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The Effects of Atropine on the Growth and Respiration of Two Species of Bacteria Normally Inhabiting the Human Gastro-Intestinal System

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THE EFFECTS OF ATROPINE ON THE GROWTH AND RESPIRATION OF TWO SPECIES OF BACTERIA NORMALLY INHABITING THE HUMAN GASTRO-INTESTINAL SYSTEM

by

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INTRODUCTION

Atropine is one of the oldest drugs in medicine (5). It is derived from the roots and leaves of solanaceous plants such as henbane and Jimson weed. Atropine is often termed a belladonna alkaloid because it can also be found in the deadly nightshade plant, Atropa belladonna.

Atropine is a highly competitive antagonist of acetylcholine at receptor sites in smooth muscles, cardiac muscle, and various glandular cells (5). Therefore, its effects are felt most strongly in the heart, intestine, and bronchial muscle (4). Although atropine is considered a stimulant, in reality its effects depend upon the dosage. A low dose causes sedation while a larger dose causes stimulation which may lead to delirium (5).

Much research has been conducted on the effects of the belladonna alkaloids on the secretory and motor activity of the gastro-intestinal tract (4). For the purpose of this study the effects of atropine upon the human gastrointestinal system are the ones considered.

Atropine is readily absorbed by the gastro-intestinal tract and is distributed throughout the body (6).

In the gastro-intestinal tract atropine causes a reduction in motility and tone of the stomach, duodenum, jejunum, ileum, and colon. It also reduces gastric acidity, pepsin secretion, and total gastric secretion (2).

There have been many studies based on the interest of using the belladonna alkaloids in the treatment of gastro-intestinal disease such as peptic ulcer (4). It would be logical to assume then, that the bacteria normally inhabiting the gastro-intestinal tract of man may be affected by the presence of these drugs. Since atropine is so readily absorbed by the gastro-intestinal system of man, its effects upon the normal bacterial flora should be investigated.

It is the purpose of this study to determine whether the drug atropine has an effect on bacteria found normally inhabiting the human body, most specifically the gastro-intestinal system.

PROCEDURE

Two different types of experiments were conducted with Escherichia coli and Staphylococcus aureus. One group of experiments determined the amount of growth of these organisms by measuring the turbidity in the Bausch and Lomb Spectronic 20 Spectrophotometer at 580 mu and recording absorbance readings. The bacteria were grown in nutrient broth and minimal media incorporating different concentrations of atropine solution. The other group of experiments involved the use of the Warburg Respirometer to measure cell respiration when using dextrose, atropine in varying concentrations, and the combination of atropine and dextrose as the substrates. Oxygen uptake and carbon dioxide evolutuon rates were calculated, then plotted on graphs.

The atropine solution used for all experiments consisted of 2.5 grams of atropine dissolved in 1000 ml. of distilled water. The atropine solution was always added to the media before autoclaving.

The same procedure was followed for each of the experiments measuring turbidity. The bacteria were grown in flasks containing 100 ml. of the growth substrate and magnetic mixers. These were then set upon magnetic

stirrers for the duration of the experiment. Absorbance readings were taken at fifteen-minute intervals by tipping the medium into the side-arm of the flask and inserting this tube into the spectrophotometer. A growth curve was plotted and the rate of increase of growth per minute was calculated for each experiment. A control flask containing sterile medium was also used for each experiment.

There were a total of sixteen experiments conducted to measure turbidity using the spectrophotometer. Experiments GA, GA-1, and GA-2 were conducted using nutrient broth as the growth substrate (Appendix I); experiments GB, GB-1, GB-2, GB-3, and GB-4 were conducted using minimal media I (Appendix I) and varying concentrations of dextrose and atropine; experiments GC, GC-1, GC-2, GC-3, GC-4, and GC-5 were conducted using minimal medium II (Appendix I) and varying concentrations of dextrose, atropine, vitamin-free casamino acids, and peptone; for experiment D bacteria were inoculated into a third minimal medium (Appendix I) without any peptone, vitamin-free casamino acids, asparaginate, or ammonium chloride and this acted as the control.

Experiment GA was used as a control experiment to determine a normal growth rate for each organism in

100 ml. of nutrient broth. In experiment GA-1 the bacteria were grown in 95 ml. of nutrient broth and 5 ml. of atropine solution; in experiemnt GA-2 92.5 ml. of nutrient broth and 7.5 ml. of the atropine solution was used as the carbon source. Table I gives the concentrations and amounts of the reagents used in the experiments conducted with minimal media I, II, and III.

Eight experiments were conducted using the Warburg Respirometer with E. coli. For experiments R-I, R-II, R-III, R-IV, R-VII, and R-VIII the bacterial cell suspensions were prepared by growing the cells in 100 ml. of nutrient broth overnight. Approximately 15-20 ml. were poured into a tube and centrifuged. The supernatant was decanted and phosphate buffer solution (pH 7.2) was added to the cells. These cells were then resuspended and centrifuged again. The supernatant was again decanted, buffer solution was added and the cells resuspended.

Cell-free suspensions for experiments R-V and R-VI were prepared by centrifuging the bacterial cells and decanting the supernatant. Aluminum oxide was added to the pellet and ground with a mortar and pestle. This lysate was then suspended in phosphate buffer.

For each experiment three Warburg flasks were used. Flask constants were determined by methods

TABLE I

EXPERIMENT	MEDIUM (ml.)			DEXTROSE (10% solution)	ATROPINE SOLUTION (ml.)
GB	Basal	I--100		--------	---
GB-1	Basal	I--100		10.0 ml.	---
GB-2	Basal	I-- 95		0.1 ml.	5
GB-3	Basal	I-- 92.5		0.1 ml.	7.5
GB-4	Basal	I-- 95		--------	5
GC	Basal	II--100	(.5% conc.)	--------	---
GC-1	Basal	II-- 95	(.1% conc.)	2.5 ml.	5
GC-2	Basal	II-- 95	(.5% conc.)	2.5 ml.	5
GC-3	Basal	II-- 92.5	(.1% conc.)	2.5 ml.	7.5
GC-4	Basal	II-- 92.5	(.5% conc.)	2.5 ml.	7.5
GC-5	Basal	II-- 92.5	(.1% conc.)	--------	7.5
GC-6	Basal	II--100	(.5% conc.)	2.5 ml.	---
GD	Basal	III-- 92.5		1 gram	7.5

described by Umbreit, et al (10). Two flasks served as reaction vessels while the third functioned as a thermobarometer.

For all experiments the two reaction flasks were loaded identically with the exception of the well; Flask I contained .2ml. of H_2O and Flask II contained .2ml. of KOH. Both contained filter paper wicks to increase the surface area absorption.

The flasks were attached to the manometers and were allowed to equilibrate for ten to twenty minutes in the water bath at 30.5 ^{o}C. The manometer stopcocks were then closed and readings were taken every ten minutes for the duration of the experiment. Oxygen uptake and carbon dioxide evolution rates were calculated as outlined by Umbreit, et al (10).

Table II gives the concentrations and amounts of the reagents used in loading the flasks for the experiments conducted on the Warburg Respirometer. Appendix II gives the concentrations of dextrose and atropine solutions used as substrates. The dextrose used in all experiments was a 10% solution which was further diluted when necessary.

Dry weights were determined for experiments R-I, R-II, R-III, R-IV, R-VII, and R-VIII. This was done by centrifuging 5 ml. of cells, decanting the supernatant,

TABLE II**

EXPERIMENT	FLASK	SIDEARM
R- I	2 ml. cells	.5 ml. dextrose solution
R- II	2 ml. cells	.5 ml. atropine solution (Low concentration)
R- III	2 ml. cells	.5 ml. atropine solution (High concentration)
R- IV	2 ml. cells	.5 ml. atropine-dextrose solution (High concentration)
R- V	2 ml. cell-free suspension	.5 ml. dextrose solution
R- VI	2 ml. cell-free suspension	.5 ml. atropine solution (High concentration)
R- VII	2 ml. cells & 5 ml. dextrose solution	.5 ml. atropine solution (High concentration)
R- VIII	2 ml. cells & .5 ml. atropine solution (High concentration)	.5 ml. dextrose solution

** Flask I and II were loaded identically except for the well.

Flask I contained .2 ml. H_2O; Flask II contained .2 ml. KOH.

resuspending the cells in 1 ml. of distilled water, placing the suspension on a preweighed phanchette, and placed in an oven to dry. Dry weight was then determined by measuring the weight per 5.0 ml. of cell suspension from the original culture. This value was then used to determine the dry weight of the 2.0 ml. suspension used in the respirometer experiments.

RESULTS
AND
DISCUSSION

Growth Experiments

Table III gives the rate of growth per minute for E. coli and Staph. aureus in the different media utilized. Neither E. coli (7) nor Staph. aureus (1) are fastidious in their nutritive requirements for growth. Experiments GB-2, GB-3, GB-4, GC-1, GC-2, GC-3, GC-4, GC-5, and GD in Table III show that the bacterial cells are able to utilize the drug atropine to synthesize the necessary substances required for cell metabolism. The rate of increase of growth E. coli and Straph. aureus in nutrient broth, to which atropine has been added, increases as the concentration of atropine solution increases and is higher than the rate of increase of growth in nutrient broth alone. Apparently atropine does not inhibit growth of the bacterial cells in nutrient broth.

E. coli

In basal medium I the highest rate of growth was

TABLE III

GROWTH RATE
$(\frac{\Delta A}{\Delta T} \times 1000)$

EXPERIMENT	E. COLI	STAPH. AUREUS
GA	33.0	3.0
GA-1	37.0	3.1
GA-2	41.0	4.6
GB	----	---
GB-1	9.3	0.7
GB-2	1.1	0.7
GB-3	25.0	2.0
GB-4	1.0	0.5
GC	6.7	3.7
GC-1	3.1	3.3
GC-2	5.0	5.8
GC-3	3.3	3.4
GC-4	3.9	4.6
GC-5	3.5	0.9
GC-6	5.3	5.0
GD	1.6	2.6

reached when the high concentration of atropine solution and dextrose were added to the growth substrate. The lowest rate of growth occured when adding only the low concentration of atropine solution without dextrose. In both cases, atropine did not inhibit the growth of the cells; but the rate of growth was not as high as in nutrient broth.

In basal medium II, the rate of growth did not vary considerably; the range was from 3.1 to 5.0. The rate of growth was higher in basal medium II than in basal medium I since basal medium II contained peptone, vitamin-free casamino acids, asparaginate, and ammonium chloride. Atropine did not inhibit the growth of the bacterial cells; the effect of varying concentration of atropine was not significant.

In basal medium III, which did not contain any nitrogen source, the rate of growth was 1.6 indicating that the bacterial cells were able to utilize atropine for the synthesis of cell materials.

Staph. aureus

In basal medium I the highest rate of increase of growth was reached when the high concentration of atropine solution and dextrose were added to the

growth substrate. This rate was not as high as the rate achieved by E. coli. The lowest rate of increase of growth occured when adding the low concentration of atropine solution to the growth substrate without dextrose. The rates of growth were not significantly lower when atropine was present.

In basal medium II the rate of growth was highest when the low concentration of atropine solution and dextrose were added to the growth medium (.5%). Apparently the concentration of the atropine solution has no significant effect upon the rate of growth. The lowest rate of growth occured when only the high concentration of atropine solution was added to the growth medium without dextrose.

In basal medium III which did not have any nitrogen source the rate of growth was 2.6; the bacterial cells were able to utilize atropine in the synthesis of cell materials.

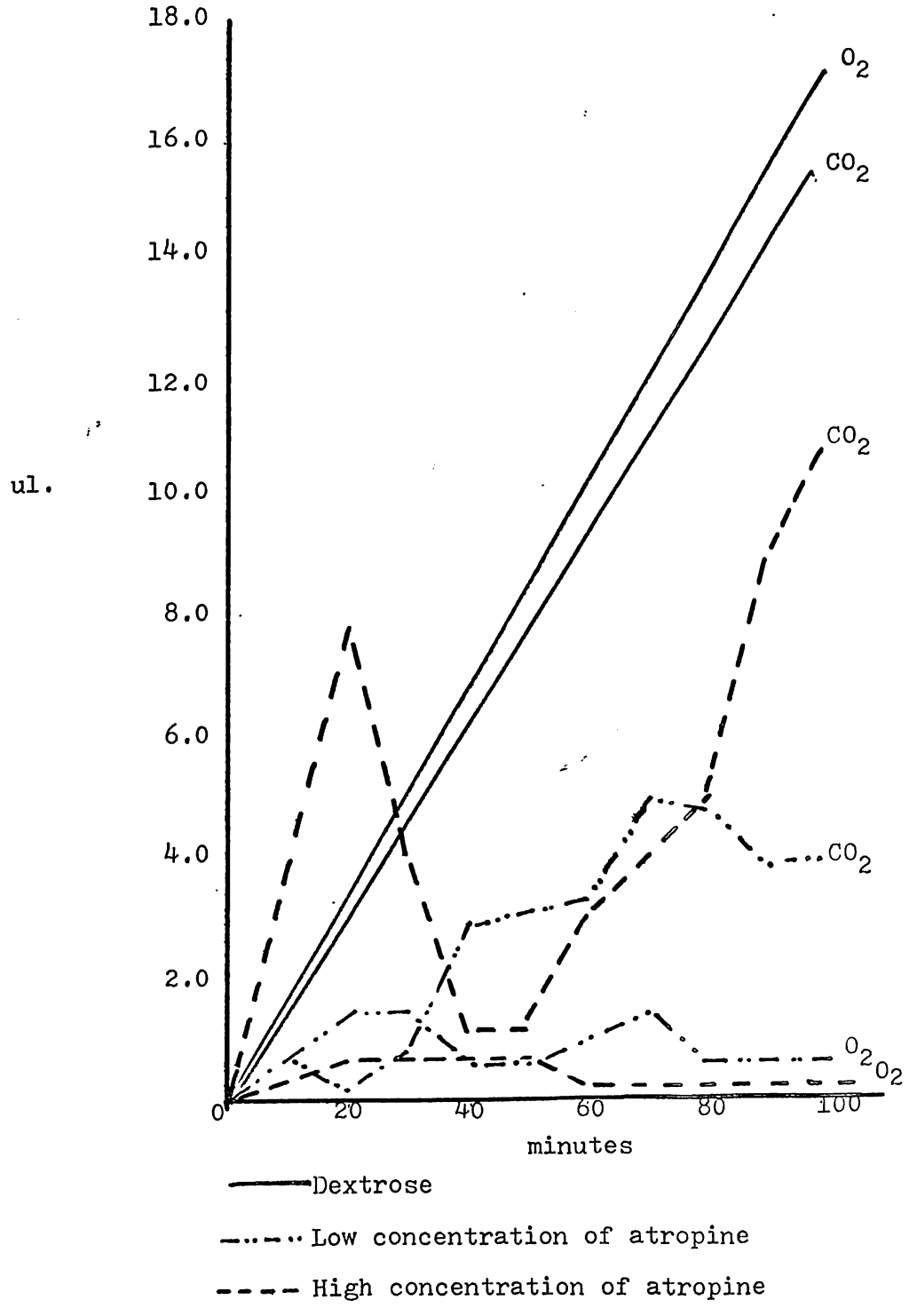
Respiration Experiments

E. coli

Graph I shows the results of bacterial cell respiration when using dextrose and atropine in high and low concentrations as the substrates. When dextrose was

GRAPH I

WHOLE CELLS



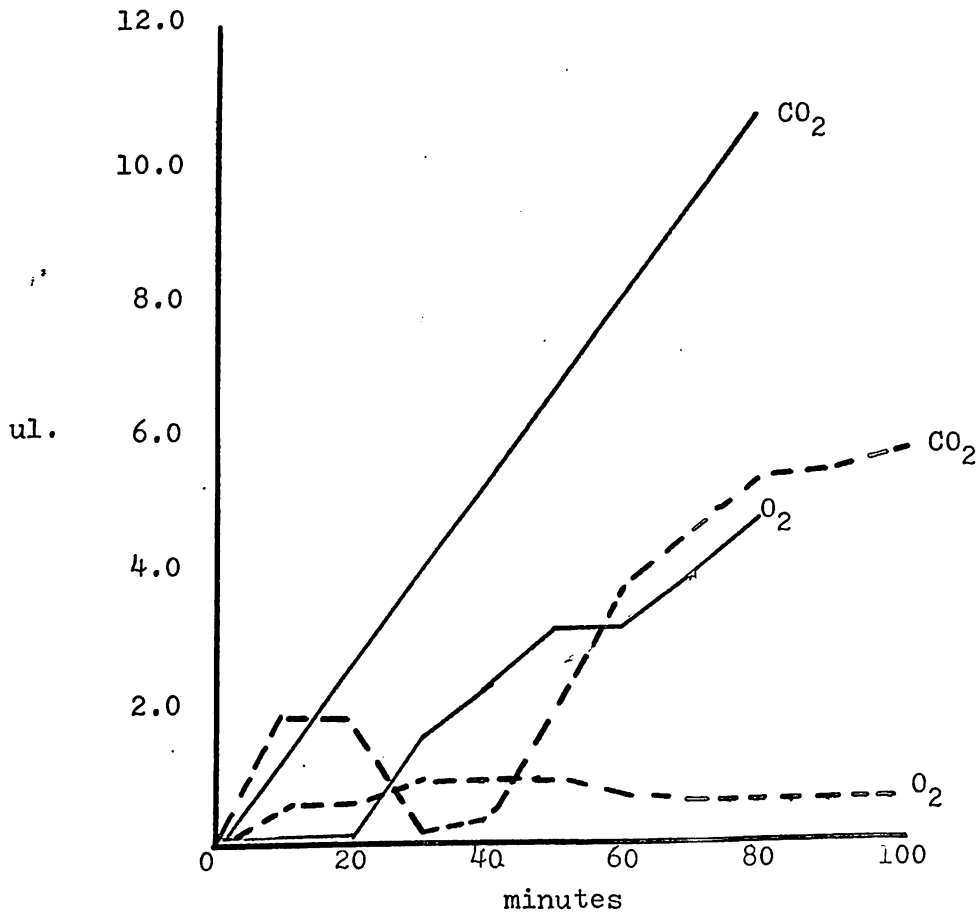
used as the substrate a normal respiration pattern was observed with more oxygen being taken up than carbon dioxide being evolved. But when atropine was used as the substrate in both concentrations, the respiration pattern was reversed; more carbon dioxide was evolved than oxygen taken up. The rate of respiration was also affected by the presence of atropine; the higher the concentration, the slower the rate of respiration.

Graph II shows the results of respiration in cell-free suspensions. Again the same pattern was observed; when both dextrose and a high concentration of atropine solution were used as substrates, there was more carbon dioxide evolved than oxygen taken up. The rate of respiration was also slower when atropine was present.

Graph III shows the results of respiration when dextrose and atropine were used in combination as the substrates. A normal respiration pattern was observed; more oxygen being taken up by the cells than carbon dioxide being evolved. There appears to be two rates of respiration; possibly the cells utilize the dextrose first and then metabolize the atropine when the first carbon source is exhausted. When the cells were grown in dextrose and atropine was used as the substrate a reversal in the respiration pattern

GRAPH II

CELL-FREE SUSPENSION

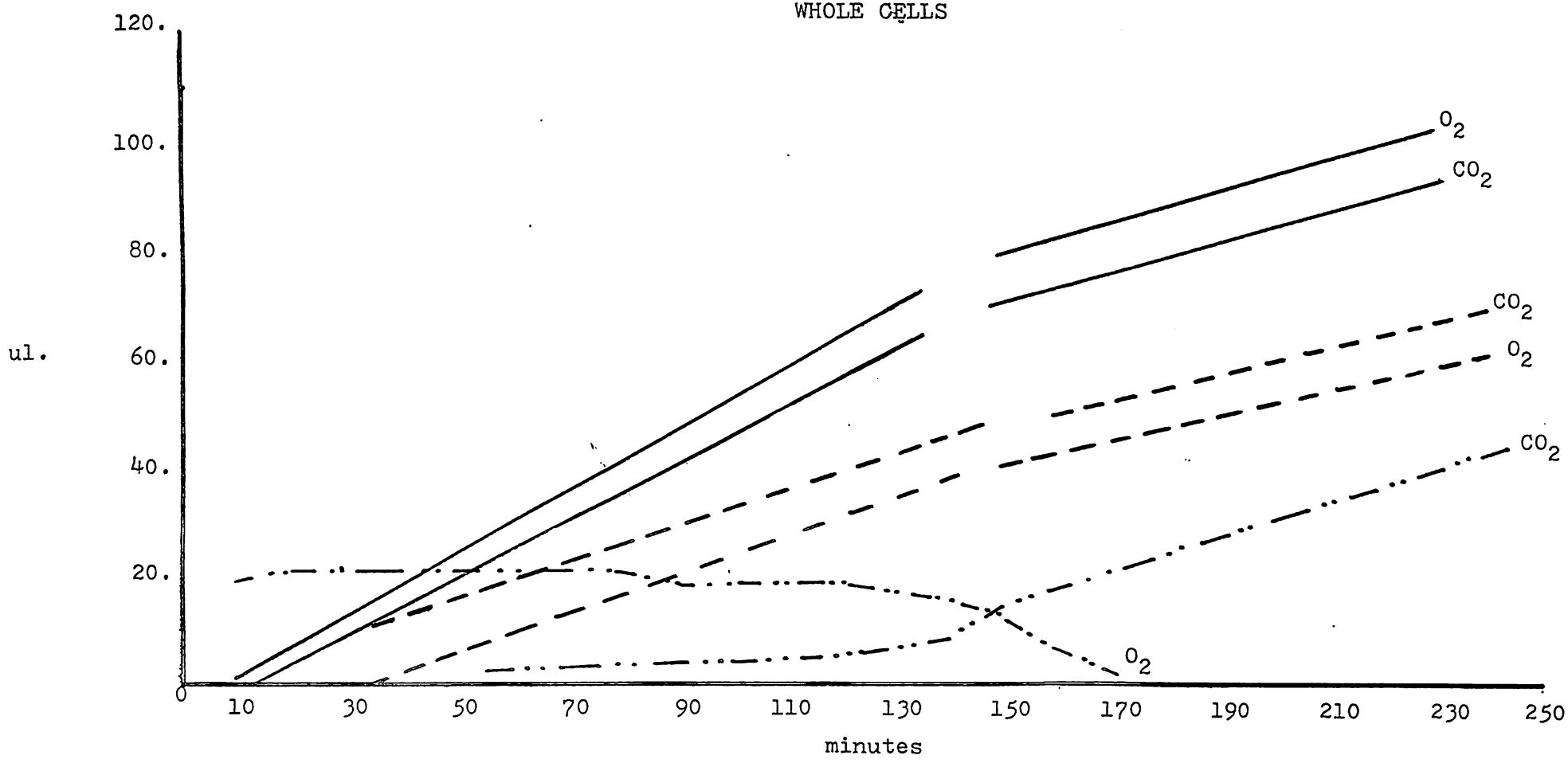


——— Disrupted cells with dextrose

– – – – Disrupted cells with high concentration of atropine solution

GRAPH III

WHOLE CELLS



Substrate - dextrose and atropine

Substrate - atropine

Substrate - dextrose

was again observed -- more carbon dioxide was evolved than oxygen taken up. When growing the cells in atropine solution and adding dextrose as the substrate, the most significant decline in the rate of uptake of oxygen was observed. The rate of uptake of oxygen is almost 0 while the rate of evolution of carbon dioxide is 46 ul. in 240 minutes. In normal glucose metabolism, more CO_2 is released than can be accounted for by the Krebs cycle (9). It is probable that another sequence of reactions is taking place when atropine is being metabolized resulting in decarboxylation with a large amount of CO_2 evolving under anaerobic conditions.

In bacteria, the membranes are the true carriers of the respiratory chain and the respiratory chain enzymes remain firmly bound with the cell membrane even when the cells are disrupted (3). The cell wall of E. coli is highly permeable to many molecules and therefore atropine must pass readily into the cell (9) and come into contact with the cell membrane and the respiratory chain enzymes.

The most significant observation made is that atropine interferes greatly with the process of cell respiration. The utilization of O_2 is almost completely prevented when the cell attempts to break down the molecules of atropine. The concentration of atropine

solution is also significant -- the higher the concentration, the more interference there is with cell respiration. I believe that the definition of a cytotoxic or protoplasmic poison (a drug that modifies an enzymic reaction essential for energy production, growth or reproduction which will affect this enzyme in any cell to which the drug can gain access) (8) can be applied to atropine and its effects on bacterial cells.

Therefore, the presence of atropine does have effects upon the two species of bacteria studied which are found normally inhabiting the gastro-intestinal system of man. The bacteria no longer are able to function normally in carrying out their metabolic processes if the respiration system is shut down. Since some species of bacteria aid in the digestive process, this can have a direct effect upon man causing gastro-intestinal disturbances. In addition atropine may affect the bacteria which live beneignly in the gastro-intestinal system causing an alteration in their metabollic processes and in turn cause gastro-intestinal disturbance.

APPENDIX I

BASAL MEDIUM	KH_2PO_4 K_2HPO_4 (ml.)	$MgSO_4$ (ml.)	NH_4Cl (g.)	Na Asparaginate (g.)	Vitamin-free casamino acids (conc.-g.)	peptone (conc.-g.)	1% inositol solution (ml.)	yeast extract (g.)	H_2O (ml.)
I	0.5	0.5	4.0	0.1	---	---	0.05	0.05	100
II	0.5	0.5	---	---	.1%--0.1	.1%--0.1	0.05	0.05	100
	0.5	0.5	---	---	.5%--0.5	.5%--0.5	0.05	0.05	100
III	0.05	0.05	---	---	---	---	0.05	0.05	100

(All three basal media were autoclaved at 121°C. for 15 minutes. End Molarity is 0.1)

APPENDIX II

SUBSTRATES	REAGENTS
I. DEXTROSE SOLUTION (Experiments I & V)	2.5 ml. of 10% dextrose solution dissolved in 100 ml. of H_2O
II. ATROPINE SOLUTION	
A. Low concentration (Experiment II)	.1 ml. atropine solution dissolved in .4 ml. H_2O
B. High concentration (Experiments III, VI VII, & VIII)	.15 ml. atropine solution dissolved in .35 ml. of H_2O
III. DEXTROSE AND ATROPINE SOLUTION (Experiment IV)	2.5 ml. of 10% dextrose solution dissolved in 70 ml. H_2O; .35 ml. of this solution added to .15 ml. of atropine solution

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