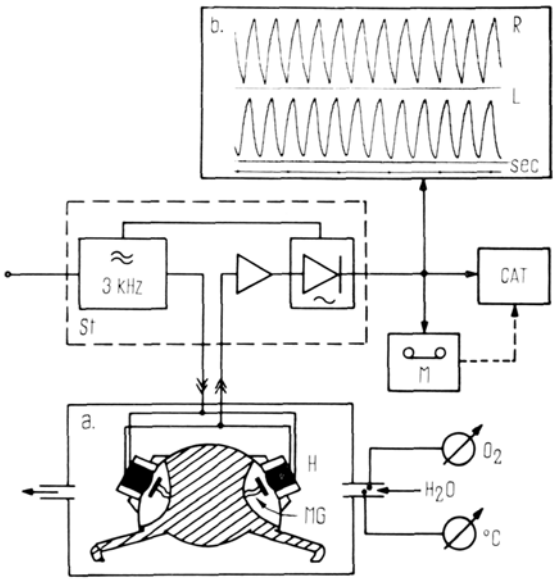
Öffnung mit Bienenwachs-Kolophonium (Mischung 1:3) befestigte Fassung nimmt den Hall-Generator auf, der zur Justierung in einem Paßstück sitzt (Figur a). Wird der den Magneten tragende Scaphognathit bewegt, so erfasst die Sonde die Veränderungen des Feldes. Die Messwerte gelangen über ein Steuergerät[3] zu den Registriereinheiten (Figur, Blockschaltbild). Während des Versuches sitzt der freibewegliche Krebs in einem durchströmten Plexiglasbecken (30 × 30 × 12 cm), das mit einem Deckel luftdicht verschlossen ist. Lediglich die dünnen Drähte verbinden das Tier mit den Anschlussbuchsen im Deckel des Gefässes.



Blockschaltbild der Versuchsanordnung. ST, Steuergerät; M, Magnetbandgerät; CAT, Computer. (a) Tier im Versuchsgefäss. MG, Magnet am Scaphognathit; H, Hall-Generator. (b) Aufzeichnung der Scaphognathitenbewegung am Schreiber. R, rechte Seite; L, linke Seite; sec, Zeitmarke.

Mit dieser Methode ist es möglich, die Bewegungen der beiden Scaphognathiten simultan zu messen und Schlagrichtung, Frequenz und Amplitude quantitativ über einen längeren Zeitraum zu erfassen (Figur b). Zur Auswertung der Phasenbeziehungen und anderer Grössen, die für eine Analyse der Koordination von Bedeutung sind, werden die Messdaten direkt oder über ein Magnetband einem Computer (CAT 400 C) zugeführt und ausgewertet. Simultan mit der Atembewegung werden Wassertemperatur und Sauerstoffgehalt im Versuchsgefäss mitgemessen.

Ergänzend sei bemerkt, dass diese Methode mit geringfügigen Veränderungen zur Registrierung von vielen rhythmischen und arhythmischen – insbesondere schnellen – Bewegungen an freibeweglichen Tieren eingesetzt werden kann.

Summary. A recording method for the ventilation movement by means of the Hall-effect in the unrestrained crayfish is described. Frequency, amplitude and direction of movement can be measured quantitatively by this method which may be applicable also to other physiological preparations.

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16. Mai 1968.

[3] Das Steuergerät wurde von Herrn Ing. Schönemann konstruiert, dem hierfür gedankt sei.

[4] Mit dankenswerter Unterstützung durch Mittel, die die Stiftung Volkswagenwerk Herrn Prof. Dr. Huber zur Verfügung stellte.

Application of the Indigogenic Principle for the Histochemical Demonstration of Ribonuclease

Recent studies in this laboratory have led to the histochemical demonstration of both acid and alkaline phosphomonoesterase[1] (orthophosphoric monoester phosphohydrolase) through the use of the synthetic substrate *p*-toluidinium 5-bromo-4-chloro-3-indolyl phosphate[2]. Enzymatic hydrolysis of this substrate liberates 5-bromo-4-chloroindol-3-ol, which is rapidly and irreversibly oxidized to 5, 5′-dibromo-4, 4′-dichloroindigo at the sites of activity.

On the basis of these findings, efforts were directed to the application of the indigogenic principle to the histochemical localization of phosphodiesterases I and II[3]. The syntheses, which were modeled after those described by Razzell and Khorana[4] for nitrophenyl-pT and Tp-nitrophenyl, proceeded from 1-acetyl-5-bromo-4-chloroindol-3-ol to give 5-bromo-4-chloro-3-indolyl-pT and Tp-5-bromo-4-chloro-3-indolyl in moderate yields. It was desirable to extend the indigogenic principle for the histochemical demonstration of ribonuclease since a majority of present techniques to demonstrate this enzyme are indirect coupling procedures or fluorescent antibody techniques[5–7]. The most recent coupling procedure involved the use of uridine 2′:3′ naphthyl phosphate[8]. A specific substrate for ribonuclease, 5-bromo-4-chloro-3-indolyl-uridine 2′ (3′) phosphate was synthesized in our laboratory modeled after the technique for the indolyl substrates for phosphodiesterases 1 and 11[3]. This substrate offers the advantage of precise enzyme localization with no or very slight diffusion. Moreover, the substrate affords a simple and direct method for demonstration of this enzyme without the need for a coupling reaction.

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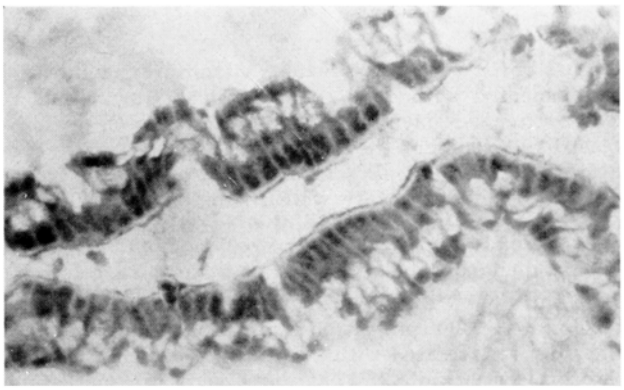

Fig. 1. Section of rat small intestine fixed with α-hydroxadipaldehyde and stained for alkaline RNase II utilizing the substrate 5-bromo-4-chloro-3-indolyl-uridine 2′(3′) phosphate at pH 7.6. A blue-green indigo is present in the cytoplasm of the small intestinal mucosa at the site of alkaline RNase II. × 800.

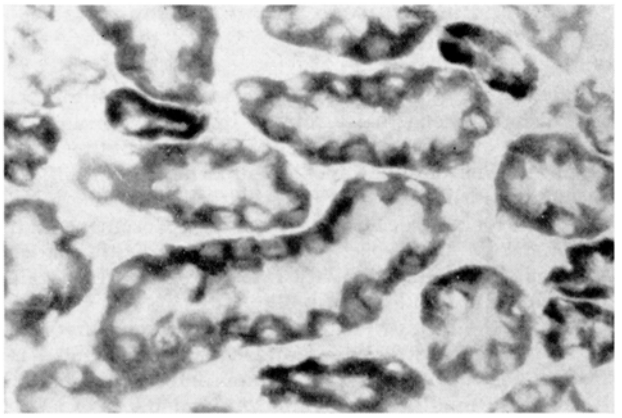

Fig. 2. Section of rat kidney fixed with α-hydroxadipaldehyde and stained for acid RNase II utilizing the substrate 5-bromo-4-chloro-3-indolyl-uridine 2′(3′) phosphate at pH 5.5. A blue-green indigo is present in the cytoplasm of the renal tubular cells at the site fo acid RNase II. × 800.

Methods. Rat tissues were used in this study. Representative pieces of tissue from each organ were removed and cut into blocks 2–4 mm in thickness, and quick frozen by placing the tissue in a glass tube and immersing it in a Dewar flask containing acetone and dry ice at − 70 °C. The tissues were embedded in optimal cutting temperature (O.C.T.) compound, purchased from Lab-Tek, composed of water-soluble glycols and resins matched to a specific cutting zone temperature of − 20 to − 35 °C. The embedded tissue was then placed on the quickfreeze bar of a Lab-Tek cryostat for 1 min until the embedding medium was frozen and became the proper consistency for cutting 6 μ sections at − 20 °C. Fresh frozen or fixed tissue sections were incubated in solutions containing 5-bromo-4-chloro-3-indolyl-uridine 2′ (3′) phosphate as the substrate. The incubation solution: for alkaline RNase II consisted of: 11 ml *Tris* buffer 0.1 m pH 7.6, 1 ml 5-bromo-4-chloro-3-indolyl-uridine 2′ (3′) phosphate 2 mg/ml buffer; for acid RNase II: 11 ml sodium acetate buffer 0.1 m pH 5.5, 1 ml 5-bromo-4-chloro-3-indolyl-uridine 2′ (3′) phosphate 2 mg/ml buffer.

Results. The histochemical reaction time for alkaline RNase II was 10 min and for acid RNase II, 2 h. A blue-green indigo was present at pH 7.6 in the cytoplasm of renal cortical proximal and distal tubular cells, pancreatic acinar cells, small intestinal mucosal and RE cells, liver, thyroid follicular cells, submaxillary acinar cells, tracheal mucosa, and adrenal cortex (Figure 1). At pH 5.5 a blue-green indigo was evident in the cytoplasm or renal cortical proximal and distal tubular cells, pancreatic acinar and ductal cells, liver, epididymus, thyroid follicular cells, submaxillary acinar (serous) and ductal cells, preputial acinar cells, tracheal mucosa, adrenal cortex and reticuloendothelial cells in spleen, lymph nodes and small intestinal mucosa (Figure 2). The enzyme localization was only cytoplasmic. Fresh frozen sections were inferior to fixed tissue sections for localization and intensity of reaction. The best fixatives were 4% calcium formol and α-hydroxadipaldehyde. Alkaline RNase II was found to be magnesium ion dependent since EDTA caused a 90% inhibition of the histochemical reaction at pH 7.6. However, EDTA did not inhibit acid RNase II activity at pH 5.5.

Our recent synthesis of the unique substrate 5-bromo-4-chloro-3-indolyl-uridine 2′ (3′) phosphate has facilitated the histochemical demonstration of alkaline RNase II and acid RNase II. Alkaline RNase II is primarily found in liver and kidney while acid RNase II is present in pancreas and other tissues. Alkaline RNase II hydrolyzes internucleotide bonds adjacent to the 3′ phosphoryl group of pyrimidine bases. Thus, RNA, poly-C and poly-U are hydrolyzed to yield small fragments but poly-A is not. Acid RNase II produces oligonucleotides from RNA and not mononucleotides. It appears to be specific for pyrimidine-3′ phosphoryl bonds since poly-A is not hydrolyzed[9]. We conclude: (a) a new unique substrate 5-bromo-4-chloro-3-indolyl-uridine 2′ (3′) phosphate has been synthesized for the histochemical demonstration of alkaline RNase II and acid RNase II; (b) this procedure was found to be more direct, rapid and precise than previous coupling techniques for the histochemical demonstration of this enzyme[10].

Zusammenfassung. Es wurde ein neues Indolsubstrat für die histochemische Demonstration von alkalischer RNase II und saurer RNase II synthetisiert. Dieses Verfahren ist besser, schneller und präziser als die bisherigen Kupplungstechniken.

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