Oxidative Phosphorylation of Guinea-Pig Aorta[1]

It is an established fact that arterial tissue exhibits a high rate of aerobic glycolysis[2]. As pointed out by LEHNINGER[3], this might indicate deficiencies in oxidative phosphorylation of the aortic wall. It was therefore considered of interest to measure the oxidative phosphorylation in arterial tissue.

Material and methods. Adult guinea-pigs were sacrificed by bleeding. The aorta was washed, stripped of adhering tissue and homogenized at 0 °C in 0.5 ml medium (0.23 *M* mannitol, 0.07 *M* sucrose, 0.02 *M Tris* buffer, pH 7.4) with a Kontes-Duall microhomogenizer. Oxygen uptake was measured with a YSI Model 53 Biological Oxygen Monitor (Yellow Springs Co., Ohio). Organic radiophosphate formation was determined by the method of NIELSEN and LEHNINGER[4]. Radioactive sodium phosphate was obtained from Nuclear Consultants Corp., Chicago, Illinois; it was heated in 1 *N* HCl for 1 h at 100 °C and subsequently neutralized. Countings were made with a GM counter (Tracerlab, Boston, Mass.).

The reaction was carried out in air-saturated medium (final volume 2.25 ml, pH 7.4) with the following millimolar concentrations: Na-glycylglycine, 15; Na-phosphate, 20 (1 μC P^{32}); glucose, 30; KCl, 50; $MgCl_2$, 5; Na-ATP, 2.5; cytochrome *c*, 0.05; Na-pyruvate, 10; Na-malate, 10; Na-EDTA, 1; and DPN, 0.5. Homogenate, 0.1 ml, equivalent to approximately 20 mg fresh tissue, was added. After 4 min equilibration at 30 °C the reaction was started by addition of 2 mg hexokinase (Sigma Type V) and readings of oxygen consumption were begun after 30 sec. The reaction was stopped after 30 min by addition of 0.1 ml concentrated TCA to a 1.0 ml aliquot. After centrifugation, organic phosphates were quantitatively extracted from the supernatant and the radioactivity of these compounds determined.

Results. In the Table the results of the experimental studies are presented, which show a notable rate of oxidative phosphorylation displayed by guinea-pig aortic homogenates in the presence of pyruvate and malate substrates. This confirms the findings recorded by WOLLEMANN and KOCSAR[5] in experiments with rat aortic tissue. These observations are of interest in view of the low Pasteur effect[2] in arterial tissue.

Oxygen phosphorylation by aortic tissue

Experiment No.	n*M* organic P^{32}/mg wet tissue	n Atom oxygen/mg wet tissue	P/O ratio
1	15.05	9.18	1.64
2	14.00	9.10	1.54
3	16.65	9.80	1.70
4	11.25	6.85	1.64
5	14.70	9.01	1.63

Zusammenfassung. Bei der Untersuchung von Aorta-Homogenaten von Meerschweinchen wurde ein hoher P/O Quotient für die oxydative Phosphorylierung gefunden.

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Washington University School of Medicine, St. Louis (Missouri, USA), July 29, 1966.

[1] This work was supported by Public Health Service Grant No. HE-00891.

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Uptake of Labelled DNA by Isolated Perfused Organs

Many cells are capable of taking up highly polymerized DNA and of retaining it for some time[1]. This phenomenon has been demonstrated in plants[2], in cells, in tissue culture[3], and in intact mice[1,4]. It is, however, difficult in the intact animal to distinguish the contributions of different organs to the uptake and the breakdown of DNA. The technique of perfusing isolated organs appeared, therefore, particularly suitable for the study of the uptake of DNA and its degradation in a given mammalian organ. By changing the perfusion liquid, after uptake has taken place, it is, furthermore, possible to determine whether the DNA is retained in the tissue.

Methods. Perfusion of the isolated liver of rats was carried out as described elsewhere[5,6,9]. Fresh, heparinized rat blood diluted with $^1/_3$ volume of Ringer solution was used as perfusion medium. In some experiments, the perfusion medium containing the active material was replaced during perfusion with non-radioactive perfusate. This washing fluid was either perfused only once or recycled continuously through the organ. The perfusion apparatus was set up in such a way that this change was possible without appreciable mixing between the two fluids and without disturbing the perfusion.

Labelled DNA was prepared from *E. coli* strain CR34 (thymine less), according to the method of MARMUR[7] using H^3-thymidine or P^{34}-O4 as precursor. The specific activity of the DNA thus obtained was 1 mCi/mg for H^3-DNA or about 0.01 mCi/mg for P^{32}-DNA.

The samples taken from the organs during perfusion were homogenized in 40 vol of water, and 50 λ of homogenate or perfusate were used for the chromatographic fractionation by centrifugation on DEAE-paper pulp according to procedures published earlier[1,8]. 6 different eluants are used for the elution. Fraction 1 (average molecular weight 200) is eluted with 0.02 *M* phosphate buffer pH 7 (P buffer), and fraction 2 (average molecular weight 2000) with 0.14 *M* NaCl in P buffer. These 2 fractions are acid soluble. Elution is then continued with 0.5 *M* NaCl in P buffer (fraction 3 average molecular

weight 50,000), with $5M$ NaCl in P buffer (fraction 4 average molecular weight 500,000), with $2M$ NaCl in $0.2M$ NH_4OH (fraction 5 average molecular weight 1,000,000), and finally with $1M$ NaOH (fraction 6 average molecular weight 5,000,000).

The radioactivity in each fraction was assayed by liquid scintillation counting. When 2 isotopes (P^{32} and H^3) were to be measured, the 2-channel method was used. The radioactivities in the various fractions of a sample were plotted as columns, thus yielding chromatographic profiles as shown in Figures 1–3.

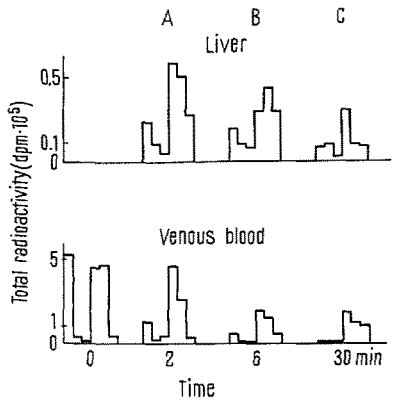

Fig. 1. Uptake of ^{3}H-DNA by the isolated perfused regenerating liver. DNA-^{3}H, dissolved in 10 ml of blood, was perfused and samples of liver and venous blood were taken (A) after 2 min. The liver was then washed with 10 ml of inactive perfusate (B), and then continuously with 40 ml of perfusate for 25 min (C). The data on radioactivity in the different chromatographic fractions of DNA are expressed as activity in total volume of perfusate or in total organ (liver weight 4.2 g).

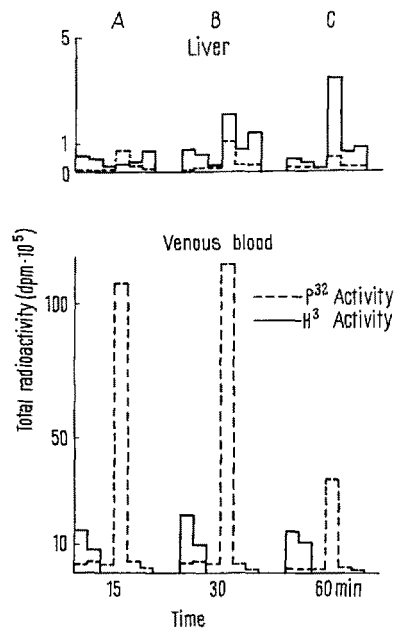

Fig. 2. Uptake of ^{32}P-DNA (broken lines) and incorporation of ^{3}H-thymidine (continuous lines) into DNA by the isolated and perfused regenerating liver. The active material was perfused through the liver in 30 ml of blood. Samples of liver and blood were taken after 15 min (A) and 30 min (B). The liver was then washed continuously with 30 ml of inactive perfusate for 30 min (C). Data on ^{3}H- and ^{32}P-radioactivity are expressed as activity in total volume of perfusate or in total organ (liver weight 3.9 g).

Results. Perfusion of the isolated liver. Polymerized H^3-labelled DNA consisting mostly of fractions 4 and 5 was dissolved in 10 ml of blood perfused once through the liver. The liver was then washed once with another 10 ml of non-radioactive perfusate. This was replaced by 40 ml of non-radioactive perfusate which was allowed to recirculate through the liver for 25 min. Data on one of these experiments using regenerating liver (24 h after partial hepatectomy) are presented in Figure 1. An appreciable % of the radioactivity (about 10% of the total activity) is taken up by the liver and most of it is retained during washing. Only 16% of the activity is lost during washing. Comparison of the chromatographic profiles shows that the high molecular fraction 6 is retained preferentially. It is noteworthy that no degradation of DNA in liver and blood takes place during the perfusion.

In order to study the relation between uptake of DNA and synthesis of DNA, experiments were carried out using a mixture of P^{32}-labelled DNA and H^3-labelled thymidine for perfusion. The P^{32}-DNA used consisted almost exclusively of fraction 4 and had a considerably lower specific activity than the H^3-DNA.

In a typical experiment, the labelled material was perfused through a regenerating liver for 10 min, and the liver was then washed repeatedly with non-radioactive perfusate. Again, the high molecular fraction 4 is retained in the liver and cannot readily be washed out. However, the amount of radioactivity absorbed is smaller than in

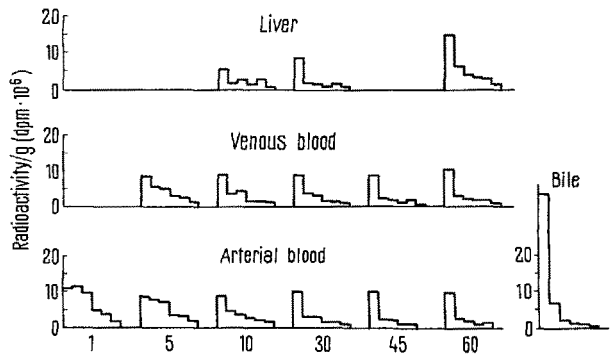

Fig. 3. Uptake of ^{3}H-DNA (partially degraded) by the isolated, perfused regenerating liver. DNA-^{3}H was added to the perfusate (10 ml) and the perfusate was allowed to recycle for 10 min. The data are expressed as activity/g blood, activity/g bile or activity/g liver (liver weight 4.1 g).

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the previous experiment (about 1%), probably due to the low specific activity of DNA which demanded a larger amount of DNA to be employed for perfusion. The thymidine-H^3 added is also incorporated into DNA; whereas retention of DNA occurs almost at once, incorporation of thymidine proceeds for 30 min to 1 h, even after the activity in the perfusate had been removed. Furthermore, the chromatographic profile of the newly synthesized DNA is different from the foreign DNA since H^3 activity appears first in fraction 6, and later in fraction 4. As in the previous experiment, no degradation of the exogenous DNA takes place during perfusion.

Only highly polymerized DNA remains intact during liver perfusion. DNA preparations which consist mostly of fractions 2 and 3 are broken down to acid-soluble fraction 1. This is shown in Figure 3, which presents data from a perfusion experiment in which degraded DNA was allowed to circulate through the liver for 1 h. It should be mentioned that in this figure the data are not expressed in terms of total radioactivity but rather of activity/g organ or perfusate.

Discussion and conclusions. The following points appear noteworthy with respect to the experiments described: DNA of high molecular weight and presumably representing native DNA is not degraded in the perfused liver or in the blood. In contrast to this, DNA already degraded in part, is broken down by the liver. Polymerized DNA is readily taken up by perfused liver and is retained even after washing with non-radioactive medium. If more DNA of lower specific activity is used, less is taken up; retention, however, occurs to a similar extent. These findings suggest that the uptake of DNA by the organ does not represent a simple diffusion from blood into cell but that the DNA is bound in the cell to active sites. The uptake of DNA does not appear to be the result of a resynthesis of DNA previously degraded. As already mentioned, the polymerized DNA is not degraded and, furthermore, incorporation of low molecular precursors, e.g. thymidine, follows a different pattern with respect to time-course of incorporation and to the chromatographic profile of the DNA formed[10].

Résumé. L'absorption et la dégradation du DNA ont été étudiées dans le foie perfusé in vitro. Alors que les DNA très polymérisés peuvent être absorbés par l'organe et y être retenus (en partie tout au moins), les DNA dégradés sont hydrolysés en composés acido solubles. Les DNA très polymérisés ne sont pas dégradés dans le sang ni dans les organes étudiés.

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Spezifität der Zinkaufnahme durch Chlorella

Bei unseren Versuchen über die Aufnahme des essentiellen Spurenelements Zink durch Chlorella pyrenoidosa[1,2] wurde zwischen stoffwechselabhängiger («aktiver») und -unabhängiger («passiver») Aufnahme unterschieden. Die passive Aufnahme hängt wenig von den Züchtungsbedingungen der Algen ab. Sie ist bei toten Algen stärker als bei lebenden und wird vorwiegend als Ionenaustausch gedeutet. Die aktive Aufnahme hängt stark von den Züchtungsbedingungen ab und wird durch Entzug von Licht und Sauerstoff sowie durch Dinitrophenol (DNP) gehemmt. Starke aktive Aufnahme zeigten am Ende einer Wachstumsperiode (10–14 Tage nach Beimpfen der Nährlösung im Stehkolben) in 1%iger Glukose gewachsene Algen. Die Nährlösung enthielt neben den üblichen Salzen 4 mM/l Kalzium, aber kein eigens zugesetztes Zink. Der Zinkgehalt solcher Algen, aus Verunreinigungen anderer Salze stammend, betrug laut Aktivierungsanalyse rund 30 ppm im Trockengewicht.

Es sollte nun festgestellt werden, inwieweit die aktive Aufnahme des Zinks spezifisch ist, das Zink also bei aktiver Aufnahme einer Verdrängung durch Fremdionen unterliegt.

Die wie beschrieben gezüchteten Algen wurden dreimal niedertourig zentrifugierend mit destilliertem Wasser gewaschen, wobei aus der Nährlösung ausgefallenes Kalziumphosphat im Waschwasser suspendiert blieb. Dann wurden etwa 50 mg Algen (Frischgewicht) in 10 ml Lösung des Fremdions eingebracht, die auch 10^{-3} mM/l markiertes Zink (^{65}Zn) enthielt. Bei den Hauptversuchen wurde kein Puffer angewendet; dennoch blieb der auf 5,0 eingestellte pH-Wert innerhalb von $\pm 0{,}2$ Einheiten konstant.

Vorversuche ergaben, dass die passive Aufnahme nach 10 min abgeschlossen ist, die aktive Aufnahme sich aber über Stunden und Tage erstreckt (Figur 1). Daher wurden Proben einerseits 10 min, andererseits 15 h nach Versuchsbeginn der in diffusem Licht geschüttelten Algensuspension entnommen. Die Messung erfolgte stets mit Szintillationszähler am algenfreien Überstand. Die Werte für die aktive Aufnahme wurden durch Subtraktion der 10-Minuten-Werte von den 15-Stunden-Werten erhalten.

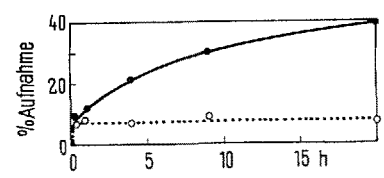

Fig. 1. Aufnahme von markiertem Zink durch Chlorella. –●– = ohne DNP, –o– = $5 \cdot 10^{-4}M$ DNP. Ursprüngliche Zn-Konzentration $10^{-6}M$; n/10 Natriumacetatpuffer, pH 5,5.

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