

active site as iron uptake. I found that  $Zn^{2+}$  has apparently a stronger affinity to the active site than  $Fe^{2+}$  (Figure 6). This is of special interest because it is known that in vivo  $Zn^{2+}$  stops iron incorporation into ferritin<sup>17</sup>.

**Zusammenfassung.** Es wurde eine neue Hypothese für die Eisenaufnahme durch Ferritin experimentell geprüft. Es scheint, dass die zweiwertigen Eisenionen in die Apoferritinhohlkugel eindringen können und im Innern an histidinhaltigen aktiven Stellen katalytisch oxydiert werden; das entstehende  $Fe^{3+}$  bildet sofort ein  $(FeOOH)$ -Mikropräzipitat, welches bald so gross ist, dass es nicht

mehr durch die Lücken der Apoferritinhohlkugel entweichen kann.

W. NIEDERER<sup>18</sup>

*Institut für medizinische Mikrobiologie der  
Universität Zürich,  
CH-8006 Zürich (Switzerland), 18 September 1969.*

<sup>17</sup> C. T. SETTLEMIRE and G. MATRONE, *J. Nutrition* 92, 153 (1967).

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## PRO EXPERIMENTIS

### Bromination of Nucleosides

Recently there has been considerable interest in the bromination of purine nucleosides<sup>1-6</sup> and nucleotides<sup>7</sup>. The reagents that have been employed for such brominations are bromine in dioxane, N-bromoacetamide and bromine water. We have now found that N-bromosuccinimide (NBS) in DMF solution is capable of brominating pyrimidine and purine nucleosides. It is known that NBS effects aromatic bromination when both reagent and substrate are in solution. DMF was chosen as the reaction medium since it dissolves NBS as well as the nucleosides (except guanosine) on slight warming.

The nucleosides in this study which were brominated are uridine, cytidine, adenosine, 2', 3'-O-isopropylidene-adenosine and guanosine<sup>8</sup>. The general procedure followed in these reactions is illustrated by the preparation of 8-bromoguanosine and 5-bromouridine. The progress of these reactions was followed by change in UV-absorptions and paper chromatography<sup>9</sup>. The structure of the bromonucleosides was confirmed by hydrolysis with *N* HCl to the corresponding brominated bases.

**8-Bromoguanosine.** Guanosine (283 mg, 1.0 mM) was suspended in anhydrous DMF (8 ml), NBS (200 mg, 1.14 mM) added and the suspension stirred overnight at room temperature. By this time all the guanosine had dissolved to a clear yellow solution. Solvent was removed under reduced pressure (40–50°), water added to the residue and the separated solid filtered and recrystallized from hot water. Yield 290 mg (80%), Rf 0.60.

**5-Bromouridine.** Uridine (244 mg, 1.0 mM) was dissolved in DMF (2 ml), NBS (200 mg, 1.14 mM) added and the clear light-yellow solution allowed to stand at room temperature for 16 h. The solution, which had turned red, was evaporated in vacuo (40–50°C). After thorough removal of DMF, the residue was crystallized from acetone to give 202 mg (62%) of the product, mp 175° (ref.<sup>10</sup>), 181°. Rf 0.63<sup>9</sup>.

5-bromocytidine, 8-bromoadenosine and 8-bromo-2', 3'-O-isopropylidene adenosine were similarly obtained in 83, 40 and 50% yields, respectively. However, when this reaction was applied to triacetylinosine or to inosine, which is insoluble in DMF, no reaction was observed during 16 h at room temperature and hypoxanthine was obtained when the reaction mixture was heated at 70–80°C for 6 h<sup>11</sup>.

**Zusammenfassung.** Mit N-Bromsuccinimid können Nucleoside in Dimethylformamid mit guter Ausbeute zu den in 5-Stellung bromierten Derivaten umgewandelt werden.

P. C. SRIVASTAVA and K. L. NAGPAL

*Central Drug Research Institute,  
Lucknow (India), 29 September 1969.*

<sup>1</sup> A. M. MICHELSON, *The Chemistry of Nucleosides and Nucleotides* (Academic Press, New York 1963), p. 34.

<sup>2</sup> M. IKEHARA and K. MUNAYAMA, *Chem. pharm. Bull., Tokyo* 13, 639 (1965).

<sup>3</sup> R. SHAPIRO and S. AGARWAL, *Biochem. biophys. Res. Commun.* 34, 401 (1966).

<sup>4</sup> R. E. HOLMES and R. K. ROBINS, *J. Am. chem. Soc.* 86, 1242 (1964).

<sup>5</sup> M. IKEHARA, S. UESUGI and M. KANEKO, *Chem. Commun.* 17, (1967).

<sup>6</sup> R. A. LONG, R. K. ROBINS and L. B. TOWNSEND, *J. org. Chem.* 32, 2751 (1967).

<sup>7</sup> M. IKEHARA and S. UESUGI, *Chem. pharm. Bull., Tokyo* 17, 348 (1969).

<sup>8</sup> All the brominated compounds gave satisfactory elemental analyses and also had the correct UV characteristics.

<sup>9</sup> *n*-BuOH-AcOH-H<sub>2</sub>O (4:1:5), descending.

<sup>10</sup> P. A. LEVENE and F. B. LA FORGE, *Chem. Ber.* 45, 608 (1912).

<sup>11</sup> Communication No. 1435 from the Central Drug Research Institute.

### Collagen Substrate Films for Localizing Collagenolytic Activity Histologically

Collagenolytic activity has been reported in animal and human tissues under both physiologic and pathologic conditions<sup>1,2</sup>. Such collagenolytic activity has been demonstrated employing the methods or various modifications of Gross et al.<sup>3</sup>. This procedure involves the use of collagen gels, obtained by extraction of mammalian skin,

as substrates. The properties of extracted collagenases have been studied by viscometry and electrophoresis<sup>4</sup>.

Substrate films on microscope slides have been employed to demonstrate proteolytic activity and to localize deoxyribonuclease, ribonuclease, amylase and hyaluronidase<sup>5</sup>. Although such substrate film techniques