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Lymphoid tissue in the nasal mucosa of primates, with particular reference to intraepithelial lymphocytes

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INTRODUCTION

A survey of the literature on the lymphoid tissue of the mucosa of the respiratory tract has revealed a few reports dealing with the situation in the lung (Miller, 1911; Andrew & Burns, 1947; Brundelet, 1965; Shields, Touchon & Dickson, 1969), but a paucity of information about lymphoid tissue in the nose. As intraepithelial lymphocytes are in fact readily observed in the nasal mucosa it seemed worthwhile to investigate their morphology in detail, using available primate material.

MATERIALS AND METHODS

The primates used in this study comprised five adult tree shrews (*Tupaia glis*), seven adult slow lorises (*Nycticebus coucang coucang*) and six adult monkeys (*Macaca fascicularis*). All animals were anaesthetized with intraperitoneal 'Nembutal'. For light microscopy, three tree shrews, two slow lorises and two monkeys were perfused with 10% formalin and then specimens were removed, decalcified in 5% formic acid, dehydrated in ascending series of alcohol and embedded in paraffin. Serial coronal sections 10 μm thick were stained with haematoxylin and eosin.

For electron microscopy, two tree shrews, five slow lorises and four monkeys were fixed by perfusing the whole animal through the heart with cold Tyrode solution followed by 4% chilled glutaraldehyde buffered at pH 7·3 with 0·1 M phosphate. The mucosa was dissected out in sheets and cut into small blocks of tissue approximately $1 \times 1 \times 0{\cdot}5$ mm in size. Fixation was continued in 4% chilled buffered glutaraldehyde for a further two hours. The blocks were then washed in buffer, post-fixed in Dalton's chrome–osmium fixative for 30 minutes, dehydrated in graded series of acetone and embedded in Araldite. Sections were cut on a Porter–Blum MT2 ultramicrotome and stained with saturated uranyl acetate and Reynolds lead citrate for 10 minutes each. They were then viewed under a Hitachi HS 8 electron microscope and pictures were taken with Kodak fine-grain positive film.

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OBSERVATIONS

Light microscopy

The nasal fossae of the three species of animals investigated could be roughly divided into a vestibule lined by stratified squamous, non-keratinized epithelium, a middle area lined by respiratory epithelium, and a posterior area lined by olfactory epithelium. Moreover, in tree shrew and slow loris, vomeronasal organs lined by both an olfactory-like neurosensory epithelium, and a respiratory epithelium, were present.

The (non-vomeronasal) *olfactory epithelium* in all three species was similar, with rounded nuclei in six or seven layers and with a clear zone superficially (Fig. 1). In none of the animals studied were lymphoid cells seen in the lamina propria of this epithelium, but, in the clear superficial zone of the epithelium, some dark rounded nuclei which may have been those of lymphoid cells, were sometimes present (Fig. 1).

The *respiratory epithelium* in tree shrew and slow loris was composed of pseudo-stratified columnar ciliated epithelium, with nuclei in two layers (Fig. 2). In the tree shrews lymphoid cells were not seen in the lamina propria of the anterior three-fourths of the mucosa, but small nodules of such cells were seen more posteriorly. Germinal centres were not present in any of these nodules. Lymphoid cells were seen in the epithelium, especially in the deeper zone.

In the slow loris and monkey aggregations of lymphoid cells were present in all parts of the lamina propria of the respiratory mucosa. These aggregations were largest postero-inferiorly. Where the lymphoid cell collections in the lamina propria were small the overlying epithelium contained lymphoid cells confined almost entirely to the deeper part of the epithelium, but occasionally a rounded lymphoid cell nucleus could be seen at the surface of the epithelium (Fig. 3): but, at the posterior end of the respiratory mucosa where there were large aggregations of lymphoid cells on the floor of the nasal cavity, such cells had infiltrated through the entire thickness of the epithelium, disorganizing the epithelial pattern (Fig. 4). Germinal

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Fig. 1. Tree shrew olfactory epithelium consisting of six to seven layers of rounded nuclei with a clear zone superficially. Bowman's glands (*bg*) and nerve bundles (*n*) are seen in the lamina propria. Arrows indicate lymphoid cells in superficial clear portion of epithelium. H. & E. × 340.

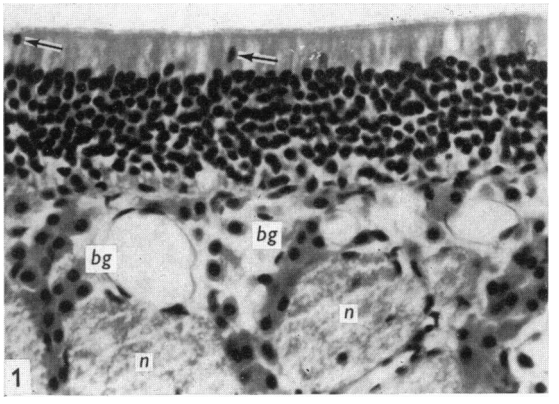
Fig. 2. Tree shrew respiratory epithelium at the floor of the posterior end of the nasal cavity. Collection of lymphoid cells (*L*) is seen in the lamina propria. Arrows indicate where these cells have infiltrated into the epithelium. H. & E. × 340.

Fig. 3. Slow loris respiratory mucosa with lymphoid cell collection in the lamina propria. Some of these cells have invaded the epithelium (arrows). Rounded nucleus at the surface of the epithelium is arrowed. H. & E. × 340.

Fig. 4. Monkey respiratory mucosa at the floor of the posterior end of the nasal cavity. The lymphoid cells have invaded through the whole thickness of the epithelium. H. & E. × 340.

Fig. 5. Lower end of vomeronasal organ of tree shrew showing junction between respiratory (*re*) and neurosensory (*ns*) epithelia. Lymphocyte collection at the lower end of the organ, labelled (*L*), has invaded the epithelium in the area between the arrows. H. & E. × 340.

Fig. 6. Respiratory epithelium of the vomeronasal organ of slow loris, showing lymphocytes (*L*) in lamina propria. Some intraepithelial lymphocyte nuclei are arrowed, including one on the surface of the epithelium. H. & E. × 340.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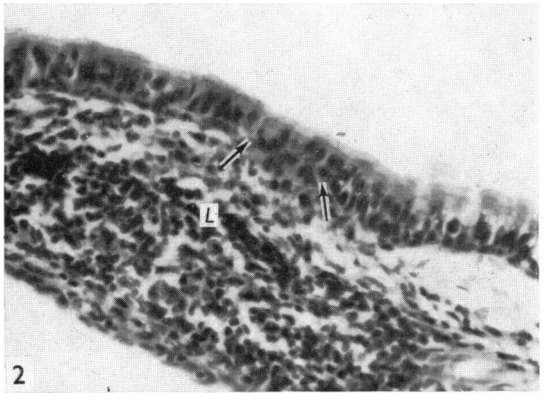
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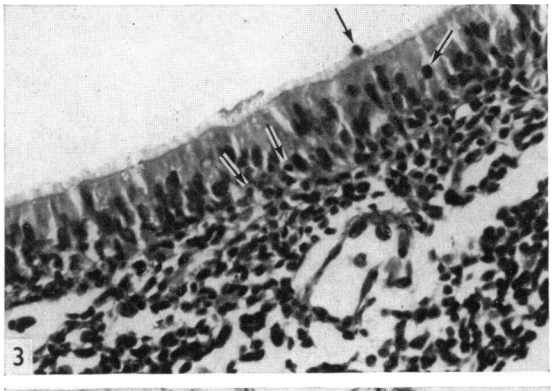
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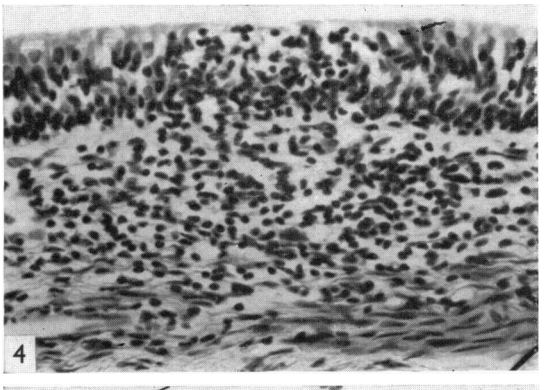
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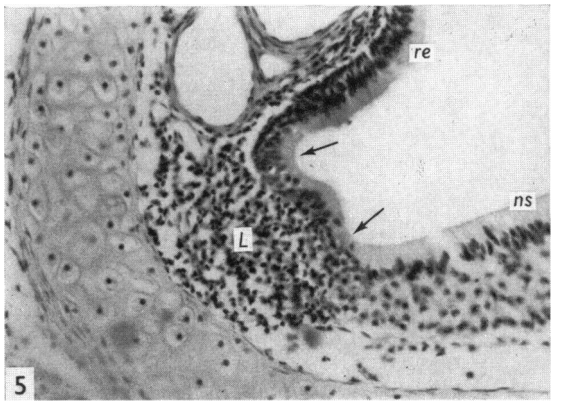
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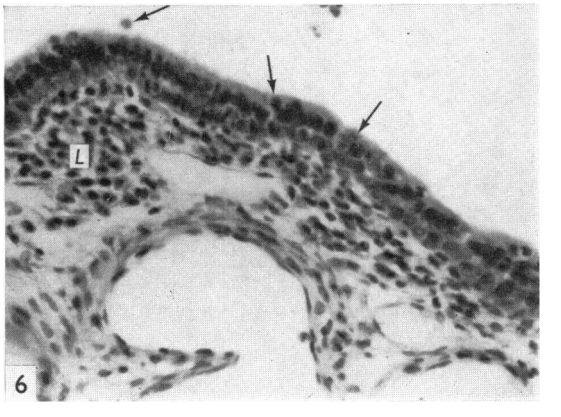
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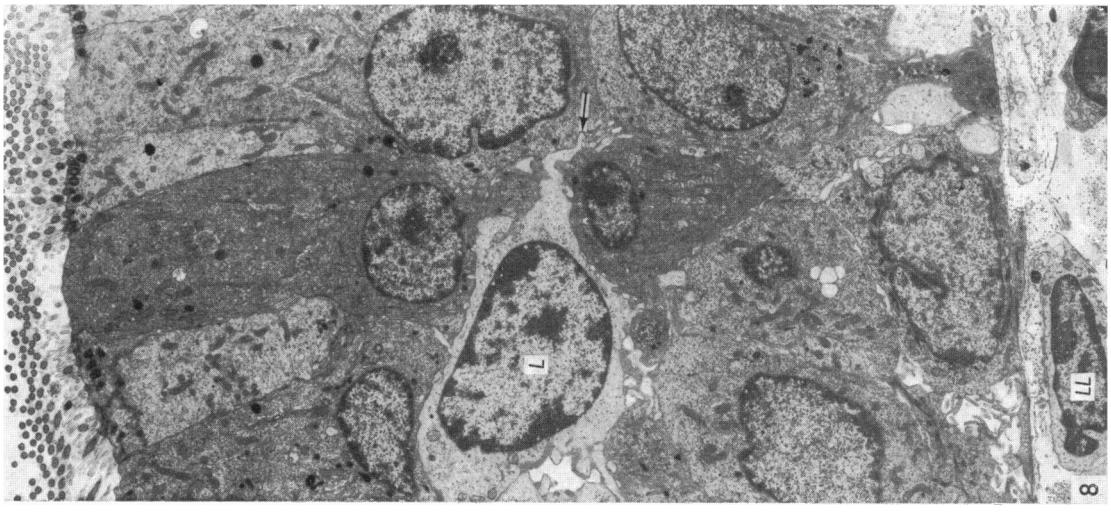
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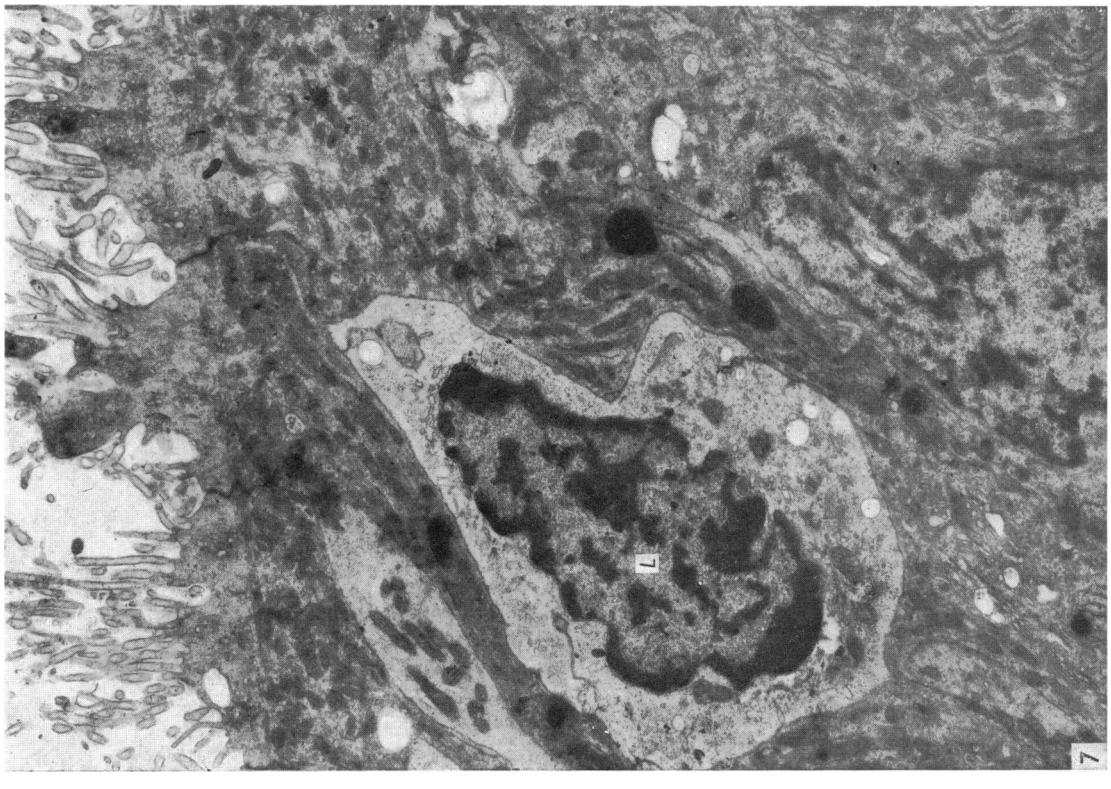
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centres were not seen in the nasal cavity of slow loris, nor on the floor of the posterior portion of the monkey nasal fossa, but they were present at the posterior margin of the nasal septum of the monkey. Here the lymphoid nodules in the lamina propria were close to the overlying epithelium.

The histology of the *vomeronasal organs* of the tree shrew and the slow loris has been described in detail (Loo & Kanagasuntheram, 1972). Lymphoid nodules were present at the upper margin of the vomeronasal organ of the tree shrew anteriorly, and at the lower margin posteriorly. Such cells had invaded the whole thickness of both the neurosensory (Fig. 5) and respiratory epithelia of the organs. Elsewhere there were only scattered lymphoid cells in the lamina propria of the respiratory portion of the vomeronasal organ and in the epithelium. In the slow loris, lymphoid cells were seen only in the lamina propria of the respiratory portion of the vomeronasal organ, with some cells in the lower half of the overlying epithelium, and occasionally throughout its whole thickness (Fig. 6). Lymphoid cells were not seen in the vicinity of the neurosensory epithelium. There were no germinal centres in the lymphoid cell nodules related to the vomeronasal organs.

Electron microscopy

As the morphology of lymphocytes appeared very similar in the different types of epithelia of the three animal species, the following description is applicable to all the intraepithelial lymphocytes seen in the present study.

The lymphocytes were readily differentiated from the epithelial cells by their smaller size, denser nuclear chromatin, scanty cytoplasmic organelles and less electron-dense cytoplasm. The nuclear outline varied from smooth to irregularly indented, but most cells showed a fairly smooth nuclear outline (Figs. 8, 9). An irregular rim of dense chromatin material was seen at the periphery of the nucleus. Occasionally a dense nucleolus was identified (Fig. 12). The double nuclear membrane was fairly regularly spaced. The outer layer of the nuclear membrane was associated with ribosomes. There were a few small oval-shaped mitochondria. Free ribosomes were scattered throughout the cytoplasm, some of them grouped in rosettes. The Golgi apparatus was poorly developed, with a few short lamellae and vesicles. There was a little rough-surfaced endoplasmic reticulum, a few vesicles, and an occasional multi-vesiculated body. In some lymphocytes (Figs. 7, 10), short lamellae of the rough-surfaced endoplasmic reticulum were in continuity with the plasma membrane. One or two small dense bodies were occasionally observed. Desmosomes were not present.

Most of the lymphocytes within the epithelium resembled those in the lamina propria (Fig. 8), except that the intraepithelial lymphocytes generally had pseudopodia containing free ribosomes. Lymphocytes were occasionally seen in the act of

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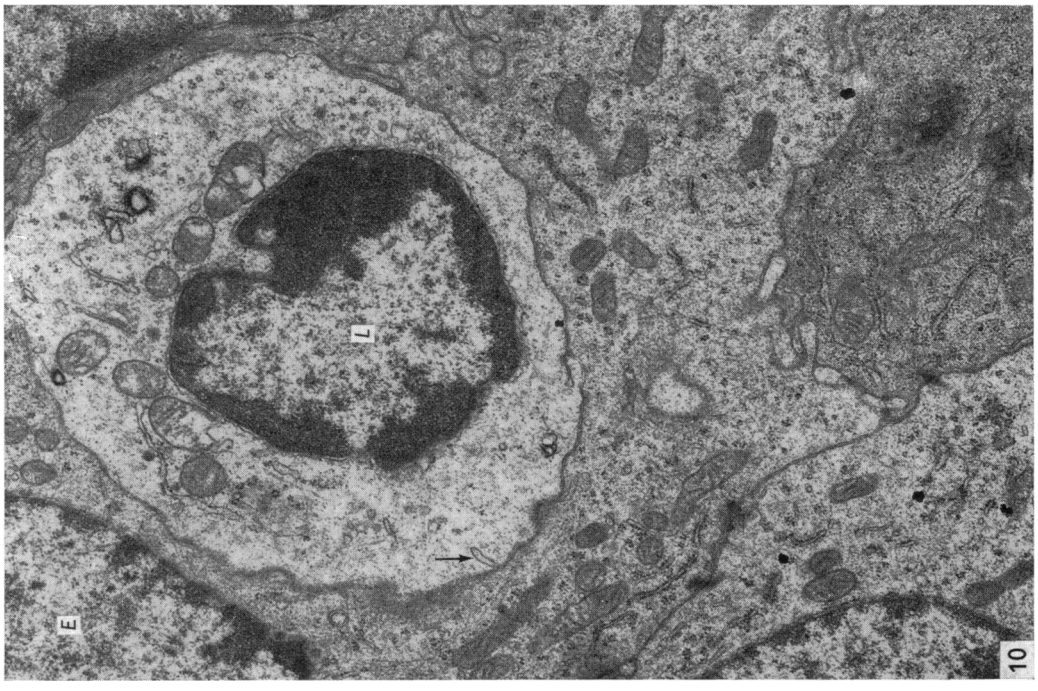
Fig. 7. Monkey olfactory epithelium. Intraepithelial lymphocyte (*L*) is characterized by dense nuclear chromatin, few cytoplasmic organelles and less electron-dense cytoplasm. × 10500.

Fig. 8. Slow loris respiratory epithelium. Lymphocyte (*L*) extends a cytoplasmic process between the epithelial cells (arrow). Note the lack of organelles in the process. This intraepithelial lymphocyte appears morphologically similar to the lymphocyte seen in the lamina propria (*LL*). × 4000.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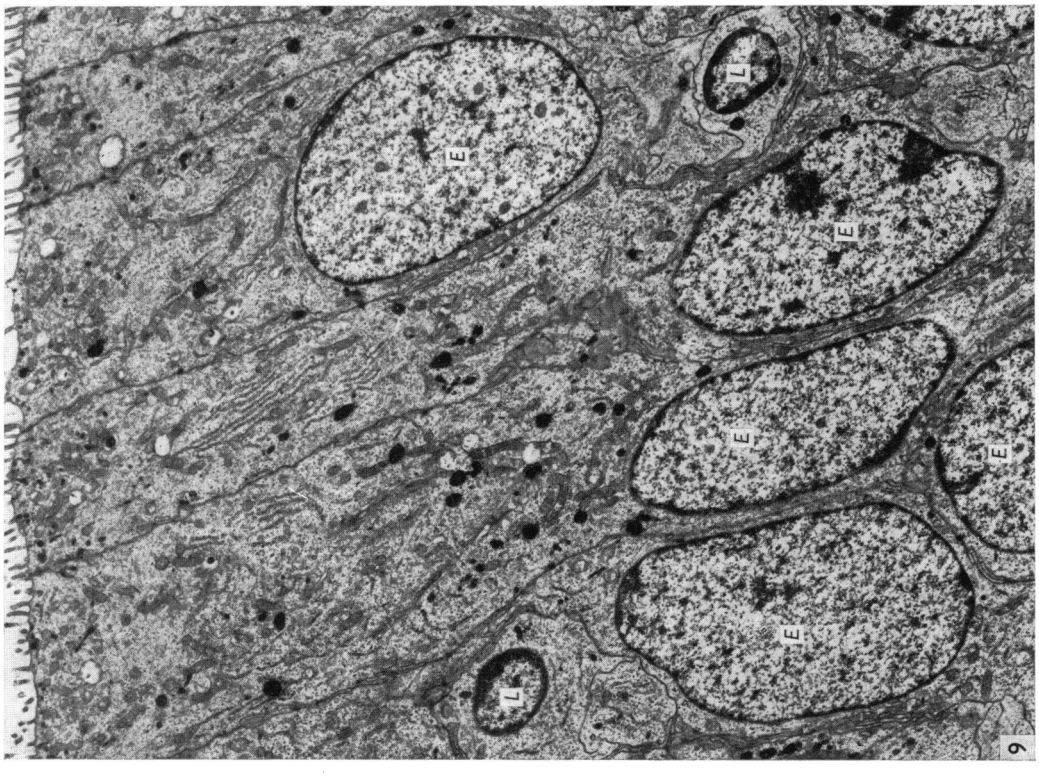
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passing through the basement membrane, lying partly between the epithelial cells and partly in the subepithelial layer (Fig. 12). In the cell illustrated the cytoplasmic projection containing most of the organelles was directed towards the epithelial side, but other cells showed the projection pointing towards the lamina propria. The basement membrane was disrupted by the passage of the lymphocytes. There was a small gap between the plasma membrane of the lymphocyte and that of the surrounding epithelial cells, confirming the view that lymphocytes do not enter the cytoplasm of the epithelial cells.

The nuclear profiles of lymphocytes near the luminal surface of the epithelium appeared to be more irregular (Figs. 7, 11) than that of lymphocytes seen near the basal surface (Figs. 10, 12), and they were occasionally deeply indented. No lymphocytes were seen entering the lumen. No degenerated lymphocytes, lymphocytes in mitosis, or lymphocytes with centrioles, were seen within the epithelium. Neutrophils, and more rarely, eosinophils and macrophages, were occasionally present in the nasal epithelium.

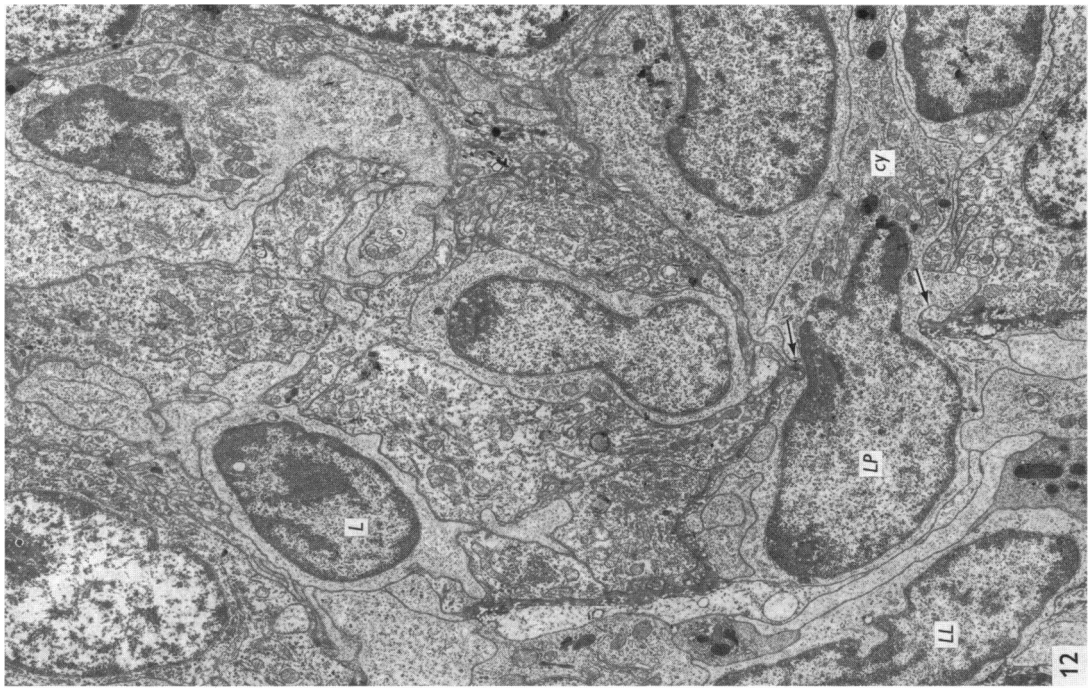
DISCUSSION

The present study demonstrates that lymphoid cells are scattered throughout the lamina propria of the nasal mucosa either diffusely or in small nodules, and that there are lymphocytes in the nasal epithelium. A close relationship between lymphoid cells and epithelium also occurs in other regions of the body, including the larynx and bronchi (Fichtelius *et al.* 1969). According to Miller (1911), lymphoid tissue in the lung is in the form of lymph nodes, lymph follicles, and small masses of lymphoid tissue. Other investigators have noticed lymphocytes in the respiratory epithelium of the trachea, bronchi and bronchioles of laboratory animals and man (Andrew & Burns, 1947; Brundelet, 1965), in the epithelium of the bronchial glands in man (Meyrick & Reid, 1970) and in the epithelium of the nasopharynx of non-human primates (Leela & Kanagasuntheram, 1973) and man (Chin, personal observations). However, there appears to be no previous mention of lymphocytes in the nasal epithelium as seen in the present study. The reason for their presence is open to speculation.

Lymphoid cells in the present study were observed at all levels of the nasal epithelium. Previous investigators (Andrew & Collings, 1946; Andrew & Burns, 1947; Andrew & Andrew, 1949; Andrew, 1965; Meader & Landers, 1967; Shields *et al.* 1969; Meyrick & Reid, 1970) state that most intraepithelial lymphocytes are present in the basal portion of the epithelium. However, Back (1972*a*) found that, in the chicken, a large proportion of lymphocytes lay near the surface of the epithelium.

Fig. 9. Respiratory epithelium of the vomeronasal organ of slow loris. Tangential sections of 2 intraepithelial lymphocytes (*L*) demonstrate their intimate relationship with epithelial cells (*E*). × 5500.

Fig. 10. Respiratory epithelium of vomeronasal organ of tree shrew. The nuclear chromatin of lymphocyte (*L*) appears denser than that of epithelial cell (*E*). A few mitochondria are near one pole of the nucleus. Some of the ribosomes are arranged in rosettes. A few short lamellae of rough endoplasmic reticulum are seen, one of which appears to be in contact with the plasma membrane (arrow). × 16800.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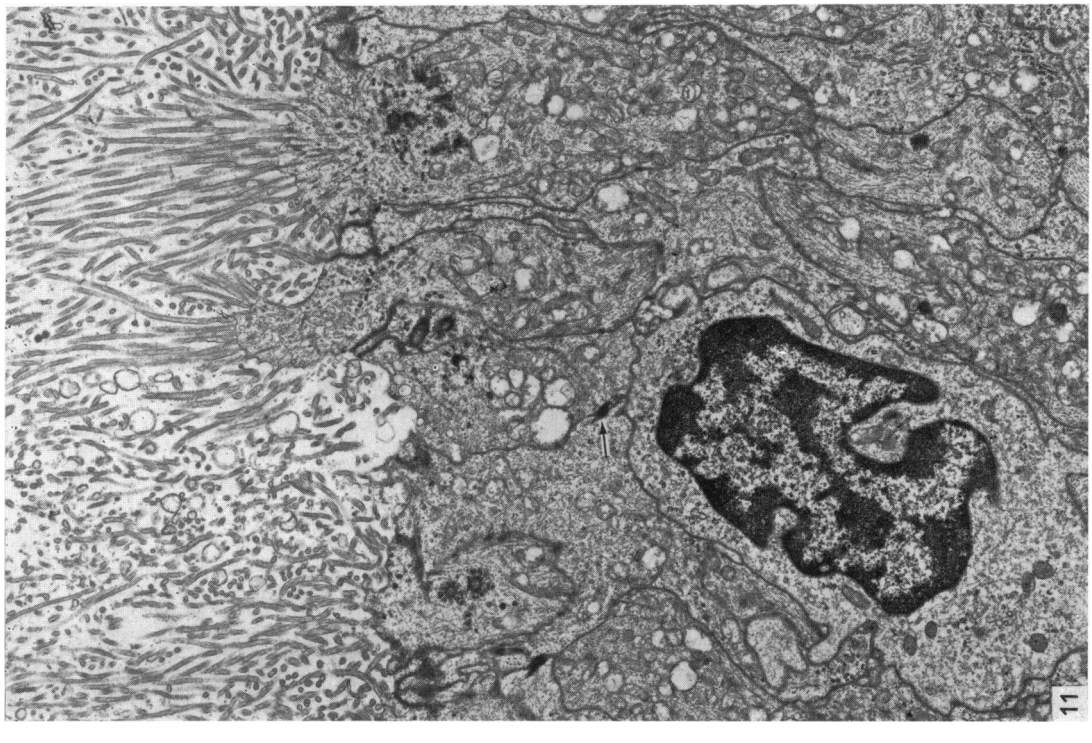
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Lymphoid cell infiltration of respiratory epithelium in rats is very extensive and reaches the lumen of the bronchi. Brundelet (1965) considered this must be of physiological significance. The present study indicates that some lymphocytes probably reach the nasal cavity because they have been seen on the surface of the epithelium. That lymphocytes can pass right through certain kinds of epithelia was demonstrated by Kotani *et al.* (1967), who found a large number of such cells in the intestinal lumen of rats. However, Kingsbury (1943) could find no evidence of lymphoid cell migration through the stratified squamous epithelium of the laryngeal 'tonsils'. According to some investigators lymphocytes change in cell size and their nuclei become pyknotic in their passage towards the luminal surface of an epithelium. This has been observed in the trachea (Andrew & Burns, 1947) and intestine (Andrew & Collings, 1946). Degenerated lymphocytes have been observed in the intestinal lumen of mice (Chin & Hudson, 1971), and within the intestinal epithelium of aged mice (Chin, 1973). Other investigators believe that most lymphocytes do not pass into the lumen but re-enter the circulation (Meader & Landers, 1967; Fichtelius, 1968; Fichtelius *et al.* 1969; Toner & Ferguson, 1971; Back, 1972*a*). Some hold that they are self-perpetuating within the intestinal epithelium (Darlington & Rogers, 1966; Back, 1972*b*) and in the epidermis (Fichtelius, Groth & Liden, 1970). Mitotic lymphocytes within the epithelium have been observed (Darlington & Rogers, 1966; Back, 1972*b*), but Meader & Landers (1967) deny this. Labelled intraepithelial lymphocytes undergoing mitosis have been seen in the trachea (Fichtelius, 1968). However, no mitotic lymphoid cells were observed in the present work.

The fine structure of lymphocytes in the nasal mucosa of primates is essentially similar to those seen in the mucous membranes generally, except that epithelial lymphocytes in the nose show no E.M. evidence of degeneration, unlike the epidermis (Andrew & Andrew, 1949) and the intestine (Meader & Landers, 1967; Toner & Ferguson, 1971). Shields *et al.* (1969) reported that a high proportion of intraepithelial lymphocytes in the bronchi, intestine and endometrium are degenerate. Nuclear fragmentation has been observed in laryngeal tonsils (Kingsbury, 1943). Various authors have noted that intraepithelial lymphocytes differ in some respects from lymphocytes in the lamina propria. An increase in cell size, together with a decrease in endoplasmic reticulum and mitochondria (Andrew, 1965; Shimizu & Andrew, 1967; Fichtelius, Yunis & Good, 1968), and an increase in lysosomes (Toner & Ferguson, 1971) have been reported in intraepithelial lymphocytes of the intestine. In the external nasal epidermis of primates, intraepithelial lymphocytes show cytoplasmic fibrils (Loo & Chin, unpublished). In the nasal mucosa of the present study intraepithelial lymphocytes had deeply indented nuclei, and poly-

Fig. 11. Neurosensory epithelium of vomeronasal organ of slow loris. Note the proximity of the lymphocyte to the tight junction between epithelial cells (arrow). The nuclear profile of the lymphocyte is irregular, and indented in places. × 10500.

Fig. 12. Respiratory epithelium of vomeronasal organ of slow loris. Lymphocyte (*LP*) is passing into the epithelium causing disruption of the basement membrane (arrows). The cytoplasmic organelles (*CY*) tend to be collected at one pole of the cell. Note the morphological similarity between the intraepithelial lymphocyte (*L*) and the lymphocyte in the lamina propria (*LL*). × 6930.

ribosomes in the cytoplasm. Perhaps intraepithelial lymphocytes become distorted, as Andrew (1965) and Hoffer, Hamilton & Fawcett (1973) suggest.

There is disagreement as to whether lymphocytes in epithelium exist between (Andrew & Burns, 1947; Meader & Landers, 1967; Shields *et al.* 1969; Toner & Ferguson, 1971; Back, 1972*a*) or within (Andrew & Collings, 1946; Andrew, 1965; Shimizu & Andrew, 1967) the epithelial cells. In the present study it was clear with the E.M. that the lymphocytes were outside the epithelial cells. However, Andrew & Collings (1946) think that intraepithelial lymphocytes in general are intercellular in the basal portion of the epithelium, but take up an intracellular position as they near the luminal surface. The direction of passage of lymphocytes seen crossing the basement membrane cannot be established from fixed material.

Some controversy exists as to the origin of intraepithelial lymphocytes some holding that they come via the blood stream (Darlington & Rogers, 1966; Fichtelius, 1968; Back, 1970) from both thymus and bone marrow (Fichtelius *et al.* 1969; Back, 1970; Ferguson & Parrott, 1972) while others think they are of local origin (Kingsbury, 1943; Kelsall, 1946; Shimizu & Andrew, 1967; Olson, 1969; Chin & Hudson, 1971; Ferguson & Parrott, 1972). The functional significance of intraepithelial lymphocytes despite much speculation, remains to be determined.

SUMMARY

Collections of lymphocytes were seen throughout the nasal mucosa and vomeronasal organs of tree shrews, slow lorises and macaque monkeys both in the lamina propria, and throughout the whole thickness of the epithelium. There was no evidence of degeneration, mitosis, or transformation into other cell types.

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