In order to study this, the rate of excretion was followed in rats in which the intestinal flora had been reduced by treatment with terramycin and phthalylsulfonazonum. In these rats the rate of excretion was much slower than in normal animals.

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Demonstration of a Thermostable Cofactor of Oxidative Phosphorylation in Rat Liver Mitochondria

Lars Ernster and Hans Löw

Wenner-Gren's Institute, Stockholm, Sweden

It has previously been reported that addition of adenosine triphosphate (ATP) and Mn^{++} to mitochondrial suspensions pretreated in the presence of 0.5 m*M* Ca^{++} and 4 m*M* Mg^{++} for 5 minutes at 30° C induces a reconstruction of the phosphorylations accompanying the aerobic oxidation of succinate [1]. Subsequently, these effects could be studied in greater detail with mitochondrial systems aged in Mg^{++} — and Ca^{++} — free media [2]. The yield of reconstructed phosphorylation in these systems shows an inverse relationship to the time of aging, and its fall can be greatly enhanced by the presence of Ca^{++} in the Mg^{++}-free preincubation medium. Recentrifugation studies of the aged suspensions have revealed that, during aging, a factor is released from the mitochondria, which is involved in the phosphorylative mechanism [3]. This factor has now been shown to be stabilized by heating the preincubation medium, obtained after recentrifugation, to 100° C for 2 minutes. Furthermore, the presence of the factor could be demonstrated in boiled extracts of freshly prepared mitochondria. Experimental data bearing on this are shown in Table 1.

Mitochondria from one rat liver were suspended in 30 ml of a buffer solution (pH 7.5) containing KCl, orthophosphate, adenylic acid, glucose and sucrose (in concentrations specified previously [2]). After removal of a sample serving as control (incubated immediately in the presence of succinate, hexokinase and Mg^{++}), the suspension was warmed to 30° C and supplemented with hexokinase and 0.5 m*M* Ca^{++}. After 5 minutes of preincubation under shaking, the suspension was cooled to 0° C, divided into four parts and each part recentrifuged for 5 minutes at 5 000 *g* in the high-speed attachement of an International Refrigerated Centrifuge. The four mitochondrial pellets (*a*—*d*) were resuspended, to a final volume equal to that before centrifugation, in *a*) a new incubation mixture of the same composition as before; *b*) its own supernatant; *c*) the supernatant heated for 2 minutes in a boiling water bath (the slight precipitate formed on heating was removed by centrifugation); and *d*) in an extract of freshly prepared mitochondria, obtained by heating a suspension (of the same composition as the original one, but twice as concentrated with respect to mitochondria) for 2 minutes in a boiling water bath, and centrifugation as above. From each of the four suspensions two aliquots of 1.5 ml were incubated in Warburg vessels, one with succinate alone, and a second one with succinate, ATP and Mn^{++}. Each vessel was furthermore supplemented with Mg^{++} and a suitable excess of hexokinase. Respiration and phosphorylation were measured for a period of 20 minutes.

Table 1. Oxydative phosphorylation in recentrifuged mitochondria aged in the presence of Ca^{++}.

Each Warburg vessel contained: KCl, 150 μmoles; orthophosphate, 50 μmoles; adenylic acid, 4.3 μmoles; glucose, 60 μmoles; sucrose, 125 μmoles; succinate, 30 μmoles; Mg^{++}, 4 μmoles; hexokinase, *ca.* 10 fold excess[2]; Ca^{++} (in pretreated systems), 1 μmole. Additions (where indicated): ATP 1.5 μmoles; Mn, 1 μmole. Final volume, 2.0 ml. Gas phase, air. Temp, 30° C. Time of incubation, 20 min.

	Additions	Respiration (μatoms oxygen)	Phosphorylation (μmoles phosphate)
Untreated system	—	14.9	27.0
Pretreated system resuspended in:			
a) new incubation mixture	—	18.0	1.0
	ATP, Mn^{++}	16.7	0.5
b) supernatant	—	16.6	2.1
	ATP, Mn^{++}	14.0	1.3
c) boiled supernatant	—	18.3	1.0
	ATP, Mn^{++}	15.9	20.0
d) boiled extract of fresh mitochondria	—	14.5	1.4
	ATP, Mn^{++}	18.0	23.2

As seen in Table I, under the prevailing experimental conditions, no phosphorylation can be reconstructed by ATP and Mn^{++} in the system resuspended in new incubation medium, or in the original supernatant. When boiled supernatant, or a boiled extract of fresh mitochondria is used as a suspending medium, reconstruction of phosphorylation again becomes possible. This indicates that the falling yield of the reconstructed phosphorylation, previously observed to occur on prolonged preincubation in Mg^{++}-free medium, may be accounted for by a destruction, probably enzymic in nature, of the released factor.

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Rapid High Precision Conductivity Recorder

Carl-Ove Andersson and Einar Stenhagen

Department of Medical Biochemistry, Institute of Medical Chemistry, University of Uppsala, Uppsala, Sweden

Olof Mellander

Department of Medical Biochemistry, University of Gothenburg, Gothenburg, Sweden

For studies of enzymatic degradation of peptides and proteins and micelle formation in bile salt solutions as well as for conductometric titrations we needed a conductivity recorder of high precision and rapid response, stable enough to follow small resistance changes over long periods of time. Several automatic recording devices have been described in the literature (*cf.* Refs.[1,2]). Most of these have been constructed for use as detectors in chromatographic analysis and none appeared to have the desired combination of high sensitivity, speed, and stability needed for our purposes. This also applied to commercially available instruments.

We have, therefore, converted a Shedlovsky precision bridge[3] into a high precision resistance recorder in the following manner.

The bridge is fed from a 1 000 c/s r.c. oscillator. The detector signal from the bridge is amplified by a three stage logaritmic amplifier which prevents overloading of the recording system for large unbalance signals. The amplified signal is rectified by a phase sensitive homodyne detector of the type described by Kinell[4]. The filtered output from the homodyne is fed to a Speedomax G recorder. To the potentiometer shaft of the recorder is mechanically coupled a linear high precision potentiometer which forms parts of two arms of the bridge, the moving contact being connected to the detector amplifier. A signal from the bridge makes the recorder pen move until the resistance balance of the bridge has been restored through the potentiometer attached to the pen movement. In order to prevent hunting in the servo system an adjustable velocity feed back to the recorder amplifier is provided from a D.C. generator geared to a balance motor of the recorder. Through a variable shunt across the potentiometer coupled to the pen, the resistance change for full scale response of the recorder may be adjusted over a 1:200 range (*e. g.* 10:2000 Ω). The speed of response is practically that of the recorder (4.5 secs. in the Speedomax G used). The lock-in amplifier detector system used gives a very low noise level which makes the recorder detect smaller resistance changes than the human ear using head phones. A sensitivity of 2 parts per 100 000 has been reached with no detectable noise in the recordings. Applications will be described.

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Studies on Pancreatic Lipase

Bengt Borgström

Department of Physiological Chemistry, University of Lund, Lund, Sweden

A simple and rapid method for the determination of pancreatic lipase activity is described. It is based on the rate of clearing of a triolein emulsion by the lipase.

Using this assay method lipase from human and rat pancreatic juice has been purified by salt precipitation and electrophoresis on starch block according to Kunkel. The purest fractions so far obtained have an activity approximately 1 500 times that of extracts from acetone powder of pancreatic glands (calculated per mg of protein) (viokase).

No evidence of any esterase activity in human or rat pancreatic juice has been found.

The effect of bile acids, fatty acids and some other substances on the rate of hydrolysis of triolein by purified lipase will be described.