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An evaluation of *adi agbon* as a clearing agent in paraffin processing of embryonic tissues - *preliminary report.*

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Summary

Adi agbon, an oily extract of the endosperm of the coconut fruit (*Cocoa nucifera, L.*) was investigated as a clearing agent for embryonic soft tissues. Paraffin sections stained with haematoxylin an eosin revealed normal tissue architecture and light microscopic details were easily identified. However, some tissue shrinkage was observed, particularly in the brain. As prepared for this study, this locally available compound is useful for routine work but appears unsuitable for quantitative histological studies.

Résumé

<Adi agbon>, un extrait huileux de L'endosperme de la noix de coco (*Cocoa nucifera, L.*) etait etudier comme un agent clarifiant pour les tissue embroyonnaires. Les sections en paraffine colorées avec L'hématoxyline et l'eosine montraient une architecture normals des tissue et les details microspiques etaient facilement identifiés. Cependant, une contraction des tissues était remarquée, surtout dans le cerveau.

Etant préparé pour cette étude, ce composé localement disponible est utile pour le travail habituel, mais apparait de ne pas etre approprié pour les études histologiques quantitative.

Introduction

An ideal clearing agent for paraffin processing of embryonic tissue should satisfactorily dealcoholize the tissues, be miscible with molten paraffin wax, and should not cause excessive hardening and shrinkage of the specimen. It will be an advantage if such a chemical is non-toxic and readily available at modest cost. None of the conventional clearing agents for paraffin processing fulfils all of these criteria[1]. Chloroform is relatively mild on tissues, but the end point of its action is not easily determined. Moreover, it is expensive. Xylene, benzene and toluene render tissues transparent, a property that enables excessive tissue exposure to be avoided. They harden and shrink tissues, particularly embryonic specimens. Cedar wood oil is gentle, non-hardening but like chloroform, expensive.

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The physcal properties and utility as a clearing agent, of *adi agbon*, an oily extract of the endosperm of the coconut fruit (*Cocoa nucifera*, L), were recently studied by Caxton-Martins, *et al.*[2] It rendered tissues transparent and resulted in crip light microscopic details in haematoxylin and cosin-stained sections. Hardening was not observed on prolonged immersion. *Adi agbon* has not been previously evaluated for embryonic tissue processing during which hardening and shrinkage are to be particularly avoided. Accordingly, we have experimented on this oil as a dealcoholizing agent for paraffin preparation of soft tissues of 18-day mouse embryos and compared it with xylene. In many parts of Nigeria *adi agbon* is widely used as a skin and hair moisturizer and in the pharmaceutical industry it is used as an oily vehicle for oral medicinal preparations[3]. It is generally accepted to be a non-toxic substance.

Materials and methods

Preparation of adi agbon

The endorsperm was removed from the coconut, grated and soaked in water overnight. The oily supernatant was decanted and heated to eliminate water. It was filtered onto anhydrous copper sulphate for further dehydration prior to use.

Tissue Processing

Embryos were obtained from pregnant Swiss albino mice on day 18 *post coitum*. They were fixed *in toto* in Bouin's solution for at least 24 hours. With the use of a stereomicroscope, the brain, lungs, liver and kidneys were dissected out of the embryos and placed for a further 24 hours in Bouin's fixative. The specimens were dehydrated in graded alcohol solutions and transferred into either xylene or *adi agbon*. Those in xylene were immersed for 30 minutes with one change and were transferred into paraffin. Specimens in *adi*

agbon were transferred into a fresh solution after sinking and into molten wax after 15 to 60 minutes in the second bath. After the first bath, some *adi agbon* processed specimens were rinsed instead for 15 minutes in xylene prior to transfer into molten paraffin. The tissues were infiltrated in two successive bath of molten wax (15-60 minutes each) and blocked. Sections were cut at 8 microns and stained with haematoxylin and eosin.

Results

Whereas xylene-soaked specimens became rapidly transparent, such a change was not observed with *adi agbon*. Hence, specimens were removed from the first bath of the latter as soon as they sank. Prolonged immersion in the second bath of *adi agbon* resulted in darkening and progressive shrinkage of the tissues. A total infiltration time in excess of 1 hour caused appreciable shrinkage of the tissues. Satisfactory infiltration without shrinkage was easily achieved by a pre-infiltration rinse of the specimen in xylene.

Histologically, *adi agbon* did not obscure tissue architecture. Nuclear and cytoplasmic details were as well demonstrated in *adi agbon* as they were in xylene. The stain-retaining property of the tissues was not diminished by *adi agbon* (Figures 1 - 5).

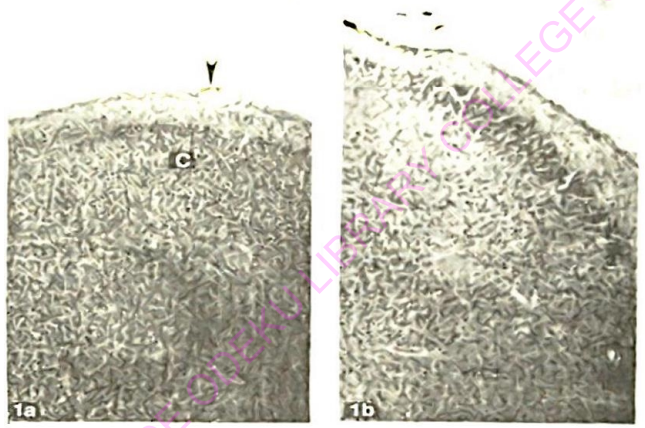

Fig. 1: Photomicrograph of sections of the cerebral cortex of an 18-day mouse embryo cleared in (a) *adi agbon*, (b) xylene. In (a), note the relative compactness of the neurons in the outer layer of the cortex (c); the pial surface is marked with an arrowhead. X 400.

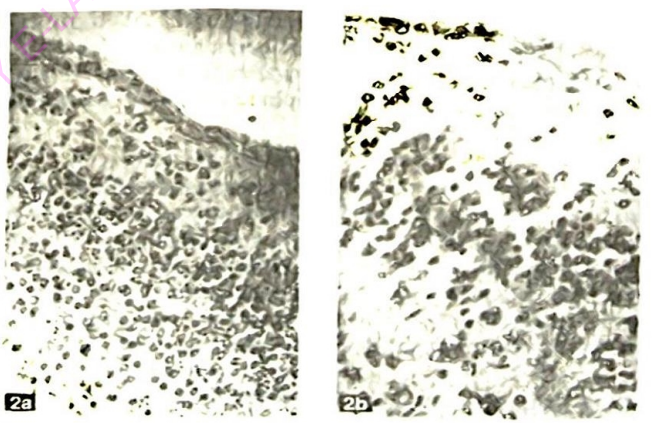

Fig. 2:High power micrograph of sections of the cerebral cortex of an 18-day mouse embryo cleared in (a) *adi agbon* (b) xylene. Note the relative looseness of the neuropil and the prominence of the extra cellular space in (b). X 1,600.

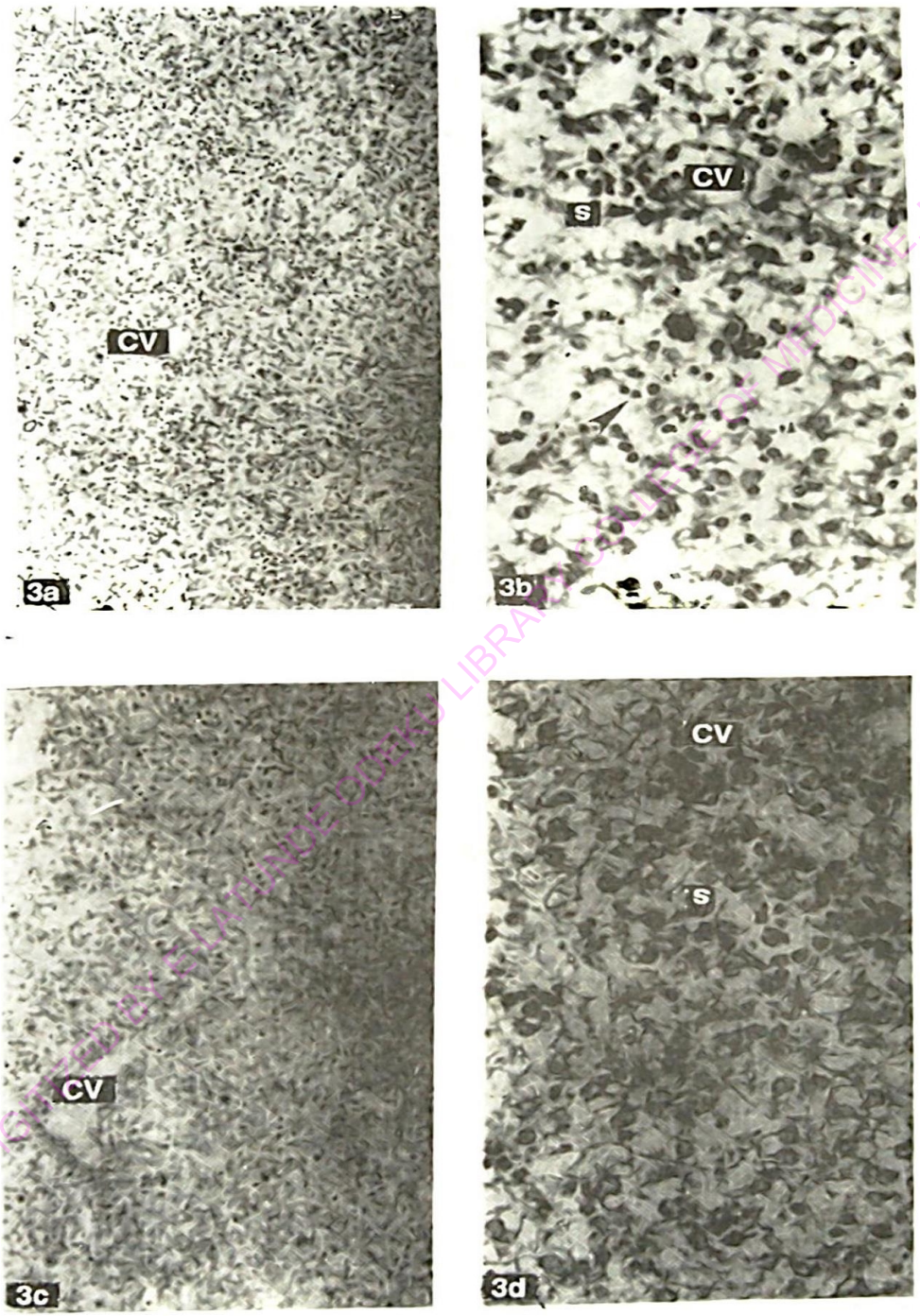

Fig. 3:Photomicrograph of sections of the liver cleared in *adi agbon* (a and b) and xylene (c and d). Note the architecture is otherwise undistorted. CV = central venule; S = sinusoids; A = hepatocytes. Mag (a) and (c) x 400; (b) and (d) X 1,600.

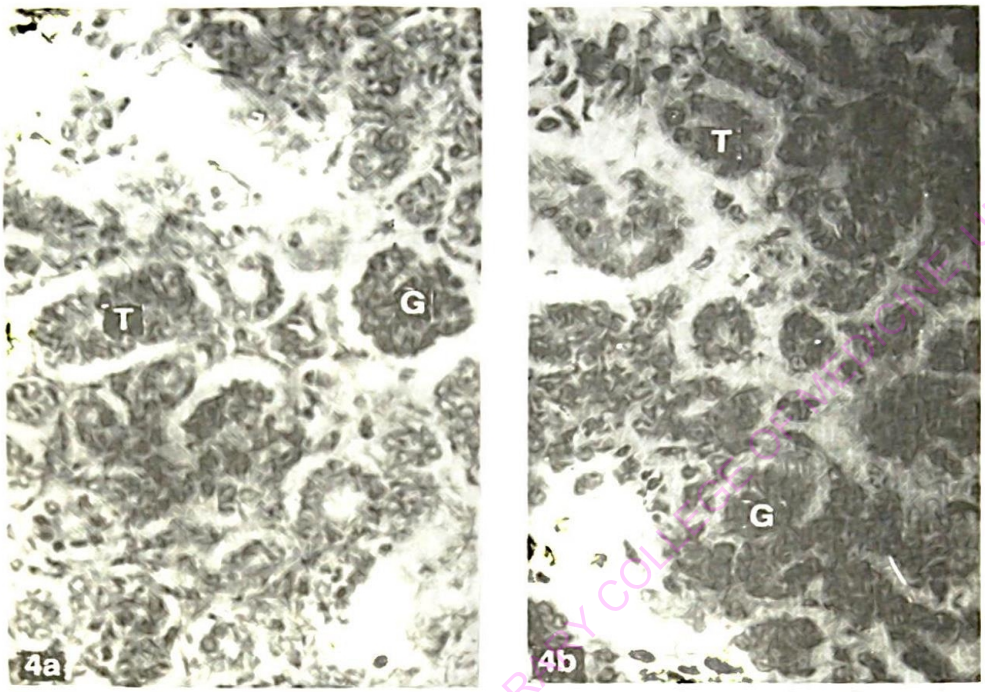

Fig. 4:Photomicrographs of sections of the kidney in *adi agbon* (a) and xylene (b). In (a) the glomerulus (G) and tubules (T) are as well demonstrated as they are in (b). X 1,600.

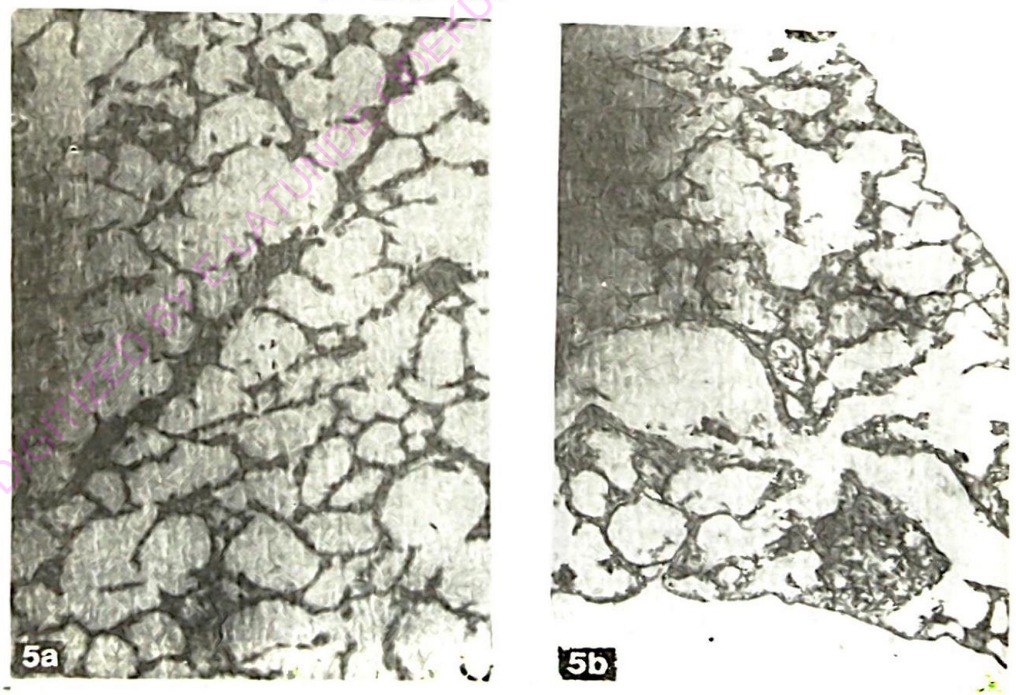

Fig. 5:Photomicrographs of section of the lungs cleared in *adi agbon* (a) and xylene (b). X 400.

However, with this agent, the tissue components were more closely packed and the extracellular spaces appeared reduced, both of which changes we attribute to some degree of tissue shrinkage. Particularly noticeable in the brain (Figure 1 and 2) these changes were also discernible in the other organs.

Discussion

This study has demonstrated that *aid agbon* adequately dealcoholizes embryonic soft tissues and permits paraffin infiltration and easy sectioning. Histological identification of these tissues was not impaired in any way. However, tissue shrinkage, not apparent on gross examination, was observed on light microscopic examination as an increase in the compactness of the tissues and reduction in extracellular spaces. Of the tissues investigated, the brain seemed to be the most susceptible to this effect of *adi agbon*. Compared to xylene, *adi agbon* did not shrink adult soft tissues[2]. Embryonic tissues are known to possess a higher water content than adult tissues and may be more sensitive to alcohol dehydration. It is unlikely that this is solely responsible for the shrinkage effect noted in this study, since xylene-cleared specimens were similarly dehydrated. What we have observed must be regarded as a specific effect of *adi agbon* on delicate tissues.

It is possible to reduce embryonic tissue shrinkage by dehydrating with more gentle chemicals. This will require a systematic trial in order to determine which of these are miscible with *adi agbon*. Alternatively, it will be of interest to vary the method of preparation since this determines the exact proportions of the various acids in the final product (unpublished observation). This manipulation may reduce the tissue-shrinkage property of *adi agbon*. Such efforts will be worthwhile because this compound is available locally and may provide a useful substitute to the relatively expensive and imported clearing agents. As prepared for this investigation, *adi agbon* is suitable for routine histological work where tissue identification is simply all that is required. It will not be a satisfactory clearing agent for embryonic tissue sections prepared for quantitative studies.

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References

1. Culling CFA. Handbook of Histopathological and Histochemical Techniques. London: Butterworths & Co. Ltd., 1974, 79-80.
2. Caxton-Martins AE, Ehilogbo G, Uche-Uwachi EO, Agbe ASA, Oluwadiya JO. Preliminary investigations into *adi agbon* as a clearing agent in routine histology. Stain Technology 1987; 62:417.
3. The British Pharmaceutical Codex. London: The Pharmaceutical Press, 1973: 122-123.

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