right to expect. In these essential phases of our national life, with all our running, we cannot even stay in the same place!

There is no ready answer, nor is there any quick solution because our present attitude towards family size will compound these difficulties in the years immediately ahead. The choice lies with each parent. The individual is the nation. The choice, however, cannot be made until we formulate an opinion on what we want for our country. In our inner thoughts we may be thankful that the problems which force this choice upon us are not as desperate as those facing the great majority of people in other countries. We can even dare to hope that they never will be. This, however, will depend on the decision we make—a decision which can serve our own interests as well as the interests of other people throughout the world.

A Social and Political Question

Above all, we should keep in mind the fact that the question of population growth, and the pressures it creates, whether considered from a national or an international point of view, is not merely a physical problem of resources and people. Even more essentially, if that be possible, it must be thought of as a social and political problem of world-wide magnitude.

Bacterial Particles in Oxidative Phosphorylation

Arnold F. Brodie and Clarke T. Gray

The biochemical function generally considered most characteristic of mammalian mitochondria is that of oxidative phosphorylation. While it cannot be said with certainty that bacteria possess subcellular elements which should be called mitochondria, recent studies (*1, 2*) have shown that cell-free extracts of mycobacteria and corynebacteria fulfill the most exacting requirements for oxidative phosphorylation. These include (i) oxidation of Krebs-cycle intermediates with P/O ratios greater than 1.0, (ii) formation of adenosine triphosphate (ATP) (*3*) from inorganic orthophosphate in the presence of a phosphate-acceptor system, (iii) absence of phosphate esterification under anaerobic conditions, and (iv) the uncoupling of phosphorylation from oxidation by known uncoupling agents. Microbial systems that couple phosphorylation to oxidation have since been described by Tissieres and Slater (*4*), Rose and Ochoa (*5*), and Hartman *et al.* (*6*) in extracts of *Azotobacter vinelandii,* while Nossal *et al.* (*7*) have obtained active preparations from yeast. In the foregoing systems the participation of subcellular particles has been demonstrated.

In contrast with the mammalian preparations, the bacterial systems studied (*4, 8–10*) have lent themselves to fractionation and reconstruction. In the latter, oxidative phosphorylation is dependent on (i) a particulate fraction which functions only as a highly organized structural unit (*9*) and (ii) supernatant factors (*4, 8, 10*) required to complete terminal electron transport and coupled phosphorylation (*11*).

Since any system which can be fractionated and reconstituted facilitates analysis of the essential components, it appears that bacterial systems will play an increasingly important role in elucidating the mechanisms involved in oxidative phosphorylation. It seems useful, therefore, to characterize the labilities and also certain of the enzymatic and chemical constituents of bacterial particles that are capable of participating in oxidative phosphorylation (*12*).

Effects of Sonic Treatment

Active cell-free extracts were obtained by treating 11-milliliter aliquots of *Mycobacterium phlei* [500 milligrams of wet cells per milliliter of 0.1*M* tris (hydroxymethyl) amino-methane (Tris) (*3*) at *p*H 8.0] in a 10 KC Raytheon magnetostrictive oscillator at 2°C for 4 minutes, followed by centrifugation of 20,000*G* (*2*). The effect of sonic vibration on cell disruption was measured by determining the rate of protein liberation. Protein was liberated rapidly during the first 4 minutes and but slowly thereafter (Table 1). The attendant solubilization of enzymes associated with the particles indicates that extended treatment served primarily to fragment the debris and particles initially released.

Prolongation of vibration not only failed to increase the yield of protein, but it was also particularly deleterious, since it brought about a continuous decline in the capacity of extracts to esterify phosphate in the presence of succinate or fumarate (Fig. 1). Losses in the capacity to oxidize these substrates, however, differed quantitatively and qualitatively. Succinate oxidation was negligible after 40 minutes of treatment because of the apparent particulate nature of this oxidase. With fumarate as electron donor, about 60 percent of the initial oxidation remained. This residual oxidation with fumarate may be attributed to the solubilization of dehydrogenases and flavoproteins which transport electrons to oxygen by a series of noncoupled oxidative reactions. Such interpretation is supported by the more rapid decline of P/O ratios with fumarate and the increased cyanide-resistant residual oxidation with preparations that were treated for 40 minutes. Similar conclusions have been reached by other workers. Nossal (*13*) noted the solubilization of the fumarase system associated with yeast granules, while Utter and Kreech (*14*) obtained lowered P/O ratios when these granules were exposed to increased disruptive treatment. These observations with microbial systems parallel those concerning the lability of the mammalian mitochondrial system to sonic oscillation (*15*).

Distribution of Dehydrogenase Activity

A comparative analysis of the distribution of oxidative enzymes between particulate and supernatant fractions was investigated after these components had been separated from 4- and 40-minute sonic extracts. Crude cell-free extracts obtained by centrifugation at 20,000*g* were recentrifuged for 90 minutes at 140,000*g* in a Spinco preparative centrifuge (*16*). The particulate fraction was

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Table 1. Liberation of protein during sonic vibration of *Mycobacterium phlei*. A suspension of whole cells (44 milliliters) was divided into four aliquots of 11 milliliters each and subjected to sonic vibration for the intervals indicated. The protein liberated was measured (*2*) after sedimentation of remaining cells and debris at 20,000*G*. The mass of remaining cells and debris from each aliquot was washed with distilled water, retreated for 8 minutes, and recentrifuged prior to determination of the protein liberated after the second treatment.

Whole cells		Remaining cells and debris		Total protein liberated (mg/ml)
Length of treatment (min)	Protein liberated (mg/ml)	Length of retreatment (min)	Protein liberated (mg/ml)	
2	26.7	8	3.6	30.3
4	30.0	8	3.1	33.1
8	31.1	8	1.1	32.2
40	31.6	8	0.83	32.4

resuspended in 0.15 *M* KCl at *p*H 7.4. Since oxygen consumption by the particles requires factors present in the supernatant (*10*), dehydrogenase activities of the particulate and supernatant fractions were measured with neotetrazolium as acceptor. The results obtained with succinate were confirmed manometrically by the phenazene methosulfate method of Singer (*17*).

Particles obtained from 4-minute sonic extracts contained most of the dehydrogenase activity for pyruvate, fumarate, α-ketoglutarate, malate, and succinate. The corresponding supernatant contained a small or negligible portion of the total dehydrogenase activity. After prolonged vibration, the enzyme activities in the particle fraction had decreased, while those of the supernatant were increased. The solubilization of particle-bound enzymes is shown in Table 2.

Distribution of Cytochrome Components

The liberation of particle-bound cytochromes during prolonged sonic vibration was also readily demonstrated by analysis of cytochrome pigments (*18*) in particles and supernatants (Fig. 2). Cytochromes *a*, *b*, and *c* were found in the particles released by brief vibration, whereas the supernatant fraction contained only traces of cytochrome *c*. After prolonged sonic vibration, the particulate fraction contained only small amounts of cytochrome *c*; the hemochromogens corresponding to cytochromes *b* and *c* were detected in the supernatant, and cytochrome *a* could not be found in either fraction. The position of the cytochrome bands in extracts corresponded to those found by Todd (*19*) in suspensions of whole *Mycobacterium phlei*.

The Nadi reaction was used for further analysis of the activity of cytochrome oxidase. Particles obtained by short-term oscillation gave a positive Nadi reaction in 30 seconds. This reaction was inhibited completely by low concentrations of cyanide. In contrast, the supernatant, as well as the particles and supernatant obtained after lengthy oscillation, were negative after 20 minutes. With the mycobacterial system a positive Nadi reaction appears to denote intact particles capable of participating in coupled oxidative phosphorylation. Preparations which gave a positive reaction within 1 minute usually gave good P/O ratios. In another microorganism tested, *Azotobacter vinelandii*, the correlation between these reactions is not as strict.

Active Particles from Extracts

The capacity for oxidation and phosphorylation is not shared by all the sedimentable components of cell-free extracts. An amorphous fraction obtained by centrifugation at 56,000*g* contained a high concentration of deoxyribonucleic acid (27 percent) and possessed no oxidative or phosphorylative capacity with succinate as substrate. Addition to this fraction of supernatant obtained by centrifugation at 140,000*g* resulted in a slight stimulation of oxidation but not in phosphate esterification. After the amorphous material had been removed, a particulate fraction was sedimented by centrifugation at 140,000*g*. This material contained 12 percent ribonucleic acid (*20*) and exhibited slight coupled activity with succinate. The functional significance of this particulate fraction in

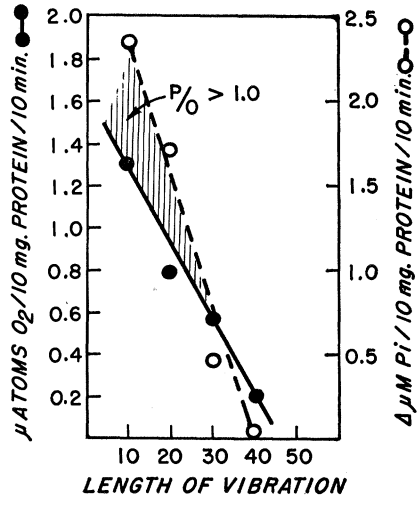

Fig. 1. Effect of sonic vibration on oxidation and phosphorylation. The system consisted of 10 micromoles of inorganic phosphate, 15 micromoles of $MgCl_2$, 25 micromoles of KF, 20 micromoles of potassium succinate, 2.5 micromoles of AMP, 1 milligram of yeast hexokinase, 20 micromoles of glucose, 0.8 milliliters of bacterial extract (*p*H 7.4), and water to a final volume of 1.3 milliliters. The extracts were obtained by sonic vibration of equal aliquots of suspended bacteria for a period of time ranging from 10 to 40 minutes. The protein concentrations were, respectively, 28.3, 27.1, 25.0 and 28.0 milligrams per milliliter. The respiration was measured by the conventional Warburg method at 30°C for 10 minutes after the addition of substrate, when it was stopped by the addition of 10-percent trichloroacetic acid; then the total inorganic phosphate was analyzed.

Table 2. Effect of sonic vibration on the dehydrogenase activity in particulate and supernatant fractions from *Mycobacterium phlei*. The reactions were carried out anaerobically in Thunberg tubes at 30°C. The system consisted of 0.1*M* Tris buffer (*p*H 8.0), 20 micromoles of substrate, 0.2 milliliter of fractionated extract, 200 micrograms of neotetrazolium, and water to a final volume of 1.1 milliliters. The diformazan formed was extracted with acetone and determined in the Klett photoelectric colorimeter using a No. 54 filter.

Preparation	Diformazan formed (μg/min 10 mg of protein)				
	Pyruvate	α-ketoglutarate	Succinate	Fumarate	Malate
Sonic treatment 4 min					
Particles (140,000*g*)	8.6	9.0	15.0	10.0	10.2
Supernatant	0	3.5	0.5	4.9	4.4
Sonic treatment 40 min					
Particles (140,000*g*)	0.0	0.2	2.5	3.0	3.0
Supernatant	3.1	8.3	1.2	9.5	11.2

Table 3. Effects of dialysis on bacterial oxidative phosphorylation. The crude extract was dialyzed for 20 hours against cold distilled water, and the values reported for the dialyzed material were adjusted to correct for dilution of the protein. Reactions were carried out at 30°C for 10 minutes after the addition of substrate. The Warburg vessels contained 13 micromoles of inorganic phosphate, 15 micromoles of $MgCl_2$, 25 micromoles of KF, 50 micromoles of potassium succinate, 5 micromoles of AMP, 3 milligrams of yeast hexokinase, 20 micromoles of glucose, 14.6 milligrams (protein) of crude sonic extract (*p*H 7.4), and water to a final volume of 1.3 milliliters. The concentration of added cytochrome *c* was $1 \times 10^{-4}M$.

System	Fresh extract			Dialyzed extract		
	Oxygen (μatom)	ΔP_i (μmole)	P/O	Oxygen (μatom)	ΔP_i (μmole)	P/O
Complete system	7.8	10.3	1.34	3.17	1.23	0.39
Complete system + cytochrome *c*	8.3	11.7	1.42	2.53	1.23	0.48
Complete system + Kochsaft	7.9	11.8	1.49	3.5	1.0	0.29

coupled oxidative phosphorylation is shown by a four- to seven-fold increase in activity on the addition of 140,000*g* supernatant. This supernatant was free of particles and, when tested separately, showed no significant oxidation or phosphorylation.

Electron microscopy revealed two distinct classes of particles sedimenting between 100,000*g* and 140,000*g*—large particles (70 to 180 millimicrons) and smaller particles (20 to 40 millimicrons). Since the large and small particles tended to sediment together, it is difficult to ascribe the coupled activity to either particle until further fractionation is achieved.

Influence of Environment

Suspension of intact cells in isotonic sucrose prior to oscillation, or dilution of the crude extract in sucrose after oscillation, offered no advantage: extracts prepared in distilled water displayed equal activity. This would seem to be in contrast with mammalian mitochondrial suspensions, which require careful control of isotonicity during preparation as a prerequisite for activity. However, the crude bacterial extracts were obtained by oscillation of thick cell suspensions yielding large amounts of native protein and lipid, which appear to exert a protective action on the particles in the crude extract. Particles isolated by high-speed centrifugation approximated mammalian mitochondria in their sensitivity to chemical agents and changes in the environment.

Dialysis of crude extracts for 24 to 48 hours had a pronounced effect on the enzymatic activity and on the integrity of the particles. Dialyzed preparations had lowered oxidative activity and even lower phosphorylative capacity (Table 3). The addition of Kochsaft (boiled yeast or bacterial extracts) or cytochrome *c* to the dialyzed extracts partially restored the oxidation with certain substrates—for example, malate—whereas phosphate esterification was not restored even by the further addition of di- or triphosphopyridine nucleotide. Harmon and Feigelson (*21*) found that the addition of cofactors to suspensions of mammalian mitochondria prepared in distilled water partially restored oxidation in some preparations. The activity was correlated with changes in the shape of the mitochondria. When dialyzed bacterial extracts were examined either by electron or phase microscopy, only a few particles could be observed.

Table 4. Effects of freezing.

Complete system*	O_2 (μatom)	ΔP_i	P/O
Fresh extract	3.52	5.2	1.48
Frozen extract (24 hr)	3.90	2.4	0.61

* Conditions were similar to those described in Table 3. Succinate was used as substrate.

The effects of freezing and storage on crude extracts are presented in Table 4. The P/O ratios obtained with preparations treated in this manner were considerably reduced. Damage by freezing followed the general pattern already reported to characterize mammalian mitochondria (*22*). Oxidation usually remained active, while phosphorylation was impaired.

Evidence that intact particles behave as reversible osmotic systems was obtained in various ways. The appearance of the bacterial particles under the phase microscope is altered when they are suspended in KCl (0.05 to 1.5*M*). Hypotonic solutions tended to swell the particles into smooth spheres many times their original size. Particles treated in this manner could couple phosphorylation to oxidation if they were used immediately. When suspended in hypertonic solutions, the particles were clumped, with an attendant loss in the coupled activity. Particles suspended in gramicidin ($1 \times 10^{-4}M$) became swollen and rough in appearance within 5 minutes and then slowly clumped into large aggregates of misshapen particles. This effect of gramicidin is correlated with our earlier observation that the uncoupling action of gramicidin is dependent on the suspending medium employed (*2*). Both the uncoupling action of gramicidin and the inhibitory action of malonate on succinoxidase occur to a greater extent in extracts prepared in water than in those made in isotonic sucrose.

When concentrated sucrose was added to water extracts or water to sucrose extracts, with tubes containing homologous diluents as controls, turbidity measurements indicated that the particles in the extracts behaved as a reversible osmotic system. The osmotic characteristics of these bacterial particles are thus similar to those described by Hogeboom *et al.* (*23*) and Lehninger (*24*) for mammalian mitochondria.

Discussion and Summary

Of the two components in bacterial extracts required for coupled oxidative phosphorylation, it is the particles which are sensitive to physical manipulation (*8, 9*) such as sonic vibration, dialysis, aging, freezing, and changes in tonicity. The supernatant factors (*10*) are resistant to such treatment. Furthermore, the physical integrity of the particles and their ability to conduct oxidative phosphorylation are far more labile than is

CYTOCHROME COMPONENTS IN FRACTIONS OF M. PHLEI

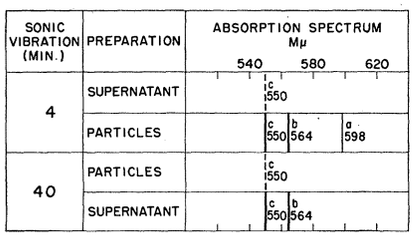

Fig. 2. Cytochrome components in fractions of *Mycobacterium phlei*. After reduction with $Na_2S_2O_4$, the fractions were examined for their cytochrome components by means of a Zeiss hand spectroscope at room temperature and immersion in liquid air. The exact positions of the bands were determined with the aid of a Cary automatic spectrophotometer. Cytochrome *b* was also analyzed as the pyridine-hemochromogen complex by the method of Pappenheimer and Hendee (*27*).

their ability to transport electrons to oxygen. Such data indicate the necessity for highly organized particles in which the spatial arrangement of the enzymes and bound coenzymes is important for coupled oxidative phosphorylation. It is apparent, however, that it is dangerous to characterize enzyme systems as particulate or soluble in nature when harsh disruptive procedures are used and when full recognition of the inherent potential of the particles is not used as a base line. We have here used a sensitive indicator of structural integrity, oxidative phosphorylation, as a measure of the degree to which the system can be assumed to represent the pattern of enzyme localization in the intact cell. We realize, however, that some pitfalls common to almost all cell fractionation procedures (*25*) may still apply.

In many respects—for example, in regard to function and degree of presumed organization—the bacterial system resembles the mammalian mitochondrial system (*26*). Certain differences do exist. These include the greater stability of the particles and the soluble nature of factors necessary for coupled activity in the reconstructed bacterial system. Because of these differences, the bacterial systems provide an excellent tool for studying certain aspects of the mechanism of coupled oxidative phosphorylation.

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C. A. Chant, Father of Canadian Astronomy

On 18 November 1956, Clarence Augustus Chant died at the age of 91. For more than 60 years he had been an enormous power behind Canadian astronomy, and the many astronomical seeds which he planted will continue, as strong trees, to bear the fruit of his labors for decades to come.

It was he who was largely responsible for the formation of the Royal Astronomical Society of Canada 60 years ago, for the founding of its monthly *Journal* and annual *Observer's Handbook,* for the setting-up of the department of astronomy at the University of Toronto, and for the establishment of a research observatory and large telescope belonging to that institution. He also made a real contribution to the wide dissemination of scientific knowledge. Textbooks of which he was coauthor—*High School Physics, Mechanics for the Upper School,* and *A Text Book of College Physics*—have instructed several generations, totaling hundreds of thousands, of Canadian high-school students. His popular book on astronomy, *Our Wonderful Universe,* has been published in three English editions and translated into five foreign-language editions. Over the years most of the Canadian astronomers, including five of the directors of the large Canadian observatories, were numbered among his thousands of students.

Chant was born on 31 May 1865, in Ontario, near Toronto. After attending Ontario high schools he was graduated in 1890 from University College, Toronto, and joined the staff there in 1891. From that time on he gave continuous service to the University of Toronto, except for a brief interval when he studied for his Ph.D. degree at Harvard in 1901.

From the time he, as a graduate student in physics, grew interested in astronomy, his life became one of unswerving devotion to the cause of astronomy in Canada. A slight, spare man, of remarkable health and strength, in his later years he typified the hale old age of excellent mental and physical powers which is the dream of many but the reward of few. His vast memory for facts and events remained with him to the end and, combined with his great erudition, made a chat with him a real treat. When the David Dunlap Observatory was built, he and his wife moved from the heart of Toronto, where they had resided for more than 40 years, to the lonely hilltop which the observatory occupies, 10 miles north of the city. Until 2 years before his death he worked daily in his office at the observatory.

A clear and concise teacher, he had a remarkable memory for his students and followed their careers with interest. He took part in five solar eclipse expeditions, including one to Australia in 1922 where photographs confirmed the Einstein displacement of starlight as it passes the strong gravitational field of the sun. Among the recognitions of his achievements were his election as president of the Royal Astronomical Society of Canada, as vice president of the American Astronomical Society, as fellow of the Royal Society of Canada, and his award of a silver medal at the Harvard tercentenary. In 1940 the Royal Astro-