The Iraqi J. Vet. Med. 16 (1992)

SOME OBSERVATIONS ON DEHYDRATION AND DRYING OF ERYTHROCYTES FOR SCANNING ELECTRON MICROSCOPY

F.Y.S. Haddad

Department of Anatomy, College of Medicine, University of Baghdad.

SUMMARY

A comparative study on air drying of human erythrocytes for scanning electron microscopy (S E M), between acetone and ethanol for dehydration and drying was carried out, using a quick method. No morphological deformity or artifacts of erythrocytes was observed. More rapid air drying in acetone (3-5 seconds) was noticed as compared to ethanol (20-30 seconds). Acetone was recommended to be used for air drying, in the absence of critical point drying.

INTRODUCTION

The most important step of S E M specimen preparation is to dehydrate and dry the tissue without damaging the morphological structure of the cells. Acetone dehydration and drying was recommended for S E M in general [1,2,3]. Critical point drying method was first developed by *Anderson* in 1951[4]. This technique has been applied successfully to S E M by *Boyde* and *Wood* in 1969[5]. But, no remarkable difference in the erythrocyte morphology between the air drying method and critical point drying was reported[6].

The aim of this paper is to report some observations on air drying of human erythrocytes for S E M, using acetone and ethanol only for both dehydration and drying.

MATERIALS AND METHODS

0.5 ml of normal human venous blood was aspirated in a syringe containing 20 ml of physiological saline and proceeded following a modified and a quick method of preparation[6,7,8]. Samples of blood without anticoagulants, were washed with physiological saline, fixed in 0.75% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, postfixed in 1% osmium tetroxide, dehydrated with graded ethanol at 25,50,65,75,85,95,99,100% and three changes of 100% ethanol or with graded acetone at 25,50,65,75,85,95,99% and 4 changes of 99% acetone. Suspensions were centrifuged at 1000 rpm for 5 minutes each except with 75% acetone and above which needed to be centrifuged for 2 minutes only.

One drop of suspension of cells in either absolute ethanol or acetone was placed on a glass slide and aluminium foil, turned obliquely on a filter paper for obsorption of the excess and quick air drying. Specimens were coated with gold and observed by Philips 515 S E M.

RESULTS

Erythrocytes were found to be well preserved and maintained their biconcave discocytosis without cellular surface changes or any other deformities such as spherocytosis, echinocytosis, elliptocytosis and knizocytosis in both acetone and ethanol when used (Fig.1). They were more

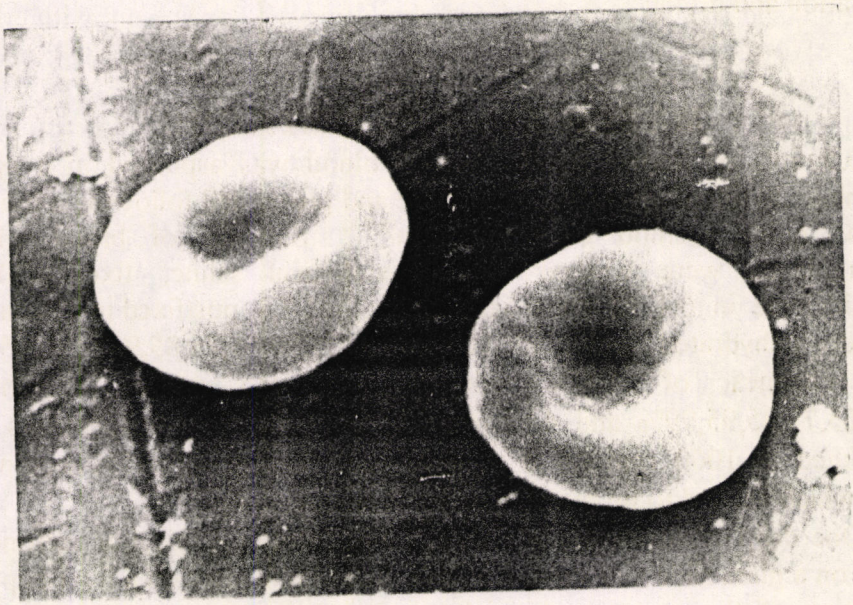

Fig. 1: Well preserved biconcave erythrocytes dehydrated by acetone . X 5,500 .

rapidly air dried in acetone, 3-5 seconds, as compared to ethanol, 20-30 seconds.

Gentle centrifugation at 1000 rpm and for not more than 2 minutes was found to be necessary to avoid stickness of the precipitated erythrocytes and to achieve easy resuspension when samples were dehydrated with graded acetone at 75% and above.

DISCUSSION

As in well known, specimens for study in high vacum of EM must be free of water or other volatile matter. Conventionally water was been substituted by acetone prior to drying in air[1,2]. Later, acetone was used for dehydration and critical point drying[9] by using F13 as solvent with critical pressure of 38.2 atmosphere at 28.9°C. While for ethanol, F23 is used as solvent with critical pressure of 47.7 atmosphere at 25.9°C or F116 with critical pressure of 29.4 atmosphere at 19.7°C. When Amyl acetate is used as an intermediate reagent between ethanol and liquid CO2, a critical pressure of 72.8 atmosphere at 31°C is required[10].

No remarkable difference in the erythrocyte morphology was found between air drying method and critical point drying[6]. This may be due to the fact that the erythrocyte has more stable structure than the ordinary cells. As mentioned above, ethanol needs Amyl acetate and liquid CO2 as intermediate reagents for critical point drying while acetone does not need that. Because of shortage of chemicals at the present time, due to economical sanctions, it is preferrable to use air drying over critical point drying and acetone is recomended for use. Moreover, we believe that rapid air drying with acetone might minimize cellular surface tension.

ACKNOWLEDGEMENT

The author is greatly indebted to Prof. *S.F. Al-Samarrai* and Prof. *M.H. Hamash* of College of Medicine. University of Baghdad and Saddam Medical College respectively who kindly offered their facilities for this study. Also thanks are extended to *Miss M. Abdal Amer*, *Miss K. Al-Hajjaji* and *Mr. T. Al-Musawi* for their technical assistance.

REFERENCES

1. Barber, V.C. and Boyde, A. (1968). Scanning electron microscopic studies on cilia. Z. Zellforsch. 84:269-284.
2. Fujita, T., Inoue, H. And Kodama, T.(1968). Scanning electron microscopy of the normal rheumatoid synovial membranes Arch. Hist. Jap. 29:511-522.
3. Fujita, T., Tokunaga, J. And Inoue, H. (1971). Atlas of scanning electron microscopy in medicine. Tokyo, Igaku Shoin Ltd.
4. Anderson, T.F. (1951). Techniques for the preservation of three dimensional structure in preparing specimens for the electron microscope. Trans. New York Acad. Sci. 13:130-134.
5. Boyde, A. And Wood, C. (1969). Preparation of animal tissues for surface scanning electron microscopy. J. Microscopy 90:221-249.
6. Al-Samarrai, S.F. (1975). Studies on preparation technique of human erythrocytes for clinical scanning electron microscopy. Arch. Histol. Jap., vol.38, No.4:321-334.
7. Al-Samarrai, S.F.(1986). Quick method for SEM. Abstract of the third Sceientific Congress of College of Medicine, University of Baghdad. April 15-17, pp. 42.
8. Al-Samarrai, S.F.(1987). Quick method for diagnostic S E M, Proceeding of the 24th Annual Medical Congress of the Iraqi Medical Society Baghdad. April 7-9, pp. 42.
9. Al-Samarrai, S.F.(1984). Critical point drying. Manual of Medical Electron Microscopy, pp. 26-27. Continuous Education College of Medicine, University of Baghdad.
10. Instruction Manual for Hitachi Model HCP-1 critical point drying apparatus . (1976), pp. 4.

بعض الملاحظات حول تجفاف ونشف كريات الدم الحمراء هوائياً للفحص المجهري الالكتروني الماسح

فرائد يوسف سمعان حداد

قسم التشريح ، كلية الطب ، جامعة بغداد.

الخلاصة

اجريت دراسة مقارنة للنشف الهوائي لكريات الدم الحمراء البشرية للأستجهار الالكتروني الماسح ، بين مادتي الاسيتون والكحول الاثيلي للتجفاف والنشف بأستخدام طريقة تحضير سريعة. لم تلاحظ تشوهات شكلية او شوائب لكريات الدم الحمراء. كما لوحظ نشف هوائي أسرع في مادة الاسيتون (3–5 ثوان) مقارنة مع مادة الكحول الاثيلي (20–30 ثانية). وعليه نوصي بأستخدام مادة الاسيتون للنشف الهوائي في حالة عدم توفر امكانية النشف عند الدرجة الحرجة.