some weak additional bands in SCHULER and REINDEL's precipitate from the crude alkaline oxidation mixture of uric acid may be caused by impurities.

Of all the structures attributed in the past to the main degradation products of the alkaline uric acid oxidation (Scheme I), only two were correct, the ones for allantoin (IV) and cyanuric acid (VII). But even in these cases the mechanism of formation was not properly understood. All the other formulas had to be corrected (Scheme II). Oxonic acid and allantoxaidine are dihydroxy-*s*-triazines (VIII and IX) and are not imidazolidones (V and VI). Uroxanic acid, on the other hand, is an imidazolidone (XIII), not an open-chain compound (III). But, in our belief, the most important new fact is that the intermediates in the degradation are symmetrical open-chain compounds (XIV, XV, X) with the former uric acid position 4 as the central carbon atom. They replace the symmetrical bicyclic compound II, which has been postulated by BEHREND and considered so far to be the key intermediate not only in the alkaline chemical oxidation but also in the enzymatic degradation of uric acid. We believe, therefore, that this new concept of the chemical oxidation of uric acid (Scheme II) will also lead to a new conception of its enzymatic breakdown.

A full account of this work will be presented in Helvetica Chimica Acta.

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Zusammenfassung

Früher veröffentlichte Arbeiten über die alkalische Oxydation der Harnsäure wurden ergänzt durch eine Untersuchung über die Struktur von Uroxansäure sowie der Zwischenprodukte, die im alkalischen Oxydationsgemisch auftreten. Die Resultate führen zu einer grundlegend neuen Auffassung über den Mechanismus der Abbaureaktion (Schema II). Uroxansäure ist ein Imidazolidon (XIII) und nicht ein offenkettiges Diureid (III); das von BEHREND formulierte Zwischenprodukt II hingegen, dem man bisher in der alkalisch-chemischen sowie auch in der enzymatischen Harnsäureoxydation eine Schlüsselstellung einräumte, muss durch offenkettige Verbindungen ersetzt werden (XIV, XV, X), in denen das zentrale C-Atom auf die frühere Harnsäureposition 4 zurückgeführt werden kann.

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Nachweis von organspezifischen Antikörpern*

Organspezifische Antigene und Antikörper wurden schon verschiedentlich mit serologischen Methoden nachgewiesen. Früher dienten dazu hauptsächlich Präzipitation, Agglutination und Komplementbindung (HENLE und Mitarbeiter[1], FURTH und KABAT[2], WITEBSKY und STEINFELD[3], WITEBSKY und ZEISSIG[4], WEIL[5], LEWIS[6] u.a.). In letzter Zeit wurden auch radioaktiv markierte Antikörper zum erfolgreichen Nachweis der Organspezifität herangezogen (PRESSMAN[7], BALE und Mitarbeiter[8], u.a.). Wir berichten im folgenden kurz über eine neue Methode, mit der es gelingt, organspezifische Antigene bzw. Antikörper zu bestimmen. Mit der gleichen nephelometrischen Methode konnten wir bereits individualspezifische Gewebsantigene bzw. Antikörper erfassen (BOLLAG[9]). Das Nephelometer, das wir verwendeten, wurde von HOIGNÉ, GROSSMANN und STORCK[10] zur Erfassung einer Trübungsreaktion zwischen Allergen und Serum allergischer Patienten konstruiert. Zum Nachweis von organspezifischen Antikörpern wählten wir folgendes System: Sensibilisierung von Kaninchen mittels Organen bzw. mittels eines transplantablen Tumors von Ratten. Da Rattenorgane kein Forssmann-Antigen enthalten, konnten wir Störungen durch den Forssmann-Antikörper umgehen. Um organspezifische Antikörper zu erfassen, wurden mittels des Absättigungsverfahrens die rein speziesspezifischen Antikörper eliminiert.

Methodik. Je 3 Kaninchen wurden mit Gewebsbrei folgender Organe von Ratten sensibilisiert: Herz, Leber, Niere, transplantabler Tumor Uterusepitheliom T 8 (Guérin). Die Homogenate wurden folgendermassen hergestellt: 500 mg Leber, Herz, Niere bzw. Tumor entbluteter Ratten wurden fein zerschnitten und mit je 5 cm³ physiologischer Kochsalzlösung im Potterapparat 2 min homogenisiert. Diese Organsuspension wurde den Kaninchen 2mal wöchentlich subkutan injiziert. Die Blut-

* Die Arbeit wurde mit Hilfe des Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung durchgeführt.

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