acetonitrile (35 ml). The mixture was kept for 2 h at 80° and was then worked up as described above. The crude amide (5.7 g) was crystallised from ethyl acetate to give mesyloxyacetamide as prisms, m.p. 118–119°. (Found: C 23.7; H 4.68; N 9.24; S 21.0. $C_3H_7O_4NS$ requires C 23.5; H 4.61; N 9.15; S 20.9). ν_{max}^{KBr} 1680, 1350, 1180 cm^{-1}.

Lichtenberger and Faure [5] report m.p. 86° for a substance claimed to be prepared from mesyloxyacetonitrile and formulated as mesyloxyacetamide, but their analytical figures agree with values calculated for ethansulfonyloxyacetamide,[6] m.p. 86°.

Mesyloxyacethydroxamic acid. Mesyloxyacetyl chloride (5.2 g, 0.03 mole) was added with stirring and external cooling, to a solution of hydroxylamine prepared by mixing solutions of hydroxylammonium chloride (4.9 g, 0.07 mole) in methanol (50 ml) and of sodium methoxide (sodium, 1.4 g, 0.06 mole) in methanol (40 ml). The reaction mixture was kept for 2 days at room temperature. The solution was filtered and evaporated to dryness *in vacuo*. The residue was extracted with ethyl acetate and the extract was evaporated to dryness. The product (3.8 g) was chromatographed on a column (4×50 cm) of polyamide (Woelm) (200 g), using acetone as eluent. The fractions (10 ml) were analysed by TLC as described below. The appropriate fractions (Nos. 70–115) were combined and evaporated to dryness; yield, 0.63 g (13 %). Crystallisation from ethyl acetate gave mesyloxyacethydroxamic acid (0.2 g) as needles, m.p. 103–104°. (Found: C 21.2; H 4.08; N 8.18; S 18.8. $C_3H_7O_5NS$ requires C 21.3; H 4.17; N 8.28; S 19.0). ν_{max}^{KBr} 3300, 3175, 1670, 1350, 1180 cm^{-1}.

TLC was performed on Polyamide (Merck) using acetone as eluent. The spots were made visible by spraying with solutions containing iron(III) chloride and an equimolar mixture of iron(III) chloride and potassium hexacyanoferrate(III), respectively, in 50 % aqueous methanol.

Methyl mesyloxyacetate. Methyl bromoacetate (b.p. 144–145°/760 mm, prepared analogously to ethyl bromoacetate [7]) (27.6 g) was added to a solution of silver mesylate (41 g) in acetonitrile (200 ml). The mixture was refluxed for 5 h and was worked up by the procedures described above. Distillation gave methyl mesyloxyacetate (20.6 g), b.p. 148–149°/14 mm, n_D^{25} 1.4400 (Found: C 28.8; H 4.81; S 19.1. $C_4H_8O_5S$ requires C 28.6; H 4.80; S 19.1) $\nu_{max}^{CHCl_3}$ 1760; 1360; 1170.

Acknowledgement. Financial support was given by the *Swedish Agricultural Research Council.*

1. Ehrenberg, L., Lundquist, U., Osterman, S. and Sparrman, B. *Hereditas* **56** (1966) 277.
2. Gichner, T., Ehrenberg, L. and Wachtmeister, C. A. *Hereditas* **59** (1968) 253.
3. Pattison, F. L. M. and Millington, J. E. *Can. J. Chem.* **34** (1956) 758.
4. U.S. Patent 3200138 (1965).
5. Lichtenberger, J. and Faure, A. *Bull. Soc. Chim. France* **1948** 995.
6. Lichtenberger, J. and Biechler, J. *Bull. Soc. Chim. France* **1938** 1586.
7. *Org. Syn. Coll. Vol.* **3** (1955) 382.

Received July 9, 1968.

Determination of the Succinic Dehydrogenase Activity of Beef and Human Heart Mitochondrial Acetone Powder with the Help of the Cartesian Diver Technique

SVEN PALÉUS

Carlsberg Laboratorium, Fysiologisk Afdeling, Copenhagen, Valby, Denmark, and Medicinska Nobelinstitutet, Biokemiska avdelningen, Karolinska Institutet, S-104 01 Stockholm 60, Sweden

During an investigation of the metabolism of the human heart there was a great need of a micromethod for the determination of succinic dehydrogenase activity. Recently, a biopsy was obtained at a heart operation which made it possible to prepare mitochondrial acetone powder according to Bernath and Singer.[1a] This material was used for measuring the succinic dehydrogenase activity with the Warburg method as well as with the Cartesian diver technique. The assay method was that used by Bernath and Singer,[1b] using phenazine methosulfate as an electron acceptor. A preparation according to the above method was made from beef heart and was used as a reference. For a detailed description of

the Cartesian diver technique, the reader is referred to Linderstrøm-Lang and Holter,[2] Waterlow and Borrow,[3] and Borrow and Penney.[4] Here only some experimental details will be given. A so called "Hauptraum"-mixture (Warburg terminology), consisting of 0.3 ml 0.01 M cyanide (pH 7.5), 0.5 ml 0.3 M phosphate (pH 7.6), 2.0 ml distilled water, and 10 µl 0.1 M $CaCl_2$, was freshly prepared before each experiment. The substrate-acceptor solution consisted of 0.3 ml of 0.2 M succinate (pH 7.6) and 0.2 ml of 1 % phenazine methosulfate solution (freshly prepared). The acetone powder of mitochondria (0.6—3.0 mg/ml) was suspended in the "Hauptraum"-mixture immediately before use with the help of a Potter-Elvehjem homogenizer.

The different reagents were introduced into the diver (of about 10 µl volume) in the following order: 0.16 µl substrate-acceptor mixture, 0.88 µl suspension of mitochondria, 0.6 µl oil seal and 3.93 µl mouth seal (this volume may vary a little during different experiments). The flotation vessel had a temperature of 20.2°C. After mixing of the substrate-acceptor mixture with the suspension of mitochondria, readings were taken every minute during the first 20 min and thereafter every quarter of an hour. A complete experiment lasted 2 h. After due corrections for the ΔV of the diver according to the formula of Linderstrøm-Lang and Holter,[2] the oxygen consumption of the different mitochondrial samples were calculated. The results are shown in Table 1.

Table 1.

Species	Amount of mitochondria (mg)	Oxygen consumption, µl/h	
		Warburg method	Diver technique
Beef	10	840	970
»	10	1380	1160
»	10		695
Human	10		11.6
»	10		26.5

The diver experiments were done at a room temperature of 20—21°C, and the experimental temperature had therefore to be in the same range, since the diver technique encounters difficulties when the temperature difference between room and flotation bath is more than 2—3°C. The Warburg experiments, on the other hand, were done at 38.1°C. In order to compare the two results, therefore, a temperature correction had to be introduced, and this was done according to Arrigoni and Singer.[5] They found that the enzyme activity increased by a factor of 1.6 between 25° and 38.1°C, using the same experimental conditions in the Warburg experiments. The values in Table 1 have been corrected by this factor. In spite of this rather arbitrary correction, the agreement of the results obtained by the two methods is fairly satisfactory.

The author attributes the low oxygen consumption in the case of human mitochondria to the anoxemic conditions prevailing at the time of the heart operation. The investigation also shows that it is possible to get rather reproducible results with the Cartesian diver technique using as little material as 0.0006 mg acetone dried mitochondria for one determination. Such a method might be of value when biopsies of less than 1 g for the preparation of mitochondria are available.

Acknowledgements. The author wants to express his sincere gratitude to Professor H. Holter for helpful discussions and great hospitality. He also whishes to thank Mrs. K. Holter for skilful assistance and discussions about the technique. The work was supported by a grant from *Statens Naturvetenskapliga Forskningsråd*.

1a. Bernath, P. and Singer, T. P. In Colowick, S. and Kaplan, N.O., (Eds.), *Methods in Enzymology*, Academic, New York 1962, Vol. 5, p. 605.
1b. Bernath, P. and Singer, T. P. In Colowick, S. and Kaplan, N. O., (Eds.), *Methods in Enzymology*, Academic, New York 1962, Vol. 5, p. 603.
2. Linderstrøm-Lang, K. *Compt. Rend. Trav. Lab. Carlsberg, Ser. Chim.* **24** (1943) 334; Holter, H. *Ibid.*, p. 399.
3. Waterlow, J. C. and Borrow, A. *Compt. Rend. Trav. Lab. Carlsberg, Ser. Chim.* **27** (1949) 93.
4. Borrow, A. and Penney, J. R. *Exptl. Cell. Res.* **11** (1951) 188.
5. Arrigoni, O. and Singer, T. P. *Nature* **191** (1962) 1256.

Received July 11, 1968.