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[17].

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.

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PRO EXPERIMENTIS

Bromination of Nucleosides

Recently there has been considerable interest in the bromination of purine nucleosides[1-6] and nucleotides[7]. 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[8]. 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[9]. The structure of the bromonucleosides was confirmed by hydrolysis with *N* HCl to the corresponding brominated bases.

8-Bromoguanosine. Guanosine (283 mg, 1.0 m*M*) was suspended in anhydrous DMF (8 ml), NBS (200 mg, 1.14 m*M*) 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 m*M*) was dissolved in DMF (2 ml), NBS (200 mg, 1.14 m*M*) 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.[10]), 181°. Rf 0.63[9].

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[11].

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

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Collagen Substrate Films for Localizing Collagenolytic Activity Histologically

Collagenolytic activity has been reported in animal and human tissues under both physiologic and pathologic conditions[1,2]. Such collagenolytic activity has been demonstrated employing the methods or various modifications of GROSS et al.[3]. 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[4].

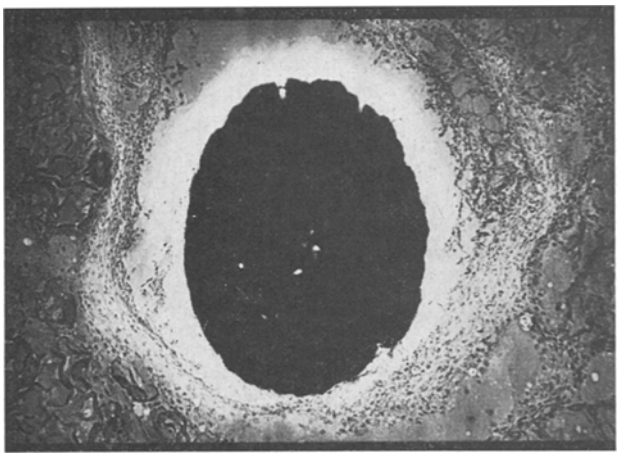
Substrate films on microscope slides have been employed to demonstrate proteolytic activity and to localize deoxyribonuclease, ribonuclease, amylase and hyaluronidase[5]. Although such substrate film techniques

do not usually give the fine enzyme localization of the dye coupling methods, the activity can be usually localized to a group of cells histologically.

Collagen substrate films were prepared in an attempt to obtain a localization of collagenolytic activity more suitable for histological application than the use of gels. A collagen dispersion was extruded through a slit onto 75×25 mm microscope slides. The concentration and amount applied was adjusted to give a dry film thickness of 5 μ. The collagen was obtained from beef tendon and is identical to the dispersion employed in making reconstituted collagen sutures[6]. These films have been employed to demonstrate collagenolytic activity in the tissue reaction zone around implanted surgical sutures.

The coated slides were soaked in sterile Tyrode's solution (containing 100 IU of penicillin and 100 mg streptomycin per ml) for 1 h. Frozen cryostat sections or tissue slices were placed on the films for 8 h, or in some cases overnight, and incubated at 37 °C and relative humidity of 95–100%. Control sections were incubated on films soaked in Tyrode's solution containing 0.01 *M* cysteine, or 0.01 *M* EDTA. At completion of incubation the slides with sections intact were gently rinsed in distilled water. Slides with tissue slices were washed to remove the tissue. The slides were then placed in acetone for a few minutes to remove any lipid material, rinsed in water and stained with picro-fuchsin. Collagenolytic activity was demonstrated by unstained areas where the collagen film had been digested (Figure). This activity was suppressed by both 0.01 *M* cysteine and EDTA. The film was digested and removed by *Clostridium histolyticum* collagenase, but not by pancreatin, pepsin or trypsin at any concentration, at pH levels from 5.0 to 8.5.

This preliminary report suggests an approach for demonstrating collagenolytic activity histologically. Studies are in progress on methods for making collagen substrate films from gels obtained from rat skins by a modification of the method of KANG et al.[7]. With these substrate films we hope to localize histologically a lower level of activity than we have found to date.



Section of size 3-0 collagen suture implanted in rat gluteal muscle for 4 days, and placed for 8 h on collagen substrate film. Stained with picro-fuchsin. Area of collagenolytic activity is shown by unstained area adjacent to suture. Magnification × 150.

Zusammenfassung. Als vorläufiger Bericht wird eine Methode zur histologischen Erfassung der Kollagenolyse beschrieben.

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A Quantitative Slide Test for ATP

The technique we describe in this short note is a modification of the well-known bioluminescence assay for ATP[1–12]. As pointed out in the literature, the sensitivity of the assay is limited by (1) the instrumentation for detecting light output and (2) the endogenous activity of the commercial firefly lantern extract (FLE). To increase the efficiency of light detection and decrease the endogenous activity of the FLE preparation, we have arranged to (1) allow the reaction to take place as close to the light detector as possible in order that a large fraction of the light be collected and (2) reduce drastically the volume of FLE required.

Our arrangement for light detection consists of a calibrated photomultiplier (RCA, 6655A) selected for its low noise, a power supply (John Fluke, 412A), an amplifier (Dymec, 2460A), and a chart recorder (Esterline Angus, AW). The photomultiplier, run at -900 V, is set with its cathode window up inside a light-tight box in a dark room.

To a microliter drop of FLE on a microslide resting on the photomultiplier window, we add a microliter drop of ATP or of the sample to be tested. Accurate delivery is achieved by means of disposable micropipettes (Microcaps, Drummond Scientific Co.). The mechanics of adding the second drop results in the mixing of the reagents. For even greater sensitivity, the reagents may be delivered and mixed right on the photomultiplier window itself. This method of delivery, however, is not recommended for routine assays, because it requires the extra precaution of cleaning the photomultiplier window thoroughly after every individual test.

Figure 1 compares the time dependence of the reaction on a microslide and that of a reaction in a tube. The volume of the reaction in the tube was 10^3 times that used on the slide and ATP was added by means of a tuberculin syringe.

The ATP used is a standard product of P-L Biochemicals, Inc. The FLE used is a commercial preparation from