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Comparison of The Staining Reaction of Lime Juice Solution with Periodic Acid Schiff Reagent on Liver and Brain TissuesFasogbon, S.A. ^{*1}, Okorie, N. ², Ajileye, A.B. ³, Otu, A. ⁴

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Abstract

The aim of this study was to compare the histochemical reaction of Periodic Acid Schiff with the histochemical reaction of lime juice Schiff on Liver and Brain tissue section of Wistar rats. Four (4) sections each were obtained from a processed Brain tissue and processed Liver tissue. The limes juice was extracted and the various pH was measured with different timing. The control tissue sections were stained with Periodic Acid Schiff technique while the test tissue sections were stained with the limes extraction obtained in a lime Schiff technique. The results show that both Periodic Acid Schiff and lime juice stained Brain and Liver sections with a similar colour (purple-magenta) and they clearly show the specific cellular features of Brain and Liver tissue. Lime Schiff technique has stained the liver and brain of the Wistar rats clearly the same way as periodic acid Schiff. This finding of this study indicates that Lime Schiff technique can possibly be used as a more cost effective and readily available alternative for the demonstration of carbohydrates in Brain and Liver sections particularly in developing countries.

Keywords: Brain, Liver, Lime Extract, Periodic Acid, Schiff reagent, Wistar rats.

Introduction

Periodic Acid Schiff Stain is a technique for the demonstration of carbohydrates in tissue sections (Baker, 1962). The purpose of the Periodic Acid is to oxidize some of the tissue carbohydrates (Culling, 1963). This produces aldehyde groups, which is then condense with Schiff's reagent forming a bright red coloration and demonstrating the tissue component to which the carbohydrate is attached (Pearse, 1968). The PAS reaction is quite straightforward. Sections are first brought to water, and then a 1% aqueous solution of periodic acid is applied for 10 to 30 minutes. The sections are then washed to remove any

traces of the periodic acid, then the Schiff's reagent is applied for 10 to 30 minutes. The Schiff's reagent is rinsed off with water, then the sections are washed with water until the water is clear and the sections look pink, usually about 10 to 30 minutes. The color deepens somewhat as the washing continues, within limits. The time for which the reagents are applied can affect the intensity of the color. Also, some substances are known to require extended oxidation, and may not be oxidized with the times given (Kiernan, 2008). The treatment with periodic acid oxidizes some of the carbohydrates in the tissue to aldehydes. The oxidation condition has to be sufficiently regulated so as to not oxidize the aldehydes further. These aldehydes then react with the Schiff reagent to give a purple-magenta color. A suitable basic stain is often used as a counterstain (Thomas, 2010).

The carbohydrates involved are 1-2 glycols. Since not all carbohydrates include this structure, the PAS is not a method for carbohydrates in general but only for those which contain 1-2 glycols or closely related structures, such as one in which an amino group replaces one of the hydroxyl groups (Kiernan, 2008). Carbohydrates which stain with this method include polysaccharides, mucopolysaccharides, glycoproteins and glycolipids (Long Island University, 2013). The essential point is that treatment must be able to produce an aldehyde on the carbohydrate component (Culling, 1963). The lime plant is characterized by thorny branches and white flowers with purple edges, while the acidic, juicy fruit is oval (egg-shaped), has an aromatic rind that is typically yellow when ripe (green as immature or under certain environmental conditions), and has a prominent nipple or bulge on the blossom end. When ripe, they have a bright yellow nose, a layer of pith underneath and a paler yellow segmented interior. Small seeds commonly known as 'floppies' are found

with the fruit; the skin can be thick or thin. Notably, the color of citrus fruits only develops in climates with a (diurnal) cool winter. In tropical regions with no winter, citrus fruits remain green until maturity (Lanzara *et al.*, 1978). The brain of a Wister rats weighs 250g. Morphologically, the brain consists of myelin sheath, ganglion cells, parenchyma cells and parietal cerebral cortices were found⁸. The histochemical assays for enzyme activities of monoamine oxidase, ATPase, glucose 6-phosphatase, acetylcholine esterase and succinic dehydrogenase in the entire central nervous system revealed values identical to those of a Wister rat (Linas *et al.*, 1998). The PAS stain is a histochemical reaction in that the periodic acid oxidizes the carbon to carbon bond forming aldehydes which react to the fuchsin-sulfurous acid which form the magenta color (Crookham and Dapson, 1991; Bancroft and Stevens, 1982). Presence of glycogen can be confirmed on a section of tissue by using diastase to digest the glycogen from a section, then comparing a diastase digested PAS section with a normal PAS section. The diastase negative slide will show a magenta staining where glycogen is present within a section of tissue (Hauser, 2005; Carson *et al.*, 2009).

The liver regulates most chemical levels in the blood and excretes a product called bile. Bile helps to break down fats, preparing them for further digestion and absorption. When the liver has broken down harmful substances are excreted into the bile or blood. Bile by-products enter the intestine and ultimately leave the body in the feces. Blood by-products are filtered out by the kidneys and leave the body in the form of urine (Eldra *et al.*, 2004).

Materials and Methods

Study Area

This research study was carried out at the Histopathology Laboratory of N.K.S.T. Len Gabrielse School of Medical Laboratory Science Mkar, Gboko Benue State, Nigeria.

Experimental Animals

Twenty (20) adult Wistar rats of both gender were obtained from the animal house of the Department of Animal Production; University of Agriculture Makurdi, Benue State. The animals were in healthy conditions and weighed between 160g-250g. On transfer to the experimental location, the rats were allowed to acclimatize for 2 weeks under a good climatic condition and were properly fed.

Ethical Clearance

Ethical approval for this research study was obtained

from the research ethics committee of the N.K.S.T. Len Gabrielse School of Medical Laboratory Science. The experiment was carried out in strict accordance with the guidelines for the care and use of animals for research.

Sample Collection and Preparation for Histopathology

At the end of the acclimatization period, the rats were allowed to fast overnight, then sacrificed by spinal dislocation. The liver and brain were harvested. The tissues were immediately fixed in 10% formol saline for 24 hours and standard manual tissue processing techniques were used. Sections of about 3-5µm were cut by a Leica medical rotary microtome. The tissue sections gotten from the liver and the brain were divided into two (2) groups. The first group of the tissue sections (control) from the liver and brain were first oxidized with Periodic Acid, then with Schiff's reagent and then counter stained with Cole's Haematoxylin stain (Avwioro, 2011), while the other part of the tissue section (test) from the liver and brain were first oxidized with extract of Lime juice and then continued with Schiff's reagent and then counter stained with Cole's haematoxylin. The stained slides of both groups were viewed and analyzed with the aid of a light microscope for histological similarities and differences, thereafter captured with a Brunel light microscope, 20 mega pixels (Brunel SP35 Digital Trinocular).

Extraction of Lime Juice

Lime fruits were plucked from the lime tree, the rind was peeled off. The juice was extracted using an electric juice extractor, which was further filtered through four-layer folds of sterile gauze and the juice pH was measured using pH meter immediately after extraction, then measured again after 24 and 28 hours.

Preparation of Slides

Four sections were obtained from a processed Brain tissue and labeled in the following order:

1. BC (Brain control at pH 2.7)
2. BT1 (Brain test 1 at pH 2.8)
3. BT2 (Brain test 2 at pH 2.8)
4. BT3 (Brain test 3 at pH 2.8)

Four sections were obtained from a processed Liver tissue and labelled in the following order:

1. LC (Liver control at pH 2.7)
2. LT1 (Liver test 1 at pH 2.8)
3. LT2 (Liver test 2 at pH 2.8)
4. LT3 (Liver test 3 at pH 2.8)

Extraction of Lime Juice

The outer skin of the lime was peeled off using a knife, they were sliced into small pieces, they were parked inside the extracting machine. The machine extracted the juice from the chaff, the chaff was discarded, the juice was placed in a beaker, the pH was measured for the first day, the juice was filtered using four layers of gauze. It was transferred to a bottle labelled with date. It was kept in a cool dry place till the next day.

DAYS	TEST	pH
28/07/2016	LT 1	2.7
29/07/2016	LT 2	2.8
30/07/2016	LT 3	2.8

Procedure for testing pH of lime juice

1. The pH meter was inserted in to the extracted lime juice
2. The result was 2.7
3. After 24 hours the pH was measured as 2.8
4. Then after 48 hours the pH was measured as 2.8

Procedure of Staining (Control)

1. The control slides BC and LC sections were dewaxed and hydrated using grades of alcohol (100%, 90%, 70%)
2. They were taken to water
3. They were oxidized using 1% periodic acid. Schiff for 10min
4. They were washed with water
5. They were differentiated with 1% acid alcohol
6. Both slides were washed with distilled water
7. They were counterstained using Cole's Haematoxylin for 10 minutes
8. They were washed with water
9. They were dehydrated using grade of alcohol (70%, 90%, 100%)

Results

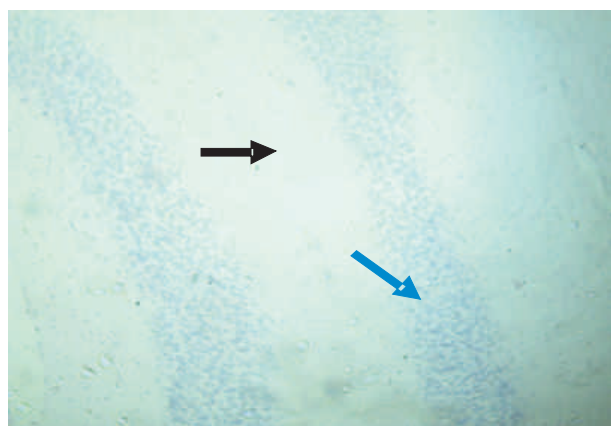


Fig. 1 Brain section (control) pH 3.9)

10. They were cleared using xylene and mounted using DPX¹⁵

Procedure of Staining (Test)

1. Sections marked BT1, BT2, BT3 and LT1, LT2, LT3 were dewaxed and hydrated in descending grades of alcohol (100%, 90%, 70%).
2. Sections were taken to water
3. Slide BT1 was oxidized with lime solution at pH 2.7 for 10 minutes
4. Slide BT2 was oxidized with lime solution at pH 2.8 for 10 minutes
5. Slide BT3 was oxidized with lime solution at pH 2.8 for 10 minutes
6. Slide LT1 was oxidized with lime solution at pH 2.7 for 10 minutes
7. Slide LT2 was oxidized with lime solution at pH 2.8 for 10 minutes
8. Slide LT3 was oxidized with lime Solution at pH 2.8 for 10 minutes
9. They were washed thoroughly in tap water
10. They were stained with Schiff solution for 20 minutes
11. They were washed thoroughly in tap water
12. They were counterstained with haematoxylin for 10 minutes
13. They were washed with water
14. They were differentiated briefly with 1% acid alcohol
15. They were dehydrated using ascending grades of alcohol (70%, 90%, 100%)
16. They were cleared using xylene
17. They were mounted using DPX
18. They were examined under the microscope.

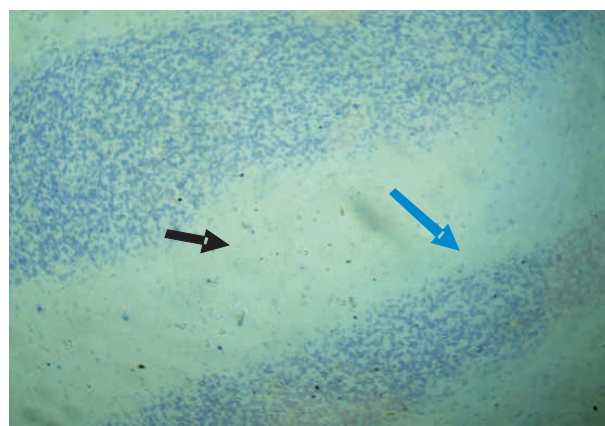


Fig. 2 BT1 oxidized with lime Schiff at pH 2.7

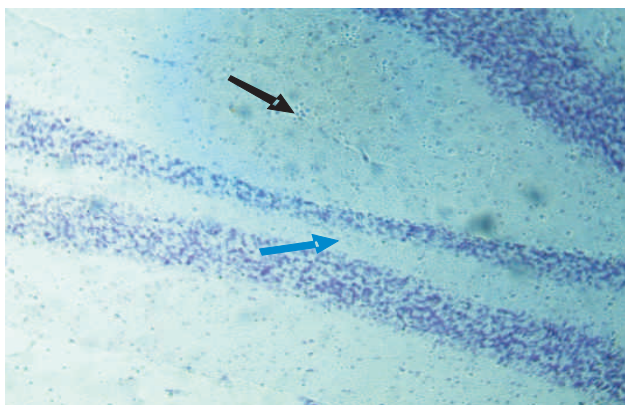


Fig 3 BT2 oxidized with lime Schiff at pH 2.8

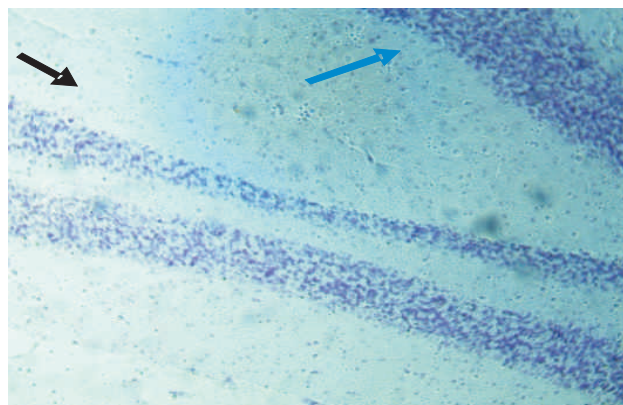


Fig 4 BT3 oxidized with lime Schiff at pH 2.8

Figure 1-4 shows the Brain section control at pH 3.9 stained with Periodic Acid oxidation showing the cerebellum with intact molecular cerebral cortex layer (Black arrow) and granular layer comprises of purkinje cells (Blue arrow) compared with Test 1, 2, and 3 demonstrated with Lime juice Oxidation at pH: 2.7, 2.8, 2.8 respectively. (x100).

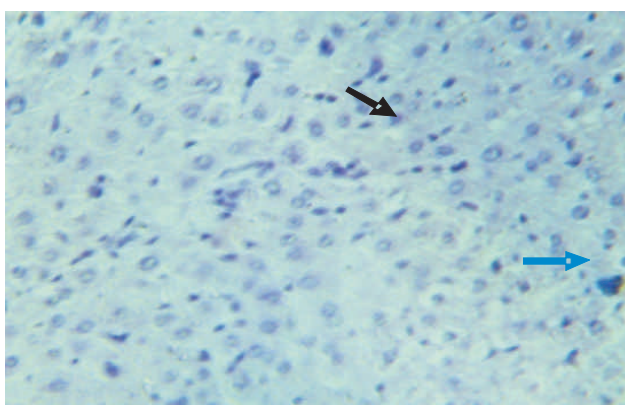


Fig 5: Liver section control pH 3.9 (x100)

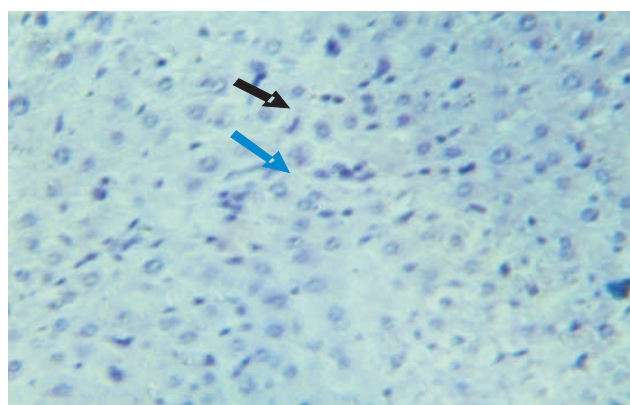


Fig 6: LT1 oxidized with Lime-Schiff at pH 2.7

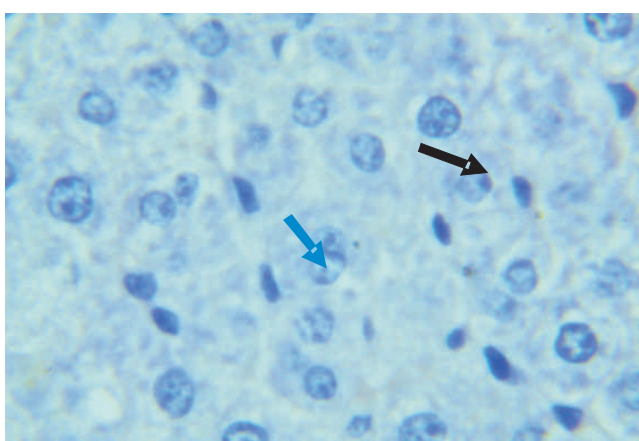


Fig 7: LT2 oxidized with Lime-Schiff at pH 2.8 (x400)

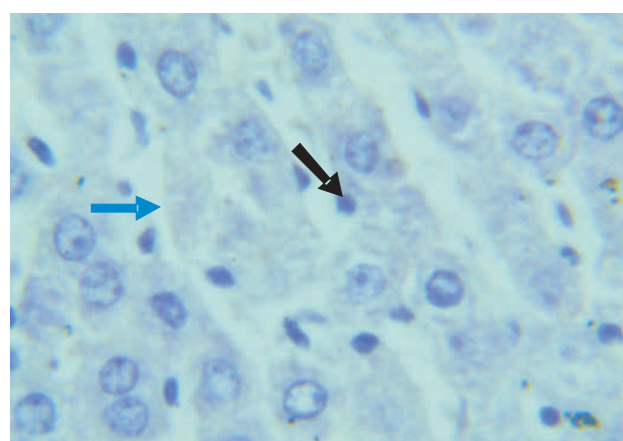


Fig 8: LT3 was oxidized with Lime-Schiff at pH 2.8

Fig. 5-8: Liver section control at pH 3.9 stained with Periodic acid oxidation showing the hepatocytes (Black arrow) and liver sinusoid (Blue arrow) compared with Test 1, 2, and 3 demonstrated with Lime juice Oxidation at pH: 2.7, 2.8, 2.8 respectively.

Discussion

It was observed that, both Periodic Acid Schiff and Lime-Schiff stained Brain and Liver sections of Wistar rat with a similar colour (purple-magenta). The lime juice which have the same pH with that of periodic acid Schiff stained the Liver at pH 2.8 pink to red, the lime juice stained the neutral and acidic mucosubstance of the liver blue due to its positive reaction with periodic acid Schiff. Therefore, periodic acid Schiff which serves as control stained the glycogen, glycoprotein and mucin of Liver and Brain purple magenta color due to it reaction with Schiff reagent. As shown by the photomicrograph, black arrow indicates hepatocytes as stained by Lime-Schiff reagent and blue arrow indicates liver sinusoid in the liver section. In the brain, the black arrow indicates the molecular layer and the blue arrow indicates granular layer when stained with Lime-Schiff reagent. The control sections BC and LC stained with Periodic Acid Schiff reagent demonstrated the cells same as in Lime-Schiff reagent. In this study, Lime-Schiff has stained the liver and brain of the Wistar rats clearly the same way as periodic acid Schiff and gave a purple magenta.

Conclusion

Lime-Schiff has stained the liver and brain clearly the same way as periodic acid Schiff and gave a purple magenta. Therefore, it is recommended that Lime-Schiff technique can possibly be used as a more cost effective and readily available alternative or substitute for periodic acid Schiff in the demonstration of carbohydrates in Brain and Liver sections particularly in developing countries.

References

- Avwioro, O.G. (2011). Histochemistry and Tissue Pathology; Principle and Technique; Histochemical Uses of Haematoxylin - A Review. *Journal of Physics Conference Series*; 24-34.
- Baker, J.R. (1962). Experiments on the action of mordants. 2. Aluminium-haematein. *Quarterly Journal of Microscopy Science*; 103: 493-517.
- Bancroft, J. and Stevens, A. (1982). *Theory and Practice of Histological Techniques*, 2nd Ed, Churchill Livingstone, NY.: 188-190.
- Carson, F.L. and Hladik, C. (2009). *Histotechnology: A Self-Instructional Text* (3rd ed.). Hong Kong: American Society for Clinical Pathology Press: 137-139.
- Crookham, J. and Dapson, R. (1991). *Hazardous Chemicals in the Histopathology Laboratory*, 2nd Edition, Anatech: 28-37.
- Culling, C.F.A. (1963). *Handbook of Histopathological Technique*; 2nd edition, London Butterworth.
- Eldra, P.S., Linda, R.B. and Diana, W.M. (2004). *Cengage Learning Biology*. google.books.com: 52-54.
- Hauser: <https://www.google.com.ng/search?q=Hausar+2005,+glycogen+PAS&dcr=0&tb=isch&2005>.
- Kiernan, J.A. (2008). *Histological and Histochemical Methods: Theory and Practice*. 4th ed. Bloxham, UK: Scion. 2008.
- Lanzara, P. and Pizetti, M. (1978). *Simon & Schuster's Guide to Trees: A Field Guide to Conifers, Palms, Broadleaves, Fruits, Flowering Trees, and Trees of Economic Importance*. Published by Simon and Schuster: 4-20.
- Llinas, R., Ribary, U., Contreras, D. and Pedroarena, C. (1998). *Theneu-ronal basis for consciousness*. *Philos. Translation of Royal Society of London. Basic Biological Science*; 353: 1841-1849.
- Long Island University (2013). *The Chemistry of Carbohydrates*" (PDF). <http://brooklyn.liu.edu>. Sited on 29th May, 2013.
- Pearse, A.G.E. (1968). The thyroid parenchymatous cells of Baber, and the nature and function of their C cell successors in thyroid, parathyroid and ultimobranchial bodies. In *Calcitonin. Proceedings of the Symposium on Thyrocalcitonin and the C cells*, London: 98-109.
- Singh, N.P.A. (2002). Simple method for accurate estimation of apoptotic cells. *Exp Cell Res*. 2002; 256:328-337.
- Thomas, J.L. (2010). *Breast*. Cambridge University Press: 55-65. Retrieved 16 November, 2010.

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