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Effect of Salinity on Glycogen Content in the Brine Shrimp, Artemia salinas, of Great Salt Lake

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INTRODUCTION

The brine shrimp, *Artemia salinas*, is one of the few organisms to be found in the hostile environment of the Great Salt Lake. Salt concentrations in the lake are found to exceed those in the oceans. In such an environment, the utilization of energy, even after it has been procured, presents a problem. The life inhabiting the lake has developed extraordinary adaptations to this and similar problems.

Electron micrographs produced by Dr. Nabil Youssef of Utah State University have revealed that unusual quantities of glycogen can be found in the muscle of brine shrimp taken from the Great Salt Lake. The glycogen was found to be associated in large packets rather than being dispersed in small quantities throughout the tissue. It was also observed that the mitochondria were small, a tendency that might indicate anaerobic rather than aerobic respiration. These observations led to the initiation of this project.

Samples of *Artemia* were collected from the Great Salt Lake and analyzed for glycogen content in an effort to determine the effect of salinity on the mode of respiration.

MATERIALS AND METHODS

Sampling methods

Artemia were collected from the lake following the respective algal blooms of the two arms of the lake. The north arm experienced no appreciable algal bloom at the collection point, but shrimp were

collected near the shore while a bloom occurred in deeper waters. Collection points were located on the causeway to Antelope Island near Syracuse for the south arm and at Roselle Point for the north arm. The specimens were collected with a small nylon net near the shoreline and placed in a large container of water taken from the same location.

Maintenance of the sample

The sample was distributed evenly between 15 separate 100 ml. collection jars. Approximately 5-10 specimens were placed in each jar along with 100 ml. of water from the large container. These were maintained at roughly 25° C and transported to Utah State University in Logan for analysis. Analysis was begun immediately so that the data would be as accurate as possible.

Preliminary procedures

Upon arriving at the laboratory, a large number of specimens were counted, placed in a pre-weighed crucible, and dried in an oven for one week at 104° C. Fifty specimens were randomly selected from each location for this purpose. From this data the average dry weight in grams was later deduced. This was useful when comparing glycogen content to dry body weight.

Reagents used

1. Deproteinizing solution: Trichloroacetic acid (5 gr.) and silver sulfate, Ag_2SO_4, (100 mg.) were dissolved in distilled water and made up to 100 ml. (cf. Mendel, Kemp, and Myers, 1954)
2. Sulfuric acid, 96% (w/w, approximately 36 N). (cf. Mendel et. al. 1954)
3. Methanol, 80% (v/v). (cf. Mendel et. al. 1954)

Extraction of glycogen

Four specimens of Artemia were selected from the available sample. These were washed individually in distilled water and then placed in a centrifuge tube containing 5 ml. of 80% methanol. These were ground with a glass rod until no large pieces of tissue remained. The tubes were then centrifuged for 2-3 minutes at 2500 rpm. The supernatant containing the methanol soluble glucose was decanted and 5 ml. of the deproteinizing solution was added to the centrifuge tube. The tubes were then placed in a boiling water bath for 14-15 minutes. The water soluble glycogen was then found in solution. After cooling in running water, the tubes were again centrifuged for 2-3 minutes at 2500 rpm.

Colorimetric reaction

One ml. of the supernatant was placed in a small test tube. To this was added 3 ml. of 96% sulfuric acid. Care was taken not to look into or stand over the tubes because of the violent nature of the reaction. The strength of the sulfuric acid is crucial to the test as noted by Kemp and Kits Van Heijningen (1954) and care was taken to see that the acid was free from discoloration and was of the proper specific gravity. The specific gravity was 1.84, as required. The colorimetric reaction was carried to completion by immersing the tubes in a boiling water bath for 6.5 minutes immediately after the addition of the sulfuric acid.

Spectroanalysis

The resulting pinkish-blue color was measured spectrophotometrically with a Spectronic 20 (Bausch and Lomb) at 520 mμ.

Blanks were prepared from 3 ml. of the concentrated sulfuric acid added to 1 ml. of the deproteinizing solution.

The glycogen content was evaluated from a previously prepared standard curve in terms of equivalent glucose units. The curve was formed from the analysis of known concentrations of glucose that were subjected to the reaction. The relationship between glucose concentration and color intensity was linear up to approximately 15 mg./100 ml., thus making this an ideal test for dilute concentrations of glucose or glycogen.

Chemical theory

Morrison and Boyd (1973) mention that particular care must be taken when working with glycogen to be certain that it is not subjected to decomposition. Glycogen is a water soluble polysaccharide composed of 12-18 glucose units. It is similar to amylopectin in structure but is more highly branched and has shorter chains.

Methods for the determination of glycogen content of large samples of tissue are fairly familiar to the biochemist. These often require large amounts of sample. Mendel et. al. (1954) and Kemp et. al. (1954) have provided the basic outline for the colorimetric determination of low concentrations of glucose and glycogen used in this project. Such a microtechnique is a great advantage to the researcher using small amounts of sample.

The colorimetric reaction is based upon the reaction of glucose with concentrated sulfuric acid. Glycogen is hydrolyzed by hot sulfuric acid to give glucose as a product. Glucose in hot acid is hydrated to form 5-hydroxymethylfurfural as shown on the following page.

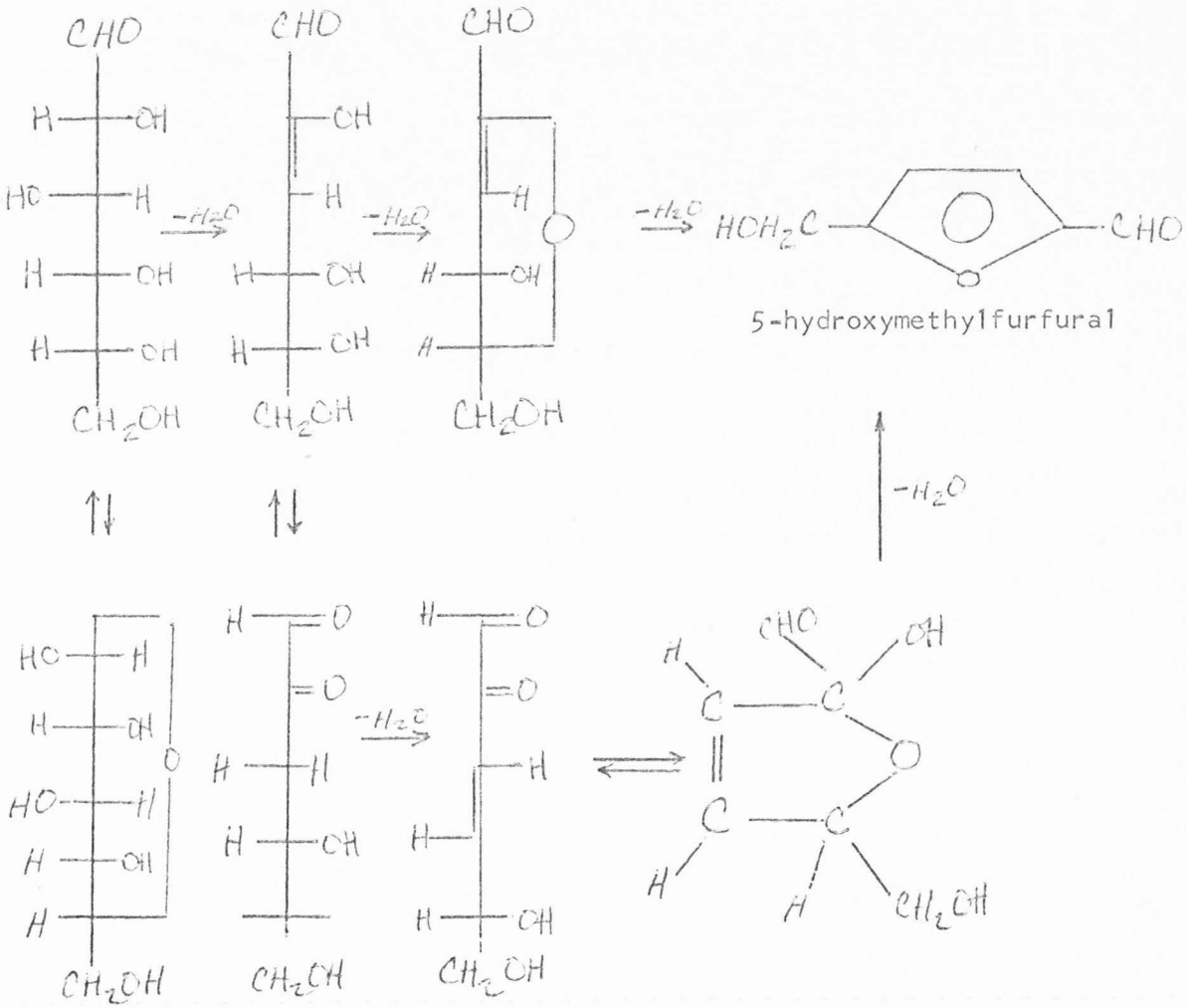

Figure 1. Hydration of glucose to form 5-hydroxymethylfurfural. After Wolfrom, Shuetz, and Cavalieri (1948).

The actual pinkish-blue color is derived from a reaction between 5-hydroxymethylfurfural (HMF) and one of its precursors.

Precautions were taken during this procedure to keep the solution free of chlorides. Only distilled water was used in the preparation of reagents. Care was taken while washing the specimens with distilled water to eliminate as much of the brine solution as possible. Kemp, et. al. (1954) state that silver sulfate is added to the deproteinizing solution to precipitate out free chloride ions that interfere with the test.

DISCUSSION

It has been theorized by Rawley and Johnson (1974), that an ancestral phyllopod was able to adapt itself to the gradual changes in the fresh water of Lake Bonneville as it was transformed into the present day Great Salt Lake, thus giving rise to the modern form of Artemia. Woodbury (1936) sheds further light on the hostile environment of the lake in the following excerpt:

> With it saline content approaching saturation, the Great Salt Lake presents a harsh environment to most living organisms, both within the water and upon the nearby shores. Those who would survive the rigors of this aquatic desert must either be equipped to meet the exactions imposed or to avoid them.
>
> The problem of meeting the peculiarities of the environment is largely physiological--that of extracting moisture from a salt solution. This problem varies not only from time to time with the rise and fall of the lake level and its consequent dilution and concentration, but also from place to place where dilution occurs by the inpouring of fresh-water streams and underwater springs is undoubtedly due to these severe restrictions.

> So far as known, no parasites have been able to follow them into the brine. Freed from parasites, these arthropods have been enabled to multiply with little or no molestation to the limit of the food supply, presumably provided principally by the blue-green algae. They occur in enormous numbers in many parts of the lake, especially near the shore where the algae are abundant.

In 1957, the Southern Pacific Railroad Company completed a causeway across the lake from Promontory Point to Lakeside which resulted in the formation of "two" Great Salt Lakes. After a period of eighteen years, it has been found that the salinities of the north and south arms of the lake differ by a factor of two, the north arm being the most saline. This has been caused by a differential in the amounts of fresh-water received by the respective arms of the lake. The salinity of the north arm has increased to the point of supersaturation during the summer months. Thus, in a very short period of time, the brine shrimp has endured quite a drastic change in the surrounding milieu of the north arm.

Previous experimentation by Jensen (1918) has shown that Artemia does much better in water of lower salinity than that of Great Salt Lake. Solutions having a specific gravity of 1.044 to 1.109 have been found best for the hatching and growth of Artemia. Rawley et. al. (1974) believe that an increased inflow of fresh-water from the surrounding mountains in the spring of the year could be a factor in the increased numbers noted in spring and summer. Temperature, as noted by Reylea (1936), is also a parameter, as is the food source. Algal blooms of the principal food source seem to initiate a corresponding growth in Artemia populations as noted by Post (1975).

Certain structural differences were observed in Artemia by Hernandorena (1974) which were raised in solutions of different salinities. Differences in the segmentation of the abdomen and in the length and hairiness of the caudal appendages were observed. These differences seemed to be ontogenetic in nature, being caused by the immediate saline environment.

Laboratory experimentation by D'Agostino and Provasoli (1968) determined that growth is best when fed ad libitum on a mixture of Dunaliella viridis and Dunaliella salina. These species require neither vitamins nor organic compounds of phosphorus and nitrogen for growth, they are photoautotrophic. Engel and Angelovic (1968) showed that algal synthetic rates are affected by sodium chloride concentrations, the rates decreased with increased salinity. The quanitity of glycerol produced at high salinities was shown to be greater in algae by Craigie and McLachlan (1964), indicating that the nature of the photosynthate varies with the salinity, i.e. more glycerol is produced at high salinity and more polysaccharides at low salinity. The production of large amounts of glycerol is most likely an adaptation by the algae for living in brine solutions.

Since Dunaliella is an energy source for Artemia, it would be interesting to inspect Artemia for any corresponding increase in glycogen content with increasing salinity. Increased salt concentrations lower the solubility of oxygen in the solution. This again affects respiration and the utilization of energy. Fresh water contains 8.38 p.p.m. O_2, the south arm of Great Salt Lake contains approximately 4.00 p.p.m. O_2, and the

north arm of the Great Salt Lake contains 1 p.p.m. or less of O_2 as noted by Seidell (1940). With such low oxygen content in the waters of the Great Salt Lake, it is little wonder that decomposition occurs so slowly and that a paucity of life exists.

RESULTS AND CONCLUSIONS

Seven determinations of four specimens each were made from both locations on the lake. Results from the tests are shown in Table 1. Per cent transmission (%T), glucose equivalents as read from the standard curve, and per cent glycogen in relation to body weights are given.

SOUTH ARM, GREAT SALT LAKE specific gravity 1.080

Tube	%T	Glucose eq. in mg./100 ml.	% Glycogen
1	80.0	9.00	18.75
2	80.5	8.75	18.23
3	85.3	6.50	13.54
4	84.0	7.20	15.00
5	85.5	6.40	13.33
6	88.0	5.30	11.04
7	79.0	9.60	19.79

NORTH ARM, GREAT SALT LAKE specific gravity 1.216

1	82.7	7.70	21.38
2	82.0	8.00	22.22
3	89.0	4.80	13.33
4	86.0	6.20	17.22
5	84.7	6.90	19.17
6	80.0	9.00	25.00
7	88.5	5.00	13.89

Table #1. Comparison of test values for specimens of Artemia taken from the north and south arms of Great Salt Lake.

Dry body weights were approximately 0.0045 gr. and 0.006 gr. per specimen for the north and south arms respectively. The resulting percentages were tabulated and found to be significantly different at the .05 level of confidence.

Conclusions

The specimens of Artemia collected from the north arm of the Great Salt Lake contained approximately 4.25% more glycogen in relation to dry body weight than those collected from the south arm of the lake. As the level of dissolved oxygen in the lake water decreases with increased salinity, the mitochondrial system of aerobic oxidation loses its efficiency. To meet its energy demands, Artemia has probably evolved a method of storing glycogen in large quantities. When needed, glycolysis occurs by anaerobic means in the tissues, principally those of the muscle. This view might be confirmed by the small mitochondria seen in the micrographs produced by Dr. Youssef and by the high quantities of glycogen found in the tissues of Artemia.

The algae common to the lake provide a rich source of glycerol for Artemia. Glycerol can be readily converted to glycogen and stored. These two factors of abundant glycerol production and low oxygen content have provided a favorable environment for the evolution of an anaerobic system in Artemia.

SUMMARY

1. The microtechnique for determination of glycogen as described by Kemp et. al. (1954) can be used for small invertebrates such as Artemia salinas.

2. A significant increase in glycogen content of the body tissues can be observed in Artemia coming from two salt solutions found in the Great Salt Lake.

3. Anaerobic glycolysis plays a major role in the energetic systems of Artemia salinas in the Great Salt Lake.

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