ratio remained unchanged. 6. In hyperthyroidism the change in the ratios was again less constant. The only significant change was the increase of the cell phospholipid phosphorus/plasma phospholipid phosphorus ratio. 7. Both the lipid values and the various lipid ratios returned towards normal following adequate treatment.

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Relation Between Metabolic Activity and Cyanide Inhibition in *Pelomyxa carolinensis* Wilson.*

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Many investigations have been made on various animal species in ascertaining the presence or absence of a cytochrome-cytochrome oxidase system in living cells. In recent years numerous reports have been published in which protozoan cells have been used as experimental organisms. Some of these unicellular forms make excellent material for this type of investigation, and all of them are sensitive to cyanides, although, formerly, there was some doubt about this, especially in reference to ciliates.

Lund,[1] Shoup and Boykin,[2] and Gerard and Hyman[3] maintained that *Paramecium caudatum* was non-sensitive to HCN. Peters[4] claimed that respiration in *Colpidium colpoda* was not inhibited by potassium cyanide and Pitts[5] working with *Colpidium campylum* and Lwoff[6] with *Glaucoma piriformis* found that there was an initial inhibition when these forms were exposed to cyanide, but that oxygen consumption soon increased to normal. Hall,[7] however, definitely proved that cyanide inhibits respiration in *Colpidium campylum* and Baker and Baumberger[8] found the same to be true for *Tetrahymena geleii.*

The apparent non-sensitivity of *Paramecium* to cyanide was questioned by Hyman in unpublished data referred to by Child[9] and Pace.[10] Saito and Tamiya[11] found cytochrome in *Paramecium,* which led to the supposition that respiration in *Paramecium* might depend, at least partly, upon cytochrome-cytochrome oxidase. Since that time, Boell[12] working with *Paramecium calkinsi,* Pace[10] with *P. caudatum* and *P. aurelia;* and Clark[13] with *P. caudatum* found that oxygen consumption of these species is inhibited by cyanide. Evidence has now accumulated to such an extent that the sensitivity of ciliates to cyanide is no longer questioned.

According to Pace,[10] sensitivity to cyanide in *P. aurelia* and *P. caudatum* is apparently dependent upon the degree of carbohydrate metabolism. He finds that inhibition is much more pronounced when glucose is pres-

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TABLE I.

Buffered Culture Solution Used in Growing and Maintaining *Pelomyxa carolinensis* and Its Food Organism, *Paramecium caudatum*.

$K_2HPO_4 \cdot 3H_2O$	65.5 mg
$NaH_2PO_4 \cdot H_2O$	40.0 "
$CaCl_2$	100.0 "
$MgCl_2$	2.0 "
H_2O (redistilled) to	1000 ml

ent in the test media, than otherwise. This, and the fact that starved specimens are non-sensitive whereas respiration in "well-fed" specimens is inhibited by cyanide, indicates a possible connection between the cytochrome oxidase system and degree of metabolism (carbohydrate metabolism) in *Paramecium*.

The following report is the outcome of further investigations based on this supposition and is concerned with the following problems: (1) the relation between temperature change and respiratory quotient, (2) respiratory inhibition by cyanide as influenced by changes in temperature, and then (3) the cytochrome-cytochrome oxidase mechanism and carbohydrate metabolism in *Pelomyxa carolinensis*.

Material and Methods. Pelomyxa carolinensis Wilson, the organism used throughout these investigations, has been cultured in this laboratory for the past 3 years. It was grown in a modification of the buffered culture solution used by Pace and Belda[14] and is given in Table I (hydrogen ion concentration = pH 6.8 to 7.0).

Pelomyxa reproduces and grows rapidly in this solution when fed *Paramecium* (usually *caudatum*) in large numbers.

The rates of oxygen consumption and carbon dioxide elimination were ascertained by means of a Barcroft-Warburg apparatus.

Hydrochloric acid (0.3 ml 3 N HCl) was placed in the onset of each of the manometer flasks to absorb any ammonia given off by the organisms (Specht[15]) and to be used in releasing bound carbon dioxide at the end of the experiment. An alkali solution (0.2 ml 1 N KOH) was placed in the inset of the manometer flasks employed to determine the rate of oxygen consumption. The so-called "direct method" was used for oxygen consumption as well as carbon dioxide elimination.

Low temperatures were maintained by means of an Aminco portable cooling unit equipped with a thermo-regulator.†

Results. I. *Effects of Temperature Upon Respiration and Respiratory Quotient.* A series of tests was conducted to ascertain the rate of oxygen consumption in *Pelomyxa carolinensis,* at temperatures ranging from 10° to 35°C. Temperatures of 40°C were not employed since Pace and Belda[14] had reported that 9 of the 12 tests which they performed at 40°C were terminated in the first hour due to death of the organisms, while the specimens in the remaining 3 tests survived only long enough to permit a single reading of the manometers.

The accuracy of the determination of the rate of oxygen consumption decreases directly with a decrease in temperature chiefly because the rate of metabolism decreases with a reduction in temperature (Pace and Belda[14]); therefore, proportionately larger numbers of test organisms were employed at lower temperatures, *i.e.,* as many as 800 organisms were used when the experiments were conducted at 10°C.

The required number of pelomyxae of average size was removed individually from the culture dishes with a capillary pipette, washed thoroughly with buffer solution and placed in 5 ml of the same buffer solution in the main compartment of 6 of the manometer flasks. These experiments were carried out in order to ascertain respiratory quotients of pelomyxae at 10°, 20°, 30° and 35°C.

It was noted that the test organisms at 10°C tended to assume a spherical and compact shape, coincidently with a decrease in the rate and amount of protoplasmic streaming. Conversely, with an increase in temperature (30° and 35°C), the organisms showed marked increase in both the rate of

[14] Pace, D. M., and Belda, W. H., *Biol. Bull.*, 1944, **86**, 146.

[15] Specht, H., *J. Cell. Comp. Physiol.*, 1934, **5**, 319.

† The purchase of a portable cooling unit was made possible by a grant-in-aid furnished by the University of Nebraska Research Council.

TABLE II.

Rate of Oxygen Consumption and Carbon Dioxide Production in *Pelomyxa carolinensis* at Different Temperatures. Average Volume of One Million Organisms, 35,145 mm³.

Temp., °C	Test No.	No. of organisms used	Duration of exp., hr	Avg O_2 consumption in mm³/hr/ million organisms	Avg CO_2 elimination in mm³/hr/ million organisms	Avg rate of O_2 consumption in mm³/hr/mm³ of cell substance	Avg rate of CO_2 elimination in mm³/hr/mm³ of cell substance
10	1	750	7	3866	1937		
	2 to 3	500	7	4453	1150		
	4 to 6	500	7½	2658	694	.070	.032
	7 to 9	800	17	1454			
	10 to 12	800	7	2199			
				Mean—2442	Mean—1119		
						R. Q. = .45	
20	1 to 6	200	6	4989	2597		
	7 to 12	200	7	6000	4410	.160	.119
	13 to 17	200	6	4660	4208		
	18 to 23	250	6	7167	6245		
				Mean—5594	Mean—4190		
						R. Q. = .75	
30	1 to 5	250	8	10166	8579		
	6 to 10	250	7	11145	10720	.332	.311
	11 to 13	250	8	13643	14147		
				Mean—11664	Mean—10920		
						R. Q. = .94	
35	1 to 3	150	7	24042	24601		
	4 to 6	150	7	34203	24391	.767	.673
	7 to 9	250	8	22668	21965		
				Mean—26971	Mean—23652		
						R. Q. = .88	

protoplasmic streaming and in the increased length and movement of pseudopodia, almost assuming stellate forms. Macroscopic (and occasionally, microscopic) examinations of the specimens immediately upon termination of the experiments, prior to the addition of the acid from the onset, showed that the specimens remained alive throughout the experiments. Organisms subjected to low temperatures were found to be in a compact and spherical shape; those at high temperatures were stretched out and formed pseudopods.

The results are given in Table II. They indicate that the respiratory quotient increases directly with temperature, along with rate of oxidation. Carbon dioxide elimination increases with an increase in temperature to a point where (at 30°C and at 35°C) the carbon dioxide output in some tests exceeds oxygen consumption.

II. *Effects of Potassium Cyanide on Respiration at Different Temperatures.* The effects of potassium cyanide upon both the structure and activity of *Pelomyxa* have already been determined by Pace and Belda.[16] They also ascertained the effects of potassium cyanide as well as other poisons on respiration in *Pelomyxa carolinensis*. However, nothing has appeared in the literature in reference to the possibility of different cyanide effects at different temperatures. Since the rate and possible type of metabolism varies with temperature, it seemed worthwhile to determine the effects of potassium cyanide at different temperatures.

Again, temperatures of 10°, 20°, 30° and 35°C were used. Potassium cyanide (10^{-4} M) was added to the culture solution in 3 of

[16] Pace, D. M., and Belda, W. H., *Biol. Bull.*, 1944, **87**, 138.

TABLE III.

Effect of Potassium Cyanide at Different Temperatures on Oxygen Consumption in *Pelomyxa carolinensis*. Average volume of One Million Organisms, 35,145 mm³.

Temp., °C	Test No.	No. organisms used	Duration of exp., hr	Avg O_2 consumption in mm³/hr/million organisms	Avg O_2 consumption KCN (10^{-4} M) in mm³/hr/million organisms	Avg rate of O_2 consumption in mm³/hr per mm³ of cell substance	Avg rate of O_2 consumption KCN (10^{-4} M) in mm³/hr/mm³ of cell substance	% inhibition
10	1 to 3	500	8	1644	898			
	4 to 6	500	8	1183	848	.052	.024	54
	7 to 8	500	8	2033	712			
				Mean—1823	Mean—845			
20	1 to 2	500	5	7725	4070			
	3 to 4	500	5	5572	1845	.209	.085	60
	5 to 7	500	6	8354	3068			
				Mean—7380	Mean—3000			
30	1 to 2	250	6	20780	4715			
	3 to 4	250	10	20610	6778	.589	.150	75
	5 to 6	250	6	20763	4346			
				Mean—20718	Mean—5280			
35	1 to 2	150	6	32416	2482			
	3 to 4	150	5	29520	4850	.928	.175	81
	5 to 6	150	6	35967	11210			
				Mean—32634	Mean—6180			

the manometer test-flasks; the remaining 3 were used as controls.

Many of the test organisms which had been subjected to cyanide action at a temperature of 35°C during the course of the experiment were found to be, upon termination of the test, fragile and susceptible to rupture upon handling by means of a transfer pipette. Following rupture, the cytoplasmic mass exuded into the surrounding solution forming a slightly cloudy mixture. However, specimens in contact with cyanide at lower temperatures of 30°, 20° and 10°C did not disintegrate upon handling.

Comparisons between the rates of oxygen consumption in the absence of cyanide and in the presence of 10^{-4} M potassium cyanide are presented in Table III.

It is obvious from these results, that the degree of inhibition of oxygen consumption by potassium cyanide increases directly with the temperature; 54% inhibition at 10°C to 81% at 35°C.

Discussion. As stated previously, Pace,[10] working with the effect of potassium cyanide on respiration in *Paramecium caudatum* and *Paramecium aurelia* noted that inhibition depends upon the degree of saturation of the respiratory system of the organisms with carbohydrate. These results, in general, were confirmed by Clark[13] for *Paramecium caudatum.* It is evident, therefore, that respiratory sensitivity to the action of cyanide is dependent in part upon the amount of carbohydrate material present in the cell. Keilin[17] proposed that the concentration of carbohydrate material within the cell is, more than any other factor, responsible for cellular sensitivity to cyanide. Commoner,[18] working with yeast, reported that the percentage of inhibition by cyanide is dependent upon the concentration of dextrose present within the cell. He noted also that, in the absence of dextrose, little or no respiratory inhibition occurred. Emerson[19] investigating cyanide-sensitivity with the alga *Chlorella,* Hall,[7] with *Colpidium campylum,* and Baker and Baumberger,[8] with *Tetrahymena geleii,* confirmed the above findings.

Pace and Belda[16] suggested that respiration in *Pelomyxa carolinensis* appeared to occur chiefly through a cytochrome-cytochrome oxidase system.

Results presented in Table II show that, evidently, metabolism is chiefly carbohydrate at higher temperatures since it was found that at 30°C, the R.Q. was nearly 1.0; that at 10°C, 0.45. Results shown in Table III upon the effects of cyanide on respiration, yield values which indicate that oxidative inhibition increases directly with an increase in temperature, in some instances approaching total inhibition. Thus, cyanides are much more effective when carbohydrate metabolism is apparently great, which, in turn, leads to the conclusion that in *Pelomyxa* the cytochrome-cytochrome oxidase type of respiratory system is concerned with carbohydrate metabolism. The greater the quantity of carbohydrate oxidized, the greater is the effect of cyanide.

Summary. Oxygen consumption and carbon dioxide elimination in *Pelomyxa carolinensis* was ascertained by means of a Barcroft-Warburg micro-respirometer. The total average oxygen consumption was found to be 2442 mm^3, 5594 mm^3, 11,664 mm^3 and 26,971 mm^3 per hour per million organisms (or 0.07, 0.16, 0.33, and 0.76 mm^3 per hour per cubic millimeter cell substance) at 10°, 20°, 30°, and 35°C, respectively.

The respiratory quotient varies directly with the temperature. It was found to be 0.45 at 10°C and 0.94 at 30°C. Consequently, carbohydrate metabolism is evidently greater at higher than at lower temperatures.

Respiration is inhibited by potassium cyanide (10^{-4} M) and the degree of inhibition varies directly with the temperature.

From the evidence presented, it can be concluded that a cytochrome-cytochrome oxidase system is the mechanism chiefly involved in oxidation of carbohydrate in *Pelomyxa* and that as carbohydrate metabolism is reduced in its extent, this mechanism becomes less and less active.

[17] Keilin, D., *Ergeb. der Enzymforschung.*, 1932, **2**, 239.

[18] Commoner, B., *J. Cell. Comp. Phys.*, 1939, **13**, 121.

[19] Emerson, R., *J. Gen. Physiol.*, 1927, **10**, 469.