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LUCAS HEIGHTS

VASCULAR INJURY IN LUNG DISEASE

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A. D. TUCKER

J. H. WYATT

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DAWN UNDERY

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ABSTRACT

Inhaled particulates which stimulate a 'delayed', cellular mode of alveolar clearance are excreted to the airways through lymphoid foci in the bronchial bifurcations. The anatomic relations and developing pathology of the tissues adjacent to these foci, including the divisions of accompanying arteries, were studied by serial sectioning and photomicrographic modelling of rat lungs. The changes are typical of classic 'delayed' inflammatory reactions and, in the rat, the fully developed stage is characterised by fibrinoid necrosis involving all three layers of the arterial wall in a linear lesion across the leading edge of the flow divider. An hypothesis was developed to relate the injury to pulsatile forces. Recent published findings indicate that similarly placed lesions, with species-specific changes in development, are universal in both cerebral and extra-cranial arterial forks of man and animals. Possible associations of the microvascular changes with human atherosclerosis and their further significance in pulmonary and systemic effects arising from industrial and environmental contaminants are explored.

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The following descriptors have been selected from the INIS Thesaurus to describe the subject content of this report for information retrieval purposes. For further details please refer to IAEA-INIS-12(INIS: Manual for Indexing) and IAEA-INIS-12(INIS: Thesaurus) published in Vienna by the International Atomic Energy Agency.

ARTERIOSCLEROSIS; BRONCHI; DUSTS; FIBRINOLYSIS; FLUID
MECHANICS; INHALATION; LUNGS; MACROPHAGES; RATS; STAGNATION;
TURBULENT FLOW; VASCULAR DISEASES

1. INTRODUCTION

We have examined, in detail, the anatomic relations and developing pathology around the lymphoid foci which form in rat lungs between the bifurcations of the bronchi and the pulmonary arteries. Being paired, these vessels divide simultaneously. Not only is the wall of the bronchus breached in the process of extrusion of detritus from the lymphoid nodule [Brundelet 1965, Tucker *et al.* 1973], but also the wall of the artery breaks on a line across the tip of the flow divider. The presence and extent of development of the arterial wall lesion at a particular bifurcation is relative to the activity in the associated lymphoid nodule.

Peribronchial, perivascular lymph node manifestations have been described in human lungs affected by industrial exposures [Cole 1944]. Dyspnoea due to vascular restriction in the gas-exchanging parenchyma is accompanied by depressed values of pulmonary ventilatory function tests. X-radiography does not necessarily detect any lung changes until much later [Balchum 1969, Trapp *et al.* 1972]. Infiltration of the vascular wall by dust- and pigment-bearing tissue was described as a feature of silicotic lungs, and pulsatile pressures have been suggested as having a significant aetiological role [Geever 1947]. Partial or complete obstruction of the pulmonary capillary circulation appeared to follow exposure to siliceous dusts [Cole 1944, Schepers 1960] and to dusts generated in the mining of uranium or other hardrock [Trapp *et al.* 1972].

2. METHODS

We have described elsewhere the difficulties encountered in the interpretation of experimental toxic effects in lungs of rats when superimposed upon the chronic respiratory disease (CRD) of laboratory rats [Tucker & Wyatt 1967]. The stigmata of CRD were eliminated by raising rats in an environment free of food-cube and mixed litter dusts and, simultaneously, there was an extension of the latent period for initiation of murine nephrosis [Tucker *et al.* 1975]. Inert particles, administered in low dosage to rats free of CRD, were cleared rapidly from the alveoli by interstitial pathways to the broncho-alveolar junctions, and without significant intervention by phagocytes [Tucker *et al.* 1973]. The same inert material administered to CRD-affected rats was taken up by phagocytes and conveyed to points of lympho-reticular hyperplasia at the bifurcations of the bronchioles. Thence excretion through the wall of the bronchus continued for many weeks [Brundelet 1965], see Addendum.

We found various stages of the interaction on the flow divider at arterial bifurcations in both control and exposed rats. Methods devised for three-dimensional photoreconstruction have been described previously [Tucker *et al.* 1973].

3. RESULTS

Essential features of anatomic and physiologic interest can be traced down to the level of the terminal bronchioles (Figure 1). Lymphocytic cells with reticular support filled the tip of the dividing spur of the bronchiole. A mucoid raft of extruded histiocytes and carmine particles clung to the surface of the epithelium in the lumen. In more distal sections, transversely through the area, lymphocytic infiltration extended beneath the epithelium along the medial walls of both bronchi to a point level with the division of the arteriole. A radicle of the bronchial vascular plexus (or rete) ran close to each bronchiolar branch on its arteriolar aspect, and broad irregular lymphatic channels were formed between the lympho-reticular focus and the arteriole, being particularly evident near the spur of the arteriolar division.

Anatomic relations and structural changes in the tissues adjacent to the paired dividing spurs become more complex about vessels of larger calibre. We reconstructed portion of the broncho-vascular bundle from the lung of a 4-month old rat in which lympho-reticular foci were only of microscopic size. The artery was 600 μm in diameter at this point (Figure 2). By comparing the effects of ageing and the development of pathology in this and other rats, a sequence of alterations was detected which:

- . began at the perivenous sheath of nearby collecting veins and involved the interstitial drainage of alveolar walls abutting on the artery (Figure 3);
- . pierced the wall of the artery from the adventitial aspect (Figure 4);
- . formed a protruding plaque of changed arterial wall tissues which extended in linear fashion across the tip of the dividing spur (Figures 5a and 5b); and
- . extended a hyaline limb through the wall towards the lymphoid focus, coming into intimate relation with radicles of the bronchial rete (Figures 5c and 5d).

Progressive stages in the formation of the protruding hyaline lesion were found during examination of lungs from rats of many ages and conditions of pulmonary health (Figures 6 and 10). The attack began from the adventitial aspect, breaking the elastic coats and pressing the successive layers into an arrowhead directed towards the lumen. At the entrance to one branch, the elastic and muscular coats of the distal lip were thinned to the point of extinction [*cf.* Figure 190, page 247 of von Hayek 1960]. At a later stage, the degenerate part of the wall became hyalinised and bulged into the lumen. After Masson trichrome staining of formalin-fixed and paraffin-embedded tissue sections, the spur lesion gave indications characteristic of degenerate collagen and elastic fibres.

Inhalation of reactive particulates stimulates the appearance of phagocytic histiocytes in the alveolar walls. These cells are characterised by lipid accumulation in the phagocytic vesicles. We used Oil Red O to stain thick, frozen sections from the lung of a rat sacrificed 24 hours after exposure to a heavy dose of cigarette smoke. As described by Brundeleit [1965], histiocytes in these sections became clustered about the adventitial aspect of the peribronchial lymphoid foci, passing through the nodules for excretion into the airway. In typical sections (Figure 7), these lipid-stained cells formed 'fronds' indicating movements along the patterns for interstitial drainage and dust clearance which we have shown elsewhere with an inert particulate [Tucker *et al.* 1973], but based here on lymphoid foci.

The second lipid-stained section (Figure 8) was taken from the lung of a rat which was sacrificed immediately after the third of a series of 1-microgram doses of finely particulate beryllium oxide (low fired at 250°C), the doses being given three weeks apart. A rosette distribution of formalin-pigment laden histiocytes about thrombosed collecting veins suggests that there had been intravascular thrombosis in the postcapillary venules. Lipid-laden histiocytes moved towards and into the lymphoid nodule over the arteriolar bifurcation and there was marked accumulation of fat deposit in large unilocular pools, closely adjacent to the focus [von Hayek 1960]. Branching radicles of the bronchial vascular plexus (rete) can be seen to have close relationship to the focus and to the pooled fats (Figure 8). Within the space of a few more-proximal transverse sections, the linear hyaline lesion across the apex of the dividing arterial spur would bring this area of reaction into communication with the lumen of the artery. This relationship becomes apparent after examination of

Figures 2d and 2e, especially since the bronchial rete is seen to flow through the focus towards the origin of the hyaline lesion at X in Figures 2e, 5c, 5d, and 10d.

That there is an interchange between the focus and the arterial lumen became quite evident when we stained sections from the lung of a rat which was sacrificed three weeks after a 1-microgram inhalation dose of beryllium oxide, fired at 900°C so that the particles were much less fine (Figure 9). The staining method was designed to be histochemically specific for beryllium [Wyatt 1972]. Some positively stained cells, which had gathered in the peribronchial focus, were excreted directly to the airway; others entered the looser tissues about the adventitia of the arterial wall which had been breached by hyaline plaque formation; a few passed into the cleft which passed through the protruding hyaline lesion to communicate with the arterial lumen; and a very few were found free in the arterial blood (Figure 9). In Figure 10a, details are shown of the tip of a protruding lesion which is similar to that in Figure 5a. The small, apparently free, hyaline body is seen to be a cross section of a fine protrusive arm of hyaline material at the tip of the lesion (see Figure 6e also); lipid-laden vacuolated macrophages are emerging from the cleft in the plaque to enter the blood flow. The semi-detached bodies at the tip of the cleft lacked only the staining characteristics of calcium to meet the classical description of Schaumann Bodies [Teillum 1949].

4. DISCUSSION

Our original purpose was the study in the rat of pulmonary reactions to particular toxic inhalants. The scope of these studies was extended of necessity to reconcile our observed results with changes due apparently to 'spontaneous' pathology in control stock kept in identical laboratory environmental conditions. The 'final common pathway' now proposed would account for commonality in reactions observed in control and experimental stock. Variations depend only on the degree of accidental environmental, and intentional experimental, exposures to potentially sensitising agents during the relevant period of time. Pathology will differ in a particular animal according to whether the predominant change is one of local vascular obliteration, local fibrotic proliferation, or the general dissemination of immune, and tissue breakdown products. This follows since tissue sensitisation always begins with histiocytic infiltration and leads to perivascular infiltration; only sometimes does it progress to necrosis in response to a particularly intense reaction [Waksman 1960].

There is continued speculation that a 'final common pathway' might account for similarities exhibited by delayed-type reactions to bacterial infection, contact (chemical) sensitivity, auto-allergy, and other forms of hypersensitivity [Turk 1967]. Since the scope of our experiments extended from controlled inhalation exposures [Tucker et al. 1973], through peribronchial, perivascular lymphatic reaction, to indications of an association between lung damage and renal injury [Tucker et al. 1975], the present work appeared to be a link between three levels of activity in the tissues (see Sections 4.1-4.3).

4.1 Tissue Sensitisation

Inhaled particles deposited in the alveoli enter interstitial clearance pathways. Unless the particles are inert and the lung healthy, clearance involves a slower histiocytic reaction through foci of lymphoreticular hyperplasia at bronchial bifurcations; that is to say, reactive particles enter the pathways from the alveoli to become a potentially sensitising inoculum.

The classical sensitisation process occurs in lymph nodes draining the inoculation site and depends upon the presence of suitable blood vessels close to the antigen-containing tissue. The term 'perivascular island' formulated by Gell, stresses the perivascular nature of cell infiltration which is the primary change. Necrosis is the secondary complication of an unusually intense reaction. 'Diseases' vary because of the spatial relationships between antigen-containing parenchyma and the mesenchymal tissues which characterise the affected part [Waksman 1960].

4.2 Vascular Damage

Vascular damage may affect (a) the immediate arterial wall; (b) the local capillary bed and/or collecting veins; and (c) the blood in circulation.

Hypersensitivity vasculitis attracted more attention following the increase in incidence of adverse sensitivity reaction when the sulphonamides were presented for clinical use. Opinions varied as to whether the lesions began at the intima, in the adventitia, or spread both ways from the media. However the predilection for lesions in the forks of arteries, large or small; was soon noted [Zeek et al. 1948]. 'Necrotising angiitis' was advanced as a convenient term for the entire group of vascular lesions in which the fully developed stage is characterised by inflammation and necrosis involving all three layers of vessel walls [Zeek 1953]. Rats appeared to suffer an increasing susceptibility by reason of an 'ageing

process' [Yang 1965].

4.3 Systemic Effects

Systemic effects could result (a) from interference with pulmonary function; (b) from injury to organs at a distance, mediated by dissemination of immune or degradation products; or (c) as a result of generalised vasculitis.

Since the reaction we observed in rat lungs to inhalants might be a particular case of the general phenomenon of local tissue reactions, we examined the literature describing the pathology of small peripheral arteries.

4.4 Features of Vascular Injury

On the basis of highly specific localisation, Stehbens [1974a] has suggested that haemodynamic vibrations rupture the wall of the artery on the apex of the dividing fork. (Such mechanical fragility would not explain the progression from adventitial fraying to an eventual outgrowth into the oncoming blood stream, nor the elaborate extravascular anatomic structural changes.) Stehbens [1974b] also raised the question of a 'final common pathway' through which the many possible atherogenic factors could conceivably act - including age, sex, endocrine glandular function, hypertension, smoking, and others.

From an extensive study of cerebral arterial lesions and of experimental arterio-venous aneurysms in sheep, Stehbens [1974b] concluded that the as yet unexplained degenerative changes in small arteries exhibit remarkable similarity to atherosclerosis. Two prominent and constant sets of features (also represented in the rat lung model) were:

(i) *Localisation*

Medial gaps (medial defects) at the apex of arterial forks appeared in the cerebral and extracranial forks of other animals and in man. Thinning of the wall and elastic tissue destruction constituted a change at the apex of arterial forks which did not happen by chance, increased in prevalence with age, and was an integral part of the atherosclerotic process. The variation in prevalence which occurred with changes in the angle of branching supported the possibility that the lesion was produced by haemodynamic forces [Stehbens 1972].

(ii) *Adventitial cellular infiltration*

Fraying and erosion of the wall adjacent to and at the point of fracture (hyaline plaque) was associated with the accumulation of:

- . siderophages [Stehbens 1974a];
- . a myriad of lipophages [Friedman & Van den Bovenkamp 1966]; and
- . homogeneous collections of cells, resembling small lymphocytes, in the adventitial tissues [Schwartz & Mitchell 1962].

Similar lymphocytic cellular concentrations appear in Figures 3a, 4a, and 4b.

Two questions must be elaborated if the arterial lesion of the rat lung were to serve as a model for a 'final common pathway' (leading from inhaled particle deposition, through tissue sensitisation, to vasculitis). First would be an haemodynamic mechanism to account for the specific localisation on a narrow line across the leading edge of the arterial flow divider and for the entry of cells from the adventitia into that flow. Second would be an explanation for observed differences in reaction in other species and for known complications in humans within that context.

4.4.1 Induction by pulsatile forces - a possible mechanism

A credible explanation must account for the highly specific localisation along the line across the apex of the flow divider. Deckker & Male [1967] concluded that the derivation of boundary conditions for unsteady flows in branched ducts from purely theoretical considerations is impractical. Massey [1970] stated that conditions determining the change from laminar to turbulent flow for differing shapes of boundary have to be determined by experiment; in the present state of knowledge they could not be calculated theoretically.

Caro *et al.* [1971] reviewed the earlier hydrodynamic theories on the siting of atherosclerotic lesions at arterial points of bifurcation and specifically rejected explanations based on the Bernoulli effect. They proposed shear dependent mass transfer of cholesterol, originating in the wall, so that it diffused into the blood stream. However, that portion of the flow divider which is subject to high shear is wider than the apical region involved by the medial defect or the hyaline plaque lesion.

Our hypothesis derives from recently observed sequences of 'small events' in fluid dynamic behaviour near the wall region and requires statistical summation of innumerable small events localised at points along the leading edge of the flow divider. Birkhoff [1955] cited Oseen for his recognition of the possibility that 'arbitrarily small events' can produce finite effects. Birkhoff also criticised, as 'plausible but limiting', the assumption that 'small causes produce small effects'.

As we see it, from the point in any cycle of pulsatile flow when a pulse is receding distally, retardation and backflow thicken the boundary layer over the flow divider. The following pulse accelerates the central core and friction between the core and the retarded, thickened boundary layer increases so that the latter is thinned by recruitment to the core flow. Schlichting [1955] described the increase in pressure which occurs along the central streamline of the plane of symmetry in the direction of flow towards the stagnation point where flow divides to pass around an object in its path. No separation occurs on the approach because there is no wall friction; nor is there separation at the stagnation point because the excess fluid there is drawn off into the downstream flows by a pressure drop along the medial walls of the branches. Thus the boundary layer over the flow divider is thinned by the arrival and passage of each pulse and flow then becomes 'entrance length' in character.

Friction at the interface between the dividing core flow and the boundary layers on the flow divider will account for the high shear regions reported there and for the sharp inflection in flow profile near the medial walls of the branches. In a recent review of physiological fluid dynamics Lighthill [1972] attributed localised turbulence, during a very small fraction of each pulse-cycle, to change in boundary layer velocity profile. 'Bursts' of turbulence follow soon after a point of inflection in the profile due to retardation of velocity near the wall; he predicted an 'enormous' increase in instability in such a situation. Thus we visualise the leading edge of the flow divider, which creates a stagnation point in the central, dividing streamline as the site for occurrence of these bursts. The 'uplifting of parcels' of retarded fluid, across the line of stagnation, will then result in a continuing, if intermittent, series of 'ejections' of the sort described by Corino & Brodkey [1969] and by Kim *et al.* [1971]. Corino & Brodkey found that 'the ejections were very energetic and well correlated to be a major contributor to total Reynolds' stress'. Ling, *et al.* [1969] confirmed that, whereas the average shearing stress at the arterial wall has a value of 20 to 40 dynes/cm², peak shearing stress at the lips of arterial bifurcations could reach 140 to 260 dynes/cm², nearly equal to the yielding stress limit of endothelial cells.

Corino & Brodkey noted that at the time of an ejection there was often a very sharp interface between the accelerated and retarded elements, thus creating a very high shear layer. Moreover, when the entering stream of high velocity fluid approached the wall at an angle (as would happen on

the flow divider), fluid particles penetrated to the region of the wall, entering the sublayer. (Massey [1970] put the thickness of the laminar sublayer in stable flow at less than 1 μm .) Corino & Brodkey found that as the mass of fluid proceeded wallwards, there was a simultaneous ejection of the fluid of the retarded wall region outwards towards the axis of flow; that is, the ejection always originated within the mass of fluid constituting the retarded element.

The concept of shear dependent mass transport by diffusion across the arterial wall [Caro *et al.* 1971] predicates that the wall be permeable and porous to some degree. Sparrow *et al.* [1971] have criticised the tacit assumption of non-slip boundary conditions in the analysis of fluid flows bounded by permeable walls and examined the association between surface mass transfer and internal flows. While the healthy arterial wall would not be of a porosity comparable with the blocks tested by Beavers & Joseph [1967], any tendency to slip-velocity over the divider would bring the origin of fluid ejections closer to the pores in that wall and so enhance the possibility that localised suction might draw interstitial fluid from the wall into the blood stream. Statistical summation of these innumerable, localised suction events, falling chiefly at points along the stagnation line across the leading edge of the flow divider, could account in time for a degenerative lesion there. Yielding to the effects of inflammatory products in the fluids drawn from the perivascular foci, the tissues would be changed to form a lesion, equivalent biologically to the proverbial effect of constantly dripping water upon the hardest stone - a physical integration of innumerable, arbitrarily small, events.

4.4.2 Complications of human vascular injury compared with the rat model

(i) *Intimal pads on lateral walls (not evident in the rat)*

Stagnation, separation, backflow, and stasis have been demonstrated on the lateral walls of the artery where it spreads into the form of a diffuser, just proximal to the flow divider [Fox & Hugh 1966]. Lateral intimal pads form in humans later in life than the apical pads [Stehbens 1972]. The differential pressures with each pulse would be much less at the lateral walls than on the apex in the path of the dividing streamline, but these pulse pressures would be accentuated at all sites by the development of hypertension [Veress *et al.* 1960, Downing *et al.* 1962, Stehbens 1974a]. Elevation of these lateral pads would accentuate the separation forces just proximal to the pads and account for the transverse formation there of linear medial gaps by the same hydrodynamic mechanism,

but later than the apical lesion.

(ii) *Lipid accumulation*

Lipid-laden histiocytes (foam cells) and pooled fats are part of the peribronchial, perivascular lymph node complex in the rat (Figures 7 and 8). Foam cells and histochemically marked phagocytes passed through the apical lesion into the blood stream (Figures 9 and 10).

The lipids in vascular lesions of treated rabbits were examined by electronmicroscopy and the principal cells involved appeared to be foam cells derived from mesenchymal remnants in the tissues or from blood monocytes [Murad et al. 1970]. Polyvacuolar and univacuolar lipid deposits were described in association with the 'neogenesis' of peribronchial lymphoid tissues, the 'pulmonary tonsil' of the human lung [von Hayek 1960].

Indeed, there has been general and longstanding agreement about this feature. As early as 1862, Virchow wrote of 'large granular cells in thickened segments of the endothelium' which 'eventually, as the ground substance between them softens, consolidate to the one communal form in which larger droplets precipitate out' [Pickering 1964]. Leary [1936] found both deposits of neutral fats and of large and granular macrophages containing fats in the form of an emulsion.

(iii) *Adventitial haemangiomas and broncho-pulmonary anastomoses*

Interstitial drainage channels were formed in the rat between sheaths of collecting veins and the site of the arterial wall lesion, becoming plexiform and angiomatous (Figures 3a and 3b). On the side of the arterial division nearest the bronchus the hyaline plaque pierced all layers of the arterial wall and came into close relation with the bronchial vascular plexus (or rete) (Figures 2e, 5b, 5c, 5d and 10d).

Nests of epitheloid cells have been described in a postnatal specimen of human lung, the situation being akin to that shown in Figure 3. Von Hayek [1960] thought that such a nest, by swelling in response to a high level of histamine, would serve to occlude part of the pulmonary circulation whilst *in utero*. The development of bronchopulmonary anastomoses was studied by Liebow et al. [1949] and was found to be accentuated by hypertension [Brewer 1955, Downing et al. 1962].

Vascular occlusion was described as an early feature in human silicosis, leading to progressive impairment of the pulmonary circulation and to eventual right-sided heart failure. Hyperaemic and proliferative adventitial vasa clustered to give the appearance of haemangiomas in relation to small and medium size arteries in proximity to margins of the nodules

[Geever 1947]. The vascular occlusion was offset by development of bronchopulmonary anastomoses to effect a supply of arterial blood regionally in the lung [Schepers 1960]. The compressed perivenous tissues, in the vicinity of experimental arterio-venous aneurysms in sheep, contained extensive angiomatous proliferation of small vessels and vasa vasorum were often prominent in the outer wall [Stehbens 1974a].

(iv) *Intimal pads, intimal tears, and thrombosis*

Since the histochemically-specific marker in beryllium laden cells indicated the passage of those cells from the peribronchial, perivascular lymph node into the blood stream, it follows that reactive tissue- and immune-products would also enter that blood flow at the apex of the arterial flow divider. The intimal pad on the apex over the medial gap might grow by coagulative accretion and resolution in response to the continued filtration of such products. Evidence now supports the concept of normal, continuous coagulation in circulating blood, whilst homostasis depends on fibrinolysis to maintain fluidity [McKay 1965]. Whether the lesion were to form a progressively protruding hyaline lesion, as in the rat, or an organised intimal pad, as in human atherosclerosis, might depend on the balance in an affected species between thromboplastic and fibrinolytic activities. Then in the rat, coagulative accretion over the medial gap would be inhibited since the thromboplastic activity level is relatively low and that for fibrinolysis is high [Astrup et al. 1970]

Adoption of serial sectioning in the longitudinal plane of the arterial fork permitted our reconstruction of the lesion and its anatomic relations. Use of that technique has also demonstrated that thrombosis complicating coronary and cerebral atherosclerosis in humans is often associated with minute intimal tears and transverse linear plaques [Chapman 1965, Constantinides 1969, Friedman & Van den Bovenkamp 1966]. Similarly, thrombi in the Circle of Willis usually contain fragments of changed arterial wall tissues as emboli [Constantinides 1967]. These embolic fragments, the Schaumann Bodies of human pulmonary hypersensitivity (Figure 10b) and of sarcoidosis [Teillum 1949], all share a similar histological appearance and staining reactions with the hyaline arterial wall plaque of the rat lung (Figures 6 and 10).

4.5 Interpretative Applications in Research and Clinical Fields

(i) *Local tissue damage mechanisms*

Since the medial gap would permit direct intra-arterial injection of immune products from the peribronchial, perivascular lymph node, antibodies

could be recycled locally to the tissues which had been subject to infective invasion or toxic inoculation. This would be more economical and more rapid in terms of humoral defence than would systemic venous distribution. Stetson [1963] suggested that tissues might be damaged by antibodies manufactured by lymphoid or plasma cells in the locality of tissue lesions, and that the local concentration of antibody in such circumstances might not be accurately reflected by the level in the systemic circulation. Yet, for this to be so, there must be an avenue for local return *via* arterioles since plasma cells are numerous in the areas to which products drain, are rarely found away from blood vessels, and almost never occur in the parenchyma [Waksman 1960].

Progression by a 'local Schwartzman reaction' following intra-vascular infiltration of these products would explain the coagulation seen in post-capillary venules of rats exposed to microdoses of inhaled beryllium oxide (Figure 8). A local Schwartzman reaction embodies the thrombotic effect of bacterial endotoxin, and perhaps other agents as diverse as intravascular haemolysis, antigen-antibody complexes, chemical or physical agents, anoxia, and endothelial damage [McKay 1965].

(ii) *Vascular obliteration in human industrial pulmonary disease*

Apart from the many parallels already mentioned above to pathological observations from humans, there are symptom complexes associated with the chronic obstructive pulmonary disease (COPD) group which suggest that local vascular obliteration contributes and occurs in response to a wide range of inhalants. Whereas the effects of siliceous dusts have been accented in the workers' compensation legislation of this century [McLaughlin 1955], the dangers to health inherent in metallic ores were known to the early Greeks and Romans when mining was the preserve of slaves and criminals. Ramazini 1700 classified industrial diseases to encompass the ill-effects of dusts in miners and quarrymen, of metallic ores and pigments in miners and artists respectively, and of musty grains in storehouse workers [Clendinning 1960]. Thackrah 1832 warned that all dusts presented some degree of hazard [McLaughlin 1955].

COPD is increasingly prevalent in urban industrial society, characterised by a long prodromal period without diagnostic radiographic change but marked by pulmonary physiologic function abnormalities detectable in the FEV₁/FVC ratios [Balchum 1969]. Similar disturbance of pulmonary function with normal or minimally abnormal chest X-ray appearance has been described in uranium and other hardrock miners. The abnormal pulmonary function detected in

uranium miners was attributed to an insufficient quantity of blood passing through a restricted capillary bed in the gas-exchanging portions of the lungs, caused by vascular obliteration and by a diffuse interstitial fibrosis affecting the very fine terminal portions of the airways that was too slight for radiographic detection [Trapp et al. 1970].

(iii) *Systemic lesions*

The highly specific localisation on the arterial flow divider is only one of the many features of the rat model which support the suggestion that small arterial lesions and atherosclerosis are remarkably alike [Stehbens 1974b]. It is not inconceivable that sensitisation in the lung might lead to systemic manifestations of generalised vasculitis due to induction of secondary reactions in capillary beds at a distance. Changes in pulmonary vessels have been compared with those in the coronary arterioles [Cole 1944].

Reduction in age-specific prevalence of 'spontaneous' nephrosis in rats, protected by measures which lowered ambient dusts levels in their environment, confirmed that a direct chain of cause and effect links chronic respiratory disease, periarteritis, and chronic murine nephrosis [Tucker & Wyatt 1967, Tucker et al. 1973, Tucker et al. 1975]. Since the homostatic balance favours fibrinolysis in the rat, increased lung sensitisation by environmental dusts might be expected to produce an excess of fibrinogen degradation product (FDP). Glomerular damage by deposition of FDP has been postulated as a cause of renal damage in humans (for citations see Tucker et al. 1975). Acute renal failure accompanies the haemorrhagic lung disease of Goodpasture's syndrome; there is an increased incidence of this syndrome in young men occupationally exposed to solvent and petroleum vapours by inhalation [Beirne et al. 1972; Medical Journal of Australia 1974]. Similarly, renal damage occurred in young women who applied ammoniated mercury in cosmetic preparations to large areas of skin [Kibukamusoke et al. 1974].

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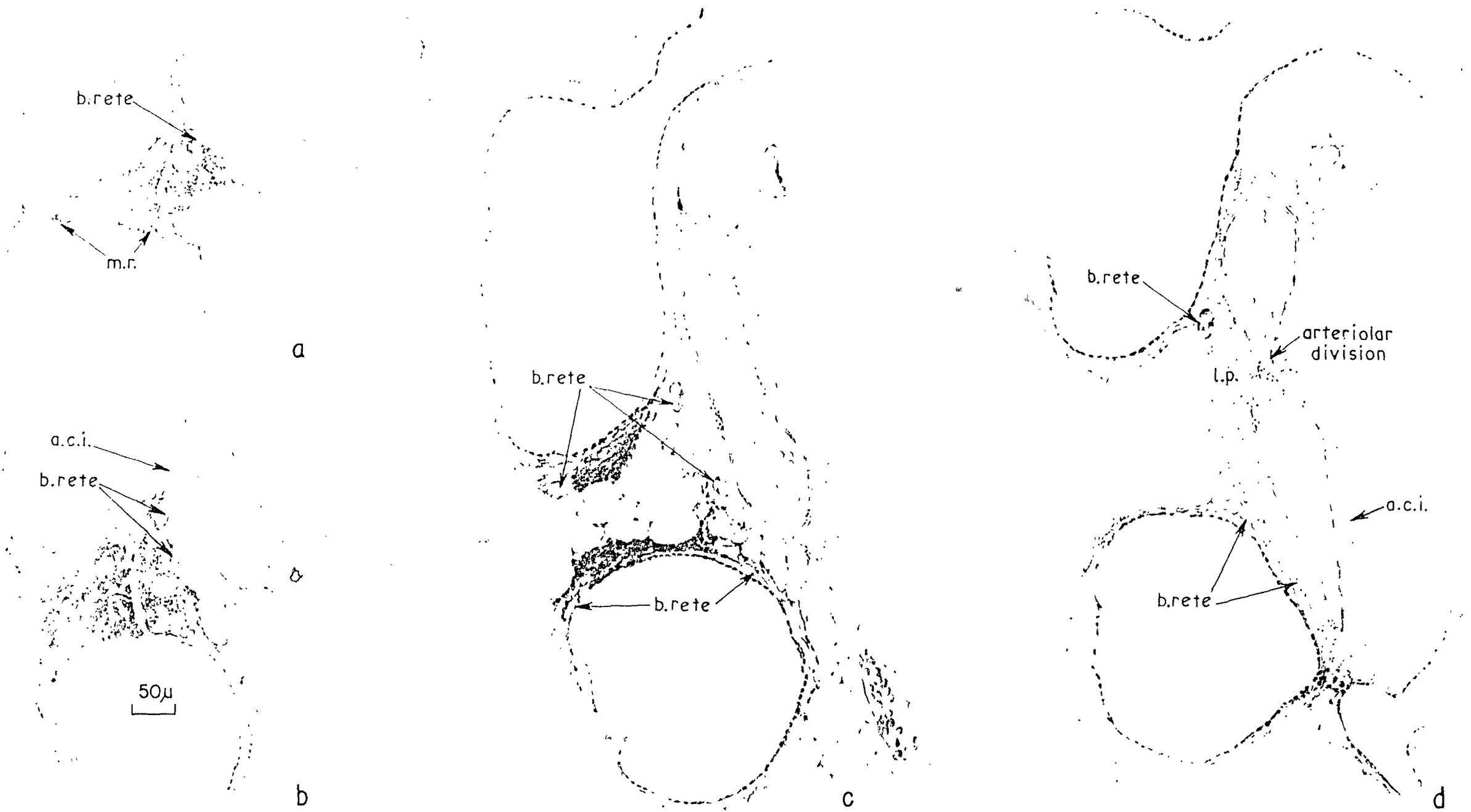
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6. ACKNOWLEDGEMENTS

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(a & b) Extruded carmine particles and cells form a mucoid raft, [m.r.] clinging to the luminal surface of the lymphoid nodule which forms the dividing spur of a terminal bronchiole.

(c & d) Radicles of the bronchial vascular plexus, [b.rete] and pools of lymph, [l.p.] are prominent between the bronchioles and the division of the artery.

ANATOMIC RELATIONS AT THE DIVISION OF A TERMINAL BRONCHIOLE IN A RAT



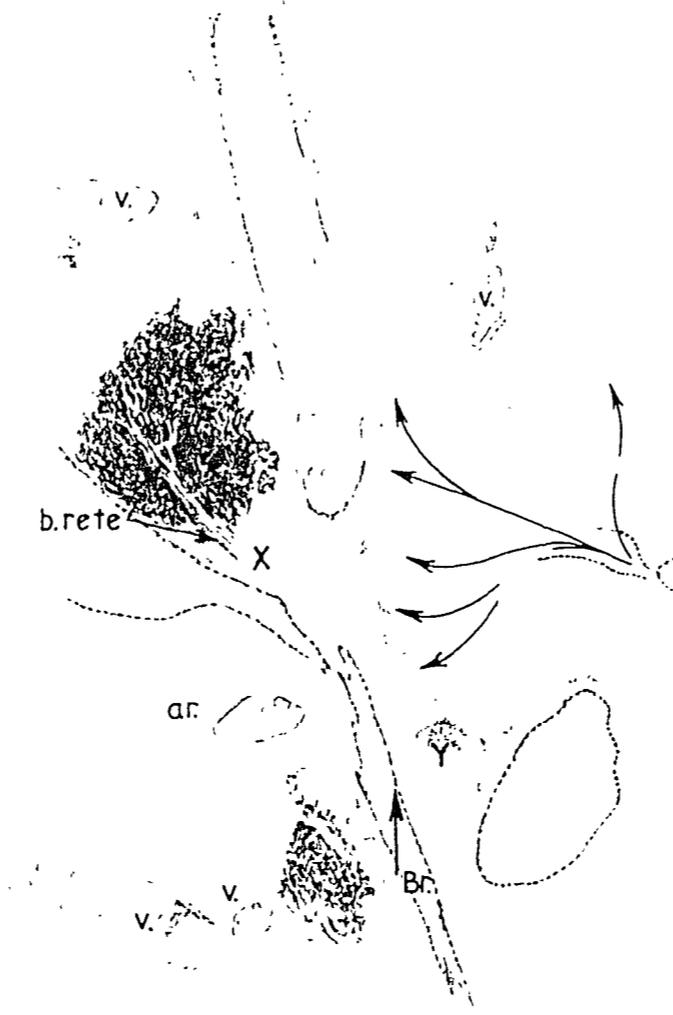
(a) Three-dimensional reconstruction from lengthwise serial sections of a 600 μm artery lying in front of its bronchus.

(b to f) Typical sections of that artery show branching points at X and Y and the relation of lesions on those arterial dividing spurs with lymphatic nodules on the accompanying bronchial bifurcations. Collecting veins, v, pass tangentially to the broncho-vascular bundle.

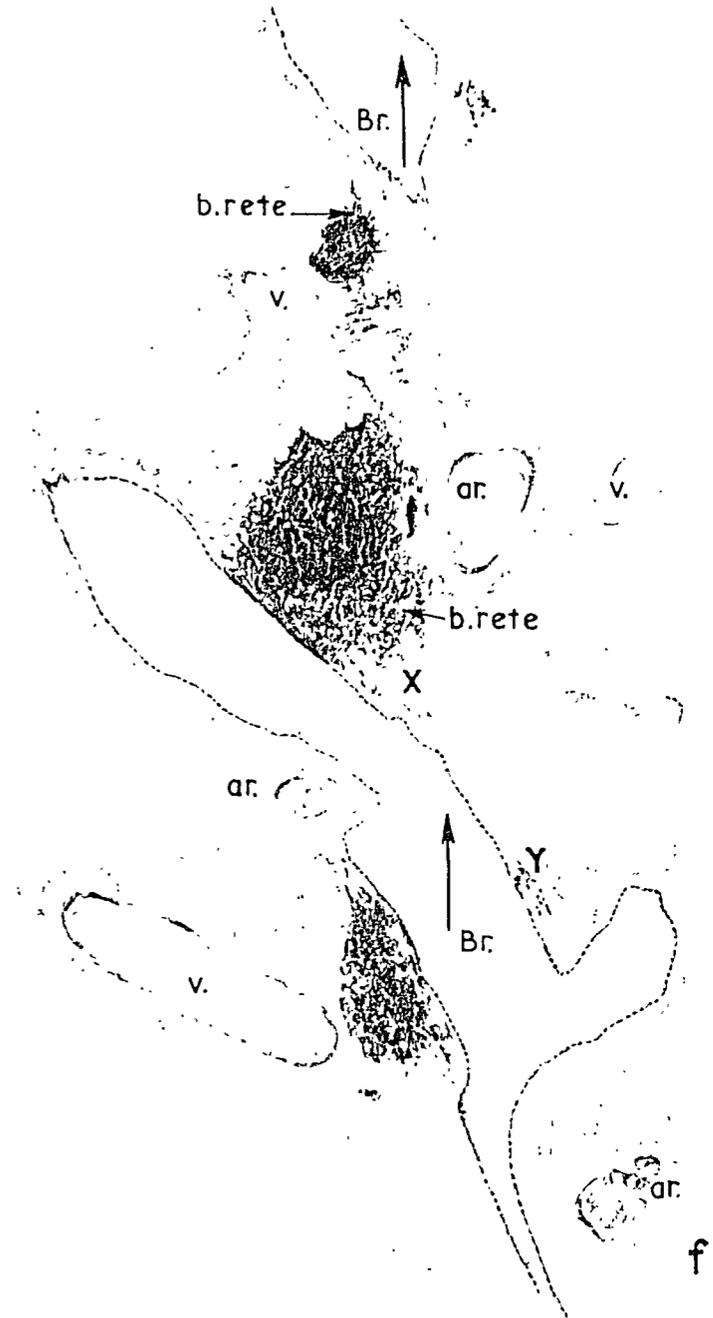
ANATOMIC RELATIONS OF A PERIBRONCHIAL, PERIVASCULAR LYMPH NODE



d



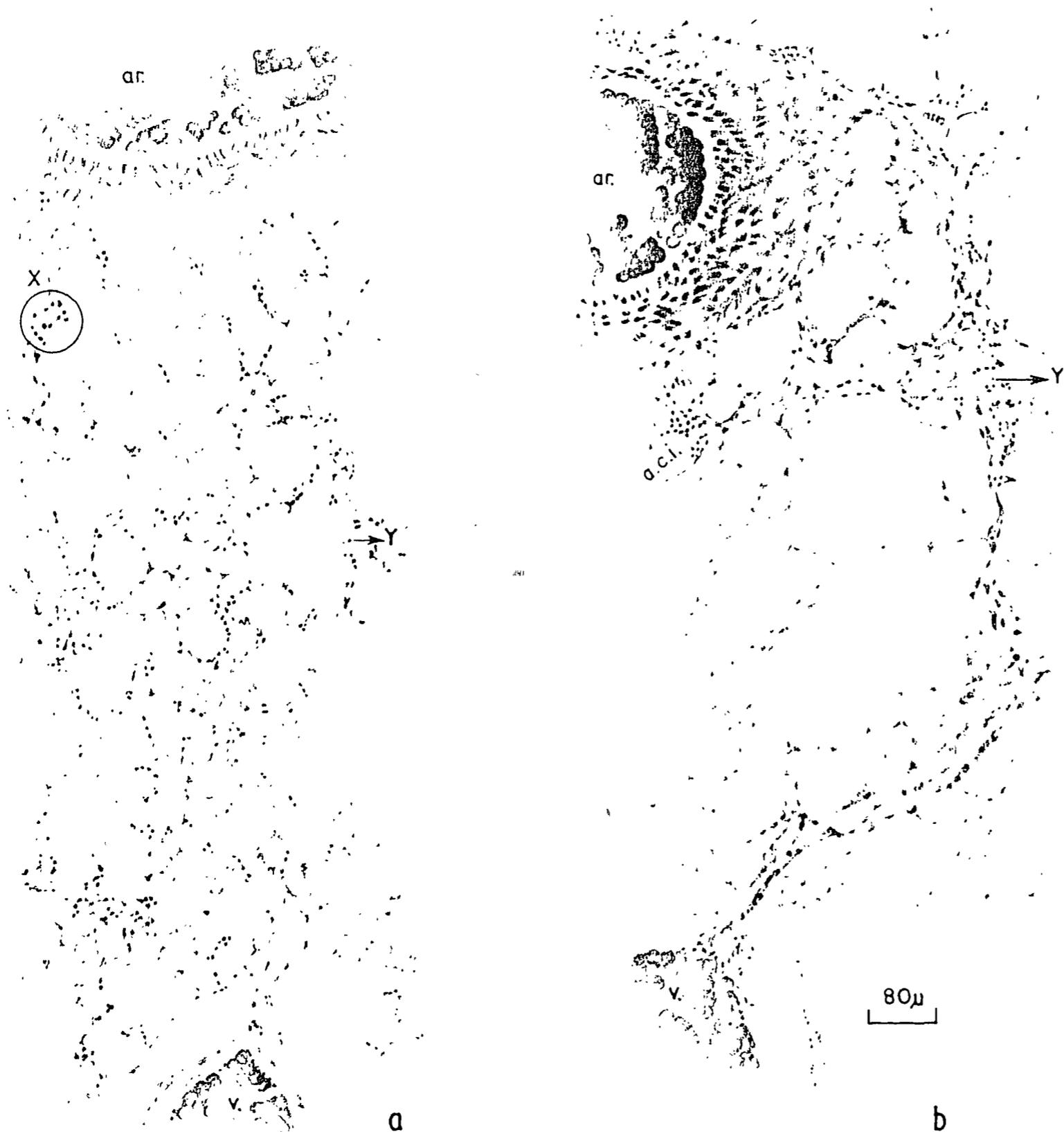
e



f

(d - f) Removal of the posterior wall of the artery reveals lymphoid nodules. These rest upon the wall of the bronchus where it opens into successive branches. (Heavy arrows indicate the course of the bronchus).

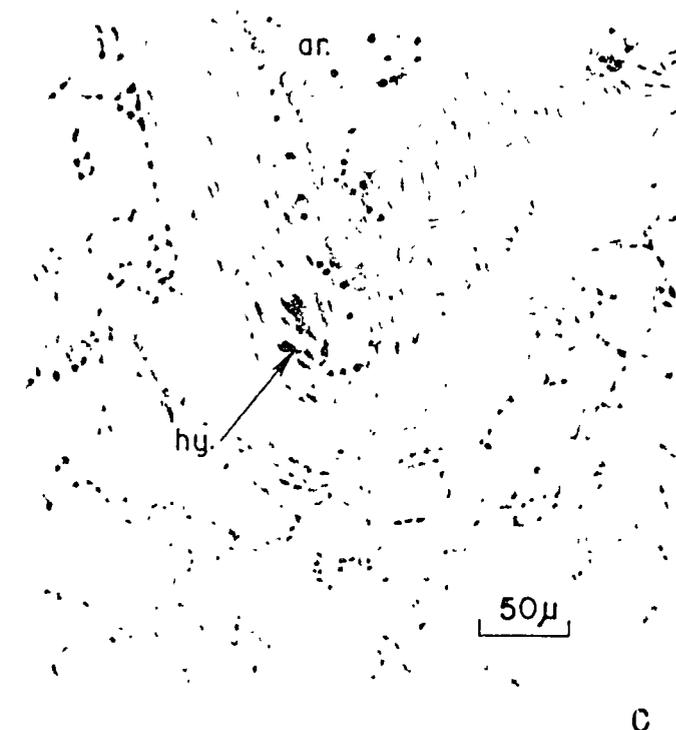
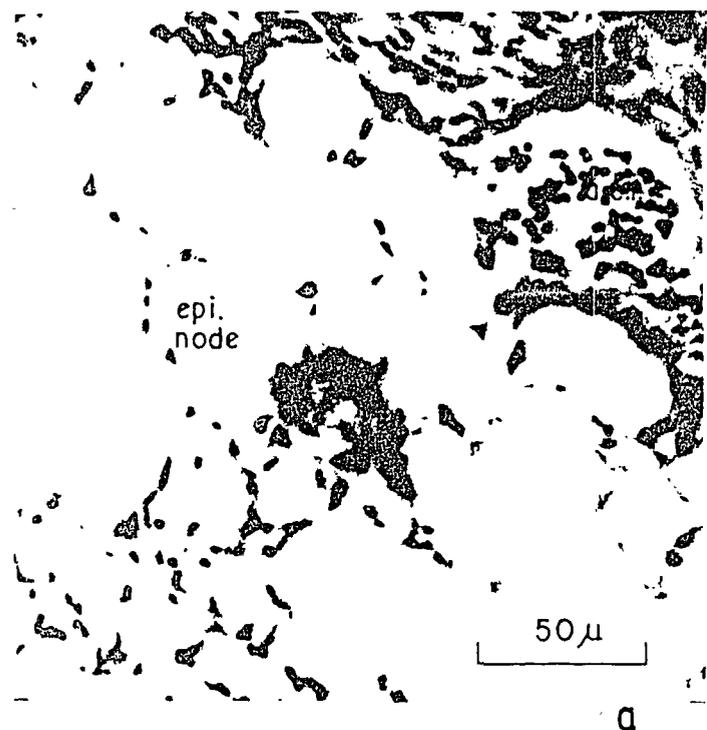
Typical alveolar ducts fan out from a bronchiole-alveolar duct junction. The bronchial vascular plexus (rete) runs through a nodule towards the situation of the lesion on the flow divider (X).



- (a) Faintly marked channels, between the sheaths of collecting veins and the points adjacent to arterial divisions (box in Figure 2b), are detectable in young healthy rat lungs.

- (b) Prominent channels form in less healthy lungs when drainage is concentrated into those branching pathways. Neighbouring alveolar septa become deficient in numbers of cells, perfused capillaries and the amount of interstitial space.

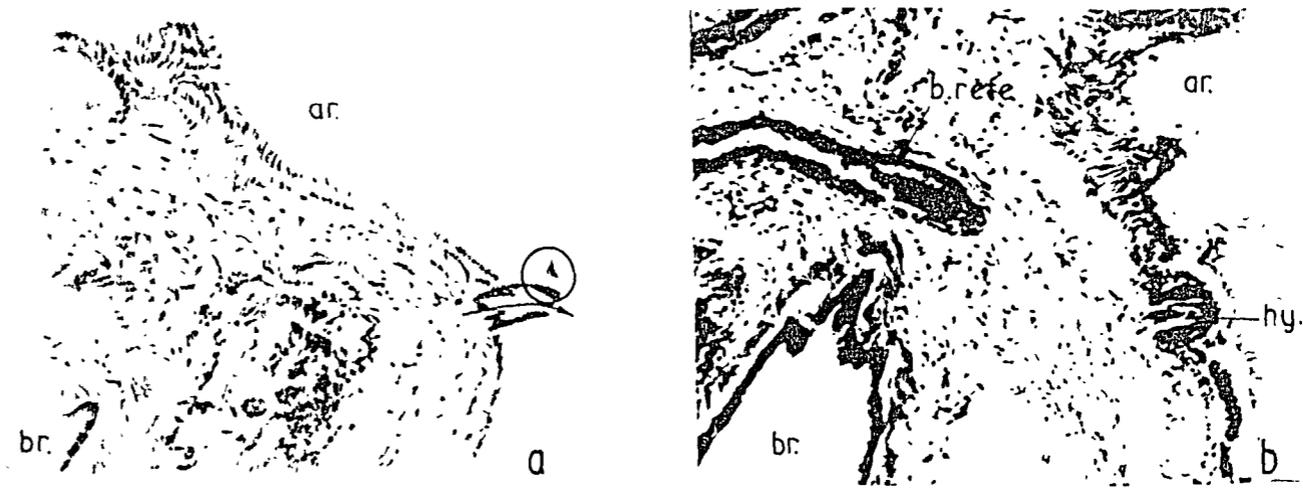
FORMATION OF ARTERIO-VEINOUS ANASTOMOTIC CHANNELS



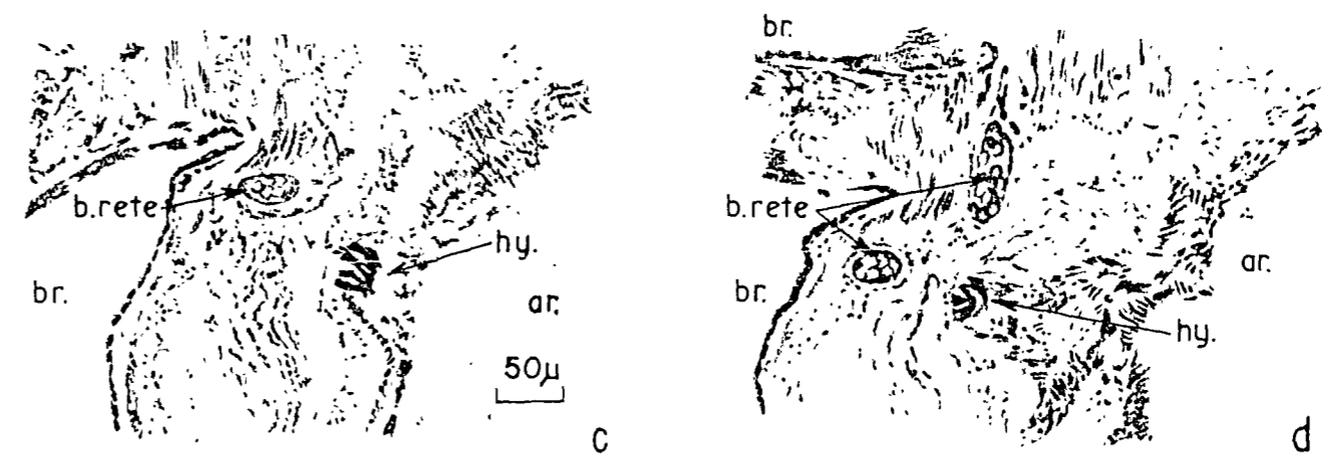
Even in the healthy lung small collections of epithelioid cells form nodes in the course of the a-v channel adjacent to the arteriolar wall (circle Figure 3a).
 (a) This photomicrograph depicts an epithelioid node developed in the less healthy lung, adjacent to an adventitial cellular infiltration, [a.c.i.,] which is coextensive with that shown in Figure 4b.

(b & c) Immediately beneath that position the channel of the a-v anastomosis penetrates the thickened arteriolar wall and joins with an extension of the main lumen. The hyaline lesion appears in this extension through the arterial wall.

JUNCTION OF A-V ANASTOMOSIS AND ARTERIOLAR LUMEN

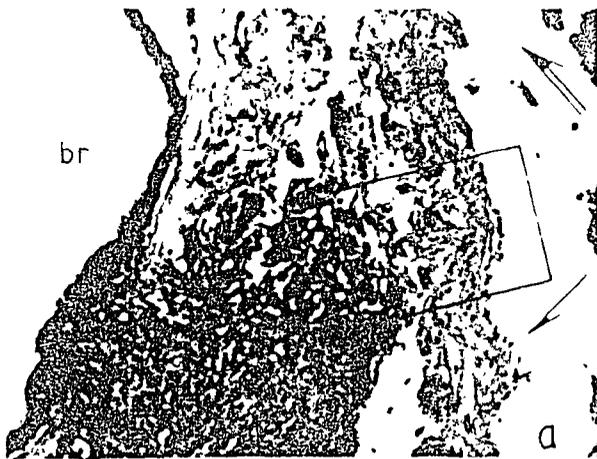


(a & b) The lesion develops symmetrically; that is, it stands out across the leading edge to meet the oncoming, dividing streamline at midstream but, nearer the side walls, draws back level with the surface.



(c & d) At the side wall nearest the bronchus, as at the a-v anastomotic junction (Figure 4b,c), the lesion penetrates the wall but comes into close relationship here with radicles of the bronchial vascular plexus b.rete. This relation is further shown in Figures 2e and 10d.

THE LESION ACROSS THE LEADING EDGE OF THE ARTERIAL FLOW DIVIDER



(a & b) In close relationship with the peribronchial lymph node, the elastic coats of the arterial wall become fragmented and compressed towards the lumen. \rightleftarrows indicates flow in the main artery; \rightarrow is flow into the branch.

STAGES IN THE DEVELOPMENT OF THE LEADING EDGE LESION



c

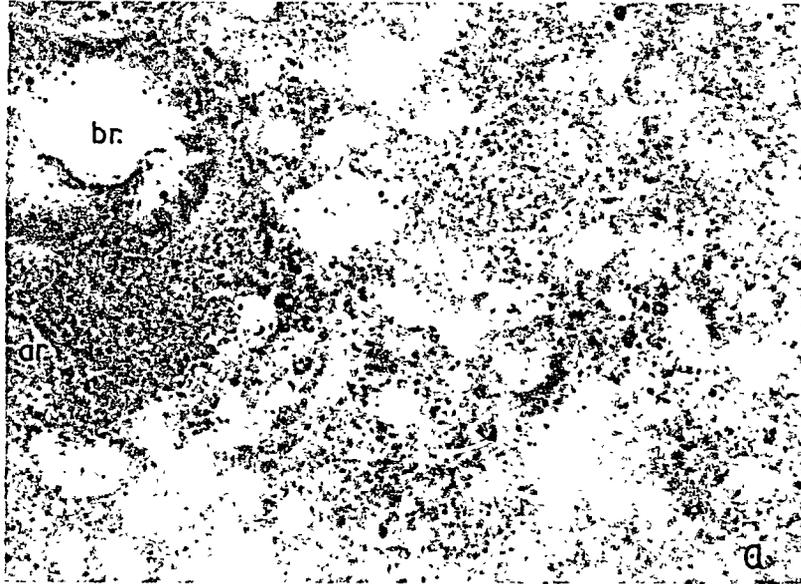


e



f

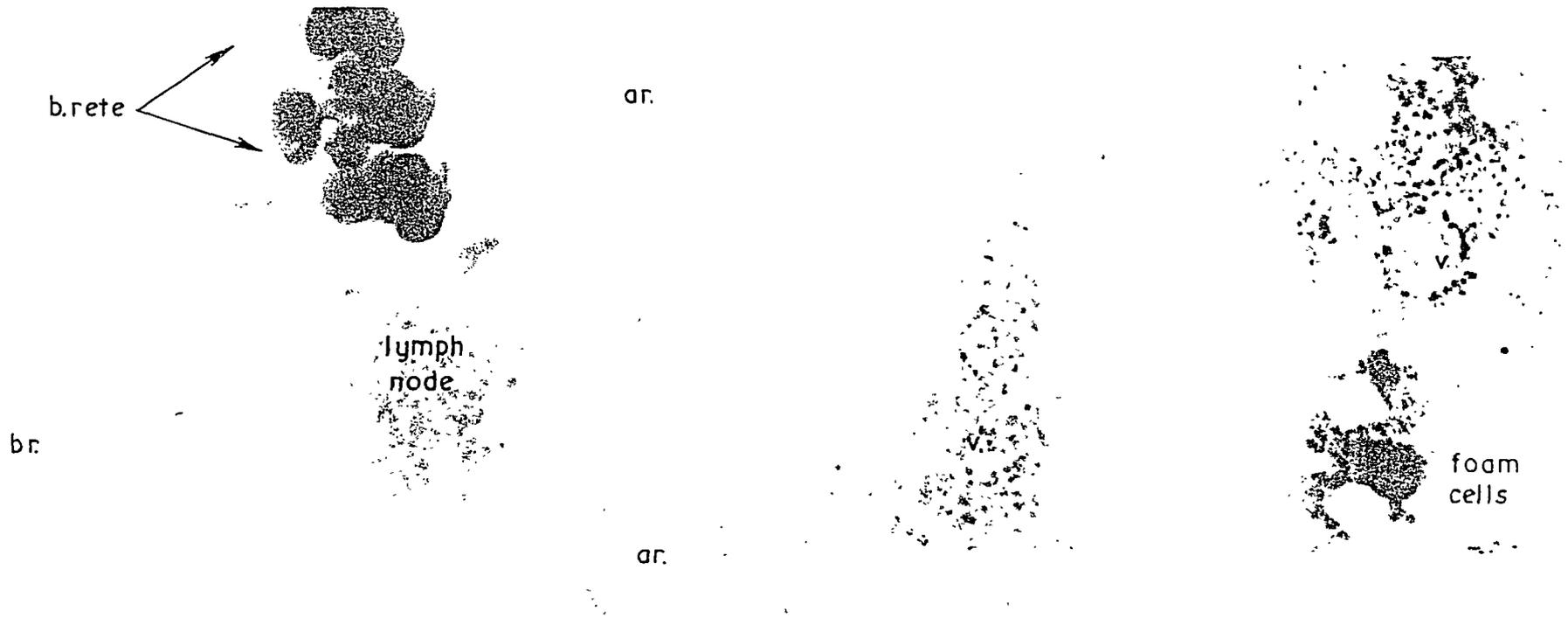
- (c & d) The wall may be thinned at the distal lip and becomes hyalinised as it bulges into the lumen.
- (e & f) Fine arms of hyaline material protrude into the blood stream, appearing as detached bodies when cut in section (see also circle Figure 5a and Figures 10a).



- (a) 24 hours after exposure of rat to heavy dose of cigarette smoke, lipid-laden macrophages stream into and mark direct interstitial pathways to a peribronchial lymph node. Cells lie within thickened alveolar septa.
- (b) After gathering around the adventitial border of the lymph node, lipid-laden phagocytes enter its substance and pass into the bronchial lumen through a rupture of the bronchial epithelium.

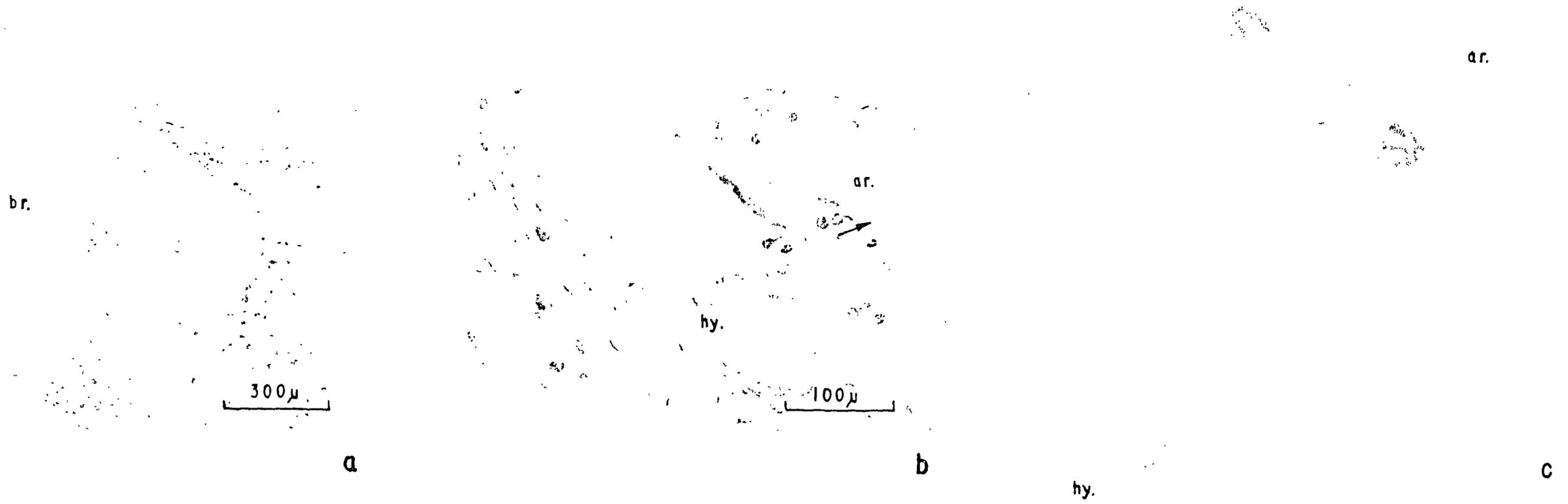
FIGURE 7.

DIRECT INTERSTITIAL PATHWAY FOR EXTRUSION OF PHAGOCYTES TO THE AIRWAY



Following several microdoses of beryllium oxide (calcined at 250°C) at three-week intervals, orange stained lipid-laden macrophages appear in alveolar septa and gather in the lymphoid tissue near the dividing spur of an artery. Red pools indicate deposits of neutral fats adjacent to the lymph node and to the bronchial vascular plexus. b.rete. The black, fine formalin pigment surrounds a thrombosed collecting vein, as emphasised by the inset photomicrograph.

SIGNS OF PHAGOCYTTIC CLEARANCE (lipid) AND OF THROMBOSIS (formalin pigment)



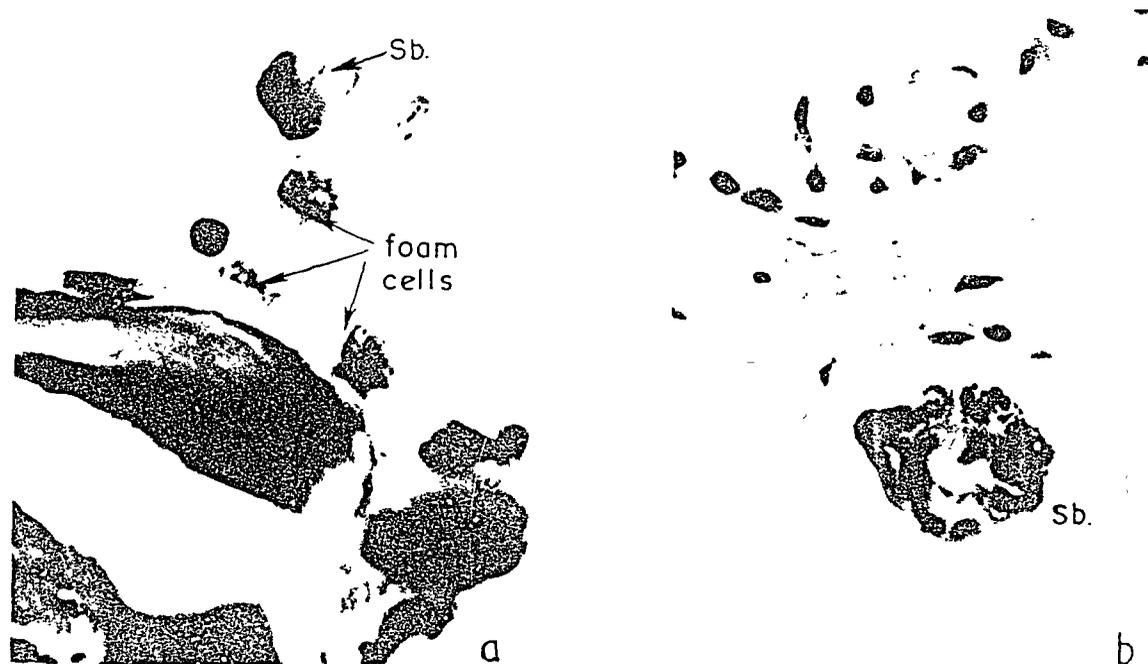
(a) Histochemically stained macrophages, reacting specifically for beryllium, have collected about the bronchiole – some have been discharged into the airway as detritus. The rat had been exposed three weeks before sacrifice to a microdose of beryllium oxide calcined to 900 °C.

(b) A few specifically stained macrophages have entered the loose adventitial interstitium in the base of the cleft which opens into the arterial lumen through the hyaline plaque. One such cell has entered the cleft.

(c) One beryllium-laden polymorphonuclear cell lies in the arterial lumen just beyond the lips of the plaque (cf. Figure 10a).

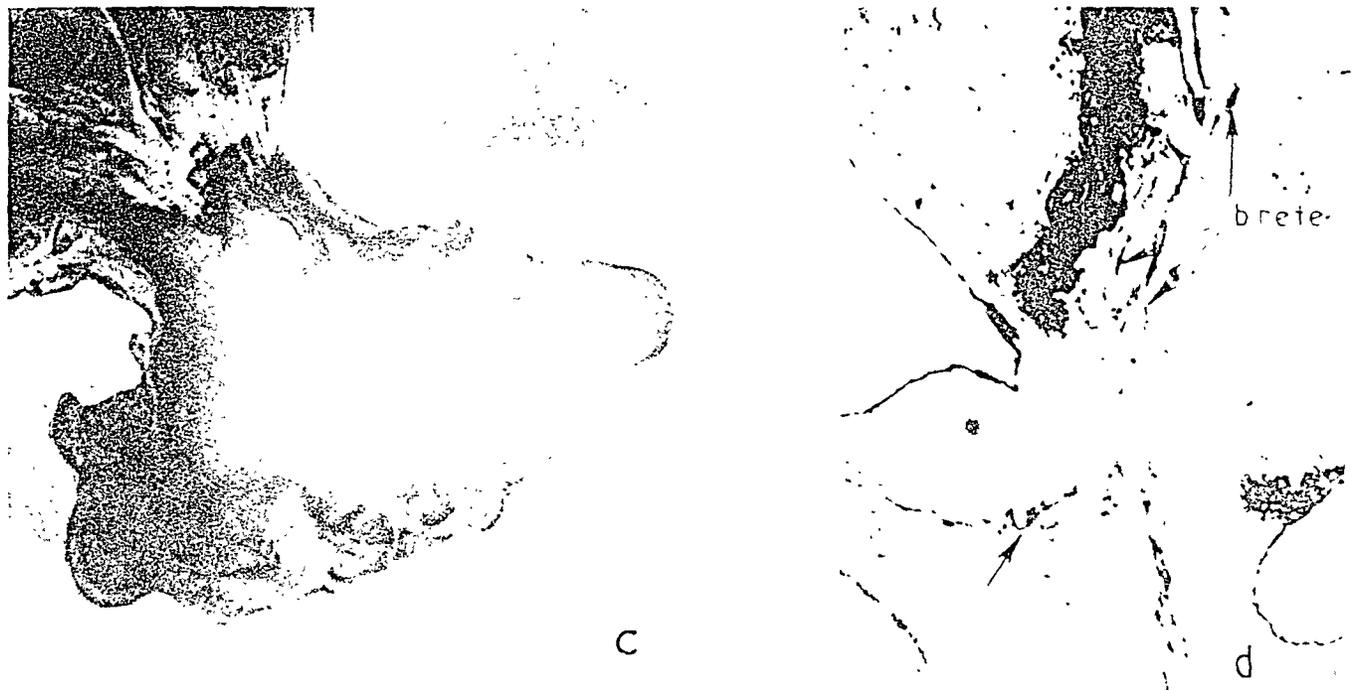
INDICATIONS FOR PASSAGE OF MATERIALS FROM LYMPH NODE INTO ARTERY

FIGURE 9.



- (a) Lipid-laden 'foam' cells enter the arterial lumen through the open tip of a plaque. The seemingly 'free' amorphous body, Sb., is the section of a protruded arm of hyaline material (see Figures 5a circled, 6e & 6f).
- (b) Photomicrograph of a Schaumann body found in a tissue specimen from a case of human pulmonary hypersensitivity (supplied by courtesy of Professor B. Gandevia, University of N.S.W.).

SIMILARITY OF THE HYALINE LESION TO A SCHAUMANN BODY



- (c) The compressed wall layers are folded in the base of this more extensively developed lesion of the leading edge (cf. Figure 6d).
- (d) When rotated 90° anticlockwise, this figure lies in conformity with Figure 5a to d. It extends the emphasis on the manner by which the bronchial end of the lesion on the flow divider is brought into close relationship with the neighbouring lymph node by the radicles of the bronchial vascular plexus, b.rete.

ADDENDUM

Sorokin & Brain [1975] described the simulation of atmospheric dusts for animal exposures with a submicrometre particulate form of iron oxide. Electronmicrographs [Brain *et al.* 1974, Figures 4 and 5] showed that the floccules were agglomerates of microcrystallites which would be of high surface area. (The beryllium oxide used in our experiments [Tucker & Wyatt 1967] (Figure 8) was calcined at 250°C and had a similar form.):

- (i) While iron oxide in this form, obtained by vapour phase oxidation of iron pentacarbonyl, might not be particularly toxic, it could not be assumed to be chemically 'inert' when introduced into the tissues.
- (ii) Though Sorokin & Brain did not stipulate the pulmonary health of their mice, they described some alveolar macrophages 'free' in alveolar spaces of control animals before any exposures. We did not find such 'free' phagocytes in the lungs of healthy control rats [Tucker *et al.* 1973].
- (iii) They did see that some uningested iron oxide particles penetrated into the alveolar interstitium.
- (iv) They also noted that peripheral alveoli were cleared before those adjacent to the peribronchium. Therefore there would be need to localise exactly the three-dimensional situation from which observations shown in photomicrographs were derived if these were to be used to contest our demonstration of a direct interstitial pathway from the periphery of the alveolar ducts to the bronchiole-alveolar duct junctions.

The interstitial pathway demonstrated with inert carmine red was postulated [Tucker *et al.* 1973] to be the physiological clearance mode in the *healthy* lung exposed to *short* exposures of *inert* particles at *low* ambient concentrations. Apart from meeting current objections, this addendum emphasises the need to establish the physical form and the chemical properties and reactivities of inhaled particulates. This need has frequently been recorded [*e.g.* Donoghue *et al.* 1972] but it has not been acted upon energetically in the past. Concurrent with the anatomic observations recorded in this paper and those directly related to it, a program of collaborative research into chemical toxicology has been in progress. A preliminary report of the increased biological reactivity of thermochemical derivatives of ammonium uranate is now

available [Stuart et al. 1975].

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