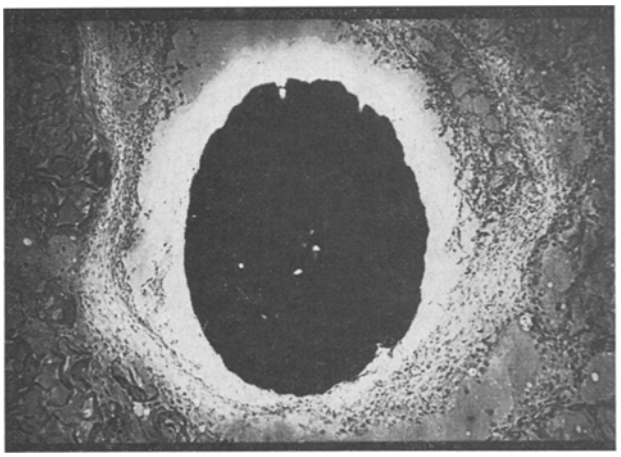
do not usually give the fine enzyme localization of the dye coupling methods, the activity can be usually localized to a group of cells histologically.

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

either Sigma Chemical Co. or Worthington Biochemical Corp. A microliter of freshly reconstituted FLE on a microslide gives at room temperature an endogenous activity below the noise of the photomultiplier at -900 V – which we calculate to be equivalent to 3×10^4 q sec^{-1}. It is not necessary, therefore, to reduce further the endogenous activity by allowing the preparation to age at room temperature or by adding apyrase[12].

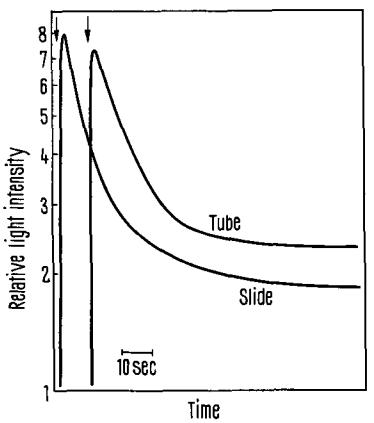

Fig. 1. Time course of firefly bioluminescence reaction. Arrows indicate the addition of ATP.

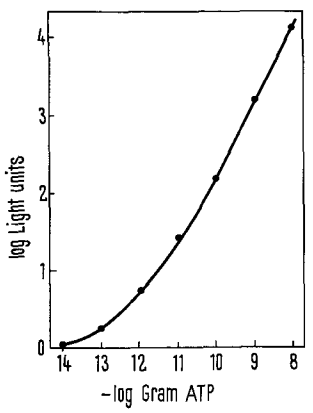

Fig. 2. Maximal velocity (or peak height) as a function of ATP concentration.

Figure 2 shows a typical standard curve. A light unit is equivalent to 3×10^4 q sec^{-1}. It is clear from the curve that the technique is sensitive enough to detect 10^{-13} g ATP.

Aside from its high sensitivity, the technique has virtue in that it is rapid, direct, uncomplicated and economical in the use of reagents[13].

Résumé. Un essai sur lamelle porte-objet a été mis au point pour la détermination quantitative du triphosphate d'adénosine. Il s'agit d'une modification de la réaction bien connue entre la luciférine et la luciférase derivées de la luciole. Cet essai permet de déceler des quantités minuscules de l'ATP pouvant atteindre 10^{-13} g.

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Eine quantitative Methode zur zahl- und volumenregulierten Übertragung von Mikroorganismen in Suspensionsmedien[1]

Es ist allgemein üblich, Medien mit leicht quantitativ bestimmbaren Mikroorganismen oder anderen lebenden Zellen nach der Methode dekadisch-logarithmischer Stufung zu verdünnen und dann in Versuchstiere oder leblose Träger- und Vermehrungsmedien zu überführen. Auf diese Weise ist etwa bei infektiösen Agentien die Bestimmung einer ID_{50} oder LD_{50} für eine Versuchstierart mit hinlänglicher Genauigkeit zu ermitteln. Zahlenmässige Exaktheit ist dabei allerdings nur entsprechend der relativ grossen Stufen des dekadischen logarithmischen Systems zu erreichen. Im folgenden soll auf die Möglichkeit hingewiesen werden, mit Hilfe eines «inversen Dreisatzes» gebrochene Exponenten rechnerisch und technisch zu umgehen, wenn in Sonderfällen ein engerer Verdünnungsraster wünschenswert ist. Dabei besteht ferner die Möglichkeit, Volumenexaktheit und -konstanz des Inokulums zu berücksichtigen. Die Methode stellt eine einfache Handhabe der Berechnung und Übertragung beliebiger Zahlen von Mikropartikeln in beliebigen Inokulationsvolumina dar, so dass über beide

[1] Mit Unterstützung der Deutschen Forschungsgemeinschaft.