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The response of the tonsil and associated lymph nodes of gnotobiotic piglets to the presence of bacterial antigen in the oral cavity

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INTRODUCTION

In calves and pigs the tonsil is one of the ports of entry into the body of bacteria and inert particles (Payne & Derbyshire, 1963; Payne, 1964).

In a detailed study Williams & Rowland (1972) demonstrated, using India ink particles, an afferent route leading from the oral cavity of the pig through the tonsillar crypt epithelium to the sub-epithelial lymphoid parenchyma. Although these authors were not able to demonstrate an efferent pathway from the tonsil to the associated lymph nodes, they postulated that the significance of the tonsil to the general body defences lay in its contribution of lymphoid cells stimulated by bacterial antigens in the oral cavity.

Germ-free piglets offer an alternative method of investigating the immunological significance of the response of the tonsil and the associated lymph nodes to antigenic challenge. Both tonsils and lymph nodes of germ-free piglets less than 4 weeks of age are devoid of germinal centres, and it has been shown that germinal centres develop in the lymph node of germ-free piglets in response to antigenic challenge (Anderson, 1973). The effect of antigenic challenge from the oral cavity should therefore be capable of study in terms of germinal centre induction in the draining lymphoid tissue. The present communication records such a study.

MATERIALS AND METHODS

Piglets. Two litters of germ-free piglets were obtained from Large White sows by hysterotomy (Tavernor *et al.* 1971). The piglets were maintained in flexible plastic isolators, and fed sterilized evaporated milk (Carnation Food Co. Ltd) diluted with sterile distilled water, together with mineral supplement. The gnotobiotic status of the piglets was determined by subculture of rectal swabs under aerobic and anaerobic conditions. Swabs were taken at weekly intervals, and from each piglet before killing.

Bacterial antigen. An overnight culture of a non-pathogenic strain of *E. coli* on glucose nutrient agar (Cruickshank, 1965) was suspended in saline (0·15 M-NaCl), washed three times, and finally suspended in saline to yield approximately 10^{10} colony-forming units per ml.

Experimental design. Each litter consisted of six piglets. At 7 days of age a piglet

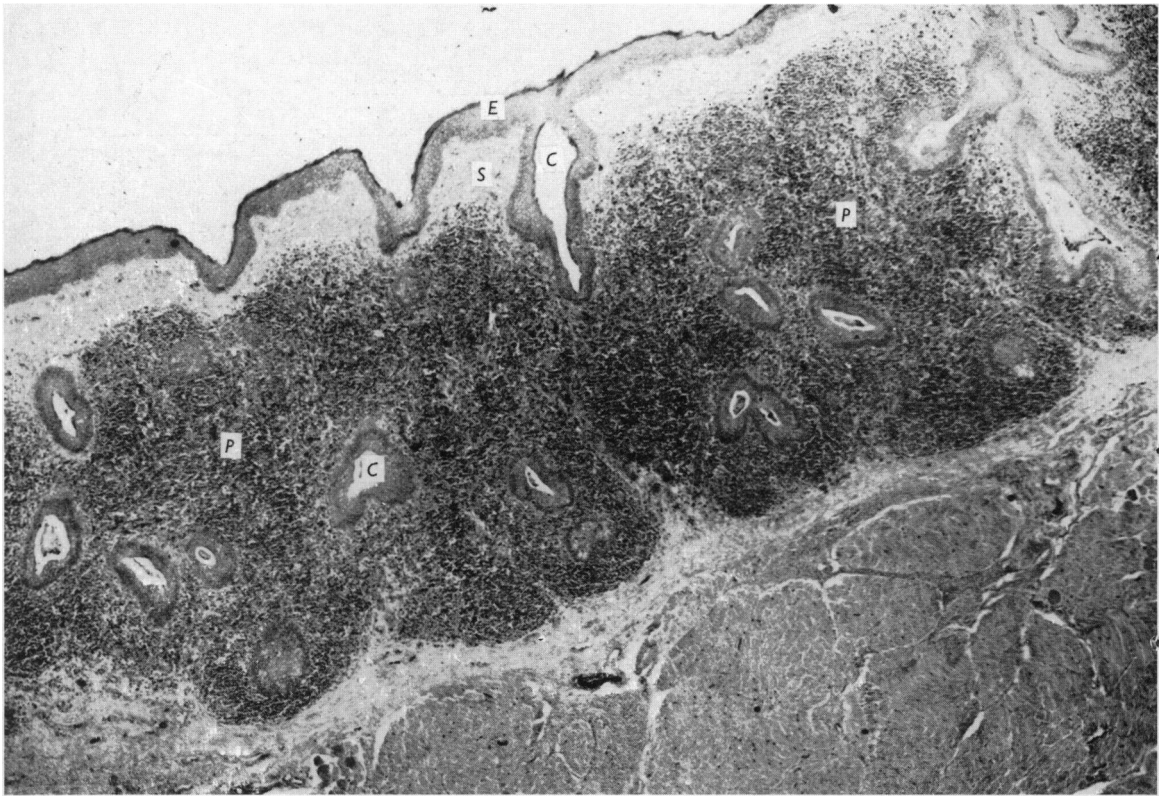

Fig. 1. Tonsil of an uninfected gnotobiotic piglet. *E*, epithelium; *S*, supporting connective tissue; *C*, crypt; *P*, tonsil parenchyma which is devoid of germinal centres. Giemsa, × 32.

from each litter was killed to provide baseline material; the remaining piglets then received 8 ml of the suspension of *E. coli* by addition to their milk (200 ml). Food was withheld for about 1 hour beyond normal feeding time to ensure that all the milk was taken: a pure culture of *E. coli* was subsequently obtained from rectal swabs from each piglet.

One piglet from each litter was killed 1, 3, 5, 7 and 9 days following exposure to antigen. The palatine tonsil and the parotid, costoaxillary and prefemoral lymph nodes were removed from each piglet. Some specimens were fixed in Carnoy's fixative, embedded in paraffin wax, sectioned at 5 μm, and stained with methyl green-pyronin (MGP). Other specimens were fixed in 12 % neutral buffered formalin prior to Giemsa or periodic acid-Schiff (PAS) staining.

RESULTS

Uninfected piglets

The tissues from the germ-free piglets killed at 7 days of age confirmed the quiescent nature of the lymphoid tissue.

The palatine tonsils of the pig are paired organs situated on each side of the

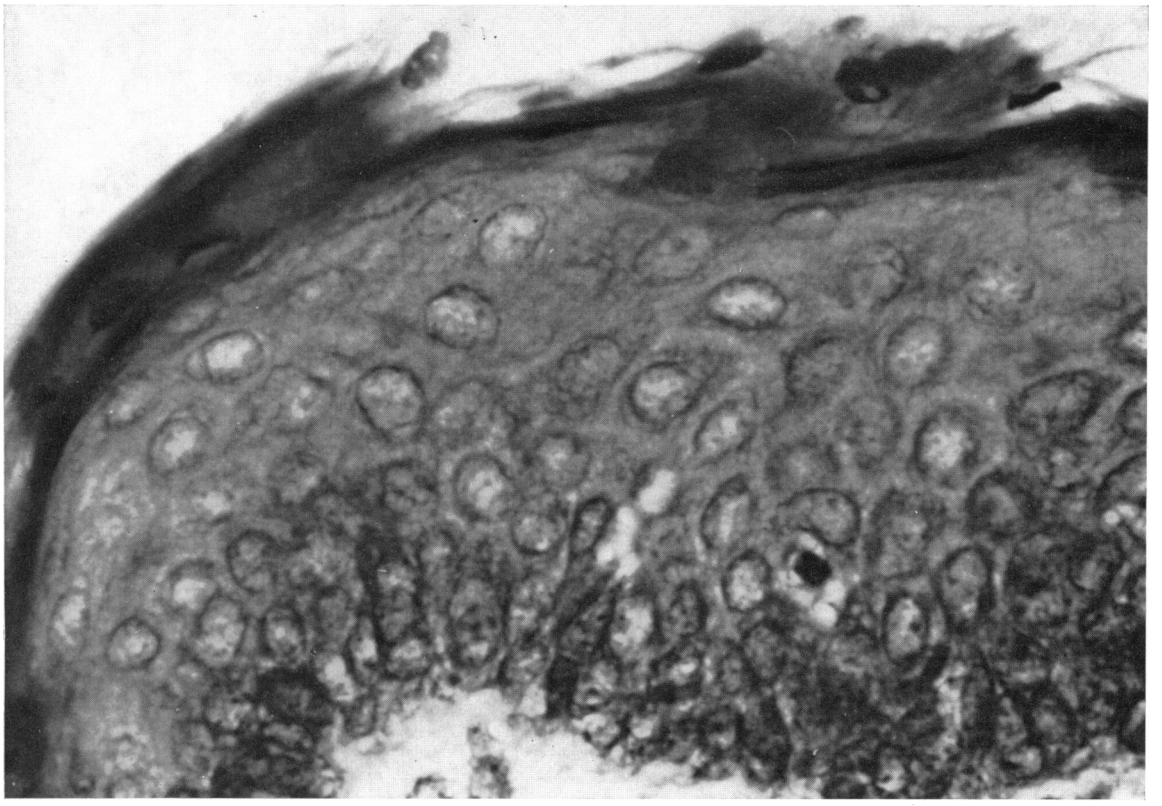

Fig. 2. Epithelium of the oral surface of the tonsil of an uninfected piglet. Polymorphonuclear leucocytes are not seen between the epithelial cells. Giemsa, × 400.

median furrow of the soft palate (Sisson, 1953) and the appearance of the palatine tonsil of gnotobiotic piglets is shown in Fig. 1. The epithelium covering the tonsil was non-keratinized stratified squamous and was supported by dense connective tissue. The parenchyma was homogeneously populated with lymphocytes, and there appeared to be no sinus system, no germinal centres and no plasma cells. The epithelium, unaccompanied by connective tissue, penetrated the substance of the tonsil in the form of crypts, so that there was an intimate relationship between the lymphocytes of the parenchyma and the epithelium. In some areas these cells were separated by a basement membrane, but in others there was no distinct boundary between the two cell types. The epithelium, even at the base of the crypts, was stratified and only rarely was a lymphocyte or a polymorphonuclear leucocyte seen between epithelial cells (Fig. 2). The debris in the crypt lumen was scant and was identified as epithelial in origin. There were mucous glands at the lateral extremities of the tonsils, and occasionally also in the connective tissue of the median furrow. These glands did not appear to drain through the tonsillar crypts to the oral cavity, but PAS-stained sections showed that the oral surface of the epithelium was bathed in mucus, and that there was mucus in the crypts.

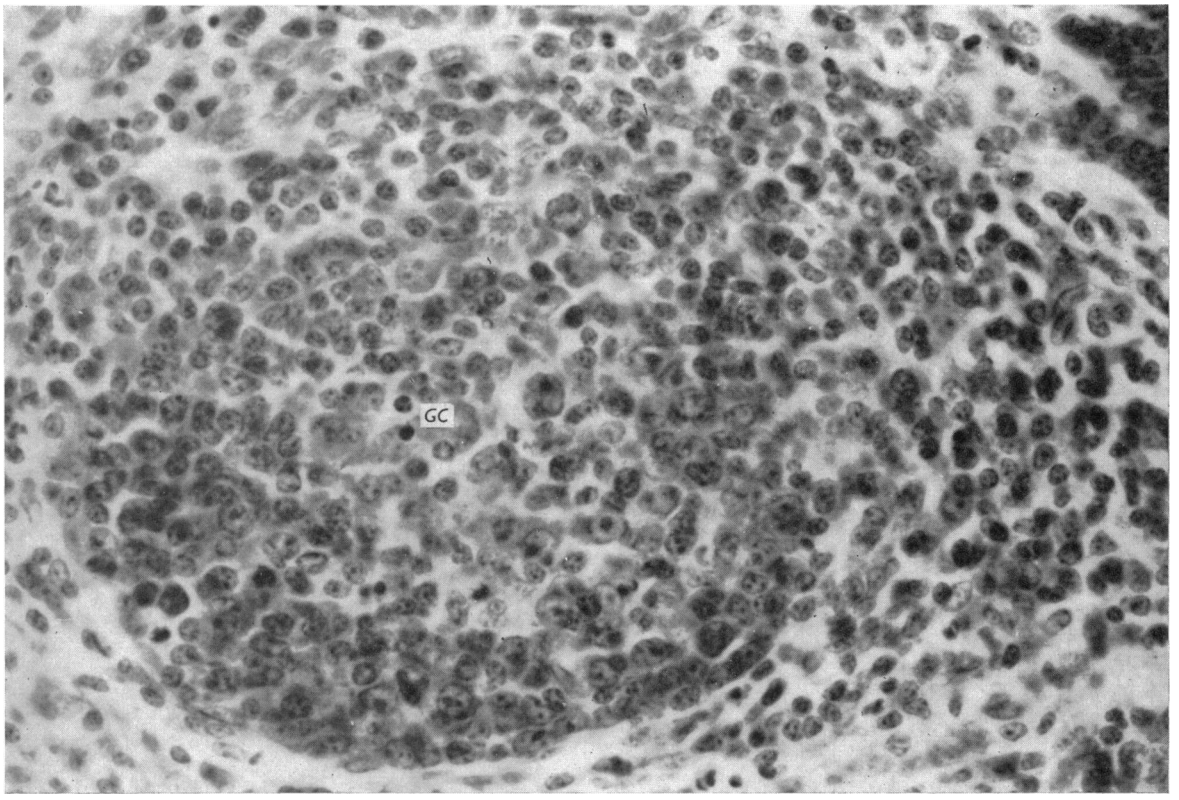

Fig. 3. Germinal centre (*GC*) in the parenchyma of the tonsil of an infected piglet. MGP, × 250.

The appearance of the lymph nodes was characteristic of the germ-free state (Anderson, 1972).

Infected piglets

Tonsil. One day after addition of antigen (*E. coli*) to the diet, pyroninophilic blast cells were seen scattered throughout the parenchyma of the tonsil. On day 3 the pyroninophilic cells had increased in number and on day 5 there were aggregates of such cells in the tonsil parenchyma. On days 7 and 9 germinal centres were present (Fig. 3), often with a corona of intensely basophilic lymphocytes, but there were no cells of the plasma cell series in the tonsil parenchyma.

Debris in the tonsil crypts was marked on day 3, and thereafter, and appeared to be of epithelial origin (Fig. 4). PAS stained sections showed that the debris was mixed with mucus. Examination of the stratified epithelium of the oral surface revealed, from 5 days onwards, polymorphonuclear leucocytes in the process of migrating to the oral cavity (Fig. 5). These cells became bound in mucus, and overflowed into the crypt lumen. There was no migration of neutrophils or lymphocytes through the epithelium at the base of the crypts. In places the epithelium was only one cell thick, and lymphocytes were seen near the crypt lumen, but this was probably an artefact of sectioning.

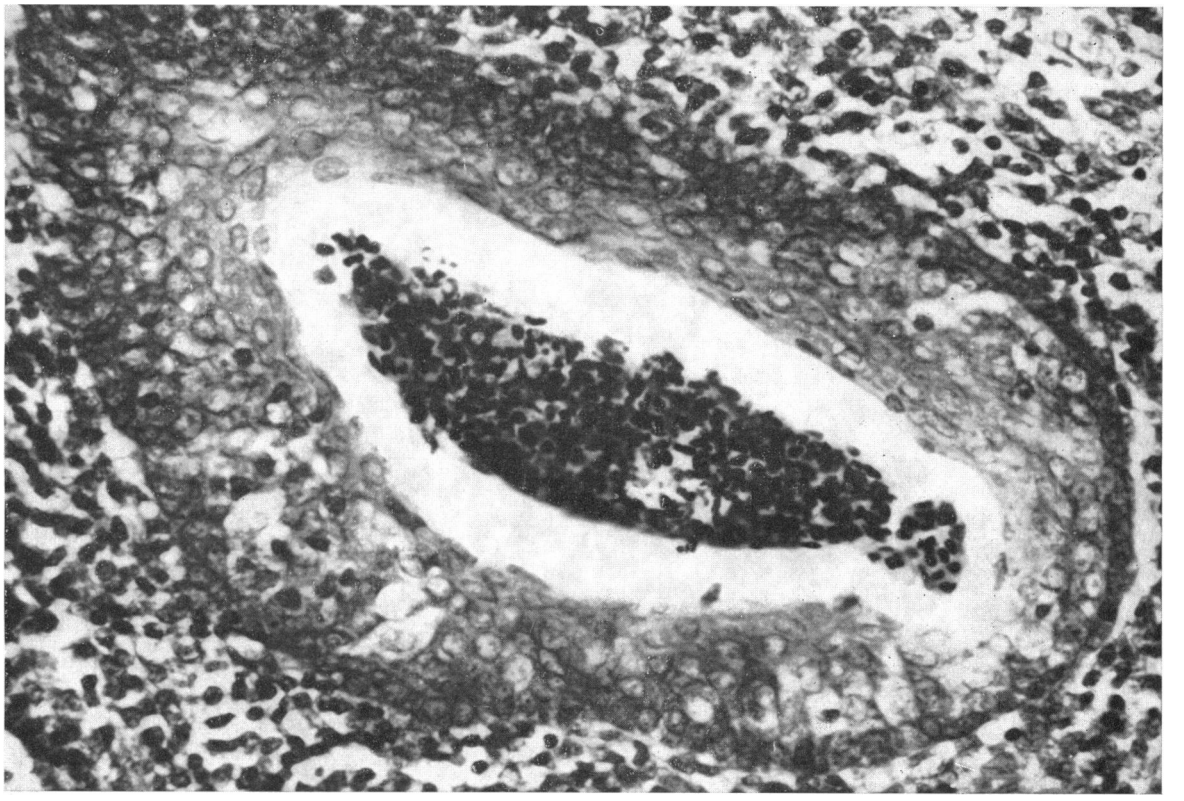

Fig. 4. Epithelial debris in the tonsil crypt lumen of an infected piglet. There are no polymorphonuclear leucocytes in the crypt epithelium. Giemsa, × 250.

Parotid lymph node. Pyroninophilic blast cells were seen in the cortex and sub-trabecular afferent lymphatic terminations on day 1. There were more pyroninophilic cells on day 3 and on day 5 they were aggregated into early germinal centres. Mature germinal centres were seen on days 7 and 9 but there were no cells of the plasma cell series in the medulla.

Costoaxillary lymph node. On days 1 and 3 after addition of antigen the costoaxillary lymph node was histologically similar to the lymph nodes from uninfected piglets. The sub-trabecular sinuses were empty of cells and the associated cortex was narrow. On day 5 a few pyroninophilic cells were seen in the cortex and in the sub-trabecular sinuses; they were aggregated into germinal centres on day 7. Only a few mature germinal centres were present on days 7 and 9 and there were no plasma cells in the medulla.

Prefemoral lymph node. On days 1, 3 and 5 there were no pyroninophilic cells in the cortex; the lymph nodes were inactive. On days 7 and 9 pyroninophilic cells were scattered throughout the cortex, but they were not aggregated into early germinal centres. Pyroninophilic cells were only rarely seen in the sub-trabecular sinus and there were no cells of the plasma cell series in the medulla.

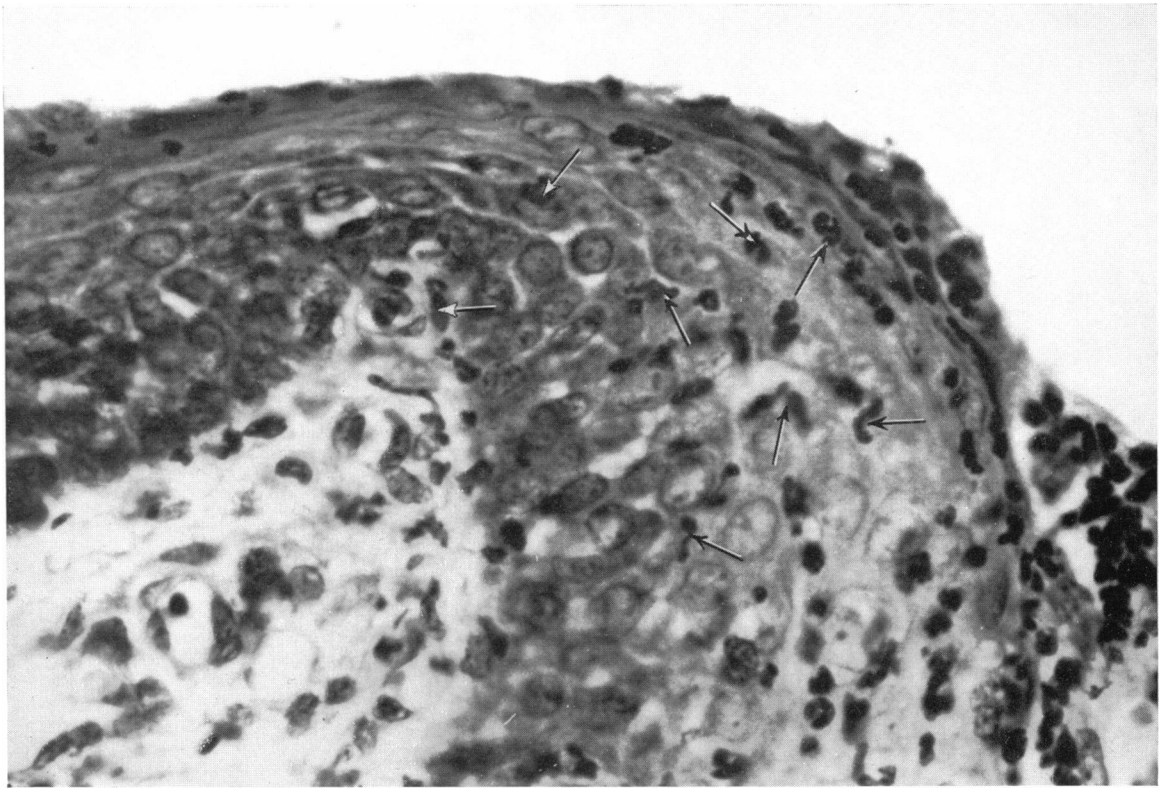

Fig. 5. Epithelium of the oral surface of the tonsil of an infected piglet. Polymorphonuclear leucocytes, some of which are arrowed, are seen between the epithelial cells, and are also bound in the mucus of the oral surface. Giemsa, ×400.

DISCUSSION

The tonsil of gnotobiotic piglets responded to bacterial antigen in the same way as the lymph node of the piglet, i.e. by the formation of germinal centres (Anderson, 1973). These germinal centres formed in the parenchyma of the tonsil by aggregation of pyroninophilic cells, and it was assumed that this occurred as the result of an *in vivo* agglutinative process (White *et al.* 1970). The reaction in the tonsil also resembled that in the lymph node in that the formation of germinal centres occurred in the absence of cells of the plasma cell series.

The palatine tonsil of gnotobiotic piglets has not previously been described. Apart from germinal centre formation, the outstanding features on addition of antigen to the diet were the marked increase in epithelial debris in the crypt lumen, and the neutrophil response in the epithelium of the oral surface. It seemed most probable that these were part of a mild inflammatory response rather than an immune response to the bacteria.

The parotid lymph node of the pig receives afferent lymphatics directly from the tonsil, while the costoaxillary lymph node is at the end of the cervical chain and

receives afferents mainly from the fore-limb; the prefemoral lymph node does not drain the tonsil (Saar & Getty, 1964). The cellular changes induced in these lymph nodes were consistent with this drainage pattern. The time-course of germinal centre formation in the parotid lymph node suggested that there was an immediate drainage of antigen and antigen-induced blast cells from the tonsil. Germinal centres were formed in the costoaxillary lymph node, but the response was less intense and slower in developing. Since there is no direct lymphatic connexion between tonsil and prefemoral lymph node, the presence of a mild pyroninophilic reaction in the latter indicates that the cells reached the node via the blood stream, however the original antigenic stimulus may have occurred in lymphoid tissue lower down the gut.

Germinal centres were induced in the tonsil of gnotobiotic piglets in response to the presence of bacterial antigen in the oral cavity, and the formation of germinal centres in the parotid and costoaxillary lymph nodes indicated an efferent pathway from the tonsil to lymphoid tissue. Considerable evidence has accumulated that associates germinal centres with the production of cells primed for an anamnestic response (Thorbecke, 1969). The series of changes described should therefore be of importance in establishing natural immunity to bacterial disease, and may be evoked in oral immunization generally.

SUMMARY

The cellular changes in the tonsil and in the parotid, costoaxillary and prefemoral lymph nodes of gnotobiotic piglets were observed following feeding with a live culture of non-pathogenic *E. coli*. Germinal centres were induced in the tonsil and in the parotid lymph node simultaneously, and in the costoaxillary lymph node after a short delay. Germinal centres were not seen in the prefemoral lymph node though there were many pyroninophilic cells in the cortex of this node. The results indicate that the tonsil of the piglet responds like a lymph node to an antigenic stimulus, and that the tonsillar response secondarily affects its regional lymph nodes. This pathway may well be activated in both natural and artificial immunization.

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