ment applies unambiguously to both reversible and irreversible enzymes, and whether the 'efficiency function' these authors derive is in fact the relevant function that is optimized under evolutionary selection pressures. However, this seminal work is an important starting point for understanding the evolutionary rationale behind the internal thermodynamic properties of enzymes.
23 Although the literature contains many discussions of the nature of microscopic intermediates in the reaction catalyzed by alcohol dehydrogenases, 2 are particularly recent: P.F. Cook, N.J. Oppenheimer and W.W. Cleland, Biochemistry *20,* 1556 (1981); K.M. Welsh, D.J. Creighton and J.P. Klinman, Biochemistry *19,* 2005 (1980). It is amusing to note that the position of the break might also be condition-dependent. For example, for organisms adapted to elevated temperatures where NADH is a weaker reducing agent than at low temperatures, one might naively expect the position of the break to be shifted to the right.
24 M.O. Dayoff, Atlas of protein sequence and structure, vol. 5. Nat. Biomed. Res. Found., Silver Spring, Maryland 1972.
25 K.I. Backlin, Acta chem. scand. *12,* 1279 (1958).
26 S. Black, Meth. Enzymol. *5,* 824 (1962).
27 R.M. Burton, Meth. Enzymol. *1,* 397 (1955).
28 D.A. Walsh and H.J. Sallach, Biochemistry *4,* 1076 (1965).
29 L.N. Cartwright and R.P. Hullin, Biochem. J. *101,* 781 (1966); see also I. Zelitch and A.M. Gotto, Biochem. J. *84,* 541 (1962).
30 M.T. Hakala, A.J. Glaid and G.W. Schwert, J. biol. Chem. *221,* 191 (1956).
31 P.D. Dawkins and F. Dickens, Biochem. J. *94,* 353 (1965); see also, I. Zelitch, J. biol. Chem. *216,* 553 (1955).
32 C.W. Shuster and M. Doudoroff, J. biol. Chem. *237,* 603 (1962).
33 S.J. Wakil, D.E. Green, S. Mii and H.R. Mahler, J. biol. Chem. *207,* 631 (1954); see also, J.R. Stern, Biochem. biophys. Acta *26,* 448 (1957).
34 A. Yoshida, J. biol. Chem. *240,* 1113, 1118 (1965).
35 P. Talalay and P.I. Marcus, J. biol. Chem. *218,* 675 (1956).
36 H.L. Kornberg and A.M. Gotto, Meth. Enzymol. *9,* 240 (1966).
37 P. Talalay, Enzymes *7,* 193 (1963).
38 R.H. Baker and H. Adkins, J. Am. chem. Soc. *62,* 3305 (1940).
39 R.E. Toomey and S.J. Wakil, Biochem. biophys. Acta *116,* 189 (1966).
40 H. Aurich, H.P. Kleber, H. Sorger and H. Tauchert, Eur. J. Biochem. *6,* 196 (1968).

PRO EXPERIMENTIS

Large scale preparation of oxidized streptolysin O using molecular filtration

G. Gazzei, F. Palei, P.L. Benanchi, S. Fabbiani, G. Antoni and P. Neri

ISVT Sclavo Research Centre, I-53100 Siena (Italy), 13 July 1981

Summary. Oxidized streptolysin O, suitable for the determination of antistreptolysin O in whole blood, has been prepared from crude broth culture of *S. pyogenes* by molecular filtration using membranes with nominal mol. wt limit 10,000.

A new hemolytic method for the determination of antistreptolysin O (ASO) in whole blood has been proposed[1]. The method is based on some characteristic properties of streptolysin O (SO): the oxidized form of the toxin can bind the specific antibodies but cannot interact with the red cell membrane. After addition of a reducing agent, both free and antibody-bound toxin interact with membrane receptors, but only the free toxin is able to cause red cell hemolysis. These principles form the basis of a new diagnostic method for the determination of ASO [1,2].

The oxidized SO was originally prepared from crude cultures of *Streptococcus pyogenes* by precipitation with ammonium sulfate, followed by centrifugation, dialysis and oxidation with hydrogen peroxide[1]. The excess H_2O_2 was then removed by dialysis and the residual unoxidized SO was inactivated by addition of $CuCl_2$, followed by extensive dialysis. Although this method gave oxidized SO which could be used in the diagnostic test, the procedure proved to be time-consuming, and unsuitable for large-scale production.

In the present paper we report the results of experiments using molecular filtration for both concentration and purification of the initial crude broth culture and for dialysis after treatment with H_2O_2 and $CuCl_2$.

Crude broth cultures of *S. pyogenes* were prepared as previously described, with slight modifications. After 10 h growth, the bacteria were removed by filtration under pressure on Seitz filters type K7 and EKS, arranged in series. The titre of the toxin was determined measuring both the hemolytic units (HU) and the combining units (CU) as previously described[1].

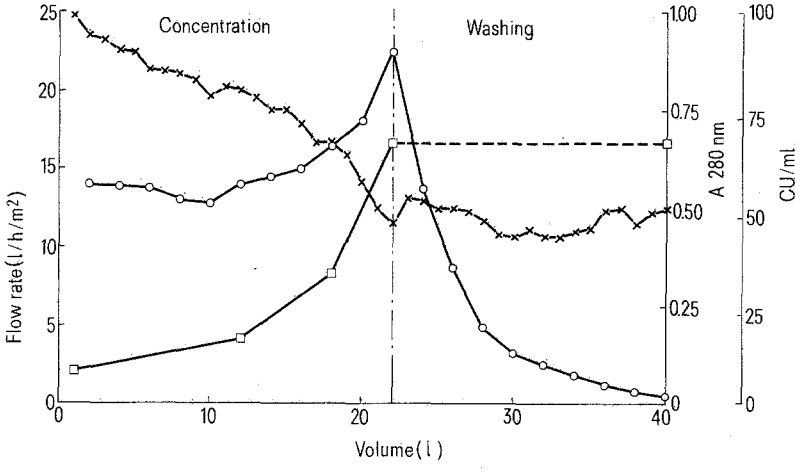
The concentration and purification of streptolysin by molecular filtration was performed with a 'high volume plastic cassette' assembled with PTGC membranes (nominal mol. wt limit = 10,000) (Millipore, USA), fed by a piston pump generating a pressure of 4 at. The total filtering surface used was 0.92 m^2.

The figure shows the results of a typical experiment, performed on a 24-l batch of filtered crude broth culture. During the concentration phase, an increase was observed in the protein concentration in the filtrate, expressed as the absorbance at 280 nm, due to the presence of proteins partially retained by the membrane, which were removed only at a higher concentration. During the washing phase, keeping the retentate volume constant at 3 l, the protein concentration decreased rapidly. The washing was interrupted when the absorbance at 280 nm reached about 1, corresponding to removal of about 84% of the proteins.

Analytical characteristics of streptolysin O during the various phases of the preparation procedure

Solution	Volume (ml)	CU/ml	Protein* (mg/ml)	Specific activity (UE/mg protein)	Yield (%)
Initial broth culture	24,000	10	18.3	7	–
Concentrated and purified SO	2580	92	26.7	48	100
Oxidized SO	5450	41	8.9	72	94.1
Oxidized and $CuCl_2$-treated SO	6400	33	6.7	60	88.9

*Determined with the method of Lowry et al.[5] using bovine serum albumin as standard.



Concentration and purification of a crude broth culture of *S. pyogenes* by molecular filtration. ○, absorbance at 280 nm, after dilution 1:40; □, titre of the toxin (CU/ml); *, flow rate (1/h/m²).

The SO titre of the retentate increased during the concentration phase, then remained practically constant during the washing, with quantitative recovery. The filtrate flow decreased in the concentration phase, settling at values of about 10–12 l/h/m² during the washing.

The material was then subjected to treatment with H_2O_2 and with $CuCl_2$ as previously reported[1]. Dialysis in tubes was, however, replaced by diafiltration; this was performed using the same system as that adopted for the initial concentration and purification phase. Removal of excess reagents (H_2O_2 or $CuCl_2$) required 5–6 volumes of water, with a flow of 14–15 l/h/m². Spot tests[3,4] were used to monitor removal of H_2O_2 and Cu^{2+}.

The table shows the analytical characteristics of SO during the various phases of the preparation procedure. The yield in CU was always approximately 90% in different experiments, with an average 8-fold increase in specific activity.

The SO obtained with the present method proved fully satisfactory for the determination of ASO titre in whole blood. The total time required for the experiment described above was about 12 h, including time for washings and general maintenance of the plant. The system is easy to operate, and being modular, its potentiality can be varied according to requirements.

1 A. Ricci, B. Berti, C. Moauro, M. Porro, P. Neri and P. Tarli, J. clin. Microbiol. *8*, 263 (1978).
2 A. Ricci, U.S. Patent No. 4, 148, 609 (1979).
3 F. Feigl and V. Anger, Spot Tests in Inorganic Analysis, 6th edn, p. 209. Elsevier, Amsterdam 1972.
4 F. Feigl and V. Anger, Spot Tests in Inorganic Analysis, p. 373. Elsevier, Amsterdam 1972.
5 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).

Instructions to authors

Experientia is published on the 15th of every month and can be obtained in any country through booksellers or from the publishers. All communications to the editors should be addressed to the publishers. All manuscripts for publication in a given number must be in the hands of the editors 3 months before publication.

Articles of general scientific interest, of interdisciplinary character: briefly stated and hitherto unpublished original reports of sufficient novelty value. In no case will papers of preliminary nature be accepted.

Experiments Papers in which animal experiments have been conducted without using the appropriate anaesthesia will not be accepted.

Manuscripts (including all figures and tables) must be submitted in English and in triplicate.

Text should not exceed 2–3 typewritten pages (50–60 lines). 1–2 relevant figures or tables. Summary of maximum 4 lines. Abbreviations should be properly explained. References should be numbered consecutively and be presented on a separate page. Name and address have to be placed directly under the title. Linguistically inadequate manuscripts will be returned. Footnotes should be avoided.

Figures Illustrations should be separate from the text. with the author's name on the back in soft pencil. The desired labelling should be shown on a second set of figures, which will be used as a model for inscriptions. Drawings for reproductions should be on good paper in Indian ink. photographs should be supplied as glossy positive prints. The illustrations should be at least one and a half times as large as the definitive size desired. Over-large figures can be easily damaged in the mail. Captions should be selfexplanatory, without reference to the text.

Tables should be provided with a title and with selfexplanatory captions.

Headings In submitting their manuscript to Experientia, authors are requested to indicate one of the headings mentioned below, under which they would wish to place their short communication:
1. Mathematics and Physics; 2. Cosmology, Astronautics, Cosmonautics; 3. Mineralogy, Geophysics, Oceanography; 4. Inorganic and Physical Chemistry; 5. Organic Chemistry; 6. Biophysics; 7. Molecular Biology, Cellular Biology; 8. Genetics; 9. Botany; 10. Zoology; 11. Ecology; 12. Biochemistry (analytic and synthetic); 13. Biochemistry (Enzymes, Metabolism); 14. Physiology; 15. Neurology; 16. Pharmacology, Toxicology, Pathology; 17. Experimental Gerontology; 18. Anatomy, Histology, Cytology, Histochemistry; 19. Embryology; 20. Endocrinology; 21. Circulation, Cardiology, Angiology; 22. Nutrition, Gastroenterology; 23. Hematology, Serology; 24. Immunology, Allergy; 25. Microbiology, Parasitology, Chemical Therapeutics; 26. Oncology, Carcinology, Cytostatics; 27. Radiology.

Reprints The authors receive 50 reprints, without cover, free of charge. Price-list for further reprints is available.