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Potentialities of Bioluminescence Analyses in Research on the Pancreatic Islets

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INTRODUCTION

The endocrine pancreas in mammals consists of millions small corpuscules. Their number, size distribution and total volume have been calculated (1,2), and it is obvious that isolation of islets offers only limited amounts of material. Although analyses of small islet samples requires micro methods, information has been obtained about enzyme activities (3-5), concentration of nucleotides (4-7) and concentration of certain substrates (7-11). High demands on sensitivity have been met by enzymatic cycling (12) and bioluminescence techniques (13). Recent comparisons show good agreement between the analytical results of the two methods (14).

ASSAY OF NUCLEOTIDES

In metabolic evaluations, data on concentrations are of primary interest, but the rate of concentration changes deserves also consideration. For example, the ATP concentrations are high both in the islets (6,15,16) and in the epidermis (17), but change more rapidly in the islets due to higher capacities of energy mobilization. The sensitivity and convenience of bioluminescence assay has been utilized for measurements of nucleotide concentrations. The use of semiautomatic technique has permitted several analyses to be rapidly accomplished so that changes in ATP concentration could be followed in experiments with hypoxia (16). As a result of the oxygen lack, NADH accumulates (18).

When added to an appropriate light yielding solution, both ATP and NADH elicit flashes. The peak height of these are proportional to the added amount of the nucleotide concerned. The use of photokinetic techniques has made it possible to carry out several analyses. The oxidized forms of the pyridine nucleotides have previously required reduction prior to the measurement. Single step, direct analyses have now also been designed for NAD^+ (19) and $NADP^+$

(20,14) by means of continuous reduction in the light yielding solution. A long lasting light emission is obtained which permits ordinary mixing instead of sample injection, and moreover, measurements using less elaborate equipment (Fig. 1). In application of the new assay of NAD^+, redox states of the islets have been calculated, and these are expressed as $\frac{[NAD^+]}{[NADH]}$ (21). The possibility of using both the reduced and the oxidized nucleotide forms as measurable products in coupled reactions have extended the applicability of bioluminescence analyses. This concerns particularly a manifold of dehydrogenase reactions in which NAD^+ is formed.

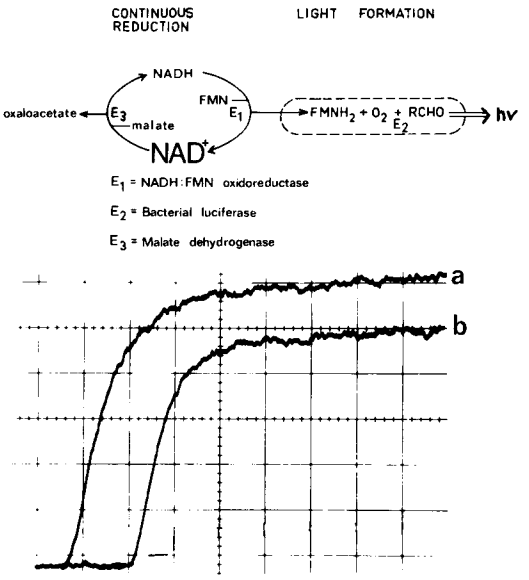

Fig. 1. *Oscillograms showing the long lasting levels of light emission as observed after addition of 12 pmol (a) and 10 pmol(b) of* NAD^+. *The reaction scheme demonstrates the malate dehydrogenase cycle which produces a continuous reduction of* NAD^+.

ASSAY OF Ca^{2+}

In research on the pancreatic islets, the potentialities of bioluminescence assay are not limited to the firefly and bacterial luciferase systems. The jelly fish *Aequorea aequorea* contains a luciferin and a luciferase which yield light flashes upon rapid addition of Ca^{2+} (see Fig. 2).

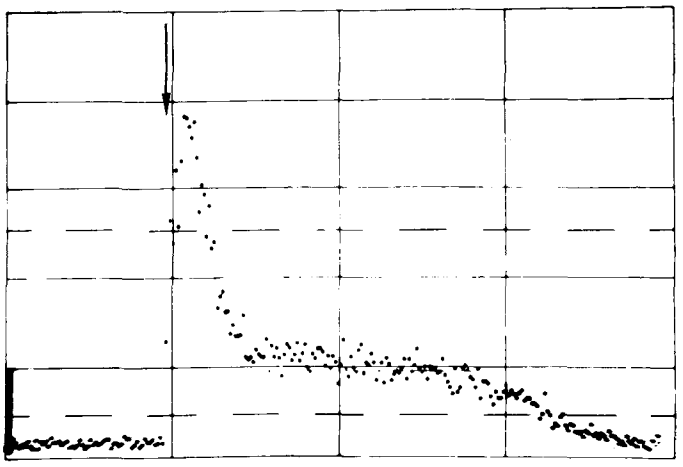

Fig. 2. *Oscillogram showing photon emission following rapid injection (↓) of 1 nmol Ca^{2+} into an Aequorin solution. The height of each dot represents the number of photon collected during periods of 6 · 10^{-2} seconds. The integral of the flash (50 periods) were used for the calculations of Ca^{2+} amounts.*
The light yielding solution was prepared by dissolving 1 mg freeze-dried Aequorin into 400 µl double-distilled water and by centrifugation for 1 h at 160,000 g in a Beckman Airfuge$^{T.M.}$. The pellet was then redissolved in 400 µl 0.1 mol/l Tris, pH 6.8. In 25 µl of this solution, 2 µl samples were injected.

In the picomole region, good linearity is obtained between the amount of calcium ions and the emitted number of photons (Fig. 3). Our calculations of the calcium concentration of the pancreatic islets from lean litter-mates of the obese-hyperglycaemic mice (gene symbol *ob/ob*) gave a value of 78.5 $\pm$ 7.6 mmol per kg dry weight. This high value is in the same range as reported values using flameless atomic absorption spectrophotometry (22).

MONITORING OF ANALYSES

In experiments leading to concentration changes, a large number of determinations may be required in order to acertain appropriate follow up. This can

be facilitated by monitoring the analytical performance with microprocessing technique (23).

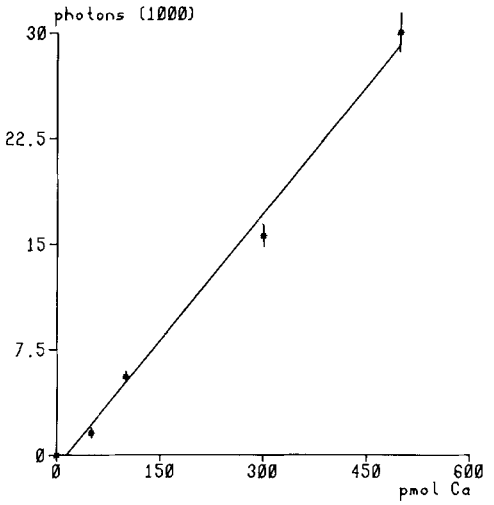

Fig. 3. *Calibration curve for the bioluminescence assay of* Ca^{2+}. *The performance of the measurements are described in Fig. 2.*

It is of particular advantage to have the computer connected to a suitable read-out device so that a series of analyses can be completed without any interruptions for recording and evaluating the results. Afterwards, single determinations can be displayed from the memory of the microprocessor for examining the time course of the light production (Fig. 4).

ABSTRACT

Progress in bioluminescence assay permits not only determinations of nucleotide and substrate concentrations, but also estimation of concentration shifts. The analyses can be extended to comprise Ca^{2+} since the *Aequorea* system is sensitive enough for applications in islet research.

By connecting the bioluminometer to a microprocessor with a suitable read-out device, it is possible to collect and evaluate large amounts of data which may be required in studies of concentration shifts. Thus, blanks, samples and standards can be processed completely within short time periods so that the light-yielding solutions remain stable.

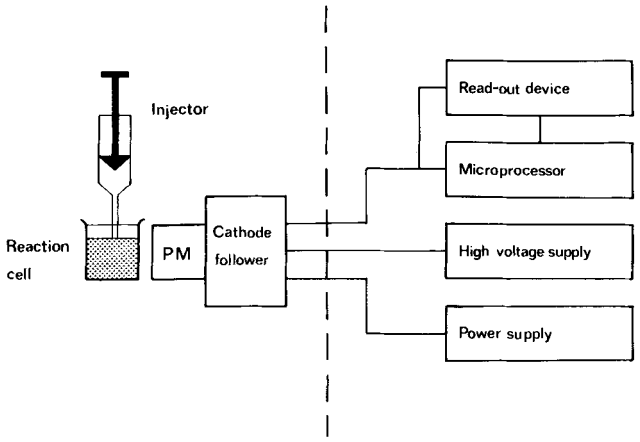

Fig. 4. *Block diagram of measuring equipment designed for bioluminescence assay.*

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