cipate that a study of preservation under controlled conditions of water vapour pressure should enable a substantial improvement in recovery following storage.

We wish to express our appreciation for the assistance of Mr. H. F. Harner and Mr. W. L. Campbell of the Maryland-West Virginia Artificial Breeders Co-operative in supplying experimental material and in carrying out the insemination.

The opinions or assertion contained herein are our own, and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service in general.

HAROLD T. MERYMAN
EMANUEL KAFIG

Naval Medical Research Institute,
National Naval Medical Center,
Maryland,
Bethesda

1 Polge, C., Smith, A. V., and Parkes, A. S. *Nature*, **164**, 666 (1949).
2 Albright, J. L., Erb, R. E., and Ehlers, M. H., *J. Dairy Sci.*, **41**, 206 (1958).
3 Luyet, B. J. and Hodepp, E. L. *Proc. Soc. Exp. Biol.*, N.Y., **39**, 433 (1938).

Fractionation of the System Bringing About Oxidative Phosphorylation in *Azotobacter vinelandii*

RESPIRATORY-CHAIN phosphorylation in a particulate fraction of *Azotobacter vinelandii* is inactivated by incubation of the suspension in salt concentrations less than 0·01 *M* potassium chloride or sodium chloride, or 0·0008 *M* magnesium chloride, manganese chloride or calcium chloride. This inactivation is partially reversed by adding salts back to the inactivated suspension[1]. It has now been found that the inactivated suspension can be fractionated by centrifugation at 50,000 *g* for 30 min. The sediment contained 85–90 per cent of the reduced diphosphopyridine nucleotide oxidase activity, but restoration of oxidative phosphorylation was not possible unless the suspension was pre-incubated with the supernatant from this high-speed centrifugation, as well as with magnesium chloride (Table 1). Although after pre-incubation this supernatant alone also catalysed oxidative phosphorylation, the oxidase activity was much too low to account for the increased P:O ratio obtained with particles pre-incubated with magnesium chloride and supernatant. It appears, therefore, that the supernatant contains a factor which is necessary for the restoration of the activity of inactivated particles. This factor is destroyed by heating for 5 min. at 100°.

Table 1. REACTIVATION OF OXIDATIVE PHOSPHORYLATION BY A SOLUBLE COMPONENT

Experiment	Pre-incubation mixture						Oxidative phosphorylation	
	WSP	WSP′	WSP″	$MgCl_2$ (0·008 *M*)	Serum albumin (0·1%)	S_3	Time needed to complete oxidation (minutes)	P:O ratio
1	+	−	−	+	+	−	<3	0·51
	−	+	−	−	−	−	<3	0·02
	−	+	−	+	+	−	<2·5	0·36
	−	−	+	+	+	−	3	0·03
	−	−	+	+	+	+	2·5	0·19
2	+	−	−	+	+	−	5·5	0·67
	−	+	−	−	−	−	<3·5	0·16
	−	+	−	+	+	−	<2·5	0·62
	−	−	+	+	+	−	2·5	0·08
	−	−	+	+	+	+	2	0·26
	−	−	+	+	+	+*	2·5	0·11
	−	−	+	+	+	+†	<3	0·10
	−	−	−	−	+	+‡	13	0·19
	−	−	−	+	+	+‡	13	0·40

WSP, washed small particles obtained by centrifugation for 2 hr., at 140,000 *g* (bottom of tube) (ref. 9); suspended in 0·05 *M* Sørensen phosphate buffer, *p*H 7·0. WSP′, washed small particles suspended in 0·005 *M* phosphate buffer, *p*H 7·0, for 30–90 min., at 0°. WSP″, sediment after centrifugation of WSP′ for 30 min. at 50,000 *g*. S_3, supernatant obtained from this centrifugation.

The mixtures indicated were pre-incubated for 90–120 min. at 0°, and were then added to a reaction medium[9] used to measure oxidative phosphorylation with reduced diphosphopyridine nucleotide as substrate.

* S_3 was heated at 100° for 5 min. and filtered.

† S_3 was not present in the pre-incubation mixture, but was added immediately before the measurement of oxidative phosphorylation.

‡ Three times as much S_3 as used in the measurements with WSP″.

This fractionation resembles that carried out by Pinchot[2] with extracts of *Alcaligenes faecalis*. The conditions leading to the reversible inactivation of the phosphorylating system in *Azotobacter* are similar to those which bring about a reversible dissociation of a two-stranded polynucleotide complex[3], or, so far as decreasing the magnesium concentration is concerned, to the dissociation of ribonucleoproteins in particles obtained from yeast[4] and *Escherichia coli*[5]. This provides some support for Pinchot's[6] suggestion that in his preparations a polynucleotide acts as a bridge holding together the necessary enzymes. It is possible that a factor necessary for oxidative phosphorylation in the particles obtained from *Azotobacter* is bound to the particles by means of such a polynucleotide complex, which dissociates on lowering the cation concentration.

These experiments with *Azotobacter* recall also recent reports on the fractionation of the phosphorylating enzymes in particles derived from beef-heart mitochondria[7,8]. Phosphorylation was obtained by bringing together a particulate fraction (containing the oxidase), a soluble fraction and magnesium. Linnane[8] found that no fractionation took place in the presence of magnesium.

H. G. HOVENKAMP

Laboratory of Physiological Chemistry,
University of Amsterdam.
July 24.

1 Hovenkamp, H. G., *Biochim. Biophys. Acta*, **34**, 485 (1959).
2 Pinchot, G. B., *J. Biol. Chem.*, **205**, 65 (1953).
3 Felsenfeld, G., and Rich, A., *Biochim. Biophys. Acta*, **26**, 457 (1957).
4 Chao, F. H., *Arch. Biochem. Biophys.*, **70**, 426 (1957).
5 Tissières, A., and Watson, J. D., *Nature*, **182**, 778 (1958).
6 Pinchot, G. B., *J. Biol. Chem.*, **229**, 25 (1957).
7 Pullman, M. E., Penefsky, H., and Racker, E., *Arch. Biochem. Biophys.*, **76**, 227 (1958).
8 Linnane, A. W., *Biochim. Bipohys. Acta*, **30**, 221 (1958).
9 Tissières, A., Hovenkamp, H. G., and Slater, E. C., *Biochim. Biophys. Acta*, **25**, 336 (1957).

Transformation Reaction of Pneumococci in the Absence of Serum Factor

CONTRARY to the general proposition that transformation reactions of pneumococci cannot take place in the absence of serum factor[1–3], our experiments[4,5] indicated that cells of *R36NC*, a pneumococcal rough strain derived from 11-*D39S*, could be transformed to streptomycin-resistant ones by means of purified deoxyribonucleate in diffusate media. But in these experiments, deoxyribonucleate was kept present throughout the culture growth and the cultivation was continued overnight before plating on streptomycin plates, so that the population change after the occurrence of transformation reaction might have distorted the results. This possibility was completely excluded in the present experiment by the use of deoxyribonuclease, which was added to the reaction mixture to stop the action of deoxyribonucleate at a definite time.

Streptomycin-sensitive *R36NC* from a blood agar slant was inoculated into Adams and Roe's medium[6] and incubated overnight. The culture was added next morning to 4 volumes of fresh medium and