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Electrolytes and Metabolites Rich Secretion from Laboratory Grown Larvae of Lucilia sericata

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Abstract
Feeding process in biological system involves release of exocrine secretion to modify the pH of the food substrate and the release of protein enzyme for digestion. Digestive secretions contain electrolytes and metabolites of varying nature and source. The aim of this study is to harvest the saprophagous secretion of larvae of *Lucilia sericata* and to quantitatively as well as qualitatively evaluate the contents. The larvae were grouped and housed in 5 different 200 ml plastic jars, each with a net cover firmly attached round the brim of the jar with elastic band. Each jar contains 300 units of the larvae and they are all kept in a larger bucket whose interior was saturated with the smell of a piece of putrid meat. Half hourly 100ml of Normal Saline was used to wash off the effluent from the larvae of each of the five groups. The analyses show the significantly ($p<0.05$) increasing and time dependent presence of Na^+, K^+, Cl^-, HCO_3^-, urea, creatinine, total protein, bilirubin and amylase secreted.
Keywords: *Lucilia sericata*, saprophagous, Electrolytes, Metabolites

INTRODUCTION

The larvae (maggots) of some species of flies are long known to exert beneficial effects on necrotic wound and have been exploited therapeutically in biosurgery, specifically Maggot Debridement Therapy, according to Pechter and Sherman (1993). These larvae administered unto wound surface release secretion that contains water and other components including proteolytic enzymes that help in the breakdown of necrotic tissue (Vistnes et al, 1981). Ammonia released from possibly the breakdown of urea has powerful antibacterial property as much as other germicidal compounds (Kerridge et al, 2005). Hassan et al (2016) further demonstrated the powerful antimicrobial nature of the secretion of larvae of *Lucilia sericata.* Previous work by Richards and Edwards (2001) has further strengthened the view that proteins synthesized and release into larval secretions have the additional function of repelling predators and thus act as part of their defense mechanism. Other proteins may have immune modulatory functions as exemplified by the larval form of the intestinal nematode *Trichinella spiralis,* which has to survive immune attack from its host as shown by the work of Valentina K. Todorova (2000). This has lead to more interest in this nematode for helmintherapy and thus can be used in management of inflammatory bowel disease which include ulcerative colitis and Crohn's disease. The eggs of a chosen helminth are administered orally to hatch and grow in the large bowel so that they may continuously provide these anti inflammatory and beneficial protein-rich secretions by the larvae.

Secretions from organisms that assist in digestion mostly contain water in a proportion of about 90% and various types of metabolites. Such constituents include Na^+, K^+, Cl^-, HCO_3^-, α-amylase, proteins and mucin etc. These substances in the secretion are used for digestive purpose among other things. Creatinine and bilirubin are metabolites still subjects of interest to medical scientists for their role in the biology of many organisms. While bile, a by-product of bilirubin metabolism, is of enormous importance in digestion of lipid in the gastro-intestinal tract, assisting by emulsifying lipids, creatinine on the other hand is a product of muscle protein metabolism and its usefulness in the body is still being evaluated outside its classification as a waste product.

Electrolytes are regularly present in body secretion of the biological system and are often incorporated into the water content of the secretion by the process of transudation or simple diffusion. Na^+ ions help in determining the tonicity of the fluid and that is important in establishing the electrochemical gradient important for trans-membrane transport.

The presence of bicarbonate is widespread all through the body systems because of the abundance of carbonic anhydrase enzyme which catalyses the hydration of carbon dioxide and that forms the unstable carbonic acid that readily dissociates into proton and bicarbonate (HCO_3^-). Modification of the pH of digestive secretion and the substrate upon which the digestive enzymes works, may be a key in activation of the enzymes often initially present in the inactive zymogen forms.

Bicarbonate may also be involved in the neutralization of acid chyme whose pH may need modification to effect the appropriate biochemical reaction.

AIM AND OBJECTIVE

The aim of this work is to stimulate the production of digestive secretion in the saprophagous larvae of *Lucilia*

sericata by saturating the air they dwell in with the offensive gaseous emission from putrid piece of beef. The effluent is timed and regularly harvested with normal saline

MATERIAL AND METHOD

A total of one thousand five hundred (1500) larvae of *Lucilia sericata*, 200 ml plastic jar each to house 300 units of the larvae, five net covers for the plastic jar with five elastic band to tighten and trap the larvae, 5 units of 200ml plastic jar, 500 gram of beef bought from the local abattoir, wide base 50 litre plastic bowl with a cover to create the atmosphere of offensive smell of putrid piece of beef, 2 litre of Normal Saline and spectrophotometer from Chemical Pathology of the Jos University Teaching Hospital.

Secretion from the saprophagous larvae of *Lucilia sericata* is stimulated by saturating the air they dwell in with the offensive gaseous emission from putrid piece of beef. The effluent is half hourly timed and regularly washed off the larvae with 100 ml of Normal Saline separately for each of the five jars. The theory behind the extraction of secretion from maggot is the Pavlov like reflex and in similarity to Sham feeding. Perfusion of maggots after 1 hour of starvation inside a well-ventilated plastic Jar with a piece of putrid meat introduced into the atmosphere of the maggots' environment stimulates feeding frenzy like behaviour. This is expected to induce by chemotaxis a Pavlov-like reflex secretion. The Normal saline solution is used to perfuse the colony of the maggots to harvest product of this stimulation. The effluent is recycled about 5 times in quick succession, through a large bore plastic perfusion tube improvised to obtain a greater concentration of the secretion from the maggots for any given harvest.

Determination of Bilirubin Concentration:

All the reagents for these tests were provided by the chemical pathology laboratory of the Jos University Teaching Hospital and were of the best quality available. *Aromatic primary amine*, 5 mmol/l; 500 µmol of aromatic primary amine was dissolved in 100ml of 0.176 mol/l Hydrochloric acid solution. Sodium Nitrite solution, 72 mmol/l, 0.5 gram of Sodium Nitrite was dissolved in 100ml of distilled water, and stored in refrigerator. This reagent was prepared every week.

Diazo reagent; 0.6 ml of the sodium Nitrite solution was added to 10 ml of the aromatic primary amine solution. This reagent was used within one hour of its preparation.

Bilirubin solution, 170 µmol/l; 10.0 mg of unconjugated bilirubin was dissolve in 100ml of chloroform and stored in a refrigerator.

Preparation of water-soluble photoproduct. 10 ml of the bilirubin solution was irradiated by a fluorescent lamp at an intensity of 1500 lux during 48 hours. Then 10 ml of distil water was added to this solution and the water-soluble photo product was extracted.

Determination of bilirubin in the maggot's secretion.

The total bilirubin in the maggot's secretion: in a test tube containing 0.1 ml of the secretion and 3.9 ml of 50% methanol, 1.0 ml of the diazo reagent was added and the solution was mixed well. Then the reaction was allowed to stand for 30 minutes at 25 degree Celsius. The absorbance was then measured at 540 nm with a Hitachi 101 spectrophotometer against a reagent blank consisting 4.0 ml of 50% methanol and 1.0 ml of the diazo reagent.

Biuret reagent is used for the protein determination and so is *flame photometric* method for determination of the ions using the standard and elaborate procedure for the laboratory and the same goes for the determination of amylase enzyme concentration.

RESULTS

The presence of and the time dependent quantity of Na^+, K^+, Cl^-, HCO_3^-, Urea, creatinine, total protein, bilirubin and amylase were qualitatively and quantitatively determined with the help of Spectrophotometer in the Chemical pathology laboratory. At the baseline, the zero minute, the measurements are reflective of the composition of normal saline used to wash off and obtain secretion from the maggots and from which 5ml sample is taken for each level.

TABLE SHOWING MEAN VALUES OF UREA-ELECTROLYTES,, CREATININE, BILIRUBIN, TOTAL PROTEIN AND AMYLASE ENZYME IN MAGGOT'S SECRETION (Mol/L)

TIME (Minutes)	Na^+	K^+	Cl^-	HCO_3^-	UREA	CREAT	TP	Bil	Amylase
0	39	0	25	<10	0	0	0	0	0
30	42	0.7	29	<10	2.4	0	1.1	0	40
60	45	0.9	34	<10	3.1	56	1.8	0	69
90	48	1.3	37	<10	3.5	7.9	1.8	0.7	74
120	60	1.4	43	<10	3.5	9.8	2.1	0.9	120
150	89	1.9	49	<10	3.9	12.9	2.2	1.2	168
180	108	2.1	87	<10	4.1	16.8	2.2	1.4	194
210	117	2.2	106	<10	4.5	18.8	2.3	1.74	216

Table I

Significant ($p<0.05$) and time dependent increase in secretion of electrolytes, metabolites and enzyme.

Sodium ions and Chloride ions were present at 39 and 25 Mol/L concentrations respectively. Other components from the maggots started showing up after thirty minutes of the stimulation by smell of putrid meat. Urea, creatinine, total protein, bilirubin and amylase were detected and assayed to the two hundred and tenth minute as shown in table I. All the components increased and analysis of variance showed that the increases in the substances were statistically significant ($P<0.05$).

DISCUSSION

Using Spectrophotometer in the Chemical pathology laboratory, the effluents from maggots (secretion) grown in the laboratory, were analyzed qualitatively and quantitatively. At the baseline, the zero minute, the measurements are reflective of the composition of normal saline used to wash off and obtain secretion from the maggots. Other components from the maggots started showing up after thirty minutes from the maggots. Urea, creatinine, total protein, bilirubin and amylase were detected and assayed to the two hundred and tenth minute as shown in table I.

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Digestive fluids secreted by organisms are usually rich in electrolytes and metabolites and these constituents are physiological. Some components of this secretion like amylase demonstrated in this work have roles in digestion while the presence of creatinine and bilirubin may be reflective of excretory function. These analytes are produced in significant time dependent manner.

CONCLUSION

The Secretion nay combination of secretion and excretory material from the larvae of *Lucilia sericata* in this work, is electrolyte and metabolite rich, as obtained in other body secretions especially from the gastro-intestinal tract. These components and their respective concentration are physiological.

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