Vol 16 No 2, p 253-257

HISTOLOGY AND ULTRASTRUCTURE OF LARYNX IN THE BACTRIAN CAMEL (*Camelus bactrianus*)

Chunhui Zhang, Chun Yang, Wenling Ye, Huifang Zhang, Baoping Shao, Shanting Zhao and JianLin Wang

School of Life Sciences, Lanzhou University,Lanzhou, Gansu, P.R. China

ABSTRACT

The light, scanning (SEM) and transmission electron microscopies (TEM) were used to observe the histology and ultrastructure of the larynx in the bactrian camel (*Camelus bactrianus*). The larynx contained five constituent cartilages, epiglottis, thyroid, cricoid and paired arytenoids. The mucous epithelium varied at different locations of the larynx, the epithelial layers of the vocal cord, arytenoid cartilage and the epiglottis (*Atrium glottides*) were composed of squamous epithelium while the other area were covered with pseudostratified ciliated columnar epithelium. Submucosal glands were tubuloalveolar with mucous (acidic and neutral) secretions. SEM revealed that most of mucosal glands had a cover on its opening, thus the openings were half-covered. TEM showed the tight junction of the epithelial cells and scattered lymphocytes and granulocytes in the epithelium. The larynx-associated lymphoid tissue (LALT) was also observed in the epithelium, some of which exhibited as typical lymphoid follicles, and others as aggregating lymphocytes.

Key words: *Camelus bactrianus,* larynx, ultrastructure

Many physiologic and anatomic studies have been done on different organ system of the bactrian camel, which include kidney (Abdalla and Abdalla, 1979; Chen and Wang, 2002; Chen and Liu, 2003), skin (Chen *et al,* 1996), nasal cavity (Wang *et al,* 2009) and trachea (Yang *et al,* 2009). In the present research, the ultrastructure of larynx of bactrian camel was studied.

Materials and Methods

Nine specimens of the adult bactrian camels of both sexes were obtained from the slaughterhouse of the Right Alasan Banner Food Company in Inner Mongolia Autonomous Region, China. The larynx was dissected free and flushed with normal saline. Samples for LM were fixed in 10% formaldehyde for 72 h, dehydrated, cleared and embedded in paraffin. Embedded tissues were cut into 5-μm thick sections and stained with hematoxylin and eosin (H&E), Alcian blue and periodic acid schiff (AB/PAS) for histochemistry of muco-substances.

Small pieces of the tissue samples for TEM were pre-fixed in 3% glutaraldehyde buffer (pH=7.2) and fixed for 3h. The tissues were then washed thrice in 0.1M phosphate buffer for 30 min before being cut into $1mm^3$ pieces and post-fixed with osmium tetroxide for 1h. The samples were washed thrice in 0.1M phosphate buffer and then dehydrated in ascending grades of ethanol before being embedded in epon 812. Semi-thin sections of each tissue were collected and stained with toluidine blue. Ultra-thin sections (50–70 nm) were then collected on copper grids. The ultra-thin sections were stained with a saturated solution of uranyl acetate for 30 min, followed by lead citrate for 7 min in a carbon dioxide-free environment. Sections were then washed in CO_2 free water, dried and examined under a transmission electron microscope (JEOL, JEM-1230).

Tissue samples for SEM were fixed as described above. After dehydration in a series of ascending grades of alcohol, samples were freeze-dried at vacuum and coated with platinum. The mounted specimens were observed under a scanning electron microscope (JEOL, JSM-680LA).

Results

LM results

Microscopic examination of tissue from 9 camels revealed that the interior surface of larynx was mainly lined by two kinds of epitheliums-squamous epithelium and pseudostratified ciliated columnar epithelium. The epiglottis (*Atrium glottides*), arytenoid cartilage and vocal cord were covered with squamous epithelium. At epiglottis, there

SEND REPRINT REQUEST TO JIANLIN WANG email: jlwang@lzu.edu.cn

were finger-like prominences of the submucosa towards the epidermis (Fig 1B) while the junction between the epithelium and connective tissue was smoother at arytenoid cartilage and vocal cord (Fig 1D). Moreover, the epithelium lining its outer surface was more keratinised. Occasionally a special structure was found at the arytenoid cartilage whre some epithelial cells formed an onion-like corpuscle (Fig 1C) in the submucosa. Other parts were lined by pseudostratified ciliated columnar epithelium with numerous globlet and basal cells (Fig 1A). Islands of squamous cells scattered in the ciliated epithelium were also found. The thickness of the epithelium varied at different locations. The epithelium of the laryngeal vestibule was thicker than other parts (10-20 cells thick) and the ciliated epithelium near the vocal cord was the thinnest (2-3 cells thick).

Transitional epithelium was seen at the junction of the two different types of epithelium in the larynx. Some cilia of pseudostratified epithelium gradually bundled together to form a wavy structure (Figs 3E and F) which gradually turned unwavy and finally became flat. The length of the cilia became shorter and shorter until disappeared and at last it became squamous epithelium (Fig 3G). However, the transition was swift at the junction of the vocal cord and ventricle of larynx.

Numerous submucosal glands (branched, coiled and tubuloalveolar) were observed in the larynx of

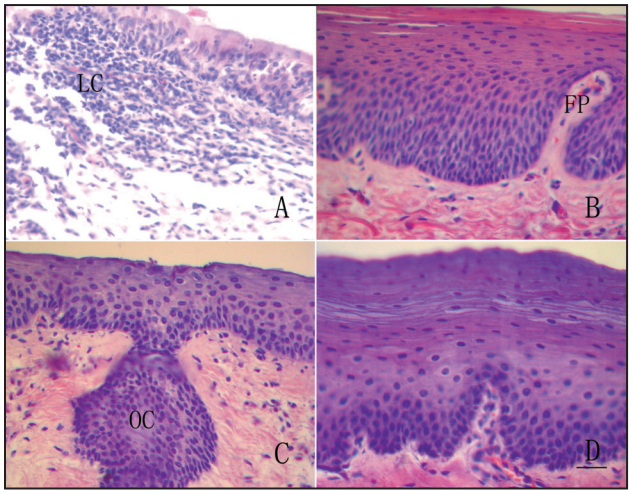

Fig 1. Different types of epithelium of the larynx of the camel, HE, bar=0.05mm. (A) pseudostratified ciliated columnar epithelium. Note the cluster of the lymphocytes (LC), (B) squamous epithelium with finger-like prominences (FP), (C) the onion-like corpuscle (OC) of the squamous epithelium, (D) standard squamous epithelium.

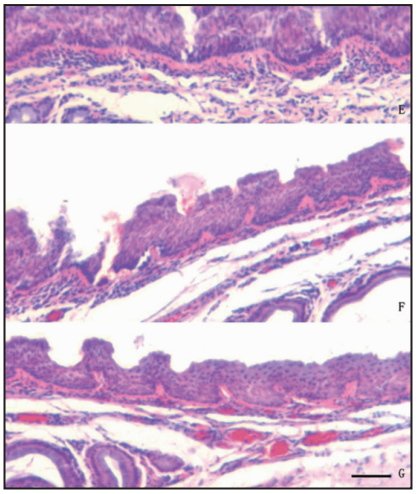

Fig 3. The transition of the epithelium, HE, bar=1mm. (E) pseudostratified ciliated columnar epithelium, (F) transitional epithelium, (G) squamous epithelium. From E to F, the transitional changes can be observed between the ciliated epithelium and squamous epithelium.

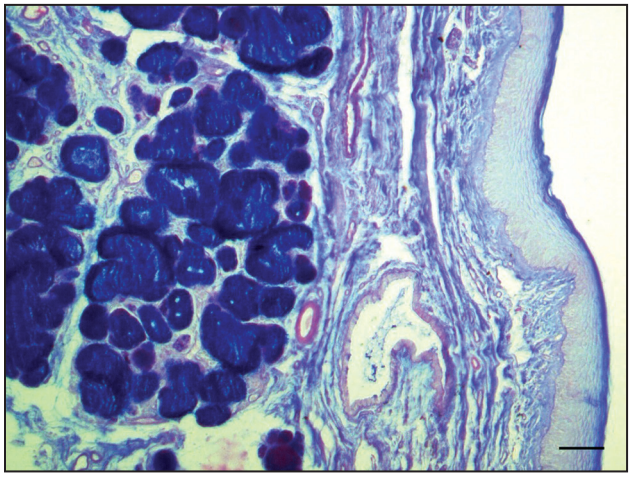

Fig 2. Tunica mucosa of the larynx of camel, Alcian blue-PAS, bar=1mm. The blue stained mucous were acidic mucin and the magenta stained mucous were neutral mucin.

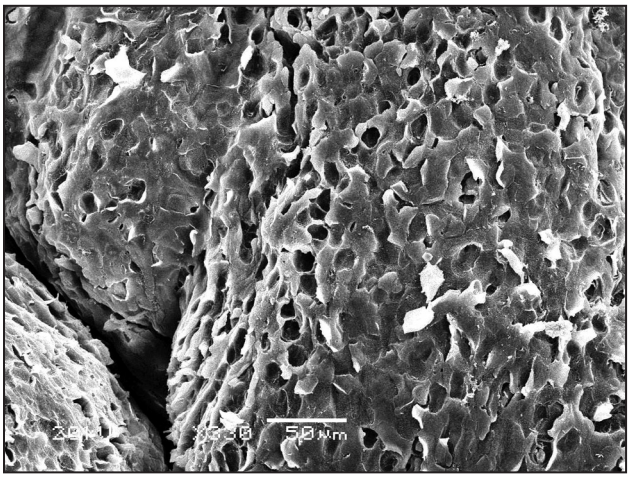

Fig 4. Scanning electron micrograph showing the irregular surface with many grooves and stomata of the squamous epithelium of the larynx.

Fig 5. Scanning electron micrograph showing a submucosal gland orifice without shell. The surface composed of short microvilli (MV).

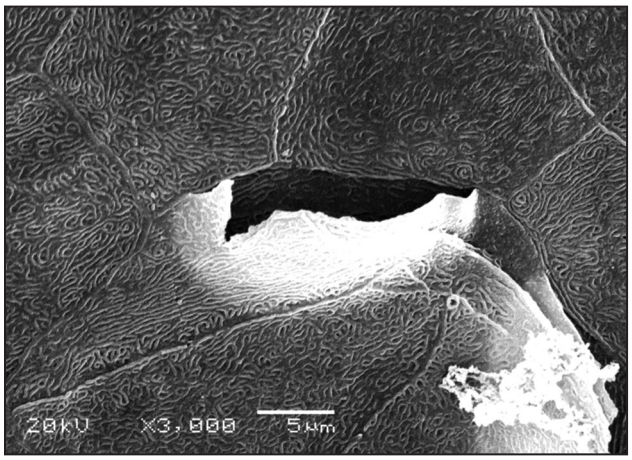

Fig 6. Scanning electron micrograph showing a submucosal gland orifice with a shell and the dense arrangement at the junctions of cells formed by microplicae.

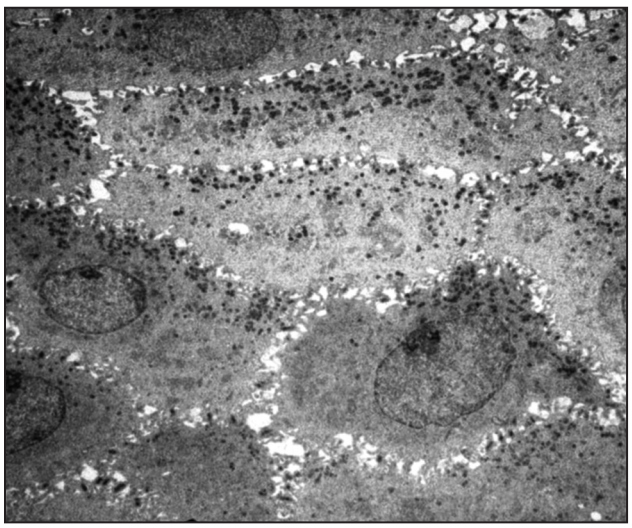

Fig 7. Transmission electron micrograph showing the squamous epithelium of the larynx was composed of layers of cells and the nuclei become flat toward superficial layer.

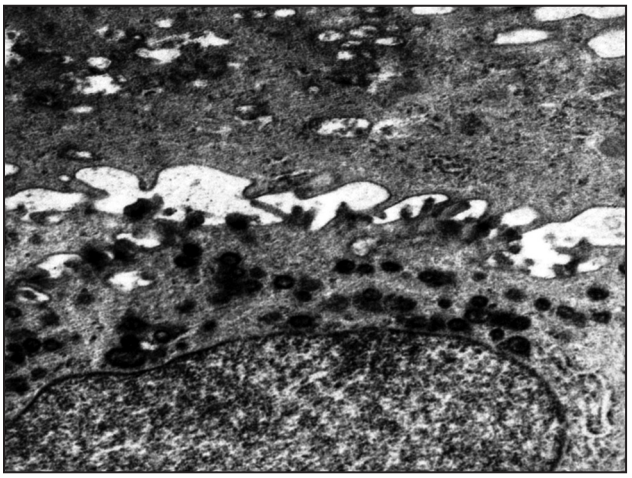

Fig 8. Transmission electron micrograph showing a squamous cell of stratum surperficiale with an elongated nuclei and many electron-dense secretory granules. Note the irregular small blunts of the cells.

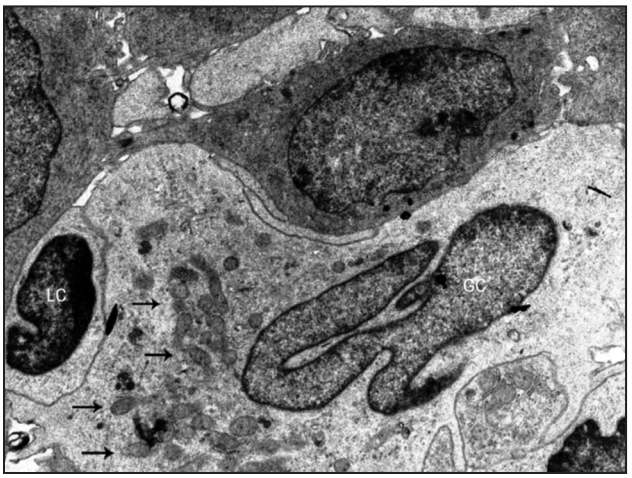

Fig 9. Transmission electron micrograph showing granulocyte (GC) with a polymorphonuclei and many granules (arrows), and lymphocyte (LC) with a large nuclei.

the camel (Fig 2). These were composed of serous tubules, mucous tubules, collector duct and final portion of this duct. Alcian blue-PAS stain revealed that mucous secretions were mainly acidic and neutral (Fig 2). Contrast to other parts, much more glands were observed near the glottis and infraglottic cavity. Lymph follicles and clusters of lymphocytes showed the same distribution as submucosal glands. The lamina propia of larynx in the camel consisted of connective tissue with numerous elastic fibres and some collagen fibres.

SEM results

SEM revealed an irregular outer surface with many grooves and stomata of the squamous epithelium. The surface was smooth except for the presence of exfoliated epithelial cells and stomata

(Figs 4 and 6). Higher magnification revealed submucosal gland orifices, most of which were half-covered by a shell, were round or oval (Fig 5). Flat squamous epithelial cells formed a 'plate-like' structure overlying each other. Varying patterns of microplicae, a feature of non-keratinised epithelium, formed a dense arrangement at the junctions of cells (Fig 6). In some areas, the cell surface was composed of short microvilli (MV) (Fig 5).

TEM results

TEM of the squamous cells of stratum superficiale showed spiny processes and enlongated nuclei (Figs 7 and 8). The epithelium consisted of 6 to 10 layers of epithelial cells and the layers were separated by inter-cellular spaces (Fig 7). The cells presented irregular small blunts which were attached to those of adjacent cells by numerous desmosomes (Figs 7 and 8). Most of the epithelial cells contained electron dense secretory granules. Granulocytes and lymphocytes were also found in the lamina propria. The granulocyte contained a polymorphonuclei and many granules (Fig 9).

Discussion

Just like other mammalian species, including man (Bloom and Fawcett, 1976), non-human primates (Leela and Kanagasuntheram, 1973), dog (Majid, 1986), goat (Kahwa *et al,* 2000) and mouse (Pack *et al,* 1981), the epithelium of the larynx of the bactrian camel was lined by two main kinds of epithelium (except for transitional epithelium) and even their distribution were similar. The squamous epithelium of the epiglottis and arytenoid cartilage thickened from posterior to anterior caused by the number of the layers of cells and this may play role in adaptive mechanism. As we know, squamous epithelium plays an important role in mechanical protection and preventing foreign body, moreover, it has a strong self-repair ability. The anterior part of the larynx may get hurt as epiglottis moves. It has been reported that 95% of the cancers of the larynx are squamous cell carcinomas (Lisa Licitra *et al,* 2003). The finger-like prominences increase the contact area between the epithelium and connective tissue, thus not only can assure the supply of the nutrition but also can make the connection firmer (Zou, 2004). Transitional epithelium found in the larynx of the camel was also seen in the nasopharynx and larynx of goats and it is also called intermediate epithelium (Kahwa and Purton, 1996).

Islands of squamous cells scattered in the ciliated epithelium is thought to be physiologic in origin and originated from the ciliated epithelium (Ke *et al,* 2005). The ciliated epithelium of the larynx was similar to that of trachea (Chun Yang *et al,* 2009). The cilia of the ciliated epithelium swing to buccal cavity which helps clearing the dust in the cavum laryngis (Duan *et al,* 2006). The half-covered gland orifice is a unique structure which has not been found in other species, like human (Bloom and Fawcett, 1976), rat (Lewis and Prentice, 1980), equine (Pirie, 1990), goat (Kahwa *et al,* 2000) and so on, and it may also be an important factor in adaptive mechanism of bactrian camel to the arid and dusty environment. Not only does the shell can prevent dust, but also can hold water. The onion-like structure at the arytenoids cartilage resembled lamellated structure at the epithelium of the tonsil (Kumar and Timoney, 2005). The origin may be the hurt of epithelium which leads to the isolation of island of epithelial cells, then they lose their polarisation, finally degenerate and form onion-like corpuscles. *Cavum laryngis* points at the rima glottides, so the air can contact larynx more effectively. The thin epithelium, abundant submucosal glands and lymphocytes are in favour of the adhesion and intake of the inhaled particles.

Acknowledgements

We would like to thank Mr Zhengxuan Zhang for sample collection assistance, and Lianlian Li and Yaoyao Liu for their help in this study. This study received financial supports from the Scientific Research Key Project Fund of Ministry of Education in 2006 (106152), Key Project Fund of State Key Laboratory for Agrobiotechnology of China in 2008 (2008SKLAB06-05), National Science Fund for Talent Training in Basic Science of China (J0630644), Open Foundation of Chinese Educational Department Key Laboratory of Magnetics and Magnetic Material (MMM200819) and Startup Fund of Collaboration Scientific Research Item of Chunhui Project of of MOE in China (z-2007-1-62012).

References

Abdalla MA and Abdalla O (1979). Morphometric observations on the kidney of the camel (*Camelus dromedarius*). Journal of Anatomy 129(1):45-50.

Bloom W and Fawcett DW (1976). Textbook of Histology, 9th Ed., Saunders, Philadelphia and London. pp 629-651.

Chen QS and Wang WH (2002). Morphologic features of renal cell related to preabsorption in two humped camel. Acta Zoology 48(02):245-250.

Chen QS and Liu Y (2003). Stereology study of fine structure of proximal and distal convoluted tubule in bactrian camel. Chinese Journal of Veterinary Science 23(3):258-260.

Chen HT, Li XM, Wang WH, Jia N and Ren AF (1996). Histomorphological research on the skin of the bactrian camel. Chinese Journal of Veterinary Science and Technology 26(3):7-9.

Gao MY(2006). Histology and Embryology-Color Atlas and Synopsis. Beijing:High Education Press.

Kahwa CKB, Balemba OB and Assey RJ (2000). The pattern of ciliation and the development of the epithelial lining of the respiratory tract in the neonatal kid: a scanning electron microscopic study. Small Ruminant Research 37(1-2):27-34.

Kahwa CKB and Purton M (1996). Histological and histochemical study of the epithelial lining of the respiratory tract in adult goats. Small Ruminant Research 20:181-186.

Ke ZY and WU ZY (2005). Characterisation of development and differentiation of human fetal laryngeal epithelial cells. Journal of SUN Yat-sen University (Med Sci) 26(4):400-403.

Kumar P and Timoney JF (2005). Histology and ultrastructure of the equine lingual tonsil. I. Crypt epithelium and associated structures. Anatomy Histology Embryology 34(1):27-33.

Leela K and Kanagasuntheram R (1973). Some aspects of the histology of the non-human primate nasopharynx. Acta Anatomica 84:374-386.

Lewis DJ and Prentice DE (1980). The ultrastructure of the rat laryngeal epithelia. Journal of Anatomy 130(Pt 3):617-32.

Lisa Licitra, Jacques Bernier, Gesare Grandi, Laura Locati, Marco Merlano, Gemma Gatta and Jean-Louis Lefebure(2003). Cancer of the larynx. Critical Review of Oncology and Haematology 47(1):65-80.

Majid A (1986). A combined histological, histochmeical and scanning electron microscopical study of the canine respiratory tract. Ph.D. Thesis, University of Glasgow.

Pack RJ, Al-Ugaily LH and Morris G (1981). The cells of the tracheobronchial epithelium of the mouse: A quantitative light microscopy and electron microscopy study. Journal of Anatomy 132:71-84.

Pirie (1990). M.E.S. Pirie, An ultrastructural and histological study of the equine respiratory tract in health and disease. In: Ph.D Thesis, University of Glasgow.

Wang HJ, Bai ZT, Yu SY and Wang JL(2009). The CT anatomy of nasal cavities and sinuses in Bactrian camel. Highlights of Science paper Online 2(11):1189-1193.

Yang C, Zhang CH, Liu YY, Xu ZH, Zhu L and Wang JL(2009). Microstructural and ultrastructural studies of the trachea in the Bactrian camel (*Camelus bactrianus*). Journal of Camel Practice and Research 16(1):121-126.

Zou ZZ (2008). A Manual Book of Histology. Beijing: People's Health Press.

MEETING OF THE CAMEL ASSOCIATION OF PAKISTAN (CAP)

A meeting of the CAP was held at the University of Agriculture, Faisalabad on 2nd November 2009. The organiser, Dr Abdul Raziq of the CAP presided the meeting. Members and the executive members attended the meeting. It changed the name of the organisation from Pakistan Camel Association (PCA) to Camel Association of Pakistan (CAP). It was decided to organise a country conference on camel in 2010. The name of an executive member Dr. Arshad Iqbal was corrected, as it was written Dr Muhammad Iqbal in previous press release. It was decided to send a camel expert group to Cholistan desert to know the ground facts about camel situation there.

THE INTERNATIONAL CAMEL SYMPOSIUM

"Linking Camel Science and Development for Sustainable Livelihoods"

The **2010 INTERNATIONAL CAMEL SYMPOSIUM** will be held in Garissa, Kenya the capital of Kenya's North-Eastern Province From **7th to 10th June 2010**. This event will be organised by the Kenya Agricultural Research Institute (KARI) under its KENYA ARID AND SEMIARID LANDS RESEARCH PROGRAMME (KASAL), funded by the European Union and by the Government of Kenya. The symposium will held in close partnership with the Kenya Camel Association (KCA), the organisation representing camel pastoralists in Kenya.

This symposium aims to not only address a scientific audience, but to bring together for the first time camel pastoralists, camel researchers and development actors. The programme will be divided into three days for presentation of scientific and development papers organised by KARI (June 8th, 9th and 10th, 2010) and two days reserved for pastoralist issues organised by KCA (June 7th and 11th 2010). Papers will be presented in English, which is the official language of the symposium. For the benefit of pastoralists there will be simultaneous translation of oral presentations into Swahili. It is hoped that the mutual exchange of information during this combined event will help researchers and development workers to better understand the problems and needs of camel keepers while at the same time enhancing the transfer and application of scientific knowledge to camel pastoralists. Submission of manuscripts and enquiries should be addressed to:

THE KENYA CAMEL SYMPOSIUM SECRETARIAT

P O BOX 57811-00200 NAIROBI KENYA

kenyacamelsymposium@kari.org

kenyacamelsecretariat@gmail.com

website: http://www.kari.org/thekenyacamelsymposium2010.htm

WORLD CAMEL ASSOCATION AND CAMEL CONFERENCE AT THE SCHOOL OF ORIENTAL AND AFRICAN STUDIES [SOAS], LONDON

In order to form a World Camel Association, the School of Oriental and African Studies [SOAS] in London is planning an international Camel Conference. The conference will be held in May 2011, with a pre-conference press conference to be held in mid-2010. This would provide a good opportunity both to consider the present state of camel culture worldwide, and also perhaps to announce the setting up of the proposed World Camel Association. Ed Emery [SOAS], conference organiser can be contacted at ed.emery@soas.ac.uk and William Gervase Clarence-Smith [SOAS], Professor of the Economic History of Asia and African can also be contacted.