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An apparatus for simultaneous measurement of 90° light-scattering, fluorescence intensity of reduced pyridine nucleotides and oxygen consumption of mitochondria

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An apparatus for simultaneous measurement of 90° light-scattering, fluorescence intensity of reduced pyridine nucleotides and oxygen consumption of mitochondria*

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Abstract

1. An apparatus for the simultaneous measurements of volume change, fluorescence intensity of pyridine nucleotides and oxygen consumption of mitochondria has been constructed. 2. Oxygen consumption is measured by the rotating platinum electrode with a modification of Hagihara's system, attached in a cuvette of the apparatus. 3. Volume changes of mitochondria (swelling-shrinkage) are measured by the 90° light-scattering at 650 mμ. 4. Relative fluorescence intensity of pyridine nucleotides is measured by the fluorometer: for the excitation, a bright light at 365 mμ. line of mercury lamp is isolated through the filter and exposed to the mitochondria suspended in a cuvette of the apparatus, and fluorescent emiion is analyzed by a grating mirror monochromator. 5. The scattered light at 650 mμ. is not affected by the excitation light and the fluorescent emiion, and fluorescence intensity is not affected by the scattered light at 650 mμ. 6. The simultaneous measurements of the oxidation-reduction of pyridine nucleotides, the respiration states and the changes in the intensity of 90° lightscattering of mitochondria are given as an example of the performance of the present apparatus.

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AN APPARATUS FOR SIMULTANEOUS MEASUREMENT OF 90° LIGHT-SCATTERING, FLUORESCENCE INTENSITY OF REDUCED PYRIDINE NUCLEOTIDES AND OXYGEN CONSUMPTION OF MITOCHONDRIA

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Studies on the structure and function of mitochondria have been made by many investigators by using oxygen electrode[1-4], 90° light-scattering[2,5], fluorometer[6,7] and double-beam spectrophotometer[1,8,9] for the purpose to elucidate the energy regulating mechanisms of mitochondria and of cancer or normal cells. It is profitable to measure simultaneously these biochemical changes of mitochondria, which usually have been done individually in many cases.

An apparatus was designated and constructed for the simultaneous measurement of 90° light-scattering, fluorescence intensity of reduced pyridine nucleotides and oxygen consumption of mitochondria or of ascitic cancer cells.

The present paper deals with the construction of the apparatus, a brief account of efficiency of this apparatus, and an example of its use are described here.

RESULTS

I. Outline of the apparatus

This apparatus consists of the following three parts: an oxymeter to measure the oxygen consumption of mitochondria, 90° light-scattering apparatus to the change of mitochondrial volume and fluorescence spectrophotometer to the state of oxidation-reduction of pyridine nucleotides in mitochondria, as shown in Figs. 1 and 2.

1. Oxymeter

Oxymeter is constructed by the authors modifing the Hagihara's system[1]. The rotating platinum electrode is inserted directly into the sample cell which is a closed system as shown in Fig. 3. This polarographic cell is made of quartz glass ($10 \times 10 \times 20$ mm). The one side of the cuvette has two small pores, and one of them is connected to a calomel half cell with agar-KCl bridge and the other one is to the piston for draw reagents into the cuvette with slender vinyl tube. The upper side has also two pores, one of them to introduce reagents

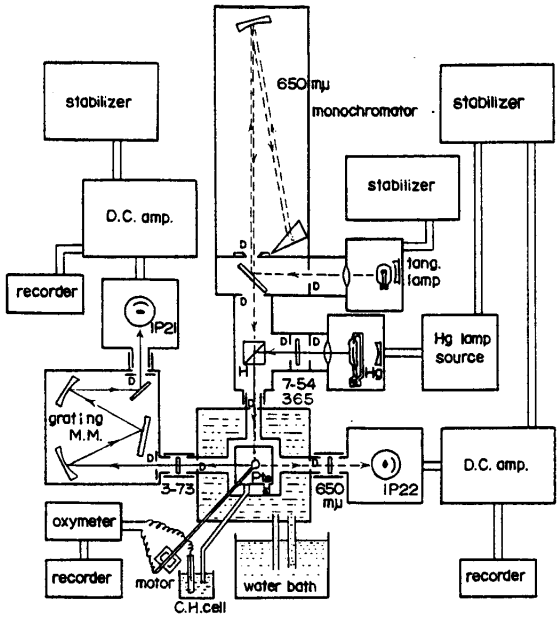

Fig. 1 Outline of the apparatus. tang. lamp: tangsten lamp, monochromator: prism monochromator, H: half mirror, 650 mμ: 650 mμ filter, 1P 22: photomultiplier, D. C. amp.: D. C. amplifier, recorder: autorecorder, stabilizer: stabilizer, Hg lamp source: source of mercury lamp, Hg: mercury lamp, 7-54: filter of Corning No 9863, 365: Hitachi filter 365 mμ, 3-73: filter of Corning No 3389, grating M. M.: grating mirror monochromator, 1P21: photomultiplier, Pt: rotating platinum electrode, motor: synchronous motor, C. H. cell: calomel half cell, oxymeter: oxymeter constructed by the method of Hagihara[1], water bath: water bath, D: diaphragm.

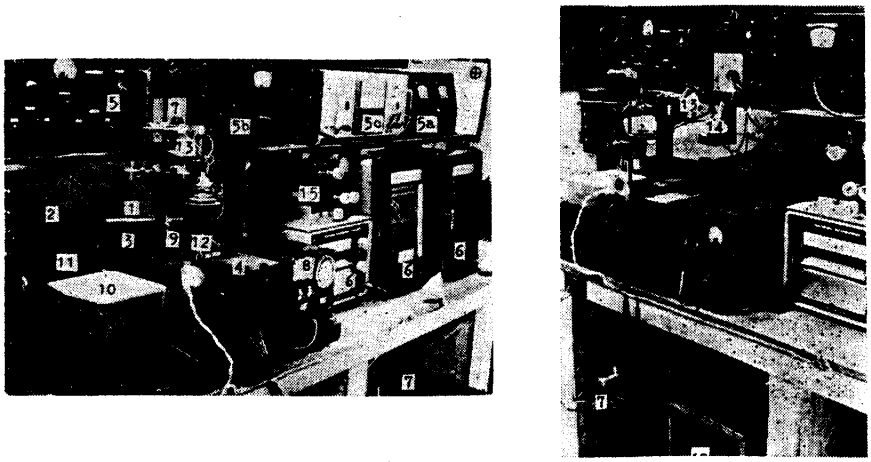

Fig. 2 Photographs of the apparatus. 1: tangsten lamp, 2: prism monochromator, 3: half mirror, 4: photomultiplier 1P22, 5: D. C. amplifier, 6: autorecorder, 7: stabilizer, 8: source of mercury lamp, 9: mercury lamp, 10: grating mirror monochromator, 11: photomultiplier 1P21, 12: rotating platinum electrode, 13: synchronous motor, 14: calomel half cell, 15: oxymeter, 16: sample room, 17: water bath.

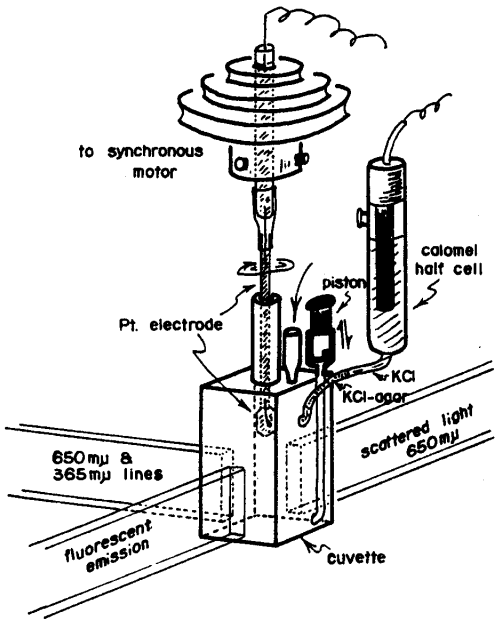

Fig. 3 Diagram of a sample cuvette for polarographic, 90° light-scattering and fluorometric analysis.

and the other one connected to the polyethylene tube which holds the rotating platinum electrode. The total volume of the cuvette is 2 ml. The rotating speed of the electrode is 600 r. p. m. and the full scale of the recorder corresponds to the oxygen concentration equilibrated with air.

2. 90° light-scattering apparatus

The monochrome light (650 mμ) of tangsten lamp is made by a prism monochromator and leads to the cuvette through two narrowed diaphragms as shown in Fig. 1. Scattered light is filtered through a glass filter transmitting 650 mμ, excluding ultra-violet and visual radiation under 600 mμ. Then the scattered light is registered by a photomultiplier tube 1P22 which is the selection of choice for the measurement of light red end of the spectrum, amplified with a DC amplifier and is recorded with Yokogawa Electric works, Ltd. autorecorder 60 type. The change in intensity of the scattered light is estimated in term of percentage of the initial intensity. The arrangement of the apparatus is shown in Fig. 1.

3. Fluorescence spectrophotometer

Relative fluorescence intensity of reduced pyridine nucleotides in mitochondria is estimated by the fluorescence intensity at 440-450 mμ, exposed to the excitation light. For the excitation, a bright light at 365 mμ line of the high

pressure mercury lamp (Olympus Co., Tokyo) is isolated from the other bright light by passing through a Hitachi 365mμ filter (No. 365) or Corning No. 7−54 (9863) which transmits ultra-violet and excludes visual radiation above 410mμ, and then it is led to a cuvette by the half mirror at the position of light path between the monochromator for light-scattering and cuvette as shown in Fig. 1. The fluorescent emission is led to the filter of Corning No. 3—73 (3389) which transmits visual radiation at approximately 440mμ at the position of 90° against the excitation light (365mμ). Then the fluorescence intensity in the spectral interval set by a grating mirror monochromator (Farrand grating monochromator) is registered by a photo-multiplier tube 1P21 which has a good signal-to-noise ratio and is suitable for the measurement of compounds that fluoresce between 350—650mμ. The relative fluorescence intensity is recorded by an autorecorder of Tōa Electronics Ltd. EPR-2T.

II. Efficacy

Fig. 4 shows the fluorescence emission spectrums of the reduced pyridine nucleotides. This spectrum is illustrated by the intensity of fluorescence of mitochondrial suspension registered by a photomultiplier 1P21. Curves A and B

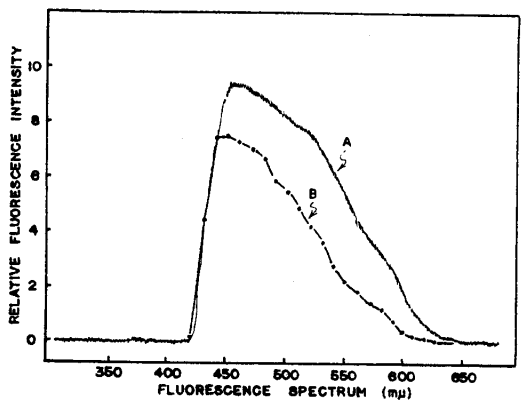

Fig. 4 Fluorescence emission spectrum of reduced pyridine nucleotide of rat liver mitochondria suspended in the medium of 0,1 M sucrose, 20 mM KCl, 5 mM Tris-HCl buffer (pH 7.4), 50 μM EDTA, 1 mM $MgCl_2$, 3 mM K-phosphate and 3 mM Na-succinate.

show the spectrums traced by a Farrand fluorometer or by the constructed apparatus respectively. Both of them show a similar pattern to that estimated by AVI-DOR[7]. In this instiance, if the colloidal solution of unsaturated fatty acid is added to the mitochondrial suspension, the scattered light by 650 mμ for 90° light-scattering or by the excitation light is not interfered with the maximum peak of fluorescence emission spectrum (440—450mμ). Moreover, the scattered light at 650mμ is not affected by the excitation light or fluorescent emission

and the fluorescence intensity is not affected by scattered light at 650 mμ (Fig. 5). Noise levels in the measurement of fluorescence is less than 0.5 per cent for a rise time (10—90%) of one second and in 90° light-scattering is less than one per cent for rise time (10—90%) of one second as shown in Fig. 5. Furthermore, the state of oxidation-reduction of pyridine nucleotides shows the same pattern in the both cases traced by a Farrand fluorometer and the constructed apparatus (Fig. 6).

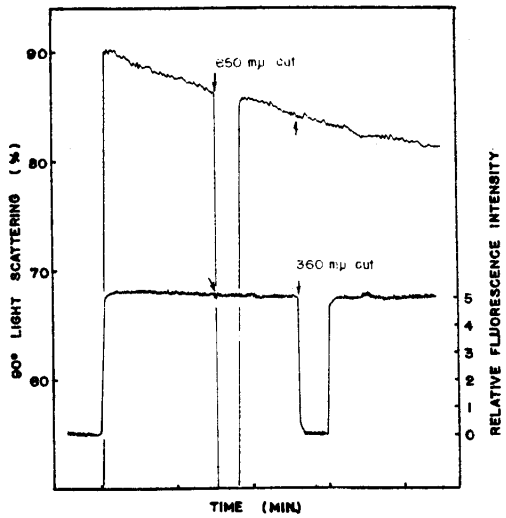

Fig. 5 Effects of excitation light at 365 mμ and fluorescent emission on 90° light scattering at 650 mμ and of the 650 mμ line for light scattering on the fluorscent emission at 450 mμ.

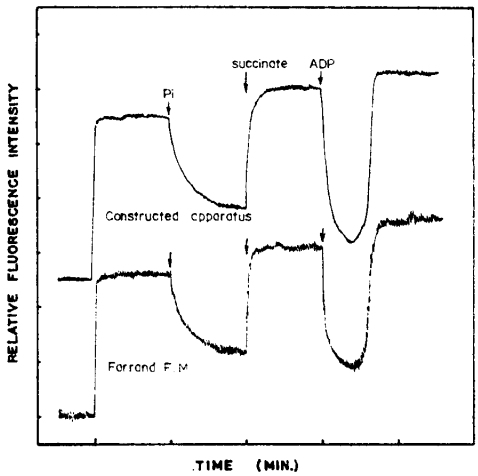

Fig. 6 Comparison of fluorescence intensity curves of rat liver mitochondria traced by using a Farrand fluorometer and the constructed apparatus. Mitochondria were suspended in the medium as described in Fig. 4 except K-phosphate. Added reagents were 3 mM K-phosphate, 3 mM Na-succinate and 100 μM ADP.

III. An example of the application of this apparatus

Fig. 7 shows simultaneous measurements of oxidation-reduction state of pyridine nucleotides, the state of 90° light-scattering and the respiration state in

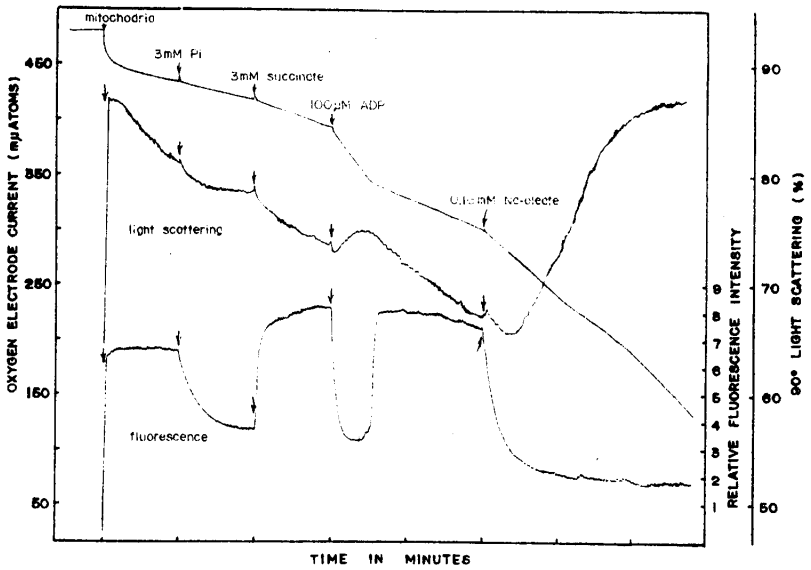

Fig. 7 Simultaneous measurements of oxidation-reduction of pyridine nucleotides, respiration state and 90° light-scattering (at 650 mμ) of rat liver mitochondria in the medium of 0.1 M sucrose, 20 mM KCl, 5 mM Tris-HCl buffer (pH 7.4), 50 μM EDTA, and 1 mM $MgCl_2$ at 25°. Arrow shows the addition of substance. Upper-trace refer to the oxygen concentration in the medium, middle-trace to the 90° light-scattering at 650 mμ and lower-trace to the fluorescence intensity of pyridine nucleotides. The time moves from left to right and downward deflections indicate oxygen consumption, swelling and oxidation of pyridine nucleotides.

rat liver mitochondria at 25°. In the medium as described in the legend of Fig. 7, pyridine nucleotides are slightly oxidized by the additon of inorganic phosphate (Pi) and is reduced remarkably by Na-succinate, accompanied with the reduction of 90° light-scattering. In Chance's state 3[9] by adding adenosine diphosphate (ADP), the increased oxygen consumption (respiratory control index is about 4), the increased light-scattering (shrinkage), and the decreased of fluorescence intensity are observed. But decreased-oxygen consumption and −90° light-scattering and increased-fluorescence intensity are brought about by changing the state of respiration to Chance's state 4 after the phosphorylation of the added ADP to ATP. The addition of a true uncoupler such as oleic acid induced the respiratory release, the increase of scattered light and the decrease of fluorescence intensity at the same time.

SUMMARY

1. An apparatus for the simultaneous measurements of volume change, fluorescence intensity of pyridine nucleotides and oxygen consumption of mitochondria has been constructed.

2. Oxygen consumption is measured by the rotating platinum electrode with a modification of Hagihara's system, attached in a cuvette of the apparatus.

3. Volume changes of mitochondria (swelling-shrinkage) are measured by the 90° light-scattering at 650 mμ.

4. Relative fluorescence intensity of pyridine nucleotides is measured by the fluorometer: for the excitation, a bright light at 365 mμ line of mercury lamp is isolated through the filter and exposed to the mitochondria suspended in a cuvette of the apparatus, and fluorescent emission is analyzed by a grating mirror monochromator.

5. The scattered light at 650 mμ is not affected by the excitation light and the fluorescent emission, and fluorescence intensity is not affected by the scattered light at 650 mμ.

6. The simultaneous measurements of the oxidation-reduction of pyridine nucleotides, the respiration states and the changes in the intensity of 90° light-scattering of mitochondria are given as an example of the performance of the present apparatus.

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