The histological diagnosis of toxoplasmic lymphadenitis

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SYNOPSIS The commonest presenting sign of acquired toxoplasmosis in man is enlargement of superficial lymph nodes. The persistence of the nodes may lead to a suspicion of malignant lymphoma and the diagnosis then hinges on the lymph node biopsy.

Three cases of toxoplasmic lymphadenitis are described in each of which the diagnosis was unsuspected clinically. The chance discovery of a toxoplasma cyst in the lymph node section of Case I led to the correct diagnosis, after an initial diagnosis of Hodgkin's disease had been made. In the other two, strikingly similar histological changes in the lymph node biopsies suggested the diagnosis, which was confirmed serologically in each case.

The histological changes are described and the clinical and pathological aspects of toxoplasmic lymphadenitis are briefly reviewed, with special reference to the differentiation from malignant lymphomatous conditions and to the specificity of the histological picture. It is concluded that the histology is, in many instances, sufficiently distinctive for a tentative diagnosis of toxoplasmic lymphadenitis to be made on the lymph node biopsy. The diagnosis should always be confirmed by isolation of the parasite or by serological tests. It is exceedingly rare for toxoplasma cysts to be found in lymph nodes and only one previous observation of this kind has been reported.

Over the past 20 years it has become evident that human infection with Toxoplasma gondii is almost world-wide in distribution and that this remarkably successful parasite has, as alternative hosts, a wide range of mammals, birds, and even reptiles. In man, infection is not nearly as rare as was formerly thought. Not unnaturally, the first cases of acquired toxoplasmosis to be diagnosed were all examples of the severe, acute form of the disease, ending fatally. This variety is undoubtedly rare, but within the past decade a number of reports have been published of a much commoner manifestation of acquired toxoplasmosis, characterized by enlargement of superficial lymph nodes, often without other conspicuous symptoms.

The importance of toxoplasmic lymphadenitis lies in its differentiation from much more serious conditions, especially Hodgkin's disease and lymphosarcoma, for which it may be mistaken on both clinical and pathological grounds. Writers on the subject have expressed different opinions about the specificity of the histological changes in the lymph nodes in toxoplasmosis. Nevertheless there is in many of the accounts a striking measure of agreement about the principal histological features, allowing for differences in terminology.

The three cases reported here serve to emphasize the relative frequency of the condition, the constancy of the histological changes in the lymph nodes, and the importance of lymph node biopsy in arriving at the diagnosis.

CASE REPORTS

CASE I A.M., a married woman of 30, was referred to St. Bartholomew's Hospital on 13 October 1960 for radiotherapy after a diagnosis of Hodgkin's disease had been made at another hospital on a cervical lymph node biopsy.

For six to seven weeks she had noticed swellings in the neck but felt otherwise quite well. Her appetite was good and weight steady.

Physical examination showed a well nourished woman. Two enlarged lymph nodes, each nearly 2 cm. in diameter, were palpable in the neck: one on the right side, at the upper end of the posterior triangle; the other, in the left supraclavicular fossa, lying just below the biopsy incision. There were no enlarged nodes in axillae or groins and neither liver nor spleen were felt on abdominal examination.

The results of laboratory investigations were as
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follows:—E.S.R. 7 mm. in the first hour, haemoglobin 86% (12.7 g. haemoglobin / 100 ml.) W.B.C. 5,000 per c.mm. (polymorph neutrophils 70%, eosinophils 2%, lymphocytes 20%, monocytes 8%), platelets 176,000 per c.mm. A radiograph of the chest did not show any abnormality.

The lymph node biopsy section was re-examined at St. Bartholomew's at the start of radiotherapy and the changes seen were then interpreted as reactive rather than neoplastic. In the course of this examination a single cyst of toxoplasma was encountered in the section and a diagnosis of toxoplastic lymphadenitis was made on the strength of this discovery. The diagnosis was confirmed a few days later by a positive dye test at a titre of 1 in 1,000.

The patient was subsequently given a 14-day course of triple sulphonamide and pyrimethamine and, three weeks after its conclusion, examination failed to disclose any palpable lymph nodes in the neck or elsewhere. She has remained well, but it was later reported that her son, aged 2 years, had an asymptomatic enlargement of cervical lymph nodes.

CASE 2 D.L., a married woman of 40, was referred to another hospital on 10 November 1960 by her doctor for the investigation of enlarged cervical lymph nodes.

Four months previously she had noticed a swelling behind the left ear, but this had disappeared spontaneously. A month later, a swelling appeared behind the left sternomastoid and grew steadily in size, while a second smaller swelling appeared on the right side too. She had not had a sore throat. The swelling was slightly tender at first but was not painful, though she experienced some difficulty in turning her head to the left. The swellings had persisted since. For a period of about three months she had also complained of indigestion and epigastric discomfort after food. She had also noticed tiredness and breathlessness but had no cough and her weight was steady.

Physical examination showed a well nourished woman. There were two enlarged, fairly soft nodes on the left side of the neck and one on the right, while two similar nodes were also found in the right axilla. The liver and spleen were not felt. On the basis of these findings and the history it was thought most likely that she had some form of 'lymphomatous neoplasia'.

The chest radiograph was normal and Wassermann, Kahn, and Price's precipitation reactions gave negative results. A blood leucocyte count before admission had shown a total of 5,000 cells per c.mm. (polymorph neutrophils 35%, lymphocytes 60%, and monocytes 5%). The E.S.R. varied between 18 and 25 mm. in one hour and the haemoglobin was 90% (13-3 g. haemoglobin/100 ml.). A relative lymphocytosis persisted for several weeks, though the total leucocyte count remained low. No atypical cells were seen in blood smears. A Paul-Bunnell screening test performed at the beginning of December was negative.

On 16 November biopsy of a cervical lymph node was carried out. The node, which measured 2-3 × 1-8 × 0-5 cm., showed a hard, white cut surface with brown streaks. The pathologist reported that the histological picture corresponded with that of lympho-histiocytic medullary reticulosis (Robb-Smith, 1947), which he regarded as a reactive response of relatively non-specific type. The section was referred to me for my opinion and, in view of the remarkable similarity of the histological appearances to those of Case 1, a diagnosis of toxoplastic lymphadenitis was suggested. Toxoplasma cysts were not found in the original section or in sections subsequently examined but the diagnosis was confirmed serologically. The patient's serum, taken on 3 December, gave a positive dye test at a titre of 1 in 4,096 and the complement-fixation test was positive at 1 in 32.

No treatment was given and on 21 December the lymph nodes were reported to be subsiding.

CASE 3 R.A.L-N., an advertising representative, aged 19, was referred by his own doctor to St. Bartholomew's Hospital on 14 November 1960 with a swelling beneath his chin which was thought to be a thyroglossal cyst.

He had first noticed the swelling, which was well to the left of the midline, two to three months previously and at that time another swelling had appeared on the right side of the neck also. The latter had disappeared following a course of sulphonamide treatment. He had had no pain, sore throat, or cough and his weight was steady. Tonsillectomy had been performed at the age of 7.

Physical examination showed a young man of healthy appearance with a visible swelling beneath the chin on the left side. On palpation, a fairly soft, doubtfully fluctuant swelling, 1 in. × ½ in., could be felt in this situation. A few soft nodes were palpable in the right axilla. The liver and spleen could not be felt.

He was kept under observation and, two months later, since the swelling showed no diminution in size, he was admitted to hospital for biopsy. Examination now showed a small palpable tonsillar lymph node on the right side, as well as the swelling on the left. Laboratory tests showed: Haemoglobin 102% (15-1 g. haemoglobin/100 ml.), W.B.C. 6,000 per c.mm. (polymorph neutrophils 65%, eosinophils 2%, lymphocytes 24%, monocytes 9%).

On 13 January 1961 the swelling beneath the chin was excised and proved to be an enlarged lymph node, measuring 3-0 × 1-6 × 0-3 cm. The consistency was firm and the cut surface of the node showed a faintly discernible follicular pattern. The histological appearances were sufficiently similar to those of Cases 1 and 2 as at once to suggest a diagnosis of toxoplastic lymphadenitis. Cysts were not found but the diagnosis of toxoplasmosis was confirmed serologically. The patient's serum, taken on 18 January, gave a positive dye test at a titre of 1 in 2,048 and the complement-fixation test was positive at 1 in 64. A course of treatment with triple sulphonamide and pyrimethamine was given subsequently and when last seen the patient was symptom free.

HISTOLOGY

The diagnosis of toxoplasmosis was made in Case 1 by the chance discovery of a toxoplasma cyst in the lymph node section. This section also showed rather distinctive reactive changes, and it was the finding of
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very similar appearances in the lymph node biopsy sections that led to the diagnosis in Cases 2 and 3. The following description of the histological changes is based upon all three cases.

The lymph node in each instance was markedly enlarged, but apart from this there was nothing remarkable about the macroscopic appearance.

On microscopical examination, the essential features are those of a subacute to chronic lymphadenitis with well-marked periadenitis. A striking degree of lymphoid hyperplasia is present which is, no doubt, mainly responsible for the lymph node enlargement. Many of the follicles are large and irregular in outline with active germinal centres (Fig. 1). These are, however, often less clearly defined than is commonly the case in reactive hyperplasia, and proliferating lymphoblasts sometimes mingle with mature lymphocytes, giving the impression of an infiltrative lesion. There are numerous mitoses of regular pattern in the germinal centres and a notable feature is the presence of many freshly necrotic cells, so that the centres are littered with karyorrhectic particles of nuclear debris, some of which have been engulfed by macrophages (Figs. 2 and 3).

Scattered through the pulp of the node, and sometimes invading the follicles, there are small clusters of very conspicuous, pale-staining histiocytes with vesicular nuclei and copious, somewhat eosinophilic cytoplasm (Figs. 3, 4, and 5). These cells resemble the epithelioid cells of tuberculosis, but multinucleate cells are scarce and none simulate Langhans giant cells. Moreover caseation is absent. The cell clusters are smaller and generally less sharply defined than the focal collections of similar cells seen in sarcoidosis. A further contrast with sarcoidosis is seen in the absence of reticulin in relation to these cells in a silver impregnation preparation (Fig. 6).

The presence of these histiocyte clusters is perhaps the most distinctive feature of the histological picture in all three cases. They are not regularly distributed, but are chiefly seen towards the periphery of the node which presents a spotted appearance on low-power examination (Fig. 4). They appear to be engaged in the phagocytosis of nuclear debris liberated by the piecemeal necrosis of cells in the follicles. Infiltration of the follicles by histiocytes is certainly one reason for the blurring of the follicular outline.

Almost as distinctive a feature as the histiocyte clusters is the dense packing of many of the lymph sinuses with free macrophages (Fig. 7). In sections of each of the three lymph nodes some of the sinuses are so completely filled that they would not be distinguishable, in the haematoxylin- and eosin-stained section, from the dense pulp of the node, but for the smaller size and stronger basophilia of the lymphocytes in the latter. Both peripheral and central sinuses may be affected, but the density of the cellular infiltration varies and, in places, discrete cells are seen against a background of eosinophilic coagulum.

High-power examination reveals a mixed population of cells in the lymph sinuses, including a scattering of neutrophil polymorphs and some large macrophages laden with cellular debris. The predominant cell, however, is a small macrophage with many of the characters of a blood monocyte. These 'monocytoid' cells have relatively large, often contorted, nuclei and moderately strongly staining, amphophilic cytoplasm (Fig. 8). They are quite distinct from the large epithelioid histiocytes described above (Fig. 7), and also from the proliferated littoral cells commonly found in reactive conditions.

Lastly, there is a marked increase of plasma cells throughout the pulp of the node, especially in the medullary cords and around the small blood vessels. The node capsule is thickened and infiltrated with cells and this infiltration, which includes many plasma cells, extends out into the surrounding adipose tissue (Fig. 9). Eosinophil leucocytes are scarce or absent in all three biopsies.

Numerous sections, from all three cases, were searched for organisms and Giemsa's method was used as well as haematoxylin and eosin staining. Apart from the cyst originally discovered in Case 1, no other definite organisms were found. The cyst, which does not appear to be intracellular, is located at the margin of a lymph follicle. It is oval in shape, perhaps due to compression by surrounding cells, and shows a definite external limiting membrane which stains with eosin. Inside the cyst, a large number of very small toxoplasms can be distinctly seen (Fig. 10).

DISCUSSION

Up to a decade ago, the only known cases of acquired toxoplasmosis in man took the form either of a severe illness resembling Rocky Mountain spotted fever (Pinkerton and Weinman, 1940; Pinkerton and Henderson, 1941), or of an almost equally fatal disease characterized mainly by encephalitis (Sabin, 1941). It was realized that there was a marked discrepancy between the rarity of these adult cases and the comparative frequency of congenital toxoplasmosis in babies. With these facts in mind, Siim in Denmark and Magnusson in Sweden began to seek for evidence of a milder form of the disease in adults. In this they were greatly assisted by the newly introduced cytoplasm-modifying antibody reaction (dye test) of Sabin and Feldman (1948).
FIG. 1. Enlarged and irregular lymph follicles (Case 3). Haematoxylin and eosin × 65.

FIG. 2. Germinal centre of follicle showing mitoses and necrotic cells (Case 1). Haematoxylin and eosin × 400.

FIG. 3. Margin of enlarged follicle showing nuclear fragments and invading epithelioid histiocytes (Case 1). Haematoxylin and eosin × 135.
FIG. 4. Clusters of epithelioid histiocytes at periphery of cortex (Case 3). Haematoxylin and eosin × 85.

FIG. 5. Detail of epithelioid histiocytic clusters (Case 3). Haematoxylin and eosin × 400.

FIG. 6. Epithelioid histiocyte clusters showing absence of reticulin formation (Case 3). Gordon and Sweet’s method × 350.
FIG. 7. Dense packing of lymph sinuses with monocytoid macrophages. Note epithelioid histiocytes at upper left (Case 2). Haematoxylin and eosin × 125.

FIG. 8. Details of cells filling lymph sinus (Case 2). Haematoxylin and eosin × 400.

FIG. 9. Periphery of node showing capsular and extra-capsular infiltration by cells, including many plasma cells (Case 2). Haematoxylin and eosin × 135.
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The first published account of glandular toxoplasmosis was that of Gard and Magnusson (1951) who described three adult patients with the disease. In the succeeding year or so, several reports of similar cases appeared from Scandinavia (Stiim, 1951 and 1952; Wahlgren, 1951; Landau, 1951; von Zeipel and Linder, 1951; Magnusson, 1951; Bang, 1953), followed by reports from the United States (Armstrong and MacMurray, 1953; Stanton and Pinkerton, 1953). The first account of glandular toxoplasmosis in this country was that of Skipper, Beverley, and Beattie (1954) and, since that time, there have been a number of further publications on the subject from many parts of the world.

The number of these reports and the fact that I encountered three cases in the space of a few weeks suggest that the condition is far from rare. Indeed, the frequency of positive serological reactions for toxoplasma among series of apparently healthy adults indicates that the cases presenting clinically still form only a small proportion of the total number of infected individuals. It is probable that more cases will be diagnosed in the future, with increasing awareness of the condition and the greater use of lymph node biopsy as a diagnostic procedure.

The clinical aspects of toxoplastic lymphadenitis have been recently reviewed by Beverley and Beattie (1958) and by Remington, Jacobs, and Kaufman (1960). The disease is commonest in older children and young adults. Some European authors (Bang, 1953; Piringer-Kuchinka, Martin, and Thalhammer, 1958; Saxén and Saxén, 1959) have observed a preponderance of female patients, but in reports from this country the sexes have been almost equally affected. Saxén and Saxén (1959) noted a seasonal prevalence of the disease in the winter months.

For a variety of reasons little is known of the epidemiology of acquired toxoplasmosis and the only cases in which the mode of infection has been definitely established have been instances of laboratory infection, where workers handling the organism have pricked themselves with infected hypodermic needles (Gard and Magnusson, 1951; Ström, 1951; Beverley, Skipper, and Marshall, 1955; Kayhoe, Jacobs, Byee, and McCullough, 1957). In the naturally occurring disease, it has been suggested that infection may occur most commonly by the respiratory route, with primary involvement of the nasopharyngeal lymphoid tissue (Beattie, 1957; Lainson, 1958). This would agree with the observation that the cervical lymph nodes are commonly affected and there is not infrequently a sore throat at the onset of symptoms (Beverley and Beattie, 1958). Commonly the lymphadenopathy extends to involve other nodes, especially the axillary and inguinal groups. Enlarged mediastinal nodes have been observed radiographically on several occasions and it has been claimed that the retroperitoneal and mesenteric nodes may be affected (Beverley and Beattie, 1958; Remington et al., 1960).

The chief importance attaching to this generally mild disease is that it may be mistaken for disorders of a more serious nature. Clinically, one of the characteristics which may lead to a suspicion of malignancy is the persistence of the lymph node enlargement, often for several months and sometimes for a year or longer. Some patients complain of marked fatigue and general debility, but loss of weight is not a feature. If there is fever at the outset, infectious mononucleosis may be suspected and the presence of a lymphocytosis with atypical lymphocytes in the peripheral blood smear may strengthen this impression, but the Paul-Bunnell reaction is consistently negative (Beverley and Beattie, 1958). Rarely, the changes in the peripheral blood may be so striking as to lead to a diagnosis of acute lymphatic leukaemia, as in Case 2 of Kayhoe et al. (1957). It should be noted that the E.S.R. is seldom much raised and anaemia is usually absent.

**FIG. 10. Toxoplasma cyst at margin of follicle (Case 1). Haematoxylin and eosin × 500.**
Although the clinical findings may arouse the suspicion of malignant lymphoma, the establishment of this diagnosis is dependent upon the findings in a lymph node biopsy. That there is a possibility of an erroneous diagnosis at this stage is shown by the history of Case 1, where initially a diagnosis of Hodgkin's disease was made on the lymph node biopsy. It is understandable that toxoplastic lymphadenitis might be mistaken histologically for Hodgkin's disease, lymphosarcoma, or reticulosarcoma, and several authors (Stanton and Pinkerton, 1953; Paton, Dick, and Beverley, 1958; Paterson, 1960) mention that the first impression gained from histological examination of the lymph node biopsy was one of a malignant condition. Pace and Babando (1960) go so far as to state that glandular toxoplasmosis cannot with certainty be distinguished from Hodgkin's disease on the histological findings.

Confusion with malignant lymphomatous conditions is liable to arise from (1) the apparent disorganization of the architecture of the node, (2) the active proliferation of large cells, variously interpreted as lymphoblasts or reticulum cells, (3) the simulation of neoplastic infiltration, and (4) the prominent histiocyte clusters, which resemble those sometimes found in association with Hodgkin's disease and, less often, with lymphosarcoma and reticulosarcoma.

The chief points which distinguish toxoplastic lymphadenitis from the various forms of malignant lymphoma are:—(1) The lymph node architecture is not, in fact, completely destroyed; (2) the proliferating cells show normal mitoses only and amongst these cells there is a high incidence of necrosis, witnessed by the numerous pyknotic nuclear fragments; (3) giant reticulum cells characteristic of Hodgkin's disease are absent (Bang, 1957); (4) the lymph sinuses are crowded with macrophages; and (5) the cellular infiltration, which involves the capsule and extends outside the node, has all the characteristics of an inflammatory infiltration.

It is too early yet to decide whether the concurrence of toxoplasmosis and lymphosarcoma, or reticulosarcoma, which has been reported twice (see references given by Remington et al., 1960) is more than a chance association. In the case of Finch (1954), the diagnosis of toxoplasmosis rested solely on the morphological interpretation of organisms seen in bone marrow smears. Certainly this protozoon does not seem to be as frequently associated with the malignant lymphomata as are some fungal infections.

Whilst it is generally agreed that toxoplastic lymphadenitis can be distinguished histologically from the various forms of malignant lymphoma, there is no unanimity of opinion on the question of whether the histological picture is at all diagnostic of toxoplasmosis. Some authors have stated that the lymph node changes are non-specific or inconstant (Gard and Magnusson, 1951; Ström, 1951; Skipper et al., 1954; Alexander and Callister, 1955; Beverley, Caley, and Warrack, 1958; Paton et al., 1958). According to others, the changes are, at least, highly suggestive of the disease (Wahlgren, 1951; Siim, 1953; Stanton and Pinkerton, 1953; Bang, 1953, 1957; Saxén and Saxén, 1959).

The prominent histiocyte clusters have attracted most attention and these have been described by almost all writers. Some have likened them to 'miniature sarcoid lesions' (Stanton and Pinkerton, 1953), but, as pointed out above, the cell clusters are generally less well defined than sarcoïd lesions and there is no increased production of collagen or reticulin (Piringer-Kuchinka, 1953). Thus there is little likelihood of confusion with sarcoïdosis.

Robb-Smith (1947) described similar reactive lesions in lymph nodes under the title of lymphohistiocytic medullary reticulosarcoma. He stated that much the same appearance was seen in Leishmaniasis, but in that condition Leishman-Donovan bodies could be identified in the epithelioid histiocytes. Later, Robb-Smith (quoted by Beverley and Beattie, 1958) examined a number of lymph node biopsies from proven cases of toxoplasmosis and reported that, in nine out of 11 cases, the appearances corresponded to his description of lympho-histiocytic medullary reticulosarcoma. He doubted, however, whether the lesion could be regarded as specific for toxoplasmosis.

Similarly, Piringer-Kuchinka (1953) gave an accurate account of the histological changes, including the macrophage infiltration of the lymph sinuses, before she was aware of the occurrence of this type of lesion in toxoplasmosis. In 1958, the same author published an account of 62 cases showing this histological picture. Among these, positive serological tests for toxoplasmosis were obtained in 46 out of the 49 individuals tested (Piringer-Kuchinka et al., 1958).

Bang (1953 and 1957) drew attention to the 'focal medullary reticulosarcoma' which he claimed to be characteristic of toxoplastic lymphadenitis, and Siim (1953) found the 'same characteristic histological picture' in all of 16 cases where lymph node biopsies were performed. More recently Saxên and Saxên (1959) and Roth and Piekarski (1959) have confirmed the constancy of the histological findings in toxoplastic lymphadenitis.

Confirmation of the diagnosis has been obtained in some instances by successful isolation of the organism from lymph nodes which have shown...
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They must be admitted, however, that, in most of the published series of cases the selection of cases has been made on a histological basis and the diagnosis has been confirmed retrospectively by serological tests. Allowance must therefore be made for the possibility that cases which did not present characteristic histological changes in the lymph node biopsy may have escaped recognition. Nevertheless, this cannot invalidate the conclusion that, when the lymph node biopsy does show this combination of changes, it provides strong presumptive evidence of toxoplasmonic lymphadenitis. This conclusion is supported by the findings in the three cases presented here, as well as by the other evidence cited above.

The diagnosis should never rest on the histology alone, for, however characteristic the changes may be, they can hardly be said to be specific. Ideally, confirmation of the diagnosis should be obtained by isolation of the organism, which may be done by intraperitoneal inoculation of the mouse with fresh lymph node suspension (SiiM, 1956). Unfortunately, the diagnosis is seldom suspected at the time the lymph node is removed for biopsy and it is hardly justifiable to perform a second operation for this purpose. Moreover, animal inoculations have not proved uniformly successful even when the diagnosis has not been in doubt. Cathie (1954) has successfully isolated toxoplasma by mouse inoculation with the patient’s saliva.

In the majority of cases the diagnosis of toxoplasmonic lymphadenitis has been confirmed by serological tests, and studies have shown that the results of these are sufficiently reliable for practical purposes (Cathie, 1957). The demonstration of a rising titre in the cytoplasm-modifying antibody (dye test) and the complement-fixation test, or the presence of a high titre in the two tests, is generally taken as evidence of an active infection. These results, taken in conjunction with the characteristic findings in a lymph node biopsy, provide sufficient grounds for a diagnosis of toxoplasmonic lymphadenitis.

Only very rarely are toxoplasma cysts to be found in lymph node sections. Indeed, a definite cyst with a clearly defined wall, such as was found in Case 1 of the present series, has been described in a lymph node on only one previous occasion (Stanton and Pinkerton, 1953). In multiple sections of the node, these authors succeeded in demonstrating two such cysts, both apparently situated in lymph sinuses. These cysts appear to correspond to the true toxoplasma cyst, described by Lainson (1958), as occurring in chronic toxoplasma infections in animals. They differ from the pseudocysts, which are found in more acute infections, in that there is a clearly defined cyst wall, apparently of parasitic origin. The contained toxoplasmas are both smaller and much more numerous than those in the pseudocysts (Lainson, 1958). Most authors have been understandably cautious in identifying vegetative forms of toxoplasma in lymph nodes, in view of their similarity to particles of nuclear debris which are regularly to be found in large numbers.

I should like to thank Dr. R. Bodley Scott, Mr. P. Stringer, and Mr. M. A. Birnstingl for permission to publish records of their patients, Dr. I. G. Williams and Dr. J. I. Pugh for the pathological material from Cases 1 and 2, Dr. D. G. Fleck for the results of toxoplasma serological tests, Mr. J. W. Miller for most of the histological preparations, and Mr. P. Crocker for the photomicrographs.

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doi: 10.1136/jcp.14.6.565

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