a Seitz filter⁸ is altered by the filtration and therefore does not become thrombin in aq. sodium citrate. Conceivably the cellulose in the filter could affect the prothrombin in much the same way as the DEAE-cellulose does. In the activation with sodium citrate or protamine sulfate only prothrombin is needed. When it does not activate under those conditions the basis is the difference in the structure of the prothrombin molecule itself, and has nothing to do with the absence of a hypothetical essential procoagulant.

TABLE I TERMINAL AMINO ACIDS IN BOVINE PROTHROMBIN AND THROMBIN

Preparation	N-terminal	C-terminal
Thrombin	Glutamic acid	Isoleucine
Prothrombin	Alanine	Tyrosine
Mg(OH), only		Glycine
Prothrombin	Alanine	Tyrosine
Mg(OH) ₂ and IRC-50		Glycine
Prothrombin	Alanine	Serine
Mg(OH), and DEAE-cellulose		

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- ¹ W. H. Seegers, Record Chem. Progr., 13 (1952) 143.
- ² W. H. SEEGERS, W. G. LEVINE AND R. S. SHEPARD, Can. J. Biochem. Physiol., 36 (1958) 603.
- 3 K. D. MILLER, State Dept. Health, Ann. Rept. Div. Labs. Research, (1957) 44.
- ⁴ S. Magnusson, Acta Chem. Scand., 12 (1958) 355. ⁵ W. H. Seegers, G. Casillas, R. S. Shepard, W. R. Thomas and P. Halick, Can. J. Biochem. Physiol., 37 (1959) 775.
- ⁶ J. Tibbs, Nature, 168 (1951) 910.
- ⁷ K. D. MILLER, J. Biol. Chem., 235 (1958) 987.
- R. GOLDSTEIN, A. LE BOLLOCH, B. ALEXANDER AND E. ZONDERMAN, J. Biol. Chem., 234 (1959)

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Studies on a paraffin-utilizing pseudomonad

A strain of pseudomonad isolated from oily mud on the underside of a motor-cycle gear box was found to grow with any one of a variety of paraffin hydrocarbons as sole carbon source. The best growth was obtained using octadecane as carbon source in a medium containing salts, buffered to pH 7, with (NH₄)₂SO₄ as nitrogen source.

Manometric uptake of O₂ following addition of octadecane was rapid when NH₄+ was also present. In absence of NH₄+ the rate of uptake was not significantly different from that in absence of substrate. Cells depleted in absence of ammonia, but with substrate, showed a lag of about 30 min before subsequent O2 uptake following addition of (NH₄)₂SO₄, but cells starved with ammonia present oxidized octadecane without lag when it was added to the system. Chloramphenicol or ϕ -fluorophenylalanine added to the cells while they were oxidizing octadecane caused a progressive fall off in rate to that of the endogenous respiration. From the above data it was deduced that at least one of the enzymes involved in the breakdown of paraffin hydrocarbons by the pseudomonad is unstable, breaking down with a half-life of about 30 min, but is normally resynthesised by the cell. In absence of NH₄+, or in presence of chloramphenical or p-fluorophenylalanine the enzyme cannot be synthesized and the overall rate of paraffin oxidation falls. These findings may be compared with those of Proctor¹, who found essentially similar results with a hexadecaneutilizing organism, but was able to detect a reduced rate of oxidation of paraffin hydrocarbon, corresponding to conversion to the corresponding primary alcohol, in the chloramphenicol-inhibited system.

Cells harvested by centrifugation following growth in octadecane--ammonia -salts medium were found to contain about 30 % lipid, 90 % or more of which was nonsaponifiable. The fatty acids obtained were methylated, and the methyl esters examined by gas-liquid chromatography. The non-saponifiable material was also analysed by this technique. Comparison of the results with those obtained from cells grown on a glucose-broth-yeast extract medium, and containing only 3 % total cell lipid, showed two major differences in the fatty acid methyl ester fractions. The paraffin-grown cells contained stearic acid, present only in traces in the control cells, and a larger amount of palmitic acid. The control cells contained virtually no volatile non-saponifiable material but paraffin-grown cells contained about 26-30 % non-saponifiable material which was shown to contain from I to 50 % octadecanol. Octadecanol was recrystallized from petroleum ether and the melting point of 58.5° was not depressed by addition of an authentic sample of octadecanol. Chromatographically the isolated material was indistinguishable from octadecanol on both polyvinyl acetate and Apiezon-L liquid phases. The conditions under which octadecanol is produced are still under investigation, but it would appear that moribund cells contain the largest amounts, thus supporting the theory that continuing protein synthesis is necessary for the further oxidation of octadecanol. The presence of traces of octadecanol in all cell preparations from cultures using octadecane as carbon source, and its total absence from broth-grown cells may be taken as evidence that this compound is an intermediate in the breakdown of octadecane by this pseudomonad.

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¹ M. H. Proctor, Biochim. Biophys. Acta, 42 (1960) 559.

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