THE CELLULAR RESPONSE IN INFLAMMATORY REACTION IN THE DUCK

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "THE CELLULAR RESPONSE IN INFLAMMATORY REACTION IN THE DUCK" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or society.

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CERTIFICATE

Certified that this thesis entitled "THE CELLULAR RESPONSE IN INFLAMMATORY REACTION IN THE DUCK" is a record of research work done independently by Smt.K.V.Valsala under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to her.

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INTRODUCTION

INTRODUCTION

Ducks have been domesticated all over the world and in many parts they form an integral part of the human food source. Although not so popular as the chicken as meat or egg producers they have established themselves as an important source of protein food atleast in certain regions. From an aquatic life over the years ducks have adopted to a semiaquatic life and of late, efforts are being made to rear them on terrestrial intensive system of management as in the case of chicken. Concerted efforts have been made to evolve breeds with a potential to produce eggs on an average 250 to 300 per annum. During the last decade there has been considerable improvement in the genetic potential of the duck for productivity. Moreover, in our country farmers have taken up rearing exotic breeds of ducks like the Khakicampbell, which are high producers.

In Kerala, rearing of ducks has all along been considered as a profitable proposition by farmers and there is a population of 4.3 lakhs of ducks in this state. The disease resistent local duck with low productivity was the backbone of the duck farmer in this state. The ducks were allowed to house in the paddy fields after harvest and they were moved from place to place, even into the neighbouring states. The traditional duck farmer in Kerala although

largely inclined to adopt classical practice of pasture rearing of ducks has also imbibed to some extent the latest technology in the form of breeding high producing stock and also practising intensive rearing. The introduction of exotic breeds of ducks with change in the management practices as in the case of other livestock has also led to change in the disease profile of ducks and new disease problems have cropped up.

In 1976, there was a severe outbreak of duck plague in the state in which a sizeable population of ducks was wiped out. This created an awareness both on the farmers as well as animal disease workers in this state regarding the necessity for proper disease control measures for ducks. This has resulted in focussing attention on duck disease, especially clinicopathologic manifestation, and prophylactic and therapeutic measures.

A proper understanding of the disease profile and manifestation is very important for undertaking disease control measures. The mechanism of the interaction between the invading agent and the host tissue system determines the disease manifestation and this has to be thoroughly understood. All pathogens basically induce an inflammatory reaction in the tissues and the intensity and type are

important factors in determining the outcome of the disease. The inflammatory process involves active participation of the different tissue components and mediators. Extensive investigations have been made on this important biological process in the case of mammals. There has not been much information on chemical, biological, vascular and cellular nature of the inflammatory response in the avian species. However, in chicken Carlson and Starr (1969), Nair (1973) and Awadhya et al. (1981) have made very valuable contributions on the cellular involvement in inflammatory process in the chicken.

There has not been any systematic in-depth study on the various aspects of inflammatory process in the duck. A clear understanding of the chronology and type of cellular involvement is very essential to fix criteria for pathological diagnosis. This would also help to clarify the mechanism in the spontaneous duck diseases. Therefore, with the objective of elucidating the basic changes involved in the inflammatory process in the duck, studies were undertaken to delineate the mechanism of inflammatory reaction in the duck using different chemical and biological agents. Besides this, with a view to understand the response involved in spontaneous diseases, taking Duck Plague and Ranikhet disease as models of viral infections a study was designed and the pathogenesis and pathology were worked out.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

1. Inflammatory response in Mammals

1.1. Views developed in ancient times

Many of the manifestations of inflammation are easily seen and felt and there seems no doubt that ever since man has emerged as a thinking animal, he has been concerned with its study. In consequence to his concern and curiosity, a vast amount of literature on this subject has accumulated dating as far back as the Egyptian civilisation.

In the recorded histories of ancient civilizations, there exists abundant evidence to indicate that atleast some types of pathological lesions, particularly those involving the skin and external organs, were well-known. The Babylonians appear to have been familiar with certain disease states, and in the code of Hammurabi which is thought to have been composed about 1950 B.C., certain specific rules were laid down on a number of operative procedures, the aim being to safeguard the life of the patients. Two important documents that have been preserved, the Edwin Smith surgical papyrus, 1600 B.C., and the Eber papyrus, 1550 B.C., provide some indication as to what the ancient Egyptians knew about medicine. These papyri deal

with a number of kinds of inflammation ie., abscesses, ulcers, carbuncles and "erysipelas".

During the Greek era, Hippocrates, the so-called "father of medicine", was apparently able to recognise and describe accurately a number of disease states such as pneumonia, tuberculosis, malaria and typhoid fever.

The outstanding work of the Roman period was written by Cornelius Celsus, (30 B.C. - 38 A.D.), in the form of a review of much of the known medical knowledge at the time. It is in this review that the well-known descriptive sentence familiar to all students of pathology appears for the first time in the literature. "Now there are four diagnostic marks in inflammation, redness and swelling with heat and pain". These have come to be known as the cardinal signs of inflammation, and obviously represent a classical description of the inflammatory process as seen by the uninitiated in every day life. Galen, (130 - 200 A.D.), another influential physician of his time has added the loss of function as the fifth cardinal sign of inflammation.

1.2. Views developed in the period of Renaissance to the end of the 19th century

Although the light microscope was available from 1624 A.D onwards, it was not widely used in the study of diseases

until the 19th century. Rokitansky and Virchow used the microscope at this time for the study of disease. Virchow appears to have been the first to have propounded the cellular concept of disease, and was of the opinion that disease was in actual fact, an aberration of the normal physiology.

John Hunter (1728 - 1793) is considered to be the father of experimental pathology. By emphasising the 'salutory" nature, as he called it, of the inflammatory process in combating disease, he appears to have stimulated speculation and experimentation into the mechanisms underlying the vascular and other local changes participating in this response.

The microscope was used during the 19th century for the <u>in-vivo</u> study of the changes associated with inflammation. Dutrochet, in 1824, and William Addison, in 1843, were the first to describe the adhesiveness of white cells with respect to the vascular endothelium. Cohnheim (1882), following his studies with the tongue and mesentery of the frog commented on the increased transudation of fluid causing infiltration and swelling of the inflamed tissues. Cohnheim also attempted to relate the changes in living inflamed tissues to Celsus' classic signs of inflammation. He correlated the redness with the abnormal congestion of

the vessels and the swelling with the transudation of fluid; pain was related to sensory nerve endings, and heat to the increased arterial flow.

Metchnikoff (1845 - 1916) was the first to emphasise the importance of phagocytosis in the inflammatory process. At this time, Klebs, Koch and several others were familiar with the presence of microorganisms within white corpuscles in the inflamed tissues but they were of the opinion that the microorganisms found this environment suitable for their growth and multiplication, and in addition it was thought that the leukocytes favoured their dissemination. Metchnikoff emphasised his view that the conspicuous vascular changes that take place in inflamed tissues are instrumental in mobilising to the site the various defensive elements of the body.

Starling, as early as 1896, put-forward a theory in an attempt to explain the exchange of fluid in the capillary bed under physiological circumstances. He considered the capillary wall to act as an inert filter relatively impermeable to proteins. In assuming this, he postulated that if the capillary pressure of the blood was in excess of its osmotic pressure, there would be as a result an outward flow of fluid, and if the reverse was true then there would be an influx of fluid to the lumen of the vessel.

Landis, in 1934 was able to demonstrate that there was in actual fact a pressure gradient higher on the arteriolar side and lower on the venous side.

Arnold, in 1876, postulated the existence of a "cement" substance between the endothelial cells, and it was thought that alteration in the natural properties of this element was responsible for the increased permeability exhibited by the inflamed endothelium. This view appears to have been widely accepted until fairly recently, when with the onset of the electron microscope, and the development of new techniques, the presence of such a cement material between the endothelial cells, as described by Arnold and a lot of other investigators, has not been confirmed. This point is however still debatable.

Alongside the postulation and experimentation concerning the increased permeability exhibited by the injured vascular endothelium, which was stimulated by the observations of Cohnheim, there was a similar interest and experimentation regarding the phenomenon of cellular emigration.

One of the most confusing and most difficult subject in the history of pathology and forming a debatable point, is the origin of cells in the inflammatory exudate. The most popular theory that persisted for many years, was

perhaps that proposed by Maximow in 1906. Maximow was of the opinion that all mesenchymal cells are derived from an undifferentiated blast cell which in the bone marrow is a free haemocytoblast and, in the other tissues, is in the form of a fixed undifferentiated mesenchymal cell. He proposed that the large lymphocyte was such a multipotential cell and that it was empowered to transform into a macrophage or even a fibroblast. Tempting as this theory may have been at the time, there are today several serious objections as to its validity.

Metchnikoff observed the behaviour of the "microphage", (neutrophil) and the macrophage. He observed that the microphage was the first cell to appear in the exudate and that it formed the first line of defence.

The macrophage, the persistent scavenger cell in inflammatory exudates, was so named by Metchnikoff but it was von Recklinghausen in 1863 who described this cell in detail. He ascribed its origin to fixed tissue histiocytes. Since that time a number of possible origins for this cell have been suggested, e.g. Merchant (1890) ascribed its origin to adventitial cells, Ranvier (1890) to lymphocytes, Mallory (1893) to endothelial cells, Kiyono (1914) to monocytes.

It is obvious from the available literature that

bacteriology and immunology had a profound effect upon the research on inflammation during the second half of the 19th century. The effect of modern trends in biochemistry on the research work on inflammation became apparent during the last few years of the 19th century and the early days of the present century. Sir Thomas Lewis was one of the first to have drawn the attention of research workers to the biochemical aspects of the inflammatory process, following his observations on the role of histamine as an inflammatory agent, and his classic description of the "triple response" (Lewis, 1927). Since that date a tremendous amount of work appeared in the literature on the possible mode of function of a number of vasoactive amines and polypeptides as chemical mediators in inflammation.

By the end of the 19th century it became clear that the inflammatory response was not a simple event, but on the contrary, a series of events that follow a more or less predetermined course. At the same time, it was also universally accepted that both the increased vascular permeability and the cellular emigration and infiltration are part of the same reaction and that they are of equal significance.

1.3. Vascular permeability

1.3.1. Introduction

The present work is mainly concerned with the cellular

aspects of the inflammatory reaction, but since increased permeability forms one of the major aspects of the inflammatory process, which is intimately linked with the cellular changes, it is considered appropriate to give a concise summary of a number of facts and postulates concerning this aspect of the inflammatory process.

This concise review is based entirely on what is known of the mammal and lower vertebrate species, as no relevant information appears in the literature regarding the avian species.

1.3.2. Normal

As stated above Starling (1896) assumed that vascular endothelium to act as an inert filter relatively impermeable to proteins and suggested that if the hydrostatic pressure exceeded the colloid pressure of the blood there would be an outward filtration and that if the reverse hold true an inward flow would result. In 1926 Landis began a series of accurate measurements of capillary pressure by using micropipettes. He was thus able to measure the hydrostatic pressure in both arteriolar and venous capillaries and he described a consistent pressure gradient higher in the arteriolar and lower in the venous side (Landis, 1934).

It was subsequently realised that the capillary endothelium is not only permeable to water and electrolytes but

to other larger molecules as well, but not as large as those of the blood proteins. Pappenheimer (1953) remarked on the similarities between the characteristics of capillary permeability and that of artificial porous membranes.

Some authors strongly support the existence of an active vesicular transport system across the endothelial cell (Palade, 1953; Moore and Ruska, 1957). Fawcett (1959) observed that such vesicles were as a rule distributed along the border of the endothelial cell and none was seen in the interior of the cell. He considered it unlikely that these vesicles play a significant role in transporting colloids from plasma to extra cellular space.

To summarise, there does not appear as yet a single hypothesis which can account for all the known facts of capillary permeability. At one extreme are the supporters of the idea of passive diffusion and hydrostatic flow of fluid and solutes through pores or slits of small but fixed size in a non-living barrier. On the other extreme are those supporting the hypothesis of the movement of fluid and solutes in and out of the endothelial cytoplasm and across the cell membrane.

1.3.3. Altered vascular permeability

One of the important and characteristic aspects of the inflammatory response is the increase in vascular permeability

Leading from the early observation of Cohnheim (1882), the nature of the defect in the vascular wall which allows the free passage of larger molecules has been and still is a subject of experimentation and speculation, and a number of theories have since been proposed in an attempt to explain it, some of which are:

1.3.4. <u>The hypothesis of the active transport apparatus</u>

The observation that the vascular endothelium was able to take up injected colloidal particles suggested to several investigators that it was actively participating in the exchange of material between the blood and tissue compartments (Biozzi, *et al.* 1948; Ovary, 1958; Gozsy and Kato, 1960; Palade, 1961). This observation however should be looked upon with suspicion as the behaviour of ferritin, a denatured protein, and colloidal gold etc. which were used as markers, may not be similar to that of plasma proteins and may in fact be treated by the endothelium as foreign (Spector and Willoughby, 1963).

The increase in the number and size of intracytoplasmic vesicles in the injured endothelial cells prompted several investigators to speculate that this increase indicates in actual fact an augmentation of the normal, physiological active transport system (Moore and Ruska, 1957). Alknse (1959) described similar changes in the endothelium of dermal capillaries of the mouse following

the topical application of histamine, and suggested that histamine acts directly on the endothelial cell to stimulate the active transport mechanism.

1.3.5. Separation of endothelial cells

Majno and Palade (1961), using the rat cremaster muscle and stimulating its vasculature by local injections of histamine and 5-hydroxytryptamine (5-HT) reached a different conclusion from that reached by Alknse (1959) and Moore and Ruska (1957). They suggested that the effect of histamine and 5-HT was to cause the separation of endothelial cells.

Haddy (1960) and Rowley (1964) observed that both 5-HT and histamine cause a rise in the intravascular pressure following venous constriction. Rowley attributed the separation of the endothelial cells at the intercellular junction to the increased intravascular hydrostatic pressure.

Landis and Pappenheimer (1963) appeared to support the idea that the increased vascular permeability following the application of 5-HT, histamine and bradykinin was not merely due to the increased venous pressure but also to a concurrent loosening of the surface bonds between the endothelial cells, which in turn may be facilitated by the

increased hydrostatic pressure.

Spector and Willoughby (1965) summarised the possible mechanisms of increased vascular permeability induced by histamine as follows:-

I. Constriction of the small veins leading to increased hydrostatic pressure in the venules with subsequent "forcing out" of protein.

II. A contractile mechanism in the endothelial cell, similar to that of smooth muscle which is contracted in the presence of histamine. The cells might thus draw apart and in doing so permit the formation of intercellular gaps.

III. An alteration in the permeability of the cells causing them to alter their shape and thus move apart (possibly by a rounding-up process).

IV. A combination of these events plus an effect on phospho-protein turn over at the surface of the cell or possible alteration of the surface cell charge leading to repulsion of adjacent cells".

1.5.6. Basement membrane in vascular permeability

Following the failure of the electron microscope to demonstrate the postulated pores of Pappenheimer in the endothelium, attention was focussed on the basement membrane as forming the limiting selective factor in

vascular permeability, assuming that the vascular endothelium yielded a free access to colloids with or without the proposed vesicular transport system (Fawcett, 1959; Palade, 1953; Bennet; Luft and Hampton, 1959; Palade, 1961).

Bennet *et al.* (1959) stated that vessels with a detectable basement membrane have a limited permeability to proteins, whilst vessels with an incomplete basement membrane, such as lymphatics and liver sinusoids, do in fact exhibit much higher permeability to protein than other vessels.

Palade (1961) was not able to demonstrate pores of any kind in the basement membrane and he postulated that if such pores do exist they must be so tortuous as to be invisible.

1.3.7. Diphasic nature of increased vascular permeability

Sevvit (1958) made the int resting observation that in thermal injury the increased permeability in the cutaneous vessels of the guinea pig is diphasic. His observations were confirmed in the guinea pig, rat and rabbit (Spector and Willoughby, 1958 and 1959; Wilhem and Mason, 1958 and 1960; Allison and Lancaster, 1959).

The delayed prolonged phase of vascular permeability involves mostly the capillaries (Cotran and Majno, 1964)

unlike the early transient histamine type of response which affects mostly venules.

1.3.8. Mediation of the vascular events in inflammation

Spector and Willoghby (1963) stated that evidence in favour of the existence of endogenous chemical mediators of the permeability response comes from the ability to demonstrate their presence at the time when they should be exerting their effects and their apparent absence from the area when the inflammation subsides or has not as yet begun. Even stronger support for this theory comes from the ability to suppress the inflammatory changes by the use of specific antagonists.

1.3.9. Increased permeability and leukocytic emigration

Hurley (1963) showed that increased vascular permeability and leukocytic emigration can be induced independently from each other.

Cotran and Majno (1964) observed that vascular leakage and leukocytic emigration did not necessarily take place from the same vessel.

Hurley (1964) suggested that leukocytes in their passage through the basement membrane in some way impair its ability to retain particles which reached its luminal surface, as a result of increased vascular permeability.

1.3.10. Conclusion

Relatively recent research work has shown that the permeability response in inflammation is sometimes biphasic, consisting of an early short lived phase and a delayed but prolonged phase. The delayed prolonged phase appears to be the essential part of the reaction. The early transient phase is probably mediated by histamine and/or 5-HT but no mediator has yet been described for the delayed prolonged response. Recent work suggested that direct injury to vessels may be responsible for the delayed prolonged phase.

1.4. Cellular Aspects of Inflammation in Mammals

1.4.1. Introduction

One of the major features of the inflammatory process is the emigration of leukocytes from small vessels, usually venules, to the injured tissues. Emigration appears to be preceeded by the margination of the leukocytes from their normal position in the centre of the blood stream to its periphery and by the sticking of such cells to the luminal surface of the endothelium. Although this process of sticking to the endothelium has been observed, described and studied for the last 150 years or so, the mechanism(s) responsible, whether physical or chemical, still remain

largely obscure. Despite the discovery of a number of agents which appear to be able to block the adhesion of the white cells, their use has not shed any more light on the mechanism(s) controlling this process. The actual mode of emigration of these cells from the vessels is perhaps slightly better understood, although some disagreement still exists on this aspect.

Leukocytic emigration is usually apparent within a matter of minutes following the application of an irritant but the major wave of it takes place two to six hours after such stimulation.

One of the debatable aspects of inflammation is the transformation of the early and usually predominantly neutrophilic exudate to one of mononuclear predominance. There are three possible explanations of the process of transformation and these will be discussed in detail later.

1.4.2. Historical data

Dutrochet in 1824 was apparently the first to describe the margination, sticking and emigration of leukocytes and considered it possible that the vessels had "lateral" openings through which the blood can discharge its elements into the tissues. Arnold (1875) was of the opinion that

both white and red cells emigrated through the intercellular cement which was thought to be present between the endothelial cells.

Addison (1843) induced inflammation in the web of the frog's foot. He noted that within half an hour the number of "globules" (leukocytes), adhering to the vessels had increased considerably and that some of them were already outside the vessels.

Cohnheim (1882) was of the opinion that molecular changes in the endothelium were responsible for the events seen in inflammation. He also seemed to be familiar with the ameboid movement of leukocytes.

Adami (1909) was in favour of a passive nature of emigrating leukocytes. Later however he dropped his argument and supported the ameboid character of leukocytes.

Clark et al. (1936) using the Sandison rabbit's ear chamber carried out a critical study on the sticking and emigration of leukocytes. Clark and Clark (1935) studied the same phenomena in amphibia. At this time they also hinted at the concurrent emigration of all types of leukocytes but they did emphasise the fact that the polymorphonuclear leukocytes appeared to make their way through the wall much faster than the other types. These investigators failed to agree with Metchnikoff's view that sticking and emigration

were in consequence to chemotaxis but on the other hand they agreed with Cohnheim's opinion that a change in the endothelium is an essential preliminary to the sticking of the leukocytes. Goodman et al. (1973) studied the inflammatory response to endotoxin and observed adhesion of leukocytes to the endothelium of arterioles and venules in three minutes. Emigration of cells was observed in three hours.

1.4.3. Intercellular cement and endocapillary layer

Concurrent with the interest in the ultrastructural study of cellular emigration, there was also an interest in the aetiology of sticking of the leukocytes to the vascular endothelium. The electron microscope failed to confirm the speculations of early investigators with regard to the presence of a cement material or a layer of fibrin covering the luminal surface of the injured endothelium.

Grant (1965) remarked that it was quite possible that the fixation and dehydration procedures employed today in preparing tissues for electron microscopy might wash out, or in some way alter, the luminal material that might have been there.

Luft (1966) was able to demonstrate that the postulated endocapillary layer does in actual fact exist and probably

consists of mucoprotein or mucopolysacharide.

1.4.4. Mode of Leukocytic emigration

1.4.4.1. Emigration through the inter endothelial junction

Marchesi and Florey (1960) were able to observe that the leukocytes passed through the endothelium by first producing a pseudopodium. The leukocyte, having passed between the endothelial cells of the vessel, was then able to move on through the basement membrane of the vessel, or it displaced the basement membrane from the endothelium and come to lie between it and the endothelial cell. They finally found a gap between the pericytes and fibrils and they were thus able to steam out into the perivascular tissues.

The observations of Marchesi and Florey differ from those of another group of workers whose results were published in the same year (Williamson and Grisham, 1960 and 1961). Working on the inflamed pancreatic vessels of the dog, they observed development of numerous cytoplasmic process, projecting into the lumen of the vessels. Leukocytes, neutrophils in particular, appeared to be embedded in these processes and were subsequently completely envelpped by endothelial cell. There was no mural defect in the endothelium communicating between the lumen and the perivascular space. In addition they comment on their observations that the vascular endothelium in this particular experimental system

has shown a striking specificity for polymorphonuclear leukocytes, although a very small number of lymphocytes have been observed passing through the endothelium. The phenomenon was not however observed in the rat and the writers concluded that it may be organ or species specific, or alternatively merely inherent to their particular experimental system.

At the present time it is considered that the intercellular route of migration is probably the more common but that exceptions occur in situations such as that described by Williamson and Grisham and by Marchesi and Gowans (1963) whose observations on the intracytoplasmic passage of the small lymphocytes through the endothelial cells of the post-capillary venules in the lymph nodes of the rat are universally accepted.

1.4.4.2. Studies on the electrical potential on the cell surface

McGovern (1957) was stimulated by the hypothesis that a change in electrical potential could account for white cell sticking and emigration, and he postulated that tissue heparin may normally function in preventing sticking under normal circumstances and that heparin may have anti-white-cell sticking properties.

McGovern and Bloomfield (1963) were however, unable to alter white cell sticking with heparin in traumatised tissues but they observed that heparin itself could initiate the events leading to leukocyte emigration. The fact that heparin could initiate leukocytosis was noted earlier by Copley (1948).

Spector and Willoughby (1963) suggested that the phenomenon of adhesion of leukocytes to endothelium could be explained in terms of the electrochemical forces operating at cell surfaces.

Bangham (1964) demonstrated that the adhesion of white cells to other surfaces can possibly be explained on the basis of calcium bridging between cells, the property being attributed to the anionic groups present on the white cells.

Thompson et al. (1967) proposed an important role for calcium in the phenomena of sticking and emigration during acute inflammation. Using a calcium chelating agent, EDTA, they were able to obtain a complete reversal of the inflammatory leukocytic sticking in rabbit's ear chambers and in the exposed rat mesenteric vessels. On injection of calcium, the sticking of leukocytes once again was observed. They also noted that the interendothelial junctions of vessels treated with the calcium chelating agent were widely open.

Grant (1965) concluded that "an altered state of the vascular endothelium was a critical event in the final common pathway of the inflammatory process, whether this was induced by trauma or micro-organisms".

1.5. Chemotaxis

Until quite recently, there has been an attempt to attribute sticking and emigration of white cells as due to chemotaxis. Florey (1962) expressed the opinion that it was quite possible that the factors controlling the sticking and emigration of leukocytes were not necessarily the same as those controlling the movement of these cells outside the vessels in the extrovascular tissues.

It was demonstrated in experimental work carried out *in vitro* that a number of bacteria, starch granules and antigen-antibody complexes are chemotactic (Harris, 1955; Boyden, 1962).

Allison *et al.* (1955), in studies of burnt tissue in ear chamber preparations, directly observed white cells emigrating from vessels and moving at random, although they have frequently been observed to follow the route of least resistance. Despite what appeared to be random movement of these cells, they finally appeared to be concentrated around the site of the injury.

Hurley (1963) was able to correlate the results of his

intradermal experiments with an *in vitro* demonstration of chemotaxis, and showed that the correlation between the *in vivo* evidence of tissue leukocytosis and the *in vitro* chemotaxis was high.

1.6. Leukocytic emigration and increased vascular permeability

Hurley (1964), following his experiments with intradermal injections of different irritants into rats, was able to demonstrate two distinct types of cellular emigration, dissociating them from permeability effects. Using histamine, homologous serum, and even physiological saline, he was able to observe a relatively non-specific emigration of leukocytes, mostly neutrophils, taking place several hours after the injection. However, by using extracts of burned skin as stimulus, and saline extracts of polymorphonuclear leukocytes, he was able to demonstrate an almost immediate emigration, reaching massive proportions by 30 minutes of the injection. When the late reaction was observed, it was not related to any increase in vascular permeability.

Logan and Wilhem (1963), studied the reaction of guinea pig skin to injury by ultraviolet light and they were able to demonstrate an early and a late permeability response and they showed a parallelism between the late phase of permeability increase and tissue leukocytosis.

In general, it appears that the evidence available today concerning the effect of increased vascular permeability on leukocytic emigration tends to support the general idea that increased vascular permeability is not the result of cellular emigration or vice versa. A vessel may show increased permeability but not emigration whilst another vessel in the same vascular bed may show emigration and not increased permeability, whilst another one near by may show both.

1.7. The early neutrophilic predominance and the transformation of the exudate to one of mononuclear predominance

A well established but rather poorly understood aspect of the inflammatory process is the transformation of the early polymorphonuclear exudate to a subsequent predominantly mononuclear cell infiltration.

The early polymorphonuclear predominance holds true in almost all reactions. Dienes and Mallory (1932) expressed the opinion that the tuberculin reaction forms an exception in that it is characterised by a predominantly mononuclear response from its initial stages. Follis (1940) observed that the cellular response in the very early stages of the tuberculin reaction is a polymorphonuclear one, and this view was certainly supported by the recent observations of

Martins and Raffel (1964). There now appears to be agreement that in most if not all inflammatory reactions, the initial change is that of a polymorphonuclear predominance.

One point on which there now appears to be general agreement is the haematogenous origin of both types of cells (Clark and Clark, 1936; Kolouh 1939; Ebert and Florey 1939; Rebuck and Crowley, 1955; [illegible] Benacerraf and McCluskey, 1963; Page, 1964; Spector and Coote, 1965).

It is possible that the change in the cellular character of the exudate may be related in some way to the fact that the neutrophil, being an end cell with a comparatively short life history, degenerates rather quickly, whilst the mononuclear cells are able to survive and proliferate.

Page <u>et al</u>. (1962), using the antimetabolite 6-mercaptopurine, were able to inhibit the lymphocytic response in inflammation, without in any way affecting the early neutrophilic exudation. This led them to the hypothesis that substance(s), released at the site of inflammation, induce protein synthesis in the circulating lymphocytes, which event appears to be essential for their margination (Page, 1964), and this perhaps indicated the existence of two different stimuli.

Paz and Spector (1962) performed a series of experiments on rats, using intradermal and intraperitoneal injections of a number of macromolecules as their experimental techniques. They were studying the response on a temporal basis and they reached the conclusion that polymorphs and mononuclear cells were leaving the vessels concurrently. They did observe however that the polymorphonuclear leukocytes were leaving the vessels very much faster than the mononuclear cells, and this obviously resulted in an early preponderance of polymorphonuclear leukocytes in the tissues. Following the cessation of emigration, the polymorphs disappeared rather quickly, leaving behind the persisting mononuclear cell population in preponderance. They observed these mononuclear cells being transformed to macrophages and then to other cell types, the nature of which appeared to be governed by the nature of the stimulus employed.

Hurley *et al.* (1966) reported an early and relatively brief emigration of polymorphonuclear leukocytes being followed by a delayed but far more prolonged emigration of mononuclear cells. They labelled the blood leukocytes by repeated intravenous injections of colloidal carbon, and they concluded that the mononuclear cells present in the later stages of the reaction were of a haematogenous origin.

Following the injections of a living suspension of *Klebsiella pneumoniae*, they observed a massive prolonged emigration of polymorphonuclear leukocytes, but no detectable escape of mononuclear cells was noted during the first twenty-four hours. Hurley and his co-workers finally concluded that the pattern of response to the various stimuli used during their experiments was consistent with the hypothesis that the two types of leukocytes under discussion migrate independently, and the results obtained suggested that a separate mechanism must control the emigration of each type of cell.

Williams and Walters (1968) made observations on the emigration of leukocytes and stated that there was a temporary inhibition of the emigration of leukocytes that exhibited intravascular phagocytosis. It is possible that the biphasic response observed by these authors may have merely been the result of a temporary inhibition in the emigration of the carbon loaded mononuclear leukocytes, whilst the unlabelled neutrophils were able to emigrate in their usual numbers and without delay.

1.8. Origin of the mononuclear cells found in the inflammatory exudate and their transformation to macrophages and other cell types

There appears to be a general agreement on the

haematogenous origin of the polymorphonuclear cells, as such cells are only found in the blood and the haemopoietic tissues, under physiological conditions. The presence of these cells in tissues was naturally taken to indicate the passage of these cells from the blood to the tissues.

The origin of the mononuclear element, however, in the inflammatory exudate has been debated for the last hundred and fifty years or so. In summary, it appears today, that there are two schools of thought about this subject. Even though the concepts of monocytic and lymphocytic origin were widely debated, it is now more or less agreed that the macrophages have their histogenesis from the monocytes.

Metchnikoff (1905) made the first detailed study of the large mononuclear elements of the blood and fixed tissue phagocytes, and he grouped these two types together, as functionally similar, and gave them the collective name of "macrophages". Awrorow and Timofejewskij (1914) are reported by Ebert and Florey (1939) to be the first to have demonstrated, in tissue culture, that macrophages and giant cells could be derived from cells in the blood. Carrel and Ebeling (1922 and 1926) cultured the buffy coat of the avian blood, and found that after an interval of a week there developed a pure strain of mononuclear cells.

Ebert and Florey (1939) provided direct and indisputable evidence as regards the monocytic origin of macrophages in inflammation.

Paz and Spector (1962), following their study of the inflammatory reaction induced by injections of macromolecules, concluded that the mononuclear elements in the lesions were derived from blood mononuclears that have emigrated concurrently with polymorphonuclear leukocytes but they did not at that time decide which type of haematogenous mononuclear cell was involved in this transformation.

Rebuck *et al.* (1964) following newer concepts of small lymphocyte peripheralisation and recirculation through the lymphocyte-forming tissues, and electron microscope studies of the lymphocyte ultrastructure, have reopened the question of lymphocytic "modulation". They have described such modulation of individual small blood lymphocytes to histiocytes in human skin window studies.

Spector *et al.* (1965) carried out a valuable and critical work on the nature of the cells that migrate from the vessels to give rise to the mononuclear cells of inflammatory exudates. From their results they concluded that almost all the mononuclear cells in the exudate must

have been derived from the monocyte type of cell from the blood, as they have noted that the percentage of labelled mononuclear cells in the exudate corresponded to that of the labelled monocytes in the circulating blood prior to the injection of the irritant.

Spector and Coote (1965) carried out a similar experiment to that of Spector et al. (1965), but in this case they used paraffin oil instead of fibrinogen as the inflammatory stimulus.

From this experimental system they deducted that almost all macrophages, epithelioid cells, and the occasional giant cell of this type of reaction were derived from cells corresponding to the circulating monocytes. Their results obtained from initial stages of inflammation confirmed the previous observations as regards the "monocytic' origin of the mononuclear cells present in the earlier hours of the reaction. They observed that following this stage there is mitotic proliferation, first of cells in vessel walls, then of histiocytes and macrophages. The histiocytic proliferation was observed to persist for twelve weeks or more, and the dividing cells were observed to have been derived from blood monocytes which however did not show mitotic activity until they acquired the characteristics of histiocytes or macrophages. The persistance of the lesion was considered to be mainly due to the

sustained proliferation and further emigration of haematogenous elements.

Spector and Lykee (1965), in a study of the cellular evolution of inflammatory granulomata, employed a similar technique to that used by Spector et al. (1965). As their inflammatory stimulus they used Freund's adjuvant, and their aim was to elucidate the events after the first two or three days, in which time it was shown previously by Spector and Coote (1965) that virtually all the mononuclears in the earliest interval were of a "monocytoid" origin.

Nair (1973) observed that monocytoid cells were seen to undergo alteration to form macrophages, epithelioid cells and giant cells. He presumed that epithelioid cells originated from macrophages which have completely degraded ingested material or from those which were not involved in phagocytosis.

Throughout the last fifty or so years a large amount of experimental work has appeared in the literature with regards to the possible transformation of small lymphocytes to macrophages and other types of cells, both in vivo and in vitro. In tissue culture studies alone, a total of over thirty different investigators were convinced that atleast some if not all of the macrophages that appeared in tissue cultures of blood or lymph have developed from small lymphocytes. But doubts have been expressed as to

whether these cells which were originally identified as lymphocytes were in actual fact, lymphocytes or not.

1.9. Transformation of lymphocytes to plasma cells

It was noted that in certain graft versus host reactions lymphocytes differentiated into large cells with pyrininophilic cytoplasm in the lymphoid tissue of the recipient animal (Gowans *et al.* 1961). Porter and Cooper (1962) injected thymidine labelled thoracic duct lymphocytes from an inbred strain of rats into recipient rats of different strain. In this way they were able to trace the labelled lymphocytes in the cortex of the lymph nodes and in the Peyers patches of the recipient animal. They noted that within 24 hours the isotope which was previously present in the inoculated cells appeared in a number of large pyrininophilic cells and they concluded that at least a portion of the inoculated cells had transformed into these cells, many of which appeared to be capable of division. Nossal and Makeda (1962) immunised rats with Salmonella flageller antigen and four weeks later a challenging dose of the antigen was administered. A single dose of tritiated thymidine was given to these animals two hours prior to the challenging dose of the antigen in order to label the cells exhibiting DNA reduplication in the lymphnode. This experimental system showed that at least 95% of plasma cells were labelled and they concluded that plasma cells were derived from small lymphocytes which

were dividing. According to Movat and Fernando (1965) a well recognised feature of immune response is the presence of blast cells. They observed an increase in the number of plasma cells in the lymphnodes draining the site of antigen administration and it was postulated that antigen recognising lymphocytes are stimulated by antigen to undergo transformation into blast cells that divide, proliferate and differentiate into plasma cells. This was supported by direct evidence that showed morphologic interaction between lymphocytes with little or no endoplasmic reticulum, blast cells with increasing amounts of endoplasmic reticulum and cell types of plasma cells with abundant amounts of endoplasmic reticulum. Cooper and Lawton (1974) observed that B cell differentiated into plasma cells after induction of specific antibody response by antigen. It was concluded that stem cell differentiation into B cells was antigen independent whereas B cell differentiation into plasma cells was antigen dependent. In birds B cell differentiation takes place in the Bursa of Fabricius and T cell differentiation in the thymus. In mammals B cell differentiation takes place by differentiation of stem cell in the liver or bone marrow (Covan _et al._ (1974).

Nossal _et al._ (1977) observed that within five to seven

days after immunisation plasma cells appeared in the germinal centre and medullary cords of the lymph nodes.

Cantour and Boyes (1977) pointed out that stem cells from the bone marrow got differentiated in the thymus and T lymphocytes and they acquired antigens like Thy and T1 antigens. The T cells were found to recognise higher immunologic determinants whereas B cells were found to recognise smaller determinants such as haptens and the macrophages were found to cooperate with T and B lymphocytes.

Katz (1978) employing phytomitogens studied the blast formation in macrophages and lymphocytes and concluded that macrophages processes the antigen non-specifically but make the antigen more palatable for the T cell. The T cell then presents the antigen to the precursors of plasma cells. The B cell was then stimulated to divide and differentiate into specific antibody producing plasma cells.

Induction of antibody formation was associated with hyperplasia of follicles and plasma cell production from the B cells (Sell, 1980).

1.9.3. Lymphocyte and Fibroplasia

The close proximity of fibroblasts at the inflammatory

site has intrigued investigators for decades. As inflammatory process progresses fibroblasts becomes evident. Leibovich and Ross (1975) depleted experimental animals of macrophages by treating them systematically with hydrocortisone and locally with antimacrophage serum. This resulted in delay in wound healing and retardation of fibroplasia. A lymphokine called fibroblast activating factor was produced by sensitised guinea pig T cells (Wahl et al 1973). The *in vitro* effects of lymphocytes and macrophage mediators of fibroblasts suggested that lymphocytes and macrophages might initiate fibroplasia and induce fibrosis associated with inflammatory lesion (Wahl and Wahl, 1980). Tsukamoto and Wahl (1980) suggested that the lymphocyte through macrophage may elicit an influx of fibroblasts into an inflammatory site by release of soluble mediators. During the last decade large number of soluble products from stimulated lymphocytes have been described such as Macrophage migration inhibition factor, mitogenic factor, leucocyte migration inhibition factor, colony stimulating activity factor, vaso permeability factor, interferon etc. (Schook *et al.* 1981).

1.9.4. Mast cells and basophils

Although in the literature there is a large amount of information concerning most of the leukocytes, there appears

to be very little concerning the properties and functions of the blood basophil. An enormous amount of information however is available on the tissue counterpart of this cell, the mast cell. The lack of interest in and experimentation on the blood basophil may lie on the fact that it is present in very small numbers only in the blood of the usual experimental animals and man, and also because it is not easily identified in routine haematoxylin and eosin preparations of tissues.

Despite the fact that the mast cell was first described by Ehrlich in 1877 the existing knowledge of its nature and function is far from complete.

The invididuality of the blood basophil and the tissue mast cell and their different mode of origins in the mammalian species are now well recognised.

It is generally agreed that fixed undifferentiated mesenchymal cells form the chief source of mast cells. It was also observed that mast cells can and do increase by mitosis and it has been conclusively demonstrated that tritiated thymidine becomes incorporated into the nucleus of mast cells, particularly so in young animals (Padawer, 1961).

It is now also well established that both basophils and mast cells contain the sulphated mucopolysaccharide, heparin and in addition histamine and 5-HT, although the

latter may not be present in significant amounts in the mast cells of several species e.g. guinea pig, dog, man, rabbit, cow and cat. Hogen *et al.* (1959), employing the fractionation - separation procedure, have shown that all these substances mentioned above are to be found within the granules seen in the cytoplasm of the mast cell.

During the course of the last seventy years or so the mast cell has been credited with a number of functions, both in health and in disease. Some of these have now been established beyond reasonable doubt, but others may still to be proven. In mammals the known functions of these cells can be listed as follows:

1. Increase capillary permeability.
2. Enhance phagocytosis.
3. Maintenance of proper fluidity.
4. Stimulation of fibrous repair.

The presence of blood basophils in inflammatory exudates has been noted upon rare occasions.

Plimpton (1940) described a biphasic migration of basophils into the subcutaneous tissues of both guinea pigs and rabbits, at 45 minutes and again at eight to ten days following the local injection of ventriculin, a defatted porcine gastric tissue preparation.

Florey (1962) expressed the opinion that the basophil may possibly participate with the mast cell in anaphylactic reactions. Rebuck, et al. (1963) using the skin window technique and an antigenic stimulus (diptheria toxoid), studied the response in two groups of human patients, one group suffering from ulcerative colitis and the other from interstitial cystitis. The large majority of these cases exhibited an increased basophilic emigration to the test lesions. Siraganian et al. (1975) observed that mast cells and basophils bear specific receptors for 1gE and bridging of cell-bound 1gE antibody molecules by multivalent antigen induces the release of variety of chemical mediators in inflammatory reaction.

1.9.5. Factors influencing chronicity of inflammation

Florey (1962) stated that there are a number of conditions in which inflammation is not characterised by the exudation of fluid and the accumulation of polymorphs, signs of the acute inflammation, but are marked by the presence of cells particularly of the mononuclear type, i.e. macrophages and lymphocytes. This latter type of lesion is the one associated with "chronic inflammation".

Spector (1967) expressed the opinion that the determination of chronicity in an inflammatory process can be made

at about forty-eight hours from its initiation. He stated that if by this time interval there is no definite enlargement of the mononuclear cells and no indication of DNA synthesis, chronicity can be excluded from the future course of the lesion, but he emphasised that the converse need not necessarily be true.

Spector et al. (1958) noted that proliferation amongst the mononuclear cells of an inflammatory exudate cannot be taken as indication of chronicity. They observed that the chronicity of a lesion is related to the persistence of the stimulus and that chronic inflammation was only found in the presence of intracytoplasmic irritants. Phagocytosis of irritant and mitotic division were seldom, if ever, exhibited by the same cell. Their results also revealed that the quantity of the irritant present in the lesion may be very small relative to the size of the reaction and they expressed the opinion that this later observation may help to explain the difficulty of demonstrating infective agents in certain chronic inflammatory lesions of obscure origin.

Spector and Heeson (1969), attempted to investigate the possibility that immune complexes could produce granulomata in the skin of the rat.

Their results indicated that granulomata occurred when the "immune complex" contained an excess of antibody

which presumably rendered the complex "indigestible". When antigen was present in excess no granulomatous lesions were seen. Brawcell *et al.* (19[illegible]9) induced non-immunological granulomata with bentonite in guinea pigs. Lay and Slauson (1982) induced pulmonary granuloma in calves. Observations were made from 24 hours to 30 days. Initially they observed multifocal vasculitis and exudative pneumonia and a granuloma was seen by seven days.

2. Inflammatory response in chicken

2.1. Heterophils

Like the mammals, birds have three granular leukocytes; the heterophil, eosinophil and basophil. The last two correspond with those of the mammal and are so named because of the affinity of their specific cytoplasmic granules for either acidic dyes (eosinophils), or basic dyes (basophils). In mammals the term "neutrophil" refers to the staining properties of the cytoplasmic granules of this cell while its counterpart in the lower vertebrates and birds, the heterophil (Lucas and Jamroz, 1961), is so named because of the great diversity in staining reactions exhibited by the specific granules of homologous cells amongst the various classes of lower vertebrates (Kyes, 1929). The specific granules of the heterophils of the fowl are rod or club shaped and strongly eosinophilic.

Hirsch (1962) reported on the phagocytic activities of chicken granulocytes and described the engulfment by them of Bacillus megaterium and zymosan granules. Glick et al. (1964) observed that chicken heterophils exhibited phagocytosis of Staphylococcus aureus organisms in vitro but they noted however, that only 30% of these cells engulfed the organism.

A number of workers have in the past carried out experimental studies on the histochemistry of avain heterophils. Merkal and Mora (1962) reported the presence of either no activity or a very weak one of alkaline or acid Phosphatase in chicken heterophils. Atwal and McFarland (1966) observed that the heterophil granules of the Japanese quail specifically lack lysosome enzymes such as acid phosphatase, acid ribonuclease and acid deoxyribonuclease. Opie and Barker (1967) reported that they were not able to demonstrate proteolytic enzymes in chicken leukocytes.

It seems possible that the reported lack of enzymatic activity of avian heterophils could account for the absence of fluid pus in this species. Nair (1973) described the ultrastructure of the heterophil and described large dense granules, small dense granules and light granules.

2.1 The monocyte and its transformation to macrophages, epithelioid and giant cells

In avian blood smears stained with Leishman's stain monocytes appear as round cells with an oval or reniform nucleus placed somewhat eccentrically surrounded by a moderate amount of basophilic cytoplasm. The chromatin appears in the form of a delicate lacey network and nucleoli are not visible (Weiss and Fawcett, 1953). This cell appears to be the most active leukocyte when observed with phase microscopy, whilst the heterophil and lymphocyte have a rather feeble amoeboid movement (Atwal and McFarland, 1966).

The transformation of the avian monocytes to macrophages, epithelioid cells and giant cells has been studied for over fifty years and indeed most of the early _in vitro_ studies of the monocytes were carried out on cells obtained from the fowl.

Carrel and Ebeling (1926) compared the characteristics and properties of the avian monocyte _in vitro_ with those of tissue macrophages from the same species. They were able to observe, photograph, and describe in their tissue cultures the increase in size and transformation of the blood monocytes to cells indistinguishable from tissue macrophages. They reached the conclusion that both the blood monocyte

and tissue macrophages are merely functional variations of a single type of cell and that their structure can be modified at will by changes in the composition of the culture medium.

Weiss and Fawcett (1953) using a more refined tissue culture technique have also commented on the *in vitro* transformation of blood monocytes to macrophages, epithelioid and giant cells.

The monocytes appear to adhere to the glass of the culture vessels early during incubation and assume the characteristics of macrophages.

Weiss and Fawcett noted that by the third day of incubation the cultured cells appeared to lose their amoeboid shape and became flattened out on the glass surface and these now were referred to as epithelioid cells. The majority of the multinucleated cells in their cultures appeared to form by coalescence of individual cells although there was evidence that some binucleated giant cells do arise as a result of division of the nucleus unaccompanied by cleavage of the cytoplasm. They did not however observe mitosis in cells containing more than two nuclei. In their opinion cell crowding and low pH of the medium appear to favour the formation of giant cells and in addition the presence of foreign matter may also assist their formation

by providing a nidus around which the epithelioid cells "cluster, cohere and subsequently unite".

Sutton and Weiss (1966) studied by means of the electron microscope the sequential transformation in vitro of chicken monocytes to macrophages. They expressed the opinion that "the multinucleated giant cell is an extraordinary structure and should not be regarded merely as a larger cell than those which fused to form it". The unusual features of these cells are the massive accumulation of mitochondria, abundant folded cell membrane, large vacuoles and the absence of lysosomes. The concentration of mitochondria and the extensive folded plasma membrane suggested to them that these cells may have active transport as their primary function. Like the osteoclast it may be involved in the movement of calcium in tissues and thus may play a role in ectopic calcification as for example in tuberculosis.

The function of the monocyte appears to be the synthesis and storage of lysosomes and its derivatives, the macrophages and epithelioid cells, function as phagocytes capable of digesting the disposing of their intake.

2.3. Avian lymphocytes and lymphoid tissue

A common feature of many of the experimental inflammatory

lesions is the presence of prominent, focal accumulations of lymphocytes. Similar lymphocyte accumulations have been observed by previous workers and have attracted interest because of their possible relationship to leukosis and to immune reactions.

In the opinion of Lucas (1949); Lucas and Breitmayer (1949); Lucas and Oakberg (1950); Lucas and Breitmayer (1950); Oakberg (1950); Denington and Lucas (1960), all lymphoid foci are abnormal.

Most of the studies on those "ectopic" lymphoid foci were made on the glandular organs of the domestic fowl and to a lesser extent on other species as well. Little attention however was given to the subcutaneous tissue, despite the fact that their presence in this tissue was reported by several investigators as far back as 1926. Lucas and Breitmayer (1950) expressed the opinion that the distribution of lymphoid foci in the dermis can be considered as reflecting reactions to invading organisms. These dermal lymphoid foci, according to the same authors, occur mostly in the feet of chickens, whilst in flying birds they were most developed at the top of the head and close to the anus and in water fowl they were usually best developed near the feather follicles.

Lucas and Breitmayer (1950), following their observations of the lymphoid foci in the pancreas, reached the conclusion that lymphoid areas can and do invade and destroy adjacent tissue. Similar observations were reported by Payne and Breneman (1952) in the case of lymphoid areas in the pituitary, thyroid, adrenal and sex glands of the domestic fowl and in their view such foci can only be considered as pathological. These structures differed however, in morphology and origin, from their mammalian counterparts and perhaps they were only modified portions of the wall of the lymphatics (Trautmann and Fiebiger, 1952).

These foci were thus found in practically all organs, but varied in amount and distribution not only between different species but between individuals of the same species as well, and if very extensive they were then usually regarded as pathological (Lucas _et al_. 1954).

The sites for lymphoid tissue in the domestic fowl were the spleen, thymus, bursa of Fabricius and intestinal tract (Biggs, 1956). Apart from these sites however, there were others where various quantities of lymphoid tissue were constantly present. In addition to the system of lymphoid nodules which were normally associated with lymphatic vessels there were present in most avian species,

including the domestic fowl (Biggs, 1956), "ectopic" lymphoid areas which have been described in connective tissue and these were often found in association with blood vessels.

Lymph nodes as seen in the mammal were not present in most avian species (Biggs, 1956), but paired structures, loosely referred to as "lymph nodes", were present in the cervical and lumbar regions of a number of amphibious species, such as the duck and the goose

Biggs (1957) described the presence of lymphoid foci ("mural" nodules) in the walls of the lymphatics and in particular those draining the hind limbs. These mural nodules appeared to be similar in development and structure to the paired nodes of the duck and the goose.

Cook and Simonsen (1953) expressed the opinion that ectopic lymphoid foci can arise <u>de novo</u> from previously free lymphocytes, in response to local infection or irritation and do not necessarily derive from pre-existing nests of cells of the lymphoid series.

Ball <u>et al</u>. (1963) considered it possible that a wide variety of pathogenic microorganisms as well as toxic drugs and metabolites such as biliverdin can all produce stimulation of lymphoid foci in organs such as the avian liver.

It is clear from this rather brief review of the

literature that despite the efforts of a number of workers some of the controversies concerning the lymphoid foci of birds have not as yet been resolved.

2.4. Transformation of the Avian Lymphocyte to other cell types

Glick et al. (1956) described the role of the bursa of Fabricius, a hind gut lymphoid organ, in the development of humoral immunity in the chicken.

Cook and Simonsen (1958) injected whole blood from pure strain (1) adult White Leghorns into newly hatched chicks of a cross between two highly inbred lines of the same breed (J and 1) and noted enlargement of the spleen and liver of the receipients. From their results they concluded that the blood leukocytes or a fraction of them were fully competent to initiate transplantation immunity and antibody production. They also expressed the opinion that these transplanted cells can proliferate and attach to the surrounding host cells in situ. Terasaki (1959) concluded that the cells referred to by Cook and Simonsen as responsible for these events were the small lymphocytes. The graft versus host reaction was indeed the same as that described for the mammal in the previous section. Thorbecke (1959) carried out a histological examination of the lymphoi

tissues in germ free chicks. He did not observe any germinal centres or plasma cells in the spleen or intestine of 2 - 6 week old germ free birds and this was in contrast to the situation in the conventional birds of the corresponding age groups.

The thymus dependent development of lymphoid tissue was represented by the small lymphocytes of the circulation and the number of small lymphocytes in the lymphoid tissues and was responsible for the ontogenesis of cellular immunity, i.e. graft versus host reactions, responses of the delayed hypersensitivity type of homograft rejection. In thymectomised irradiated chickens the germinal centres, plasma cells and immunoglobulin synthesis remained intact (Cooper et al. 1966b; Aspinal et al. 1963).

The bursa dependent system was represented morphologically by the larger lymphocytes of the germinal centres and the plasma cells and functionally by immunoglobulin production (Cooper et al. (1962); Aspinal et al. (1963).

Cooper et al. (1966) referred to the thymus and bursa of Fabricius as central lymphoid organs in the chicken essential for the ontogenic development of adaptive immunity in this species.

Clawson et al. (1967) studied the fine structure of

the lymphocyte from the bursa of Fabricius and thymus. Most striking of the fine structural differences of these two cell types was in the cytoplasmic ribosomal population and distribution. Lymphocytes indistinguishable from bursal lymphocytes were found by them within the splenic germinal centres. They observed that the bursal type of lymphocytes were undergoing transformation to "haemocytoblasts" which were in turn the precursors of plasma cells. This evidence tended to link the bursal lymphocyte to the plasma cell as a single line of cell differentiation. Clawson et al. (1967) observed that the bursal type of lymphocytes, preplasmacytes and plasma cells were absent from the spleens of bursectomised, irradiated, antigen stimulated birds.

It is well-known that the chicken, unlike mammals, has no organised lymphoid tissue but possesses instead numerous extra-vascular foci of cells of the lymphoid series throughout their body. Duffus and Allan (1968) assumed that, "if cellular reactions similar to those in sheep occur in the chicken following antigenic stimulation, the cells involved would be distributed via the circulation in order to propagate an immune response". To prove this theory they immunised chicks with killed Salmonella gallinarum organisms and attempted to detect the type of cells involved in the immune response amongst the leukocytes of circulating blood by means of the immunocyto-adhesion method.

Duffus andAllan classified the "immunocytes" (cells

involved in specific immune reaction) which appeared in the circulating blood of the immunised chickens into the following three categories.

a) Haemocytoblasts, which made their appearance after the first day were in maximal numbers between the 3 - 5th day and minimal by the 7th day.

b) Cells of the plasmacyte series. Present from the 3rd to the 9th day.

c) Cells of the lymphocyte series.

They concluded, from the extent of immunocyto-adhesion, that cells of the plasmacyte series were the more potent antibody producers amongst the immunocytes. In their opinion the cells of the lymphocyte series were not actively secreting haemaglutinin but may have been involved in cellular immunity or with immunological memory.

The same authors considered it possible that the extravascular foci of lymphoid tissue in this species may have been involved in antibody production in addition to the spleen where most of the cells of the bursal dependant system are known to reside.

2.5 Thrombocytes

In birds the thrombocytes develop as mononuclear cells which have a blast stage like that of other blood cells

and remain as such throughout their life span.

The avian thrombocyte is an elongated blood cell slightly larger than an erythrocyte with an oval nucleus and a cytoplasm composed of a framework with large spaces (Lucas and Jamroz, 1961).

The function of this cell is considered by Lucas and Jamroz to be similar to that of the mammalian platelet in haemostasis.

Sweeny and Carlson (1969) have carried out extensive electron microscope and histochemical studies on these cells and reported the presence of "lysosomal like" cytoplasmic inclusions, golgi complexes, mitochondria and endoplasmic reticulum, as well as acid phosphatase positive granules. They stated that from their observations one could conclude that these cells are probably capable of carrying out extensive synthetic activities.

Carlson *et al.* (196[illegible]), following fluorescent and electron microscope studies on these cells, reported that the chicken thrombocytes have a phagocytic activity as evidenced by their ability to segregate vital dyes such as trypan blue, neutral red and acridine orange in cytoplasmic vesicles and their ability to engulf, segregate and degrade staphylococcal organisms. In addition they also studied

untreated cells by means of phase contrast microscopy and noted the presence of perinuclear vacuoles containing one or more granules. They observed that those cells over a period of a few minutes to three or four hours gradually assumed a spindle shape and the vacuoles migrate slowly towards the cytoplasmic membrane where they rupture and thus liberate their granules to the exterior of the cell. Several new vacuoles were forming at the perinuclear area. This process was considered by Carlson and his colleagues as indicating a possible trephocytic function of the avian thrombocyte, the significance of which is still unknown. Chang and Hamilton (1979) also made similar conclusion. Awadhya et al. (1980) observed engulfed carbon particles in the thrombocytes and confirmed phagocytic role of these cells.

2.6 Mast cells and basophils

In the lower vertebrates there seems to be a close genetic relationship between the basophils (mast cells of the blood) and those in the tissues in the sense that basophils may leave the blood and enter the tissues under both physiological and pathological circumstances. Once in the tissues, by means of hypertrophy and reduction in the size of their granules, they transform into histogenous

mast cells. Morphologically the histogenous mast cells resembled those of mammals although they are as a rule smaller in size and with finer granules. It was possible that such a relationship between the blood basophil and the tissue mast cells was present in reptiles and birds as well (Michels, 1938).

They were normally the same size and shape as the heterophils. Their nucleus was usually round to oval in shape and very rarely indented. This type of nucleus in the basophils of lower vertebrates may be indicative of its lower position in the evolutionary scale (Michels, 1938).

In avian blood, basophils were usually more abundant than eosinophils and on average form 2% in a differential count, although in some species such as the pheasant they may form upto 10% (Lucas and Jamroz, 1961).

The granules of both the histogenous and haematogenous elements were basophilic and stain metachromatically with metachromatic stains such as Toluidine blue and thionine.

The variable structure and density of the granule matrix ranging from homogenous and electron dense to coarsely particulate and more electron lucid, as well as the occurrence of para-crystalline arrays and myelin like

figures were considered as well-known characteristics of mast cells and basophilic leucocytes (Flood and Krüger, 1970). Wight (1970) described the fine structure of the mast cell in chicken. He described cell surface pseudopodia which were parallel to the cell surface, diffusely distributed ribosomes, a small golgi complex, and numerous specific granules of oval or spherical shape which were either electron opaque or less dense with fine granular matrix. He ascribed the varying appearance of the granules to the different stages of maturity. Wight and Mackenzie (1970) observed that the granules of the mast cells of the chicken are relatively in soluble in water and they contained heparin but they could not demonstrate histamine and other biogenic amines and several enzymes which occur in mammalian mast cells. Similar features were described for the mast cells, pigeon (Bowers *et al.* 1981) and ducks (Valsala, 1984).

2.7. Experimental studies on inflammatory reaction in chicken

There has been no study describing the basic changes in the inflammatory reaction in ducks. However, there are various reports detailing the inflammatory response in chicken.

2.7.1. Turpentine

Allen (1969) described the cellular response in acute inflammatory reaction in chicken. There was pronounced infiltration with heterophils, mononuclear cells and basophils

during the initial stages. Lymphoid hyperplasia was detected in six hours. Carlson and Allen (1969) injected trypan blue, Staphylococcus aureus and bovine serum albumin into the wing webs of chicken and studied the inflammatory reaction. Upto 3 hours post injection the inflammatory reaction was dominated by exudation of heterophil cells. The peak of exudation and the most acute reaction were at approximately 12 hours post injection. The most numerous cells were the heterophil and a phagocytic mononuclear cell which appeared to develop from monocytes.

Jortner and Adams (1971) studied the ultrastructural features of turpentine induced inflammation. The initial response was heterophilic and later there was macrophage, epithelioid and giant cell reaction. Nair (1973) made a detailed light and electronmicroscopic study of the inflammatory reaction in chicken employing different agents. He studied the inflammatory reaction following the injection of turpentine and described the sequence of changes that followed. Thirty minutes after the injection the heterophils and monocytoid cells were seen adhering to the endothelium of venules and capillaries. The perivascular and intervascular areas were free from infiltrating cells. At two hours heterophils and monocytoid cells were seen

in the lumen and wall of the vessels and in perivascular locations. At four hours these cells were found emigrating. The number of heterophils was more than the monocytoid cells. Basophils were seen in the lumen and outside the vessels. Many of them had degranulated. Lymphoid collections were observed in the lumen of many of the venules and as small collections in the perivascular locations. At 12 hours the lymphoid foci became very prominent. At 15 hours giant cells were seen. At 24 hours intensity of emigration of heterophils diminished and many heterophils were necrotic. The lymphoid foci around the venules were numerous and such vessels showed enlarged endothelial cells. Around the vessels there was slight proliferation of reticuloendothelial cells. At two days around the necrotic areas macrophages formed a rim and appeared to form a syncitium. The giant cells were seen arranged in a palisade fashion. Perivascular lymphoid aggregation was prominent. At three days p.i a mantle of giant cells were seen around the necrotic area. The lymphoid cells showed tendency to spread out into surrounding areas. At five days p.i. there was growth of granulation tissue into the necrotic area. At seven days p.i. and after, granulation tissue formation was prominent. In many locations the lymphoid nodules were found circumscribed. At 14 days p.i. the necrotic areas were completely replaced by granulation tissue. Many

lymphoid nodules still persisted. By autoradiographic studies it was demonstrated that epithelioid cells originated from macrophages. The cells in the lymphoid nodules were heterogenous. Cells with the morphology of thymic lymphocytes and bursa type lymphocytes were seen along with many cells showing blastoid transformation. Transitional stages from the blast cells to plasma cells were observed. The transformation to mature plasma cells was characterised by reduction in the free ribosomes and an increase in the rough surfaced endoplasmic reticulum which almost filled the cytoplasm of the mature plasma cell.

Awadhya _et al._ (1980) studied the inflammatory reaction in chicken using mesentery as the test system and turpentine as the inflammation inducing agent. It was documented that heterophils and monocytes emigrated concurrently. Participation of basophils was also demonstrated. A biphasic pattern of vascular permeability was characterised by an immediate transient reaction and a delayed more prolonged response. The increased permeability was confined to venules. Awadhya _et al._ (1981) while studying the inflammatory reaction following thermal injury observed that there was concurrent emigration of heterophils and monocytes. Basophilic response, giant cell formation and perivascular lymphoid foci were seen in turpentine induced inflammation.

2.1.2 Staphylococcus aureus

Carlson (1972) described the inflammatory response in chicken caused by *S. aureus*. The inflammation was dominated by an exudation of heterophils, mononuclear cells and basophils. The most characteristic observation was the appearance of lymphoid hyperplasia as early as six hours post infection, increasing in intensity until at 36 hours. Nair (1973) observed that by thirty minutes the heterophils were adhering to the endothelium of the capillaries and venules. At one hour monocytoid cells and heterophils were seen in the perivascular area.

At 24 hours both in the intervascular area and perivascular area there was predominance of heterophils. The heterophils had accumulated in large numbers around the inoculum and many of these cells were degenerated and necrotic. Basophils were seen within the small vessels and in small numbers in the perivascular areas from the third hour onwards. From six hours lymphocytes were seen within the lumen, in the wall and around many venules. Proliferation of reticuloendothelial cells was seen around these vessels from 24 hours p.i. By 48 hours numerous macrophages and giant cells formed a rim around the lesion. Four hours after the injection the heterophils around the inoculum had become completely necrotic. The lymphoid cells

were seen spreading out. Immature and mature plasma cells were found within the secondary lymphoid nodules and elsewhere. By the 5th day p.i. heterophils were more or less absent in the intervascular area. A continuous zone of giant cells surrounded the necrotic zone and around these were lymphocytes macrophages and fibroblasts. More peripherally the granulation tissue was seen and it contained many lymphocytes and macrophages. The perivascular lymphoid foci were reduced in size. By seven days after injection the zone of giant cells around the inoculum began to break down with the ingrowth of proliferating granulation tissue. At 10 days the lesion had regressed and there was moderate fibrosis with occasional islands of lymphocytes.

2.7.3 Freund's complete adjuvant

Nair (1973) studied the tissue response following injection of Freund's comple e adjuvant and described the sequence of changes. At four hours there was heavy exudation of heterophils. Basophils were seen from the fourth hour byt they were few in number. The transient predominence of heterophils in the intervascular area was replaced by that of mononuclear leucocytes, by 24 hours. At 48 hours ninety per cent of the cells in the intervascular area were monocytoid cells. Droplets of inoculum were

surrounded by concentrically arranged monocytic epithelioid cells and fibroblasts. The lymphocytic emigration was seen from the eighth hour. At first they appeared as small perivascular cuffs but from 48 hours they spread out. After the fourth day plasma cells also made their appearance. On the third day a second wave of emigration of heterophils was seen. Many small vessels were filled with heterophils and few monocytes. The recurrent waves of emigration were seen upto the 12th day. After two weeks the granuloma contained numerous epithelioid cell collections surrounded by cellular zone of macrophages, giant cells, lymphocytes and plasma cells.

2.7.4 Dextran sulphate

Nair (1973) studied the sequence of inflammatory response following the injection of dextran sulphate in chicken. By one hour the endothelium of the venule was lined by the monocytoid cells and heterophils and an occasional cell was seen emigrating. By six hours large numbers of heterophils and monocytoid cells were seen perivascularly. Both the mononuclear cells and heterophils emigrated concurrently. There was evidence of transformation of monocytoid cells with the blood monocytes into typical macrophages with enlarged acidophilic cytoplasm. At about the 16th hour the monocytic cell formed 60 to 70

per cent of the cells in the intervascular area. In touldine blue stained sections almost all cells were seen to have ingested dextran sulphate. At 24 hours giant cells were seen and the heterophils in the intervascular area had declined in number. Collections of lymphocytes were present near the vessels and by 48 hours numerous lymphoid foci were present. By day four the heterophils had completely disappeared and the inflamed area consisted of round or spindle shaped macrophages. Proliferation of reticuloendothelial cells was seen around the vessels. Fibroblastic proliferation was insignificant. By seven days only the well oriented macrophages along with very few fibroblasts were seen. The lymphoid foci were seen surrounded by a narrow zone of granulation tissue.

2.7.5. Talcum

Nair (1973) studied the inflammatory response by injecting talcum. The early cellular response was similar to the reaction induced by dextran sulphate. After 24 hours the intervascular exudate consisted of monocytoid cells with an admixture of normal and necrotic heterophils. At 48 hours the heterophils were scanty. Away from the inoculum there were numerous well developed macrophages and few heterophils. In places where talcum was present

there was mainly a giant cell reaction. Presence of giant cells of various sizes with numerous nuclei mostly arranged in the periphery and containing crystals of talc was the common finding the granuloma. There was massive necrosis with the inoculum admixed with necrotic material and fibrin. This necrotic mass was surrounded by a rim of heterophils. By four days there were many giant cells. At day seven, the granuloma consisted of mainly giant cells and all of them were containing crystals of talc. The granuloma became encapsulated by fibroblasts by this time. By the tenth day the reaction site was completely encapsulated.

2.7.6. Fibrinogen

Nair (1973) studied the cellular response to fibrinogen and stated that the cellular response to fibrinogen during the first 24 hours was almost similar to that seen after injection of turpentine. After 24 hours numerous giant cells were seen around the inoculum. By seven days most of the heterophils had disappeared and large macrophages with eccentric nucleus were common. Perivascular lymphoid accumulation was seen at 24 hours. At four days many of these had fused to result in large sheets of lymphoid cells. Occasionally small secondary nodules containing large lymphoid cells were seen.

2.7.7 Ascaris suum and Toxocara canis

Nair (1973) studied the cellular response to hetero-genous metazoan parasites in the chicken. No basic difference was noticed between A. suum and T. canis larvae. The changes observed were the same as that seen with other agents during the first 20 hours p.i. The larvae were surrounded by a mass of cells consisting mainly of macrophages, heterophils and giant cells. At 24 hours prominent collections of lymphoid cells were seen. At four days necrotic changes were noticed in locations where the parasites were located. The necrotic mass was surrounded by multinucleated giant cells and around these a zone of fibroblasts was seen. Perivascular lymphoid foci were characteristic. From the fourth day there was marked reduction in the number of heterophils and by tenth day marked fibroplasia was evident. At fifteen days the reaction was characterised by remnants of parasites, giant cells, few macrophages, fibroblasts and plasma cells. There was no evidence of any eosinophilic reaction at any stage of the reaction.

2.7.8. Red blood cells

Toth and Norcross (1931) inoculated young duckling with chicken blood cells intravenously. Breeder ducks were inoculated twice with an increased dose of chicken

RBC. The young ducklings and the breeders responded with very low titres to chicken RBC and sheep RBC.

2.7.9. Hypersensitivity reactions

Luoma and Benedict (1977) studied the changes in reversed passive anaphylactic reaction in chicken and observed that heterophils were the earliest infiltrating cells. Dhodapkar *et al.* (1981) studied the reaction and documented concurrent emigration of monocytes and basophils following heterophilic reaction. By twelve hours perivascular lymphoid aggregations were seen. By 48 to 72 hours they observed fibroblastic proliferation. They suggested that early emigration of heterophils and monocytoid cells and participation of basophils were characteristic features of acute inflammatory reaction. Maxwell and Burns (1982) by giving multiple intra-peritoneal injections of horse serum to chicken induced sensitisation and this induced eosinophilic response.

2.7.10 Dynamics of cellular involvement

Nair (1973) observed that adherence of leucocytes was seen in venules and occasionally in capillaries and this was noticed independent of the irritants employed. But the intensity and onset of emigration of leucocytes varied with the irritants used. With turpentine emigration was

observed by 30 minutes p.i. and with talcum it did not become pronounced until about 4 to 6 hours. During early phase of inflammation heterophils emigrated. The hetero-phils was shown to have greater mobility than monocytoid cells. The heterophils disappeared soon but there were fresh waves of migration of cells.

The majority of monocytoid cells present was shown to have characters of blood monocytes. They were seen to change into macrophages. The macrophages were shown to be efficient phagocytes. The epithelioid cells were demonstrated to originate from large undifferentiated mononuclear cells. From sequencial studies it was concluded that giant cells were formed by the fusion of macrophages. The giant cells were shown to be capable of ingesting a variety of particulate matter.

The lymphocytes were seen emigrating by about six hours and secondary lymphoid nodules were observed when immunogenic agents were used. The lymphoid foci formed was seen to spread to surrounding area by about 3 to 5 days. This feature was not observed with dextran sulphate and it was concluded that sulphated polysacchride like dextran sulphate could inhibit this. The secondary lymphoid nodules seen were considered anatomical parallel to germinal centres in the spleen. Plasma cells were seen by 2 to 3

days. The plasma cell lines were associated with proliferating reticulo endothelial cells.

In the investigation conducted by Nair (1973) in chicken basophilic emigration was seen in all types of inflammatory reactions induced by different agents. But the response was more when turpentine and or bovine fibrinogen was used. The arrest of lymphocytes in the area of inflammatory reaction was suggested to be due to liberation of heparin by basophils at the site of reaction. There was no involvement of eosinophils in any of the inflammatory reactions. Therefore it was suggested that chicken eosinophil may not have the same function as that of the mammalian eosinophils.

Fibroplasia was recorded by Nair (1973) by 3 to 5 days. The only instance in which fibroplasia was not recorded was associated with inflammation induced by dextran sulphate. Transitional stages from the blast cell to mature plasma cell was observed. The transformation was characterised by reduction in free ribosomes and an increase in the rough surfaced endoplasmic reticulum.

3. <u>Duck plague</u>

The disease was first reported in a commercial flock on the east coast of United States and was designated as

Duck Virus Enteritis. The disease has been subsequently reported from China, Belgium, France, Netherlands and India. Duck plague was first reported from Netherlands by Janson and Knust (1949). Jansen (1961) gave a detailed account of the disease. In India Mukherjee et al. (1963) first described the incidence of duck plague in West Bengal. Mukherji et al. (1963) observed listlessness, lethargy drooping wings, swollen face and sticky eyelids and nasal discharge in affected ducks. There was pronounced greenish diarrhoea. There was enlargement of the liver, scattered petichiae and greyish white spots. Streaks of greyish white necrotic spots were seen in the oesophagus. The mucosa of the gizzard was thickened and there were well defined patches of haemorrhages in the intestines. Oophoritis and peritonitis were also observed. Intranuclear inclusions were described in the liver of ducks. Jansen (1964) observed that the severe mortality observed in Madras during 1944 - 45 where no bacterial organisms could be isolated from affected ducks and experimental transmission in chick embryo was not successful might have been outbreaks of duck plague. Dardiri (1974) gave a descriptive account of the causative agent. He reported that after the 12th egg passage in chicken eggs the virus becomes lethal to chicken. Rajan et al. (1976) and Nair and Sulochana (1981) described outbreaks of

duck plague in Kerala and lesions observed by them were similar to that described by Mukerji *et al.* (1965). In addition to these they also described necrosis of the musculature of the gizzard and heart muscle fibres. Leibovitz (1977) considered enanthematous lesions in the intestine as pathognomonic of duck plague infection.

4. Ranikhet disease

Ranikhet disease is a highly contageous infectious disease which mainly affects chicken and turkeys. The disease was first described by Doyle in England in 1927. Edwards in 1928 first described the occurrence of the disease in India. Subsequently, it has been reported from many parts of the world. Hansen (1968) described four pathologic forms of the disease. According to him in Doyles form haemorrhagic lesions in the digestive tract was a prominent feature and this was caused by certain velogenic and Asiatic strains. In Beaches form also caused by velogenic strains losion was prominent in the respiratory tract and nervous system. The Beaudette's form was an acute respiratory and lethal nervous infection of growing chicken. In Hitenner's form the disease was a mild transient respiratory infection caused by lentogenic strain.

According to Asplin (1947) ducks and gheese infected did not develop clinical disease although they possessed antibodies. Reports on natural and experimental Ranikhet disease in ducks were controversial. Iyer (194[illegible]) could not infect ducks with virulent strain of the virus. Sharma et al. (1977) infected ducks with a virulent strain of the virus and concluded that ducklings were susceptible to the infection and they observed pneumoencephalitis in them. Sriraman et al. (1980) infected ducklings with a velogenic strain of R D virus experimentally but could not produce the infection in them. They concluded that ducklings were resistant to RD infection. Sulochana et al. (1981) isolated a strain of Newcastle disease virus from ducks ailing from respiratory infection and it was found to be pathogenic to chicken.

MATERIALS AND METHODS

MATERIALS AND METHODS

1.1. Stock

Birds used in this series of experiments were Desi ducks of 4 - 10 weeks old. When studying the response of Ranikhet disease virus and duck plague virus birds of 12 - 16 weeks were employed.

1.2. Agents used

Turpentine - (commercially available)
Dextran sulphate (Mol. wt. 500,000)
Freund's complete Adjuvant (Difco)
Homologous erythrocytes
Talc (commercially available)
Staphylococcus aureus
Ranikhet disease virus
Duck Plague virus.

1.3. Preparation & administration of inocula

The different inocula were prepared and kept in sterile utensils and sterile techniques were used throughout. The quantity of inoculum, the route of administration and biopsy schedule are summarised in table 1. Subcutaneous injections of inocula were made by means of 1 ml tuberculin syringe and

24 gauge needles. The site for subcutaneous inoculation was in the web of the leg except for the viruses which were administered I/M. Biopsies were taken at intervals as indicated (Table I).

1.3.1. Turpentine

Turpentine (0.1 ml) was inoculated subcutaneously in the web of the foot.

1.3.2. Dextran sulphate

A 2% solution prepared in physiological saline and 0.15ml was employed for inoculation.

1.3.3. Freund's complete adjuvant was inoculated subcutaneously (0.15ml) and biopsies were taken at the predetermined intervals.

1.3.4. Homologous erythrocytes

Erythrocytes were prepared from blood drawn from the wing vein of ducks. Five ml of blood were drawn in a 10 ml syringe containing EDTA at the rate of 1 mg/1 ml of blood. The samples were centrifuged at 3000 rcf for 15 minutes for packing the erythrocytes. The plasma was discarded and the buffy coat was removed by a wide mouthed Pasteur pipette. The erythrocytes were then resuspended in 1 ml of

TABLE I

Details of ducks used, agents injected and schedule of collection of materials for studies.

Number of ducks	Agents injected	Quantity	Time at which lesions were examined	No of injection sites/duck	Route of injection
10	Turpentine (commercially available)	0.1 ml	30 min 1 hr, 3 hr, 6 hr, 12 hr 1 day, 2,4,6,8,14 and 21 days	2	Subcutaneously
10	Dextran sulphate 2% suspension in distilled water	0.15 ml	30 min 1 hr, 2 hr, 3 hr, 6 hr, 12 hr 1 day, 2,4,6,8,14 and 21 days	2	"
10	Freund's complete adjuvant (Difco)	0.15 ml	30 min 1 hr, 2 hr, 4 hr, 6 hr, 12 hr 1 day, 2,4,8,14 and 21 days	2	"
10	Homologous erythrocytes	0.2 ml	30 min 1 hr, 3 hr, 6 hr, 12 hr 1 day, 2,4,6,8 and 14 days	2	"
10	Talc (commercially available)	0.2 ml 0.5% suspension in distilled water	30 min 1 hr, 2 hr, 4 hr, 12 hr 1 day, 2,4,8 and 14 days	2	"
10	*Staphylococcus aureus*	0.2 ml 12×10^8 Bacteria	30 min 1 hr, 2 hr, 3 hr, 4 hr, 6 hr, 12 hr, 18 hr 1 day, 2,3,5,8,14 and 21 days	2	"
10	Ranikhet disease virus	0.5 ml 1 in 50 s/c	Sacrificed on 5th, 6th, 7th 8th and 9th day p.i.	1	Intramuscular
10	Duck plague virus	0.5 ml	Sacrificed on 5th, 6th, 7th, 8th and 9th day p.i.	1	Intramuscular

physiological saline and recentrifuged. The supernatant was discarded. This procedure was repeated twice. A portion of the suspension was diluted to 4 times and 0.2 ml of this suspension was employed for inoculation.

1.3.5. Talc

Commercially available talc was mixed with physiological saline to contain 0.5 g of talc in 100 ml of normal saline. This was sterilised and stored in sterile containers before use and 0.2 ml was inoculated subcutaneously.

1.3.6. Staphylococcus aureus

An avian strain of *Staphylococcus aureus* was used. 0.2 ml of an 18 hour broth culture was inoculated subcutaneously.

1.3.7. Ranikhet disease virus

The Komorov strain of virulent virus obtained from the Veterinary Biological Institute, Palode was used. The vial containing freeze dried material from 0.5 ml of the original suspension was diluted with 5 ml of distilled water. From this diluted viral suspension 0.5 ml was transferred into another test tube containing 5 ml of distilled water. The final dilution was 1 in 50.

Ten ducks were employed for the study and 0.5 ml of the viral suspension was given I/M. In this case tissues were collected from visceral organs after sacrifice as indicated in the table to study the cellular response after systemic spread of the virus.

1.3.8. Duck plague virus

The virulent virus was obtained from the Veterinary Biological Institute, Palode. The freeze dried virus was diluted in physiological saline to make a final dilution of 1 in 50. 0.5 ml of the viral suspension was inoculated intramuscularly. The ducks were sacrificed at the intervals as indicated. Tissues were collected from organs for histopathological study.

1.4. Histopathological investigation

Tissues collected for histopathological examination were preserved in 10% neutral formalin. Routine paraffin embedding technique was adopted and sections were cut at 4 - 6 μ in thickness and stained with Harris Haematoxylin and Eosin. Wherever necessary sections were also stained with Van Gieson's picrofuchsin, PAS stain, Perl's stain for iron (Luna, 1968) and toludine blue (Padawar, 1959).

1.5. Electron microscopy

Pieces of tissue collected from the web of the

experimental birds were fixed in a mixture of 1.5 % paraformaldehyde in water and 1.5% glutaraldehyde in 0.2 M phosphate buffer at 4°C. The tissues were then washed three times in 0.1 M phosphate buffer and post fixed in phosphate buffered 1% osmium tetroxide for one hour at 4°C. The specimens were rapidly dehydrated in graded series of ethanol, passed through propylene oxide and embedded in Epon. Ultra thin sections were cut in an LKB ultratome and mounted on copper grids and double stained with aqueous solution of uranyl acetate and lead and examined in Philips EM 420 electron microscope.

RESULTS

RESULTS

1. General observation

The cellular dynamics of response to the various irritants was studied by light and electronmicroscopic observations. Details of cellular involvement were documented at specific time intervals. Changes at all time intervals were not described if the pattern of cellular reaction was qualitatively same.

2. Light microscopic studies

2.1. Turpentine

Inflammatory response was studied on the tissue biopsies from the subcutaneous sites of inoculation of turpentine. Tissues were collected at 30 min, 1 hr, 3 hr, 6 hr, 12 hr, 24 hr and on days 2, 4, 6, 8, 14 and 21 days p.i.

At 30 min p.i. there was severe congestion. A few numbers of heterophils and monocytoid cells could be seen adhering to the endothelium of venules and capillaries and an occasional cell had emigrated out of the blood vessels. There was margination of heterophils and erythrocytes on the wall of the capillaries and venules. Fibrinous oedema was seen perivascularly. Heterophils and a few monocytoid cells were seen in the perivascular space (Fig. 1, 2).

At three hours p.i. emigration of leucocytes was now

Fig.3 Preponderance of heterophils in the cellular aggregates. Turpentine 3 hr p.i. H & E x 400.

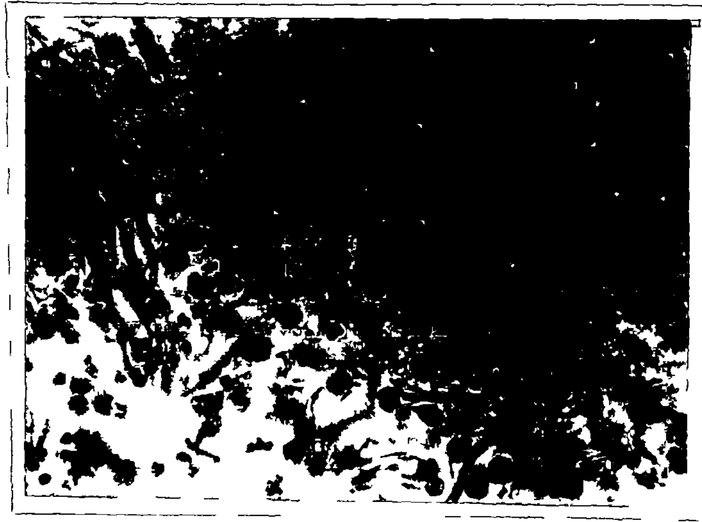
Fig.4 Heterophilic predominance in the exudate. Turpentine 3 hr p.i. H & E x 400.

well-established and heterophils and mononuclears, appeared to emigrate concurrently, but the former seemed to do so in much higher numbers than the latter. The majority of the mononuclears seen adhering to the endothelium of venules or in the perivascular and intervascular area was of the monocytoid type. An occasional cell with deep staining nucleus and with a narrow rim of cytoplasm could also be seen admixed with the monocytoid type. A large number of heterophils, most of them necrotic were found accumulated at the periphery of the bleb formed by the inoculum. Some of these heterophils were devoid of granules or found to contain agglomerated granules. Quantitatively there was a preponderance of heterophils over monocytoid cells, approximately four to five heterophils to one monocytoid cell (Fig. 3, 4).

At six hours after injection oedema was still prominent and there was deposition of fibrin within the bleb of the inoculum. Many of the vessels were thrombosed. Leukocytic emigration and infiltration continued to be prominent. The heterophils were found still emigrating in higher numbers than the mononuclears and in the intervascular area there was accumulation of heterophils, many of them necrotic along with monocytoid cells (Fig. 5). A few of the monocytoid mononuclear cells in the intervascular and perivascular locations were larger than the other cells, with abundant

Fig.5 Monocytoid cells and heterophils many of which are necrotic. Turpentine 6 hr p.i. H & E x 1000.

Fig.6 Monocytoid cells in the intervascular and perivascular locations with abundant cytoplasm and open type of nucleus. Turpentine 6 hr p.i. H & E x 1000.



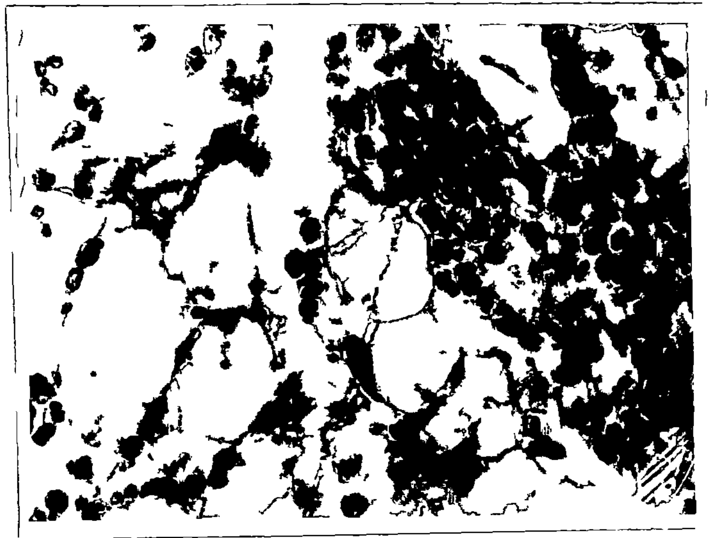
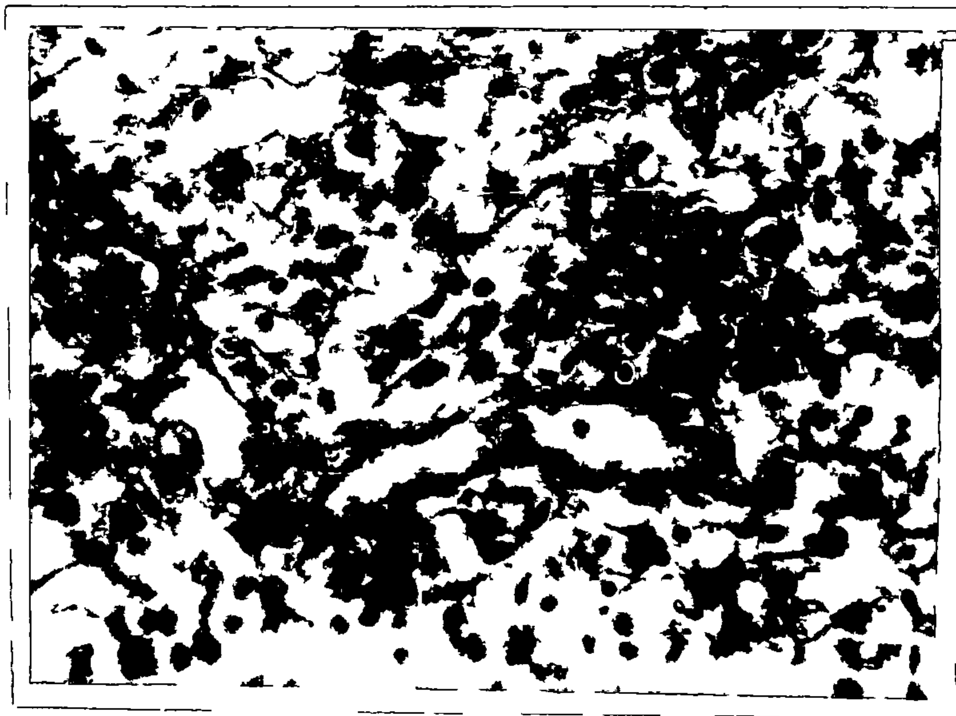
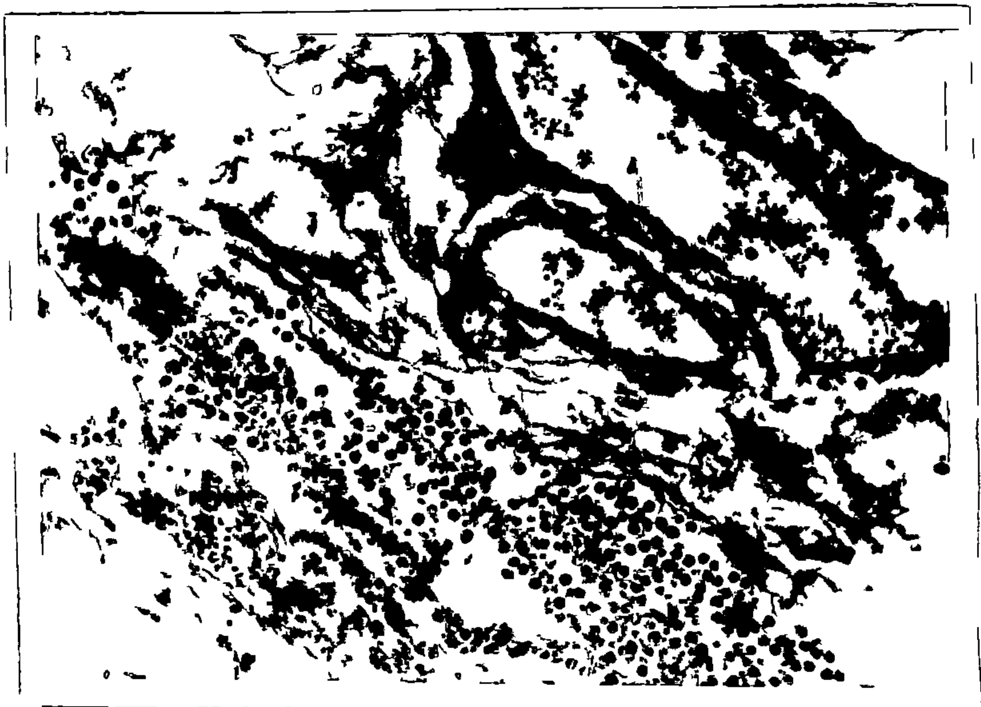


Fig.7 Large number of mononuclear cells in the [illegible]-vascular area. Turpentine 12 hr p.i. H & E x 400.

Fig.8 Large number of heterophils. Turpentine 24 hr p.i. H & E x 400.





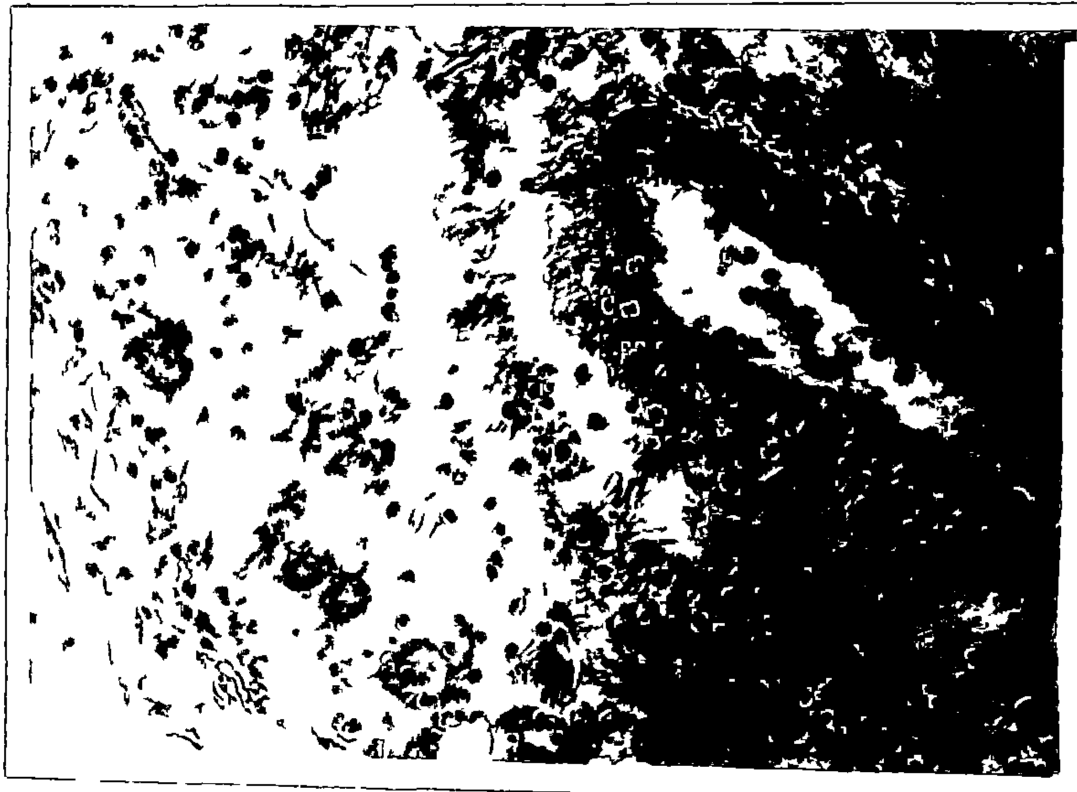
acidophilic cytoplasm and open type of nucleus (Fig.6). Some of these cells were vacuolated while a few others had contained heterophilic granules. Cells with the morphology of basophils, were noticed in the sections stained with toluidine blue which did not reveal typical basophilic metachromatic granules, but had a metachromatic colouration of the cytoplasm.

At 12 hr p.i. emigration of both heterophils and monocytoid cells were still taking place. Even though the heterophils were still emigrating in large numbers, in the intervascular area the number of mononuclears were found to be more (Fig.7). The monocytoid mononuclears continued to increase in size, many of them assuming an irregular form. At the border of the necrotic zone there was a sheet of monocytoid cells, some of which showed tendency to form multinucleated giant cells. Basophilic type of cells were not prominent.

At 24 hr p.i. there was a decline in the emigration of leukocytes; heterophils were still found in large numbers (Fig.8). The monocytoid mononuclears showed a tendency to further increase in size. The cytoplasm was abundant, eosinophilic and some of them contained ingested cellular debris. Small lymphocyte like cells were seen emigrating

Fig.9 Swollen vasculer endothelium, lymphocytes and Macrophages seen perivascularly. Turpentine 24 hr p.i. H & E x 1000.

Fig.10 Cellular exudate consisting of macrophages, lymphocytes and heterophils. Turpentine 4 days p.i. H & E x 400.



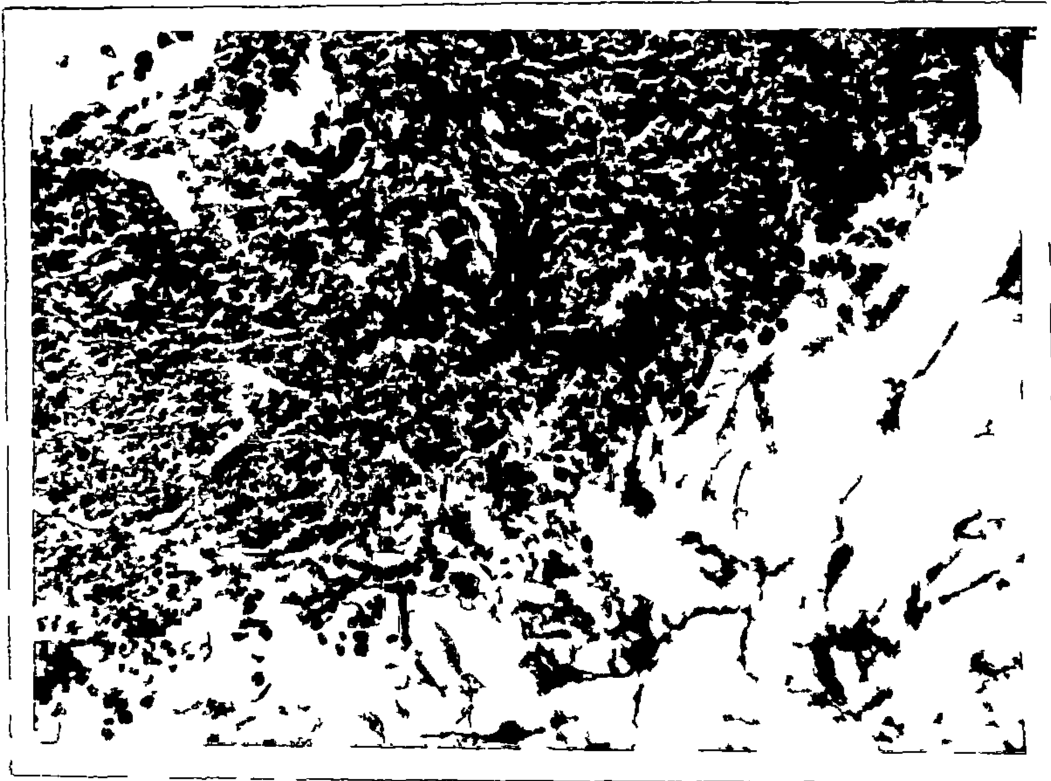
from vessels whose endothelium was found swollen and had taken the form of cuboidal cells instead of a flattened appearance (Fig.9). The pericytes of these vessels appeared swollen and had a basophilic cytoplasm. A few fibroblasts were also seen in the intervascular area.

Leukocytic emigration was not a characteristic feature at 48 hr p.i. even though many newly formed capillaries were clogged with heterophils. Heterophils were still seen in the inflammatory exudate, but the predominant feature was the presence of large number of regularly arranged monocytoid - phagocytes and proliferating fibroblasts. There was also evidence of collagen deposition. Some of the phagocytic cells were in the process of mitosis. Lymphocytes were seen in groups around blood vessels. Near the necrotic zone the number of giant cells had increased.

At four days p.i. there was not much evidence of emigration. Cellular exudate consisted mainly of mononuclear macrophages, lymphocytoid cells and few heterophils (Fig.10). This inflammatory zone was surrounded by a highly cellular and vascular granulation tissue. Focal areas of granulation tissue have extended and merged with each other to form a highly vascular zone. Away from this merging zone, loosely arranged mononuclear macrophages and fibroblasts were still

Fig.11 Massive accumulation of necrotic leucocytes. Turpentine 6 days p.i. H & E x 200.

Fig.12 Predominant accumulation of lymphocytes and monocytes. Turpentine 6 days p.i. H & E x 400.



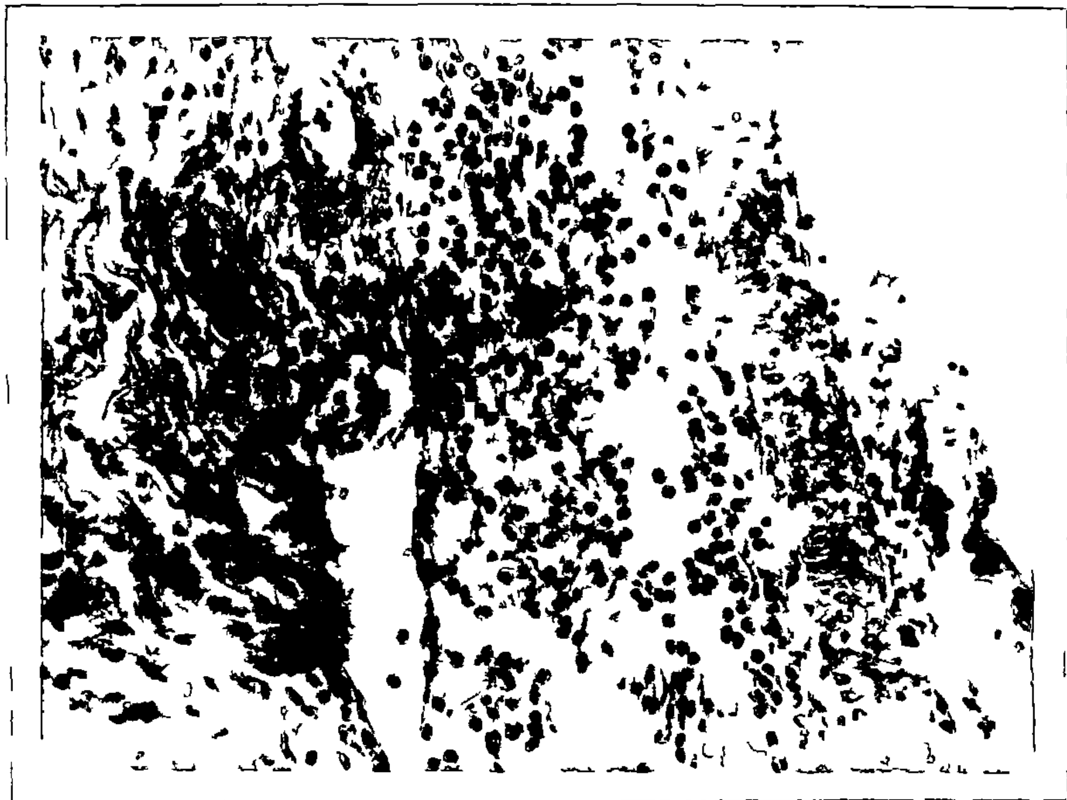


Fig. 13 Collagen deposition in the inflammatory zone. Fibroblasts and macrophages noticed. H & E x 200

Fig. 14 Concurrent emigration of heterophils and mononuclear cells. Dextran sulphate 1 hr p.i. H & E x 200.

present. Among the vascular groups, small collectionsof lymphocytes were present.

At six days p.i., the lesion contained massive accumulation of necrotic leukocytes (Fig.11). Most of the macrophages appeared large with a round pale staining reticular type of nucleus. The zone of granulation tissue bordered by the giant cells was being replaced by more fibrous type. Increasing numbers of lymphocytes were seen along this compact tissue. In the intervascular area there was predominant accumulation of lymphocytes and macrophages (Fig.12). Some of these lymphocytes could be seen infiltrating in the walls of veins and sometimes arterioles. At eight days there was not much qualitative difference in the lesion except that there was more collagen deposition (Fig.13). At 14 days p.i. the composition of the lesion was similar but the granulation tissue was more compact with increased number of lymphocytes. Fibrosis was very prominent.

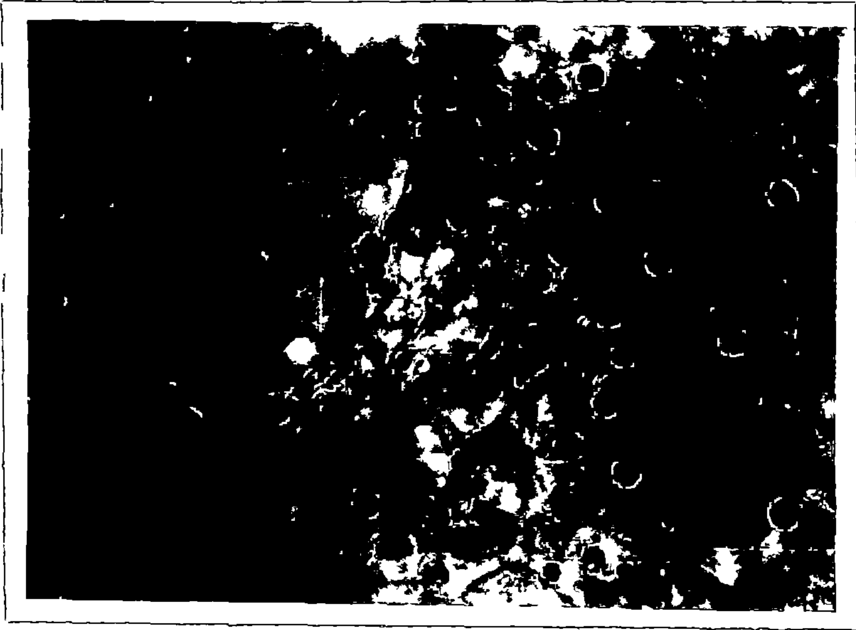
At 21 days p.i. fibrous tissue had completely replaced the necrotic tissue. The cellular component was very sparse with occasional groups of lymphocytes and a few macrophages. The lymphocytes did not show any evidence of infiltration. There appeared to be a tendency of proliferation of pericyte like cells around blood vessels.

Fig.15 Heterophils and monocytoid cells with high proportion of heterophils. Dextran sulphate 2 hr p.i. H & E x 1000.

Fig.16 Perivascular accumulation of monocytoid cells and heterophils. Dextran sulphate 3 hr p.i. H & E x 400.

Fig.17 Large number of heterophils in the intervascular zone. Dextran sulphate 3 hr p.i. H & E x 250.

Fig.18 Heterophils and monocytoid cells with preponderance of monocytoid cells. Dextran sulphate 6 hr p.i. H & E x 400.



2.2. Dextran sulphate

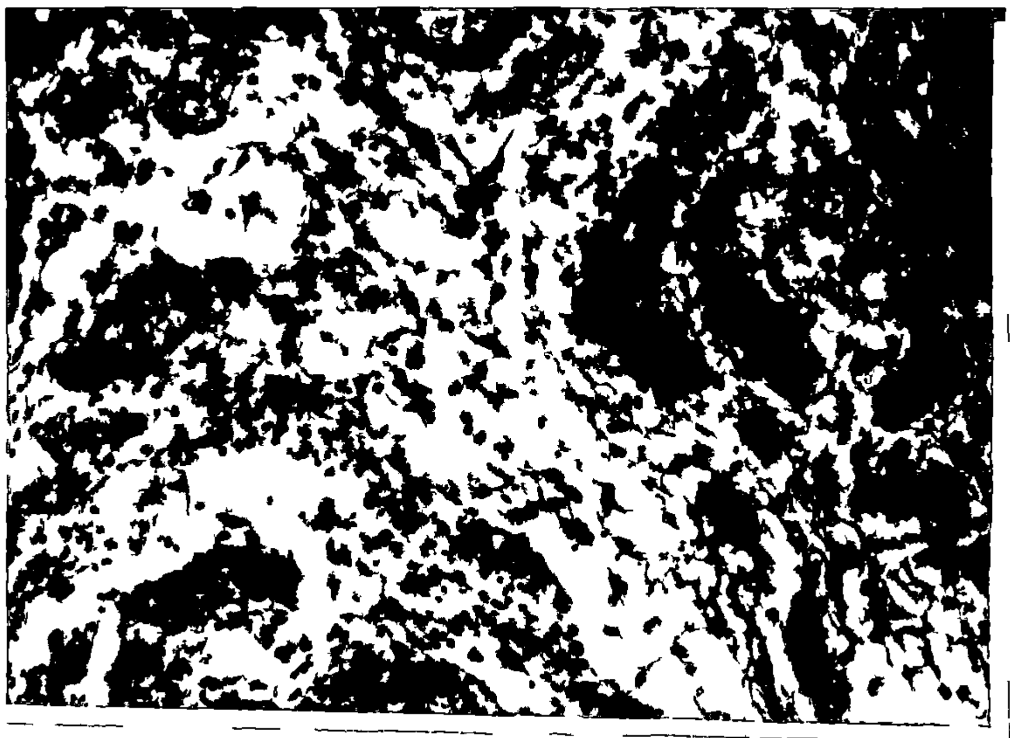
From table 1 it would be seen that 0.15 ml of a 2% solution of Dextran sulphate was injected subcutaneously in the leg web and biopsies taken after 30 minutes, 1 hr., 2 hr., 3 hr., 6 hr., 12 hr., 24 hr., 2 days, 4 days, 6 days, 8 days, 14 days and 24 days.

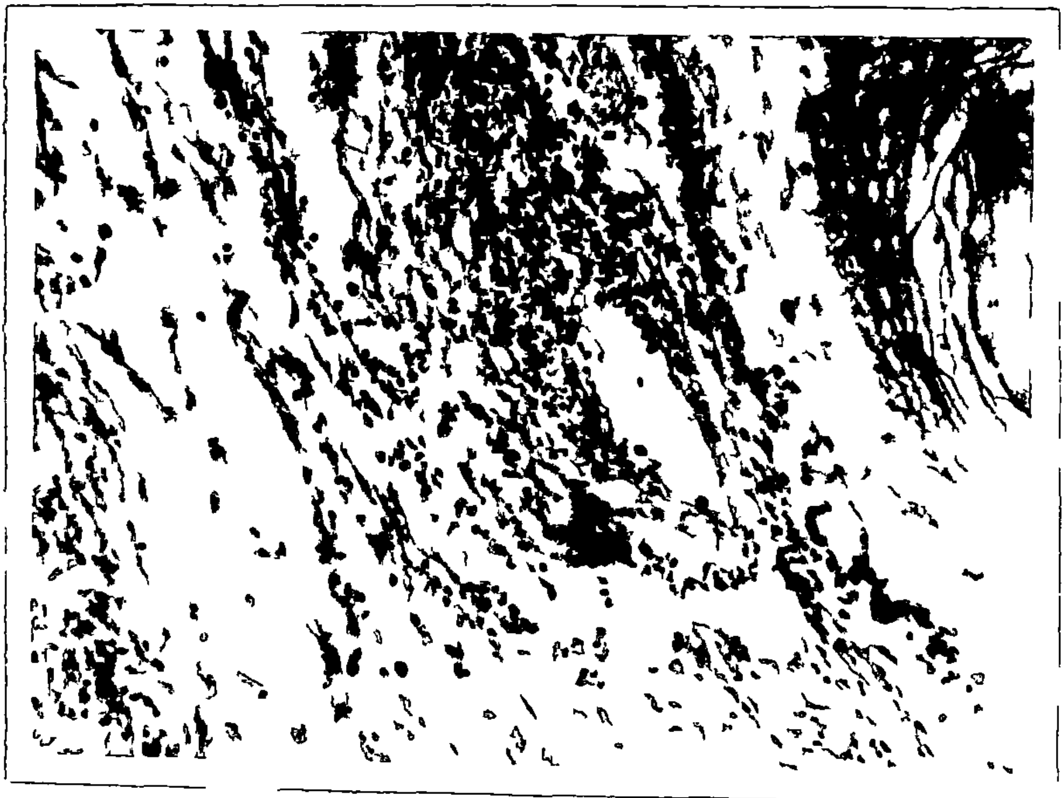
At 30 minutes p.i. there was slight congestion of blood vessels. An occasional heterophil was seen emigrating. At one hour p.i. emigration was little more pronounced. The vessel walls were paved with leukocytes and there appeared to be concurrent emigration of heterophils and monocytoid cells although the number of monocytoid cells were very few (Fig.14).

Congestion still persisted at two hours and three hours. Both heterophils and monocytoid cells were emigrating in large numbers, the percentage of heterophils being higher (Fig.15). The mononuclears, many of which were of the monocytoid type tended to accumulate in the perivascular locations (Fig.16) while the heterophils were found in large numbers in the intervascular areas (Fig.17). Both the heterophils, some of which exhibited degranulation and the monocytoid mononuclears appeared swollen. Numerous vacuoles of varying sizes were seen in the monocytoid cells.

Fig.19 Pronounced emigration of heterophils and monocytoid cells. Dextran sulphate 12 hr p.i. H & E x 200.

Fig.20 In the intervascular and perivascular locations predominant infiltration with monocytoid cells. Dextran sulphate 12 hr p.i. H & E x 150.





Concurrent emigration of both heterophils and monocytoid cells was still going on at six hours p.i. in larger numbers. But both in the perivascular and intervascular areas there was a monocytoid cell predominance (Fig.18). A few of the heterophils were necrotic.

At 12 hours p.i. emigration of leukocytes, mainly heterophils and monocytoid mononuclears, was still going on in large numbers and the reaction appeared maximal at this stage (Fig.19). Both in the perivascular and intervascular areas there was a mononuclear predominance, approximately constituting 70% of the cells (Fig.20). The monocytoid mononuclear cells showed varying levels of morphological alterations, in size, shape and tinctorial properties. The cytoplasm became foamy and the nucleus became large and pale staining. Cytoplasm also revealed numerous granules. A few giant cells were also seen. Both in the macrophages and giant cells phagocytic vacuoles were seen in plenty. A few lymphocytes were seen lying on the endothelial surface of the venules, but hardly any of them could be observed in the perivascular area.

At 24 hours p.i. there was decline in the emigration of heterophils and monocytoid mononuclears. The cellular composition was mainly of heterophils, monocytoid cells and few lymphocytes (Fig.21). Some of the macrophages

have become large measuring upto 20 μ in diameter with acidophilic coarsely vacuolated cytoplasm and with irregular and ruffled cell membranes. Cells in different stages of morphological transition from the typical blood monocyte to large macrophages were found scattered in the perivascular and intervascular areas. Many heterophils were necrotic, some with complete disappearance of granules and pyknotic nucleus. Small lymphoid accumulations were noticed in the perivascular area.

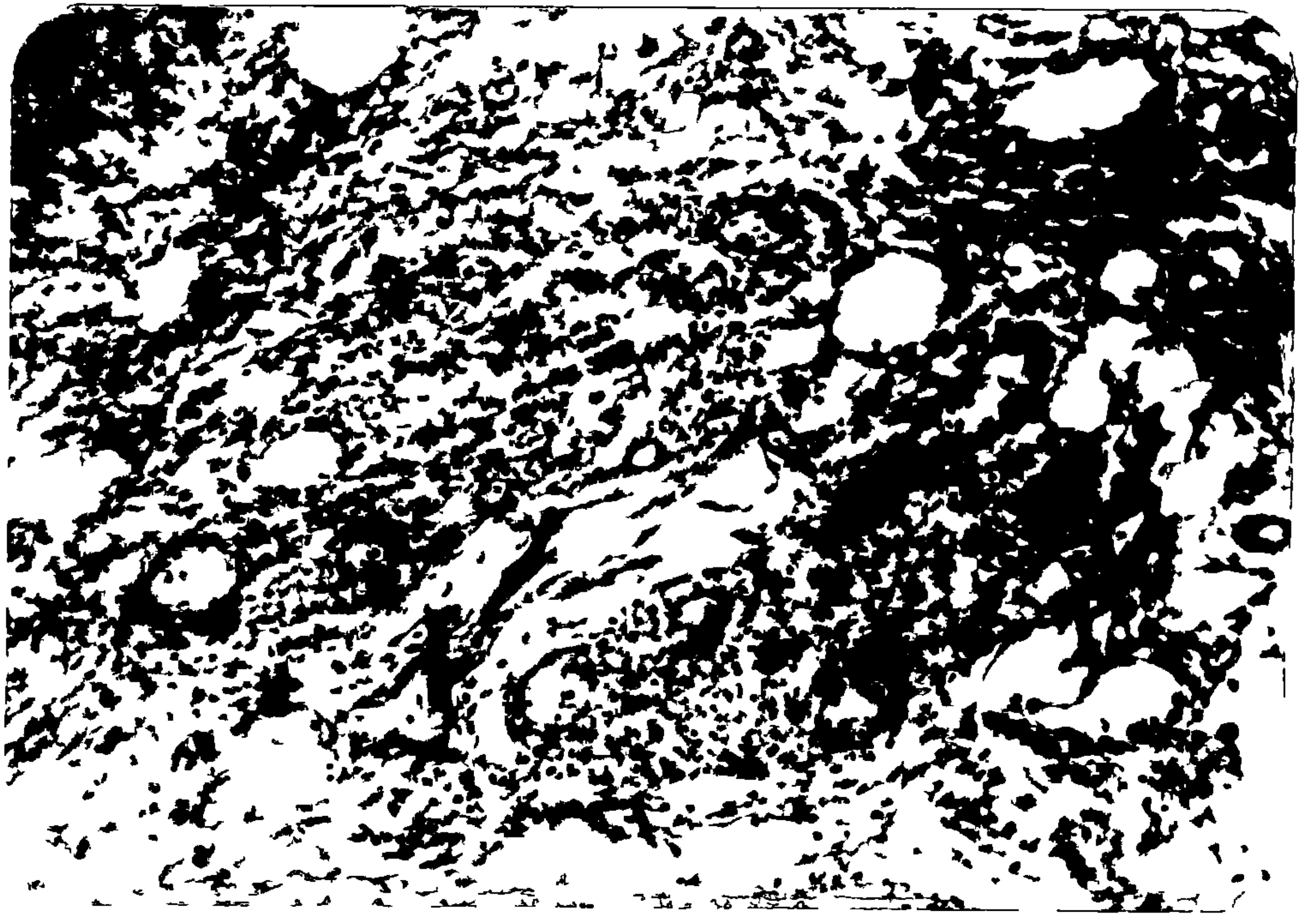
Two to four days p.i. emigration of heterophils and monocytoid cells was absent but lymphocytes could be seen still emigrating to form lymphoid foci perivascularly when the lesion was examined during 2 - 4 days p.i. These lymphocytes did not show any tendency to spread out into the inter vascular area. Cells with the typical morphology of macrophage some of them reaching sizes upto 30 μ were observed. Many of these cells were in stages of mitosis.

The macrophages in the peripheral rows were more uniform in size, but with indistinct cytoplasmic outline and pale staining nuclei, majority of them being located eccentrically. In the deeper areas the macrophages were smaller and were arranged without any definite orientation.

At six and eight days p.i. the lesion was mostly

Fig.21 The cellular exudate consisting of heterophils and monocytoid cells and lymphocytes. Dextran sulphate 24 hr p.i. H & E x 400.

Fig.22 Lymphoid aggregates forming lymphoid nodules. Dextran sulphate 6 days p.i. H & E x 100.



composed of macrophages. Lymphoid cells have aggregated into nodules (Fig.22). Emigration of all types of leukocytes was completely stopped at 14 days p.i. Lousely arranged macrophages and fibroblasts were seen. Even though a large number of the macrophages were intact a few of them appeared as if rupturing. Fibrosis was not very prominent. Numerous lymphoid collections were seen. A compact zone of granulation tissue was found at the periphery of these foci.

2.3. Freund's complete adjuvant

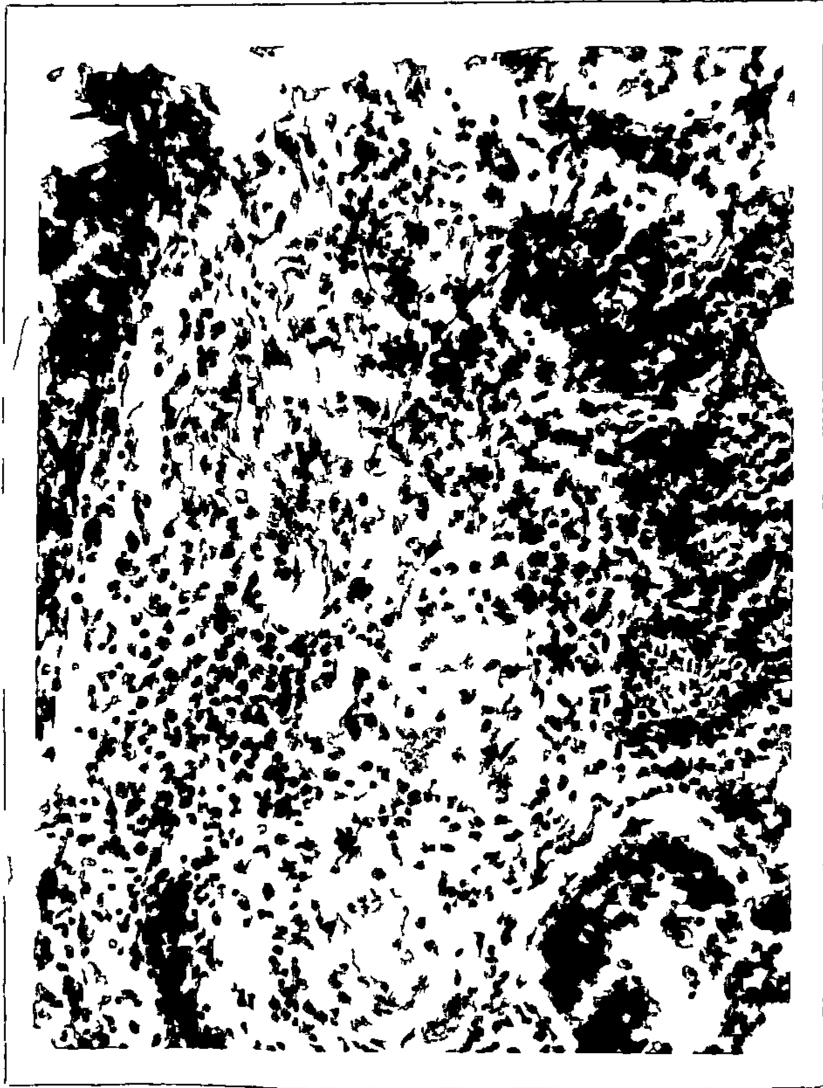
Freund's complete adjuvant (Difco) was injected (0.15 ml) subcutaneously in the foot web. Biopsies were taken at time intervals as indicated in table I.

Emigration of leukocytes was well established at one hour after injection and both types of leukocytes, heterophils and mononuclears appeared concurrently emigrating at all stages. The reaction was maximal at 12 - 24 hr and minimal by the 14 day of injection. There was heterophilic predominance in the intervascular areas until the 24 hr. During this time the heterophils appeared to be emigrating in higher numbers than the mononuclears, the majority of which were of the monocytoid type (Fig.23). From the 12th hr onwards the mononuclear cells in the intervascular areas started exhibiting some morphological

changes. The cells gradually increased in size with pale staining eosinophilic cytoplasm. The nucleus was pale staining. Some of them with a vacuolated appearance. A few of them were in mitosis. From 24 hr there was a preponderance of mononuclear cells in both the perivascular and intervascular areas. After two days a large number of the monocytoid cells became elongated with a relatively small round pale-staining nucleus and acidophilic cytoplasm. They showed a tendency for close apposition and resembled epithelioid cells. A number of these cells as well as fibroblasts exhibited mitotic proliferation. At two days leukocytic emigration was still seen and lymphoid foci were present (Fig. 24). At four days the differentiation and orientation of the mononuclear cells became more definite. Peripherally ie., beneath the base of the dermis thin fibres of collagen have been laid down along the rows of mononuclear macrophages. Branching trabeculae of granulation tissue were seen to arise from this area and join each other. The cellular constituents were mainly of mononuclear cells (Fig.25) with large irregular cytoplasm and round to spindle shaped epithelioid cells with indistinct cytoplasmic outlines. A number of fibroblasts was seen amongst the mononuclear cells. Lymphoid foci were numerous and prominent and there was further an increase in the number

Fig.23 Large number of heterophils along with monocytoid cells and lymphocytes. Freund's complete adjuvant 24 hr p.i. H & E x 400.

Fig.24 Formation of lymphoid nodule. Freund's adjuvant two days p.i. H & E x 400.



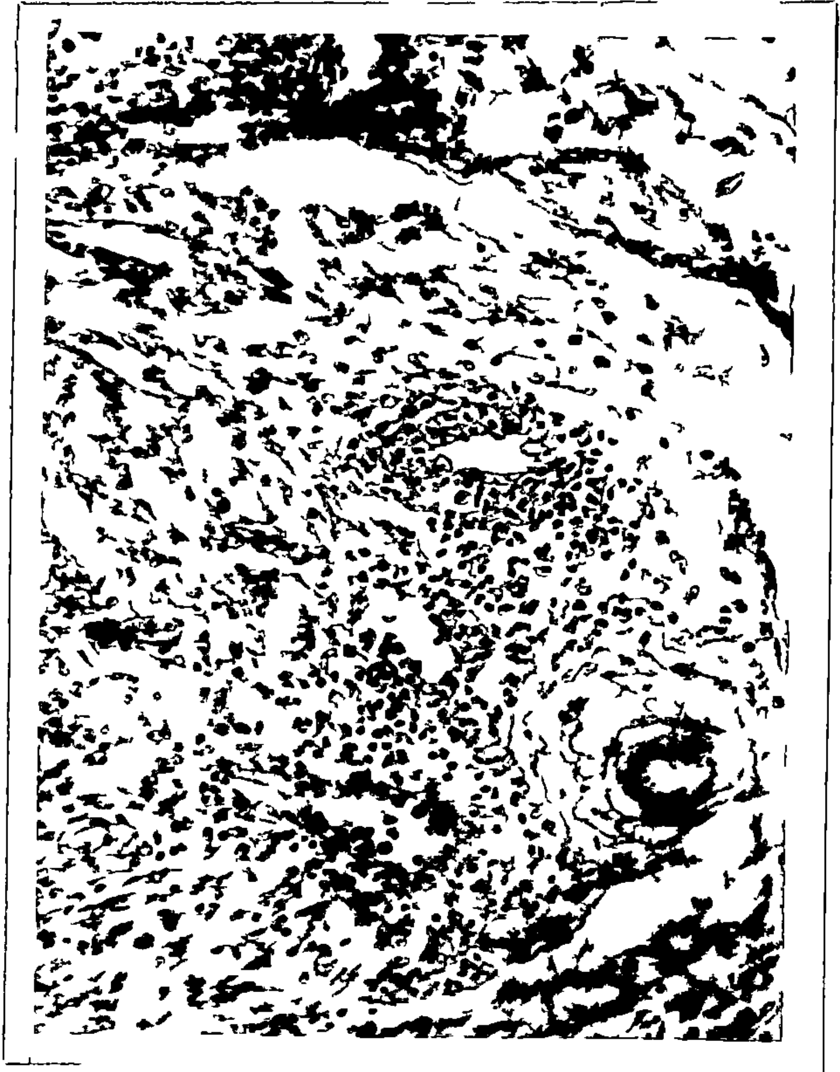
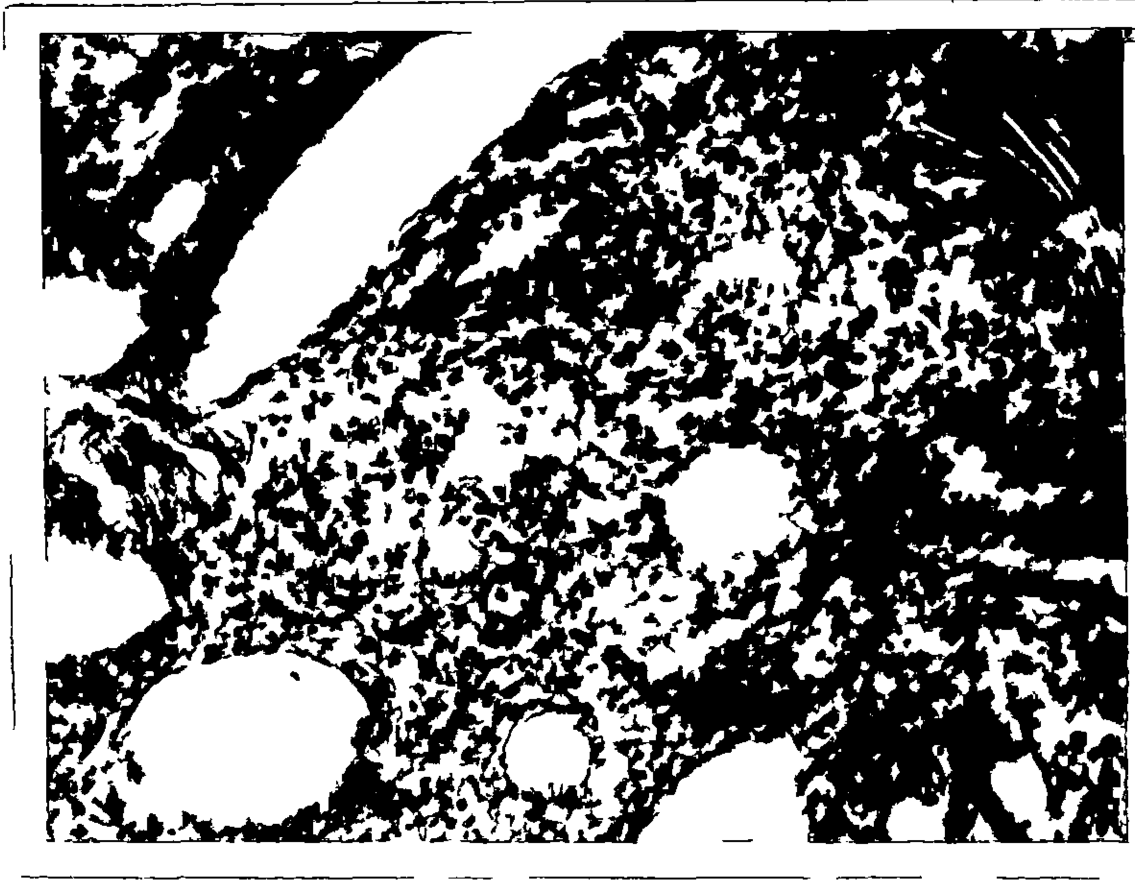


Fig.25 Mononuclear cells and epithelioid cells around the inoculum. Freund's complete adjuvant 4 days p.i. H & E x 400.

Fig.26 Cuff like aggregation of lymphocytes around blood vessels. Freund's complete adjuvant 4 days p.i. H & E x 100.



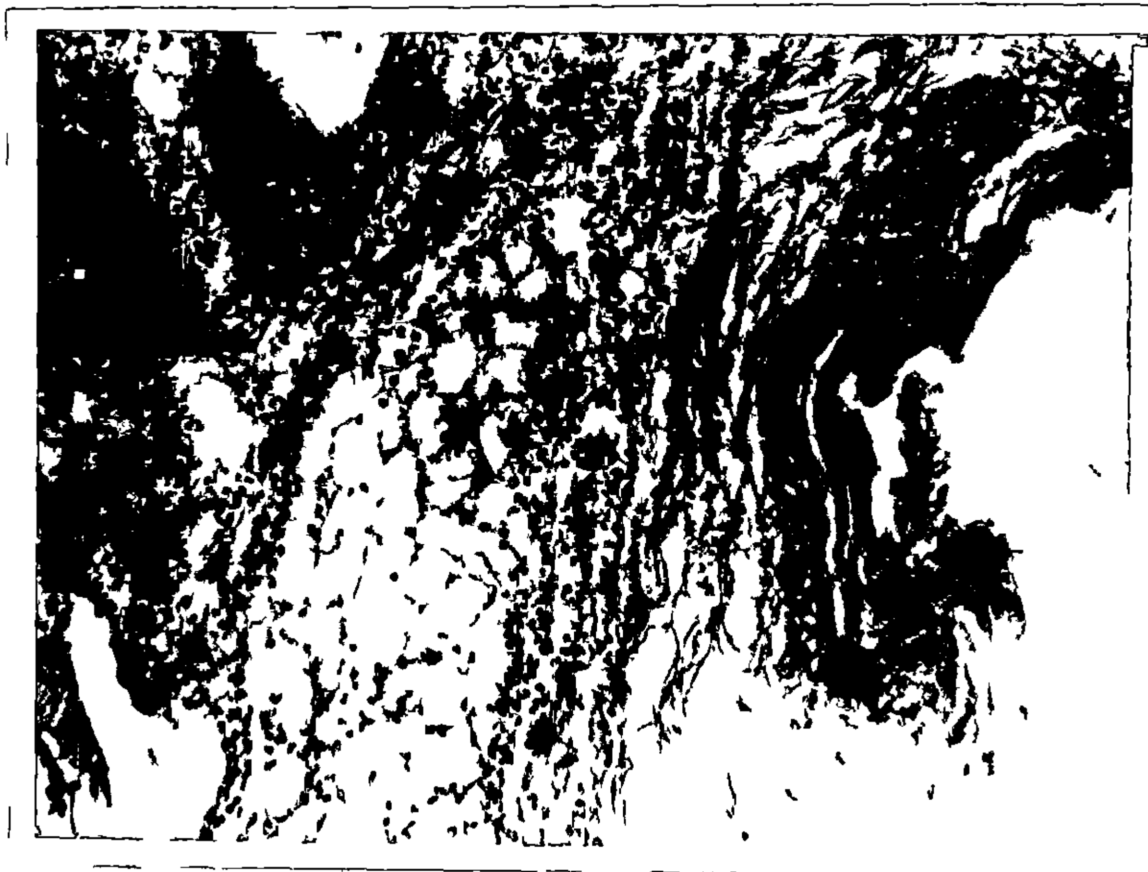
of blast cells amongst the small lymphocytes. Small lymphocytes appeared to migrate from and form small cuff like aggregations around newly formed blood vessels (Fig.26). These cells were also seen spreading into the intervascular areas (Fig.27).

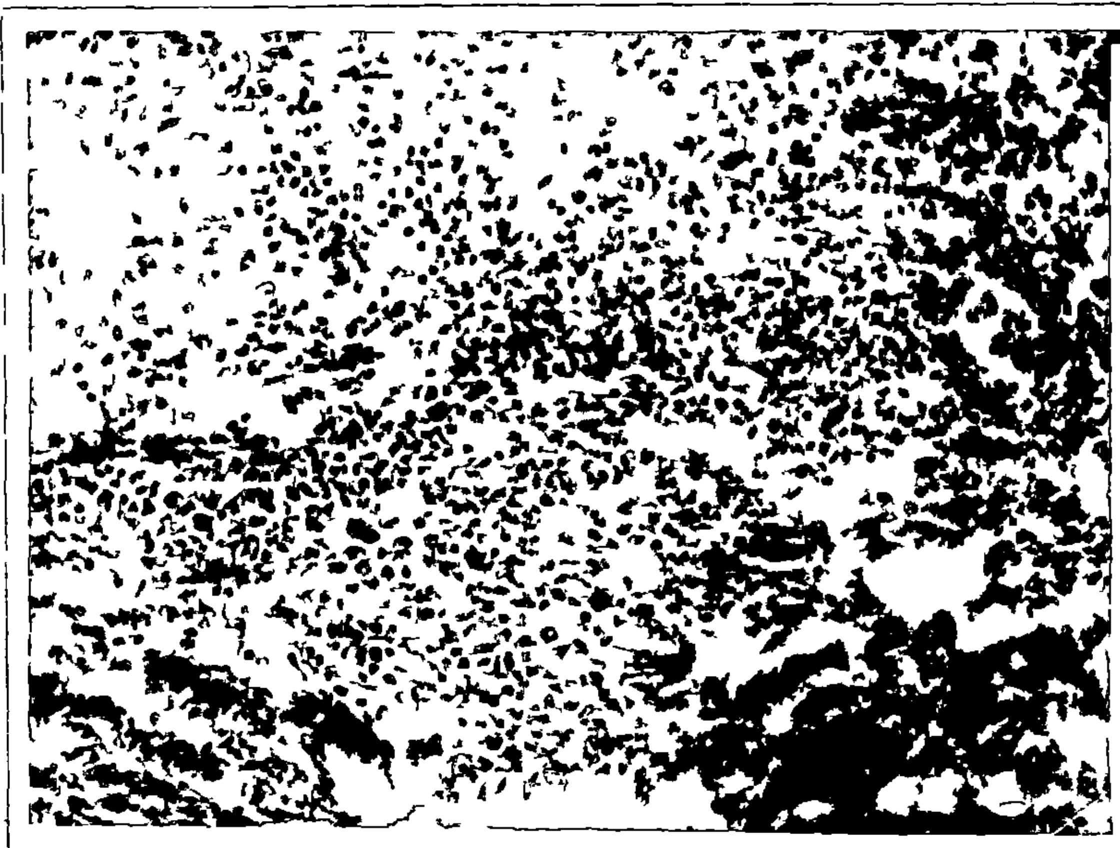
At eight days, emigration of leukocytes appeared to have declined but the mononuclears appeared to be emigrating in significantly higher numbers than heterophils. Epithelioid cells and a few giant cells were seen around the inoculum and outside macrophages, fibroblasts and more epithelioid cells were seen. The trabeculae of granulation tissue were highly vascularised. A number of diffuse lymphoid foci appeared to be forming around newly-formed vessels in the inner zone of the advancing granulation tissue. A few well circumscribed lymphoid foci were also seen. A large number of mature and immature plasma cells was noticed amidst the lymphoid cells. A few of them contained Russel - bodies in their cytoplasm.

At 14 days the zone of the granulation tissue was more extensive but not as cellular and vascularised as it was earlier. The regular and compact arrangement of the bundles of collagen was absent and there was some indication of their resorption. The macrophages continued to enlarge

Fig.27 Small lymphocytes infiltrating into the inter-vascular area. Freund's complete adjuvant 4 days p.i. H & E x 150.

Fig.28 Macrophages and lymphocytes as a dense collection. Homologus erythrocytes 6 days p.i. H & E x 400.





in size some of them reaching upto 20 - 25 μ. Large giant cells were seen loaded with phagocytosed material. Lymphoid cells both in the form of nodules and as free cells were seen, but the secondary lymphoid nodules in the more peripheral areas were now smaller in size. Mitotic proliferation of cells within the most peripheral nodules was no longer appreciable. Similarly, the number of mature and immature plasma cells in the peripheral areas of the granulation tissue had declined significantly although their number in the deeper areas was still very high.

At 21 days, the peripheral fibrous zone was more extensive than previously, and in its peripheral areas the collagen was in the process of resorption and remodelling. The inner zone was still highly vascularised and leukocytes were still seen emigrating from the newly formed vessels, even though the number of such emigrating cells was few. These cells after emigration were more or less intact, only few of them showing degranulation as was seen during the early phase when most of the heterophils which emigrated had degranulated. Few secondary granulomas consisting of fibronecrotic material and surrounded by giant cells, epithelioid cells, macrophages and lymphocytes were also seen. The lymphoid foci were still seen, but there appeared to be a reduction in the number of blast cells. The number

of mature and immature plasma cells appeared to have declined even further, but they were still abundant in the deeper zones of the granulation tissue.

2.4. Homologous erythrocytes

As seen from the table I 0.2 ml of suspension of washed homologous erythrocytes were injected subcutaneously in the web of the foot and the lesions were examined at different time intervals.

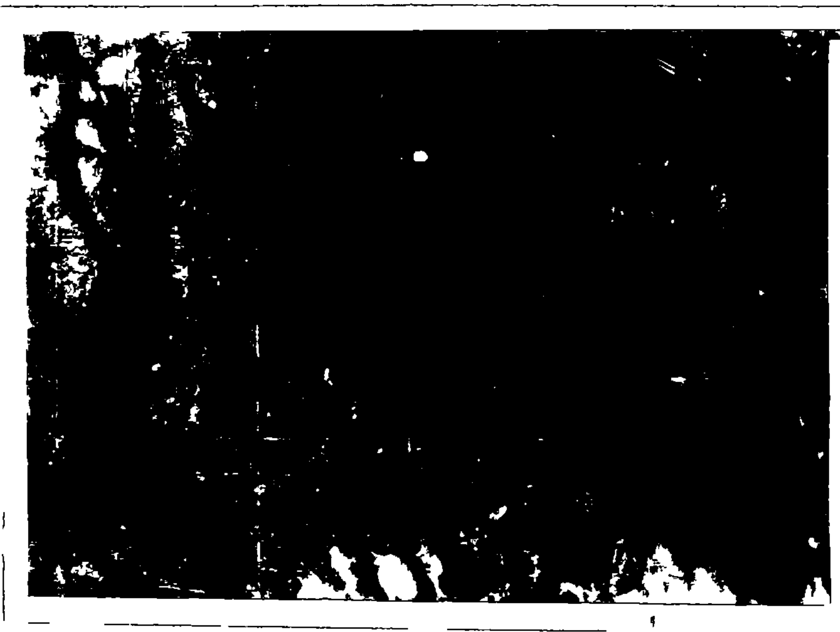
At 30 minutes there was mild congestion and leukocytes were seen pavementing the small vessels. At six hr emigration of leukocytes was well established. The emigration was concurrent and the heterophils were still predominating over the monocytoid type of cells in both the perivascular and intervascular areas. A few degranulated cells probably basophils were noticed. At 12 hr the emigration of leukocytes appeared maximal. A few small lymphocytes could be seen forming cuffs around venules, mostly within vascular groups. At 24 hr the emigration of leukocytes appeared to have declined. The mononuclear cells were seen in large numbers in the perivascular areas while the heterophils continued to predominate in the intervascular areas (Fig.2a). The monocytoid cells showed morphological alterations in the form of increase in size,

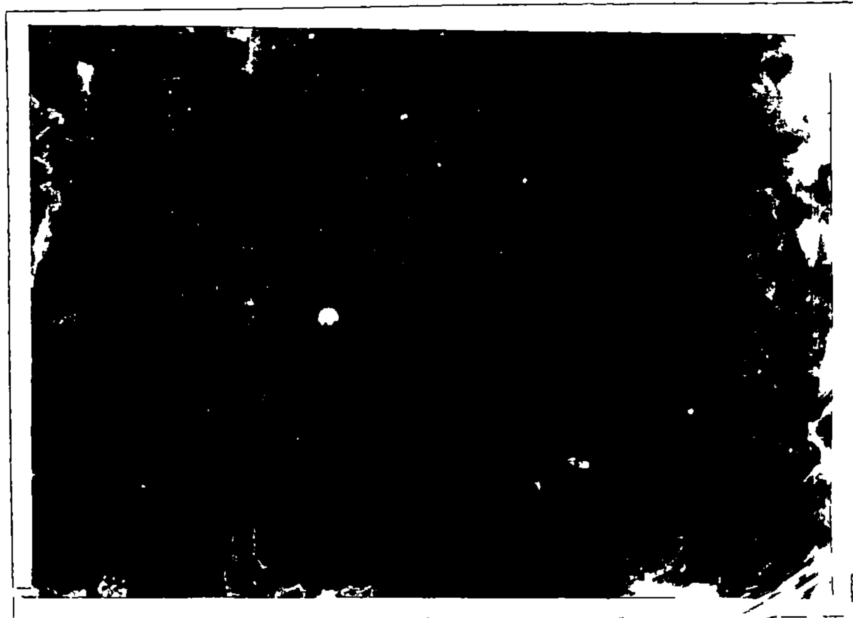
change of shape and presence of phagocytosed material in the cytoplasm. The nucleus was large, pale staining and irregular, sometimes with a reniform contour. The erythrocytes in the intervascular areas were in various stages of disintengration, some of them presented a picture of a cell with complete karyolysis while some other cells retained a distorted nuclear membrane. Many of the phagocytes were seen to have ingested disintegrated or intact erythrocytes. Lymphoid foci were present usually within vascular groups.

Forty-eight hours after injection leukocytes were still seen emigrating concurrently but only in small numbers. The macrophages were round in shape, upto 25 μ in diameter with a foamy cytoplasm and an eccentrically placed pale staining nucleus. Giant cells were present around pools of fibrinoid like material. Lymphoid foci were numerous. During the period of 4 - 6 days most of the erythrocytes had disintegrated or were removed. Rows of compact macrophages and fibroblasts were seen (Fig. 29). The macrophages appeared large and most of them contained haemosiderin. A number of macrophages and fibroblasts exhibited mitotic proliferation. Lymphoid foci appeared diffuse and the lymphocytes were seen spreading away into the intervascular areas. At eight days, leukocytic

Fig.29 Macrophag s and fibroblasts arranged in a compact manner. Homologus erythrocytes 6 days p.i. H & E x 600.

Fig.30 Mononuclear cells in the intervascular areas. Talc 24 hr p.i. H & E x 1000.





emigration was minimal. Macrophages were seen arranged in well-defined rows. Most of these cells were loaded with haemosiderin. Cells with the morphology of epithelioid cells were absent. Well circumscribed lymphoid foci were present but blast cells were scanty.

After 14 days only an occasional vessel showed evidence of leukocytic emigration. Heterophils were absent in the perivascular and intervascular areas. Numerous haemosiderin laden macrophages were still present along with unorganized collection of lymphocytes.

2.5. Talc

Talc (0.2 ml of c 0.5% suspension) was injected subcutaneously in the web of foot. The tissues were examined at different time intervals as indicated in Table I.

The cellular reaction was very minimal upto two hours and significant emigration was noticed only after four hours. The emigration was concurrent, but the number of emigrating cells was not massive. A few cells with the morphology of basophils, but with no metachromatic granules were noticed. These cells were probably degranulated basophils. After 12 hr the population of heterophils was very scanty and most of the cells which had moved away to the intervascular areas had degranulated. After 24 hours there was predominance

of mononuclear cells in the inter-vascular areas (Fig.30) and the monocytoid cells exhibited morphological alterations in assuming the size and shape of typical macrophages. In the locations where talcum particles were present there was a preponderance of large macrophages, along with a few giant cells (Fig.31). Small lymphoid accumulations were seen away from the perivascular location. At four days the number of heterophils both in the perivascular and intervascular areas was only very few even though some venules were seen pavemented with heterophils. Many macrophages were seen clustered around the talc particles and giant cells were seen arranged in a palisade fashion where there was accumulation of talc crystals. Some giant cells were seen to contain talc strands of fibrin in addition to crystals of talc.

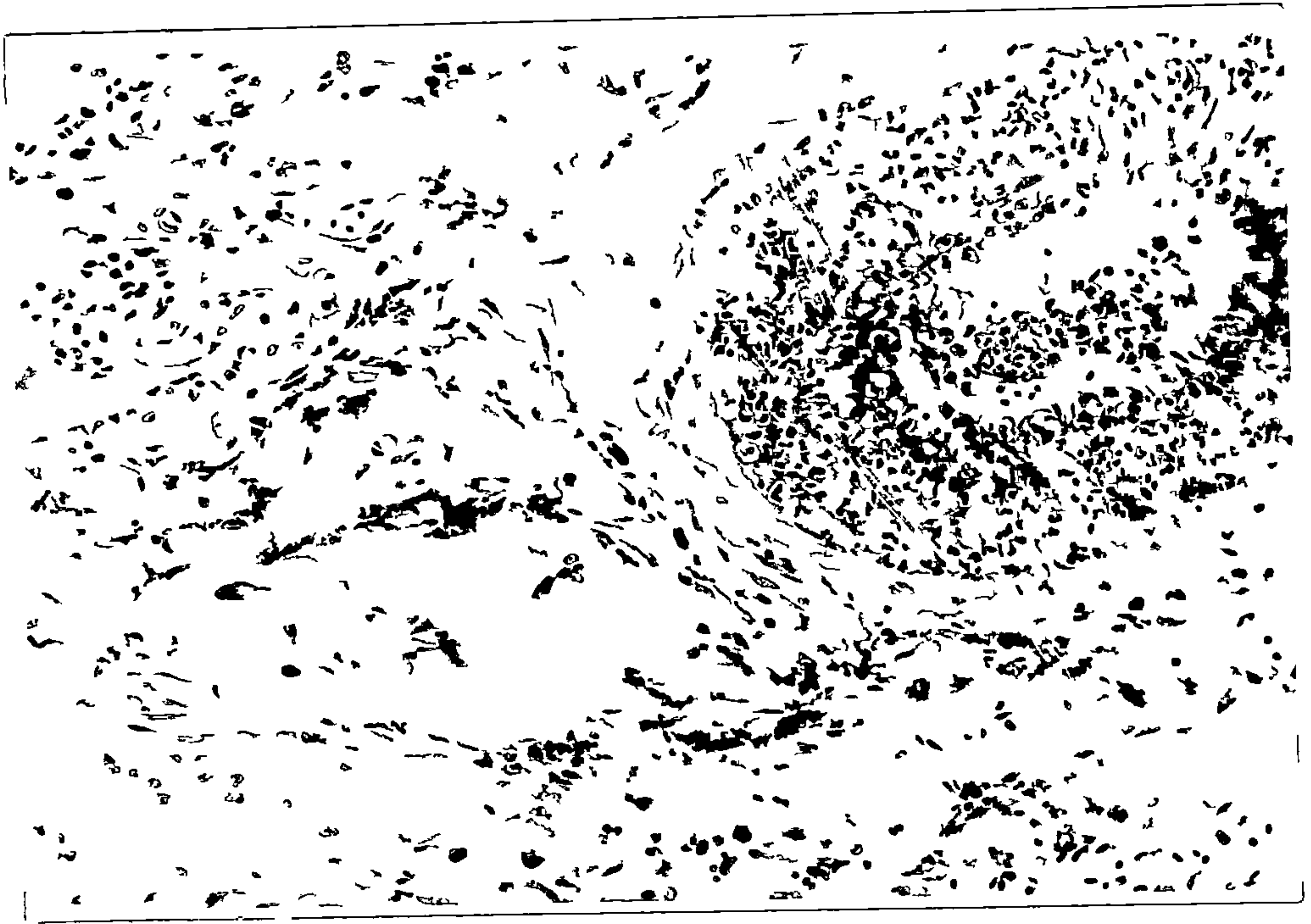
At eight days, the lesion was that of a granuloma with macrophages and giant cells many of them containing talc crystals. Numerous fibroblasts had encapsulated the granuloma. After 14 days the cellular reaction of the lesion was sparse except for the compact encapsulated granuloma with few talc crystals, giant cells and few macrophages. Except for an occasional lymphocyte there was no other characteristic cellular reaction away from the granuloma. But around the zone of fibrous tissue numerous

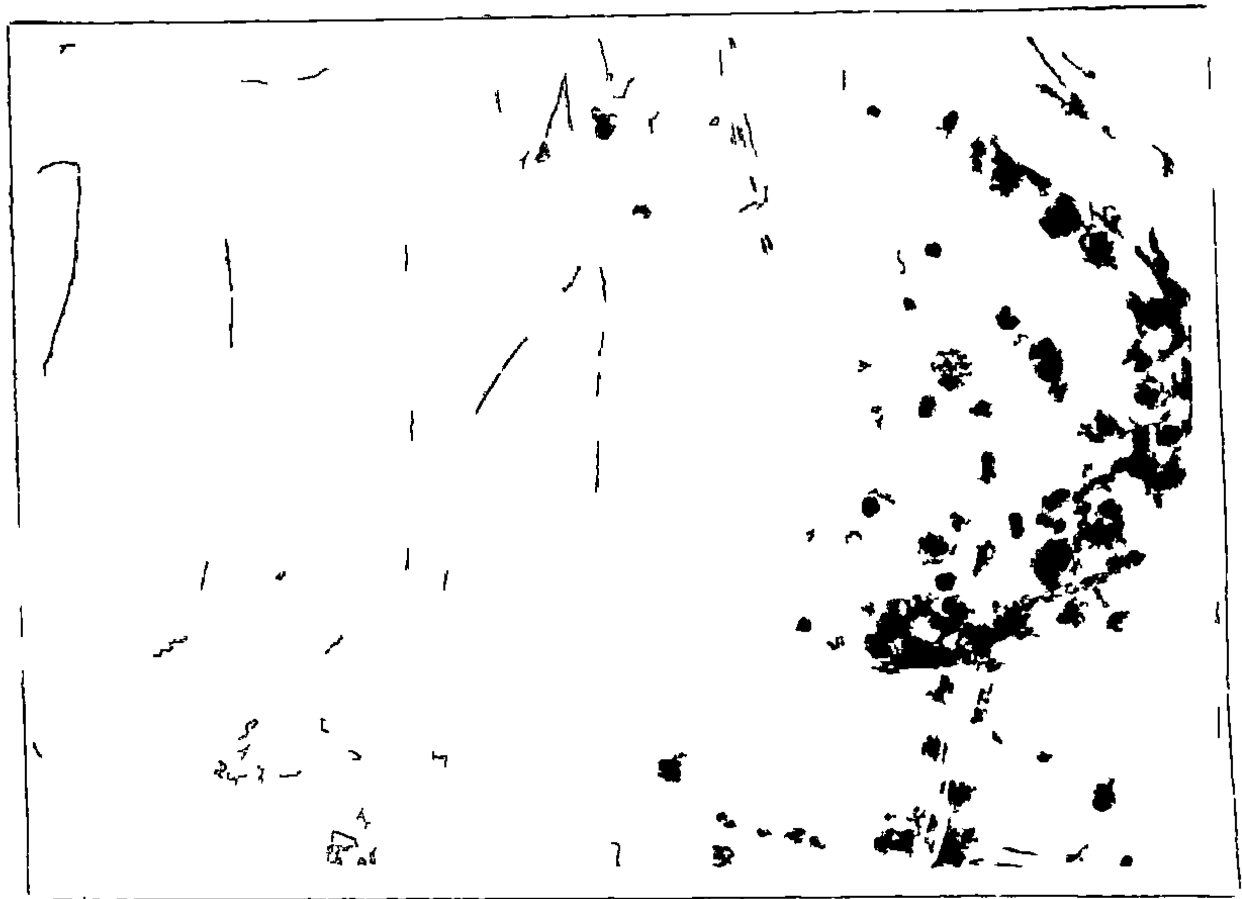
Fig.31 Large numbers of macrophages and a few giant cells. Talc 24 hr p.i. H & E x 600.

Fig.32 Emigration of heterophils and monocytoid cells. Predominance of heterophils in the perivascular locations - Clumps of blue staining cocci are also seen. Staphylococcus aureus 3 hr p.i. H & E x 200.

Fig.33 Exudate consisting mainly of heterophils. *Staphylococcus aureus* 3 hr p.i. H & E x 1000.

Fig.34 Concurrent emigration of heterophils and mononuclear cells. *Staphylococcus aureus* 6 hr p.i. H & E x 1000.





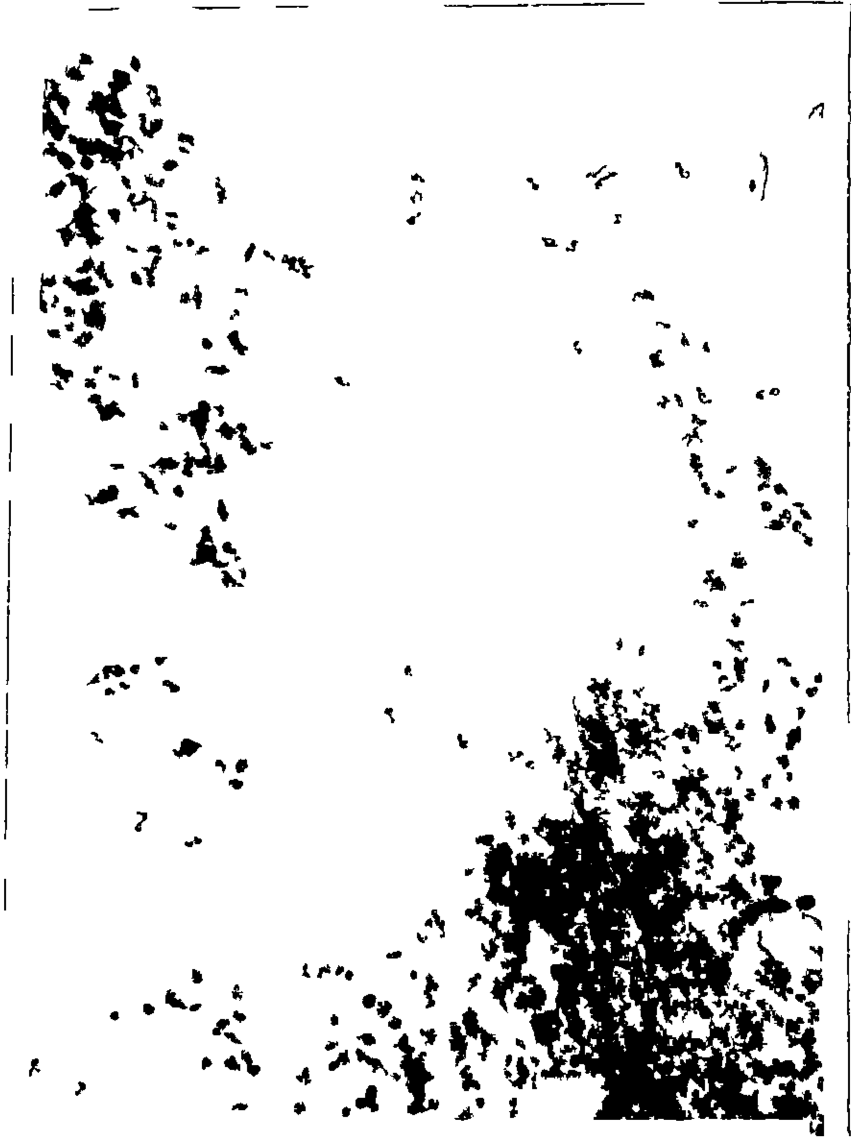
well formed blood vessels were noticed.

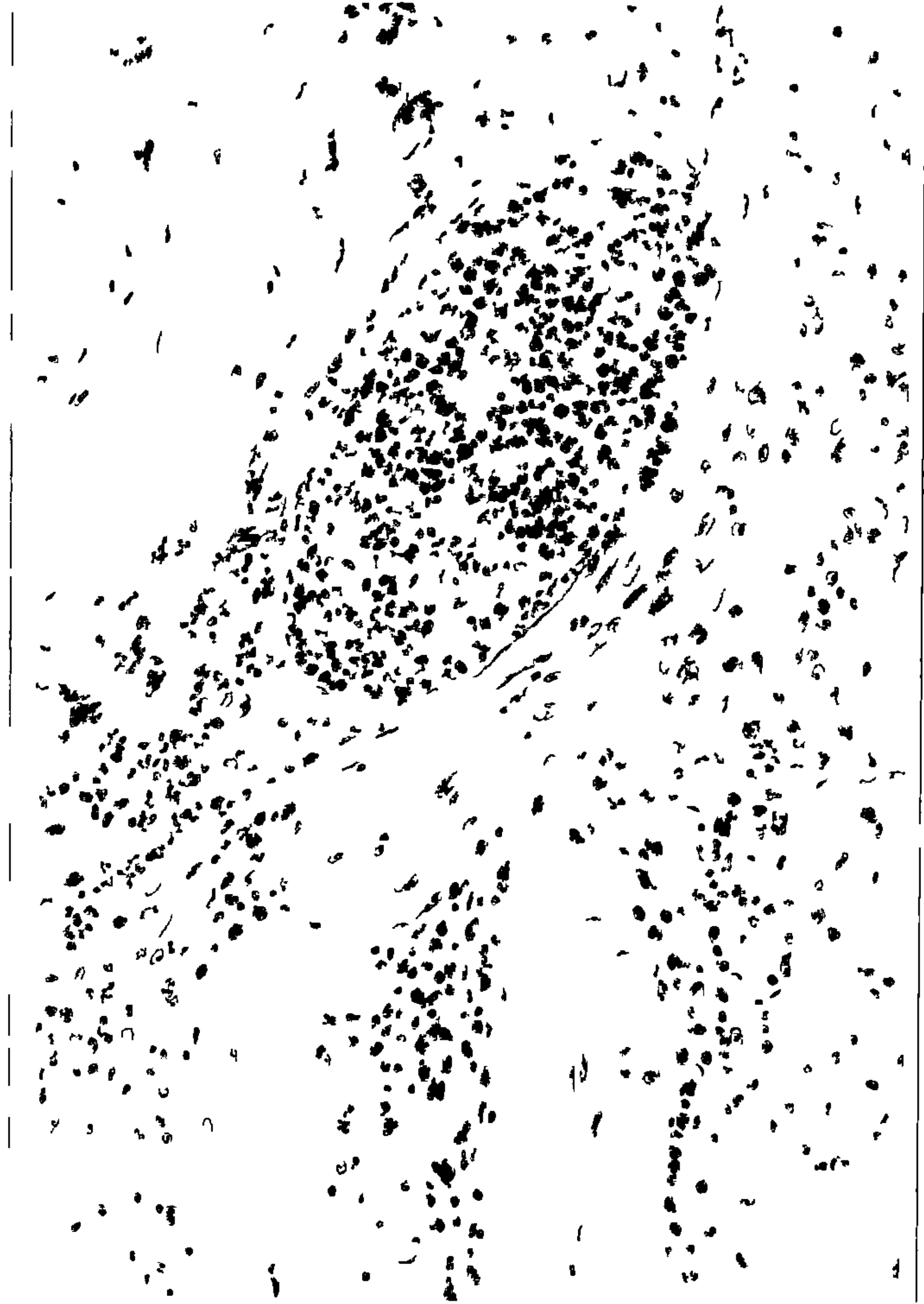
2.6 Staphylococcus aureus

An avian strain of Staphylococcus aureus in broth (0.2 ml) was inoculated subcutaneously in the web of the foot and biopsies were taken as per the schedule given in Table I. At 30 min and one hour congestion and oedema were prominent and most of the small vessels were pavemented with leukocytes mostly heterophils. Concurrent emigration of heterophils and monocytoid cells was seen; but the number of heterophils noticed perivascularly was much more than the monocytoid cells. A few basophils were also seen emigrating and most of them in the perivascular areas had a degranulated appearance. At three hours after injection, the emigration of leukocytes was well established (Fig. 32 & 33). At the perivascular areas the monocytoid cells predominated while in the inter-vascular areas the number of heterophils was about 3 - 4 times that of monocytoid cells. In the perivascular areas many of the heterophils had degranulated and some of them had become necrotic. Large groups of bacteria were seen in the tissues. Phagocytosis of bacteria by heterophils and monocytoid mononuclears was also prominent. At six hours after injection, emigration of heterophils and monocytoid cells was still concurrent but was more intense than was seen earlier (Fig.34). Now the number of heterophils in the perivascular area was

Fig. 35 Predominant infiltrating cell is heterophil. The capillaries are engorged with heterophils and mononuclear cells. _Staphylococcus aureus_ 6 hr p.i. H & E x 400.

Fig. 36 Large number of leucocytes, many of them necrotic around the inoculum. _Staphylococcus aureus_ 6 hr p.i. H & E x 400.

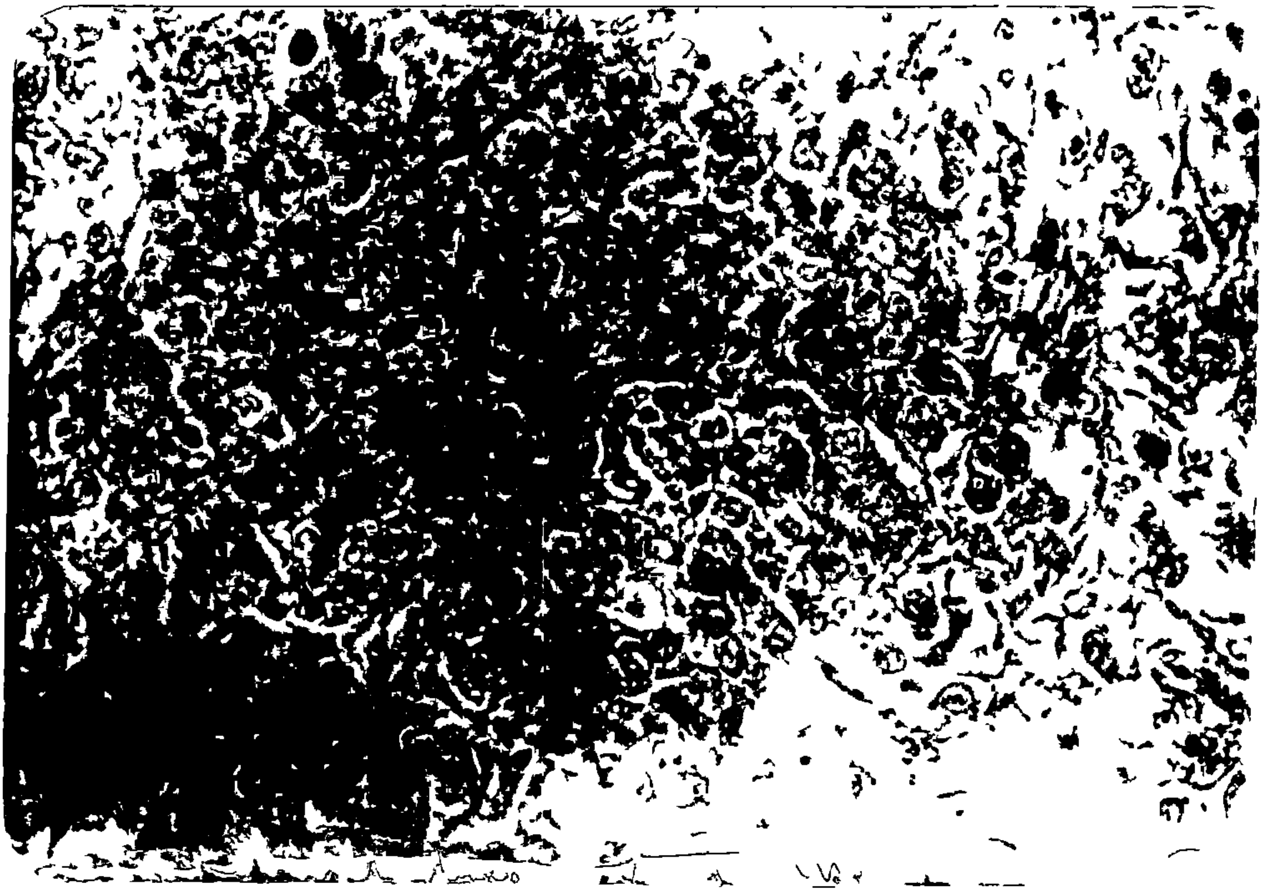


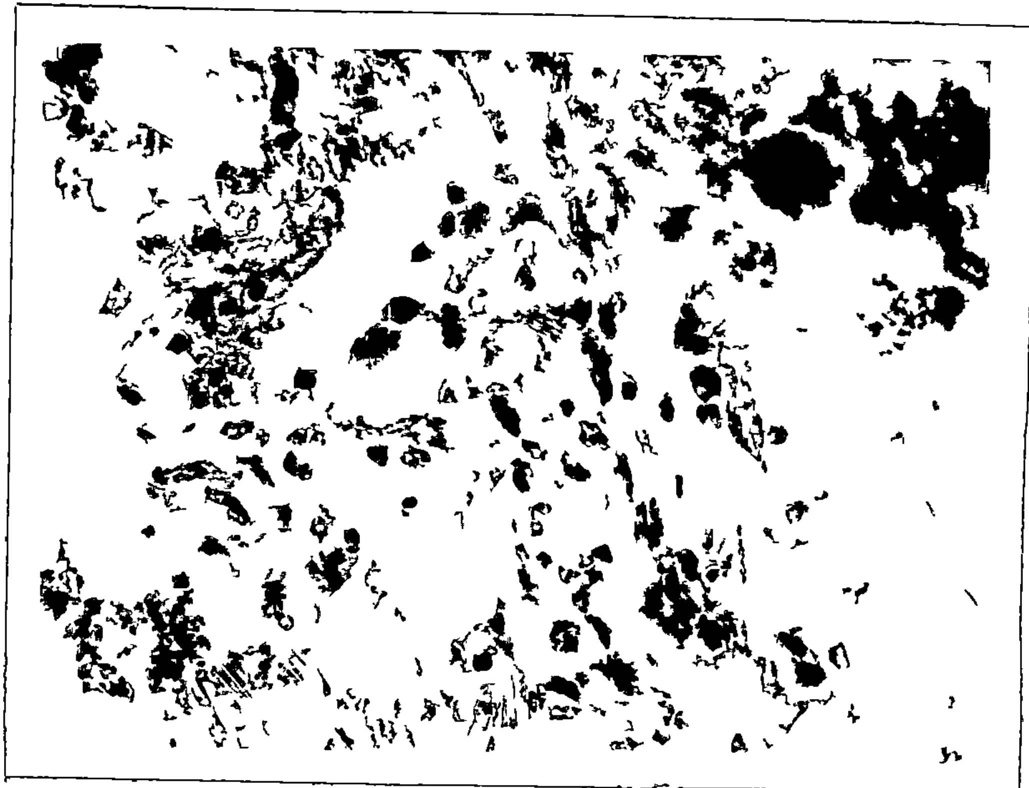


more than the monocytoid cells (Fig.35). A large number of leukocytes, many of them necrotic heterophils, were seen accumulated around the inoculum (Fig.36). Around the inoculum strands of fibrin had been seen deposited. Many of the heterophils and macrophages were seen loaded with bacteria. At 12 hr the qualitative nature of the reaction was same except that there was massive infiltration of emigrated cells. In the intervascular areas there still was a preponderence of heterophils. But the monocytoid cells had become larger with an increased quantity of basophilic foamy cytoplasm and large pale staining nucleus. There were foci of necrosis and surrounding these zones there was infiltration of heterophils, macrophages and few lymphocytes (Fig. 37) along with proliferation of reticuloendotheliable cells. A few lymphocytes were seen emigrating from venules and forming cuffs around them. These lymphocytes demonstrated a tendency to accumulate around the vessels than moving away as did the heterophils and macrophages. At twenty-four hours after injection there was decline in the emigration of leukocytes even though there was still evidence of concurrent nature of emigration of heterophils and mononuclear cells (Fig. 38 & 39). Many of the venules where emigration was going on had swollen prominent endothelial cells. In addition, proliferation of reticuloendothelial like cells was seen around

Fig. 37 Foci of necrosis surrounded by lymphocytes, macrophages and lymphocytes. *Staphylococcus aureus* 12 hr p.i. H & E x 1000.

Fig.38 Concurrent heterophilic and mononuclear cell emigration. *Staphylococcus aureus* 24 hr p.i. H & E x 400.





the vessels. The monocytoid cells exhibited further morphological changes, many of them assumed a large profile with plenty of cytoplasm and reaching a size of about 20 - 25 µ. Among the large macrophages were also found smaller cells with the morphology of blood monocytes. Some of the macrophages exhibited mitotic proliferation. The size of the lymphoid foci was larger than at 12 hr. A number of large pale staining cells with the morphology of blast cells could be seen in the lymphoid foci. At three days there was marked decline of emigration of cells, with the exception of lymphocytes. There were prominent lymphoid accumulation. Apart from the characteristic lymphoid cells, these foci contained large irregular cells with pale staining nucleus. Eventhough these collections of lymphocytes appeared as well defined foci, there was no encapsulation. At six days there was no significant emigration of leucocytes except for an occasional lymphocyte which could be seen adhering to the blood vessels. There was proliferation of fibroblasts in the intervascular area and they were seen arranged in rows along with the macrophages. The macrophages were large, some of them reaching a size of 25 µ and having an eosinophilic cytoplasm. Collagen fibres had been laid down along the rows of macrophages. Around the necrotic fibrinous mass at the site of inoculum there were a few

giant cells. The lymphoid foci were prominent and they contained in addition to the cells with the typical morphology of lymphocytes, numerous blast cells and mature and immature plasma cells. The venules inside the lymphoid foci contained large number of lymphocytes. After eight days the lymphoid foci appeared diffuse and contained large number of blast cells and plasma cells. The plasma cells were found within the secondary nodules as well as around vessels. The zone of necrotic tissue was soon surrounded by granulation tissue, consisting of infiltrating cells, newly formed blood vessels, fibroblasts and collagen. At 14 days most of the necrotic tissue had been removed. The lesion appeared as foci of granulation with only few cells. Comparatively lymphoid cells were more than in other types. Plasma cells were also evident. When the lesion was examined at 21 days, it appeared as a compact granulation tissue with only few cells.

2.7. Infection with Ranikhet Disease virus

This study was conducted to assess systemic inflammatory response and cellular dynamics after intramuscular inoculation of Ranikhet Disease virus.

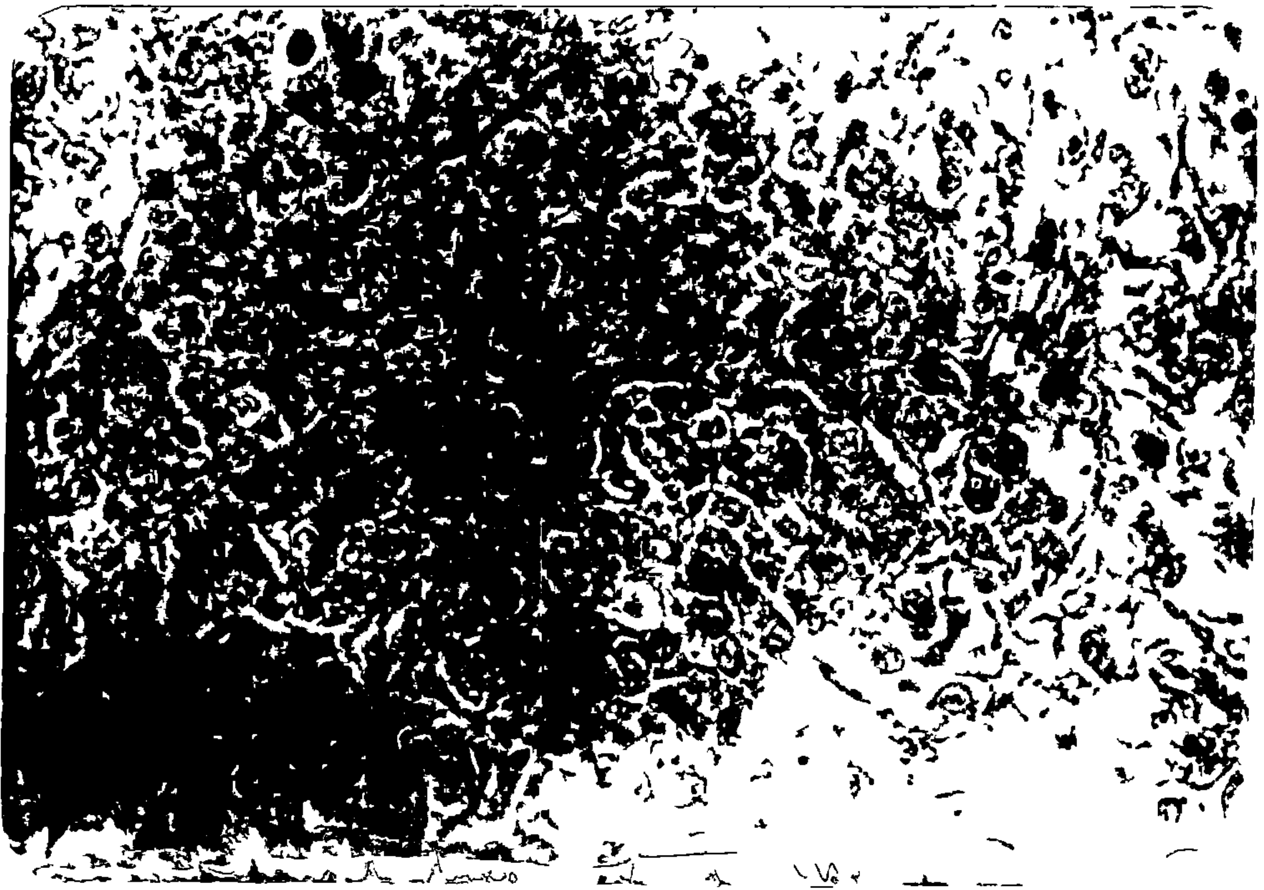
Clinically the inoculated ducks appeared normal. Grossly there was slight to moderate congestion of internal organs from the fifth to the ninth day, but thereafter the organs appeared normal.

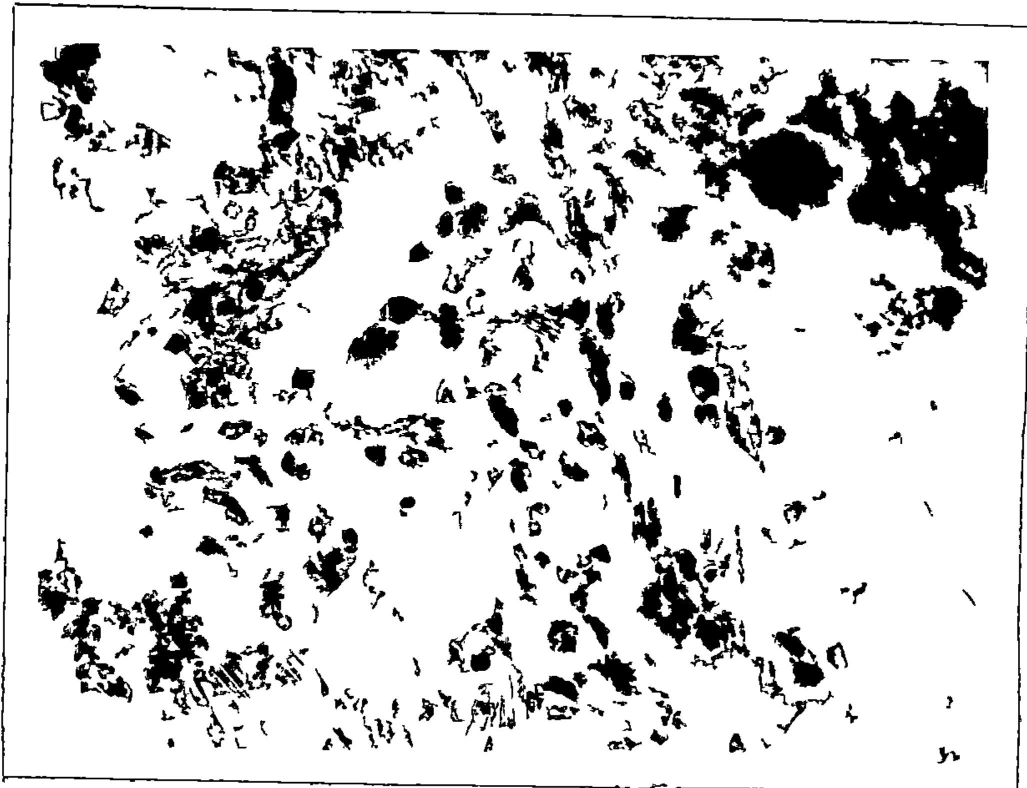
Histologically in the liver there was marked congestion of the central veins and sinusoids from the fifth to the ninth day. The space of Disse was prominent and there was accumulation of moderate quantity of oedematous fluid. There was slight and perceptible oedema in the perivascular areas especially in the portal tracts. A sprinkling of neutrophils and a few mononuclear cells were seen round central and sublobular veins and in the portal areas (Fig.40). An occasional cell in the portal area possessed the morphology of a degranulated basophil. After the fifth day, there was an increase in the number of monocytoid mononuclear cells and lymphocytes in the portal area. This became gradually reduced. Initially the hepatic cells showed slight granular degeneration and some of the cells showed fatty change. At about the eighth day an occasional cell showed coagulative necrosis. Around these necrotic cells there was no infiltration of cells. On the ninth day the liver presented a normal histological picture except for a few lymphocytes in the portal area.

From the fifth to ninth day, the kidneys were congested. The tubular epithelial cells, more specifically in the proximal and distal convoluted tubules showed degenerative changes on the sixth day. The glomeruli showed increased cellularity as evidenced by the increase in the number of mesangial cells. In scattered areas there was slight

Fig.39 Few heterophils around the blood vessels. Clumps of cocci are seen. *Staphylococcus aureus* 24 hr p.i. H & E x 100.

Fig.40 Liver - Sprinkling of heterophils and lymphocytes. RD virus. H & E x 400.





perivascular infiltration of heterophils. There was no indication of any pathologic change after the eighth day except for an occasional lymphocyte in the interstitial location.

The spleen appeared congested from the fifth to the seventh day. In the red pulp there was a relative increase of heterophils and monocytoid mononuclear . The mononuclears gradually assumed the morphological appearance of macrophages and these cells were seen in larger number in the subcapsular and trabecular sinus. After the eighth day the splenic corpuscles appeared very prominent with increased number of small lymphocytes with hyperchromatic nucleus. At this stage the penicillar arteries, central arteries, and small veins contained large number of lymphocytoid cells. On the ninth day the increased cellularity of the red pulp that was observed earlier was absent even though the splenic corpuscles appeared very prominent. Amidst the lymphocytes were seen large pale staining cells with irregular nucleus and cytoplasm. A few mature and immature plasma cells were present.

The lungs showed slight diffuse congestion from the second day. The vascular reaction gradually became less and on the eighth day it became imperceptible. On the sixth day a few heterophils and mononuclear were seen

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emigrating. But this reaction appeared transient. The tertiary bronchial mucosa showed slight oedema and scattered heterophilic infiltration from the fifth to the seventh day. After the eighth day there was moderate infiltration of lymphocytes which did not show the tendency to form nodules. On the ninth day a few lymphocytes were seen in the interstitial tissue.

Various parts of the alimentary tract did not show significant histological alteration except transient congestion. The brain did not reveal any significant change.

2.3. Infection with Duck plague virus

Experimental infection was induced with the Duck Plague Virus to assess the dynamics of pathological changes with special reference to vascular changes and cellular response. Ducks in various stages of the disease were sacrificed on the fifth, sixth, seventh, eighth and ninth day p.i.

2.3.1. General appearance and gross lesions

Clinically the infected birds did not show any symptoms till four days post infection. From the fifth day onwards the ducks appeared dull and they had diarrhoea and larchrymation. The mucosa appeared congested. Necrosis was seen from the sixth day and gradually became very intense. By

the eighth day the ducks were almost prostrate.

On the fifth day there was diffuse congestion of liver, spleen, kidneys and lungs. The mucosa of the alimentary tract showed patchy foci of congestion. On the fifth day, the liver showed diffuse congestion and enlargement. A few peticheae were also noticed. On the sixth day the liver had a pale bronze colour and showed scattered pin head sized greyish white spots. The gall bladder was slightly distended and contained dark green bile. The changes seen during the later part were also similar except that the enlargement of the liver was more pronounced.

The heart was found slightly enlarged from the fifth day. This enlargement was more pronounced in the ventricles which appeared dilated. There were a few peticheae in the epicardial and endocardinal surfaces. A few ill defined pin head sized foci were also noticed in the ventricular wall. After the 7th day these changes were very pronounced as evidenced by large blotches of endocardial haemorrhage and patches of myocardial necrosis.

The spleen was slightly enlarged and congested on the sixth day. The enlargement increased till the ninth day.

The kidney was severely congested on the fifth day. On the sixth day the kidney was severely enlarged and few

petichoae were also seen. Pale areas indicative of necrosis were seen all over the kidney. The changes were qualitatively same till the ninth day.

On the fifth day, the mucosa of alimentary tract showed scattered areas of congestion. By day six there were a few small erosions and linear greyish white slightly raised streaks of necrosis in the buccal and oesophageal mucosa. The proventriculus showed mild catarrh on the fifth day and on the sixth day linear erosions were seen. Areas of erosions and necrosis persisted till the ninth day.

No gross lesions were seen till the fifth day in the gizzard. On the sixth day, the keratin layer appeared thickened, wrinkled and became firmly adherent to the underlying musculature. The gizz ard musculature showed irregular greyish white patches of necrosis which later coalesced.

The intestines were moderately hyperaemic on the fifth day. On the sixth day focal erythematous patches were seen scattered in the intestine, particularly in the region of the jejunum and ileum. By day eight the erosions were also seen and the mucosa had a thickened appearance with copious amount of mucin.

On the fifth day the peritoneum and the ovarian follicles were moderately hyperaemic. By the eighth day

the follicles became inspissated and had an irregular contour. The peritoneum became opaque, intensely hyperaemic and was adherent with fibrinous exudate. The mesenteric vessels were severely congested. In some of the birds the follicles had ruptured releasing the contents into the peritoneal cavity.

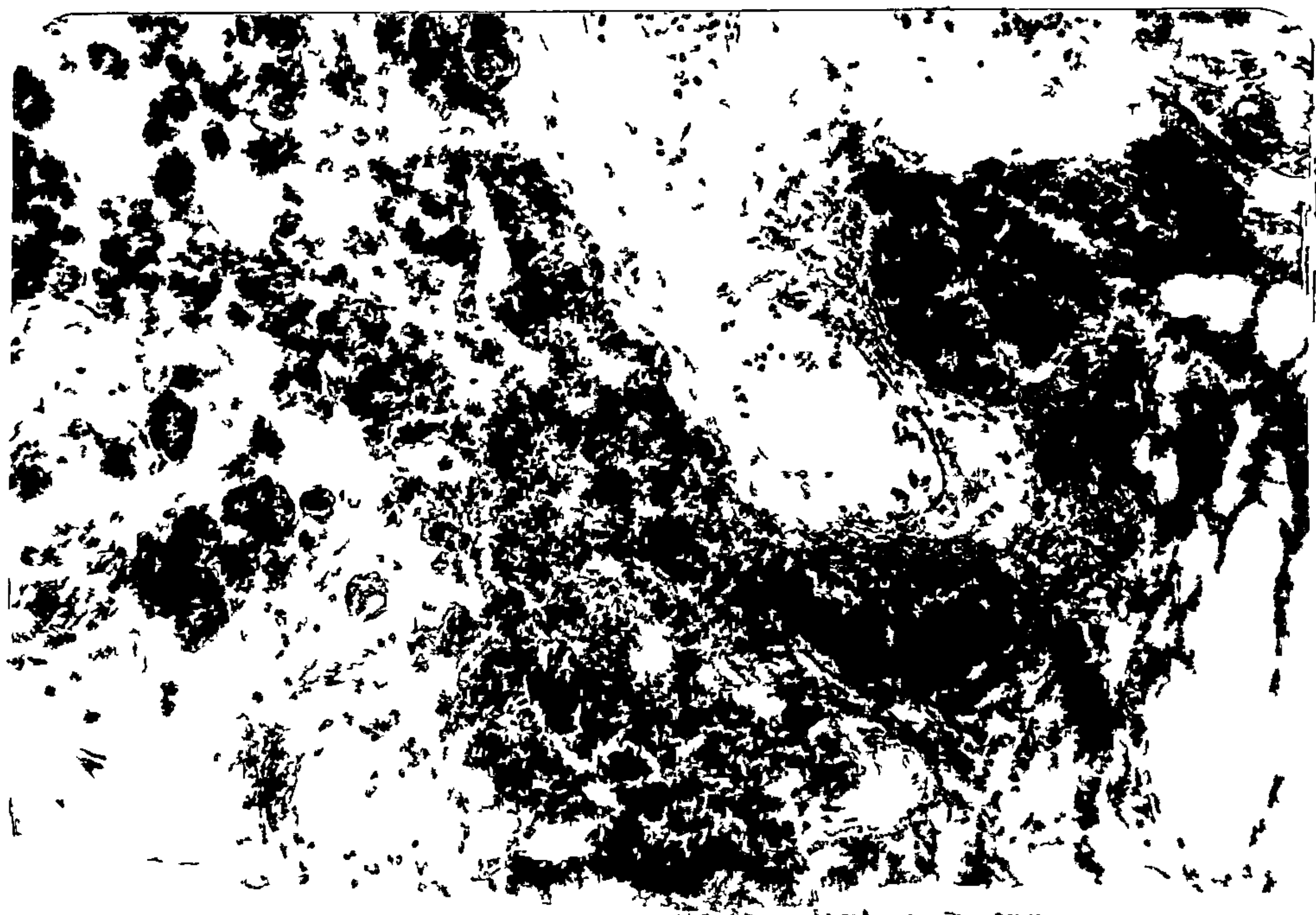
2.8.2. Histopathology

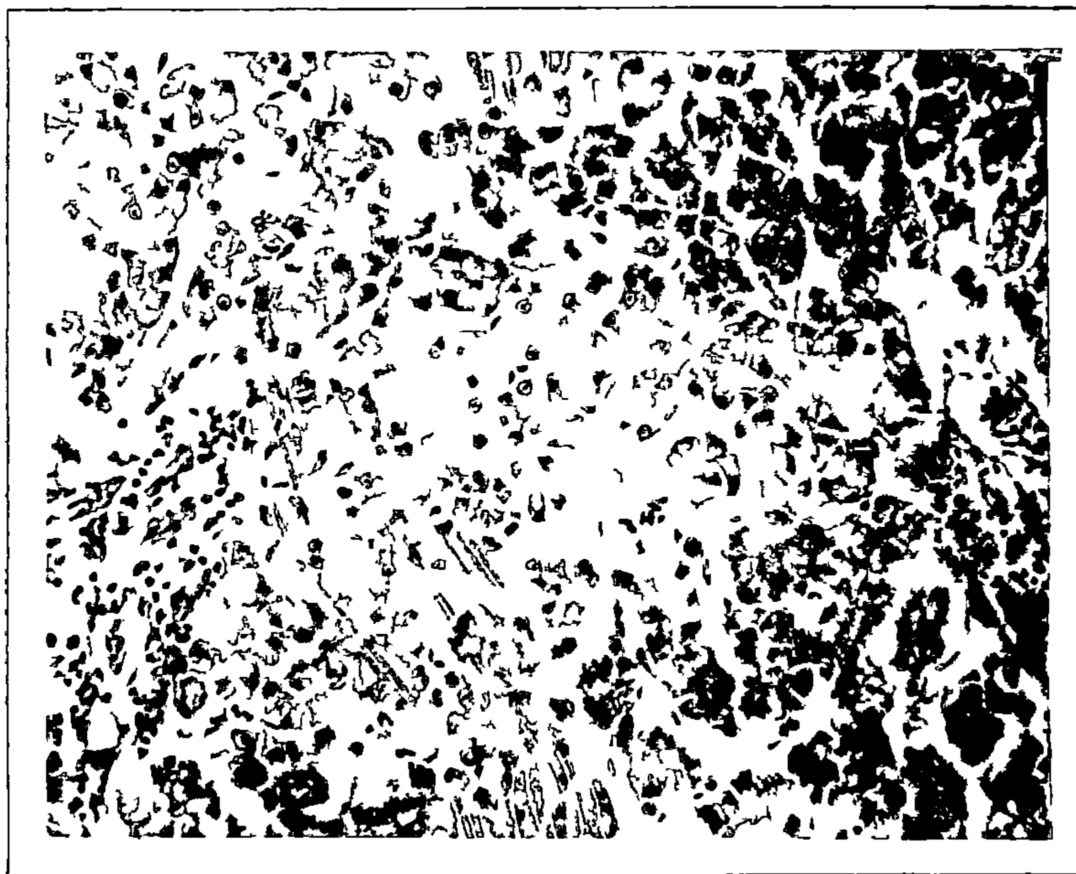
2.8.2.1. General reaction

On the fifth day the hepatic sinusoids and central veins were severely congested. There was slight oedema in the perivascular areas and the space of Disse. The endothelium of the sinusoids appeared prominent and the Kupffer cells were distinctly evident. Focal areas of haemorrhage were also seen. Many of the hepatocytes were swollen and had a granular appearance. On the sixth day these changes were very pronounced and in addition minute foci of coagulative necrosis of hepatocytes were seen. On the eighth day the necrosis became more pronounced and large areas of hepatic parenchyma could be seen undergoing coagulative necrosis (Fig.41). The hepatocytes had vesicular nucleus and few of the cells showed the presence of intranuclear inclusions. Inclusions were not seen in cells which were necrotic. The biliary canaliculi were

Fig. 41 Liver - severe necrosis of parenchymatous cells. Duck plague virus. 8th day p.i. H & E x 400.

Fig.42 Liver - Heterophils in the periportal and perisinusoidal areas. Duck plague virus. 5th day p.i. H & E x 800.





prominent and some of them were seen plugged with bile casts. The involvement of hepatic parenchyma with necrosis was more in those ducks which were in extremis on the eighth day.

In the heart initially few areas of haemorrhage were seen in the pericardium. By the sixth day the myocardial fibres had undergone degeneration and necrosis. This involvement increased gradually and on the eighth day it was found that large groups of muscle fibres had become necrotic. No specific site-predilection was noticed for this.

In the kidney there was moderate to severe congestion of the vessels and glomeruli on the fifth day. On the sixth day, in addition, focal areas of haemorrhage were also noticed. Tubular cells, more specifically, of the convoluted tubules showed varying grades of degeneration and necrosis. Many of the epithelial cells had become swollen and were seen occluding the lumen. Necrosis was seen involving more areas during the subsequent days.

There was pronounced engorgement of the capillaries of lungs on the fifth day. Petechiae were seen subsequently. By the eighth day focal areas of pneumonia were noticed. The reaction was very severe when examined on the ninth day. The spleen was intensely congested on the sixth day. On the eighth day there were foci of necrosis.

The lesion in the gastro intestinal tract was one of mucosal necrosis associated moderate cellular reaction. Peritonitis and oophorits were consistent lesions and they became intense from the sixth day onwards. Strands of fibrin were seen adhering to the peritoneal surface.

In the brain there was congestion of the meninges by the sixth day. From the eighth day onwards the neurons, especially the Purkinje cells showed chromatolysis and degeneration. Occasional foci of neuronophagia were also noticed. Moderate diffuse gliosis was seen.

2.8.2.2. Cellular involvement

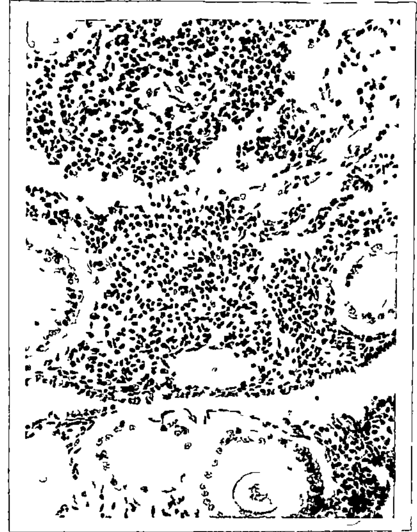
The cellular involvement in the various tissues during the early phase of the reaction was very minimal. There was hyperaemia in most of the organs and few number of heterophils could be seen pavementing the arterioles and venules. Emigration of cells was sparse and the parenchyma of the organs contained only a few of these emigrated cells. The periportal areas and perisinusoidal locations had comparatively larger number of cells. The intensity of emigration was more after the fifth day when there was marked destruction of parenchyma (Fig.42). Emigration of heterophils and monocytoid mononuclears were concurrent during the early phase. By the sixth day emigration of lymphoid cells had

become very prominent. In the portal areas there were nodular accumulation of lymphoid cells which could be seen gradually spreading into the hepatic parenchyma (Fig.43). On the eighth day, there was intense infiltration of these cells into the lobules. Amongst the lymphoid cells, could be seen mature plasma cells as well as cells in stages of maturation into the plasma cell. Only few macrophages were found free among the infiltrating cells even though the Kupffer cells appeared very prominent. Cellular involvement in the myocardium was very scanty even though the intermuscular areas revealed congested capillaries and perivascular accumulation of few leukocytes, mostly lymphoid cells and macrophages. During the later stages when the muscle fibres showed varying grades of degeneration and necrosis, large macrophages and few fibroblasts were seen. The cellular involvement in the kidney was also minimal. But in later stages lymphoid cells had accumulated in the interstitial tissue. Macrophages were conspicuous by their absence.

In the spleen, the blood vessels of the capsule and trabeculae appeared congested. The venous sinuses appeared engorged and numerous heterophils could be seen admixed with erythrocytes. Heterophils and monocytoid cells were emigrating from the capsular and trabecular vessels and even from the central arterioles. Accumulation of cells around

Fig.43 Liver - Portal areas showing lymphoid nodules. Duck plague. 6th day p.i. H & E x 400.

Fig.44 Ovary - Infiltration of lymphoid cells and plasma cells. Duck plague virus 6th day p.i. H & E x 400.



the venous sinuses indicated emigration from arterial capillaries as well as from venous sinuses. After the fifth day, the splenic nodules increased due to accumulation of cells with blastoid features. Numerous such foci of necrosis were noticed. The central arteries in some locations were clogged with lymphoid cells. After the sixth day the lymphoid nodules became very prominent extending into the red pulp. The outer dark staining lymphoid cells had increased in number. Numerous cells of plasma cell lineage were present in the lymphoid nodules. The intensity and extend of necrosis had increased by this time. The subcapsular and trabecular sinuses contained few numbers of macrophages. Admixed with the erythrocytes and associated with the Billroth cords, a few macrophages and other cells with the features of epithelioid cells were present. Erythrophagocytosis was noticed in some macrophages. By the eighth day some of the lymphoid cells showed degeneration and necrosis. The germinal centre in many locations contained cellular debris.

The peritoneum and ovary revealed massive infiltration of heterophils. The smaller vessels were plugged with heterophils and a few monocytoid cells. The emigration was concurrent and the number of emigrated heterophils was 3 to 4 times the number of monocytoid cells. On the sixth

Fig.45 Electron micrograph - Electron dense core (C) in the large dense granules. Mature heterophils. Two types of granules, large dense (LD) and light granules (LG). Irregular contour with numerous villous and pseudopodial projections x 19200.

day the heterophilic component of the cellular exudate was replaced by massive infiltration of lymphoid cells (Fig.44). Immature and mature plasma cells were present. Many of the infiltrating cells had become necrotic.

The cellular involvement in other organs was scanty at different time intervals except for scattered lymphoid reaction. In the brain there was moderate gliosis and in locations where there was neuronal necrosis, accumulation of microglial cells was noticed. Perivascular lymphoid accumulation was not a characteristic feature at any stage.

3. Ultrastructural studies

In order to ascertain the ultrastructural changes of the inflammatory cells, the normal features of heterophils, basophils, macrophages, and lymphocytes were studied by electron microscopic examination of bone marrow from normal healthy ducks and of the inflammatory lesions induced by Dextran sulphate and *Staphylococcus aureus*. It was not possible to study the eosinophils because of the non-availability of these cells in the sections examined.

3.1. Heterophils

The mature heterophil had an irregular contour with numerous villous and pseudopodial projections (Fig.45). The

Fig.46 Electron micrograph - Exudate heterophil showing numerous large dense granules (LD) and light granules (LG) - Glycogen particles present in the cytoplasm - Nucleus (N) - Phagosome (P) x 19200.

P
LD
LD
LD
LG
N

nucleus was prominent with irregular lobed appearance and with prominent nuclear membranes. Nuclear pores were few. The chromatin appeared mostly as clumped heterochromatin mostly arranged along the nuclear membrane. Small clumps of heterochromatin were also found along with light staining euchromatin. Nucleolus was not evident in most of the cells and when present was not prominent. The immature heterophil as found in the bone marrow had moderate number of mitochondria with well developed cristae. But the mature heterophil had only a few small oval or slightly elongated mitochondria. Similarly while the myelocytic stage contained numerous strands of rough surfaced endoplasmic reticulum containing floculent electron dense material, the mature heterophil had only an occasional strand of rough surfaced endoplasmic reticulum. Free ribosomes were also not evident. Glycogen was present both as alpha and beta particles, but only in small quantities. In the myelocytic stage, the Golgi zone was well developed with stacks of cisternae. There was evidence that some cisternae and vesicles of the Golgi apparatus contained electron dense contents. Some of these vacuoles were seen budding off from the concave surface of the Golgi lamellae. Numerous large electron dense granules mostly spherical were seen at this stage. Gradually as the cells matured and passed into the

Fig.47 Electronmicrograph - exudate heterophil showing mitochondrial damage (M) x 19200.

M
M

metamyelocyte stage and into the adult stage, these granules became large, probably due to fusion of the pre-existing smaller granules. During the immature stages many of the granules presented well defined oval or circular electron-lucent areas. But in the mature heterophils these areas became electron dense. Most of the mature granules which were either oblong, and cucumber shaped and numerous electron dense core. The granules varied in size and some of them had a length of 40 nm. Another type of granule smaller in size, with less electron dense and a fibrillary matrix was also seen. These two types of granules were similar to the large dense granules and the light granules of chicken heterophil.

The heterophils, soon after emigration from the blood vessels had almost the same ultrastructural features as the normal heterophils. Subsequently when exposed to the inflammatory agent showed varying grades of morphological alterations (Figs. 46 and 47). There was an increase of glycogen in the cell. Endocytic vacuoles were numerous and in the case of staphylococci, phagosomes containing the bacteria were noticed. Mitochondria showed varying degrees of damage. Most of these heterophils exhibited prominent pseudopodia. Many profiles of fusion between the granules and between the endocytic vacuole and the granules were seen. Large configurations indicative of such fusion

PL

were observed. The emigrated heterophil had an increased amount of glycogen in the early stages but subsequently this was reduced or was absent. After exposure to the irritant the heterophils showed varying degrees of alteration of nucleus and cell organelles. At one extreme there were cells with almost a washed out appearance of the cytoplasm with only a few granules remaining (Fig.48) while other cells showed a homogenous matrix with swollen mitochondria and with all the granules remaining intact. On occasions free heterophilic granules liberated from necrotic cells were found extracellularly.

3.2. Macrophages

The macrophages associated with the inflammatory reaction showed considerable morphological variation. They varied in size, shape and in organellar contents (Fig.49). During the early stages the cells were small and round but at later stages the cells increased in size with irregular contents and with numerous villous projections. Nucleus showed a gradual increase in the euchromatin. The perinuclear cisternae became prominent and some of them contained flocculent material. There was a gradual increase in the number of profiles of rough surfaced endoplasmic reticulum apart from a dilatation

PERINUCLEAR CISTERNAE
ne
ER

of the cisternae (Fig. 50 & 51). These cisternae contained slightly granular or floculent contents. There were occasional lipid particle. But, there was no consistency in their shape and number in a cell. In the early stages the Golgi complex was not prominent but in later stages it was well developed with numerous stacks of lamella with a corresponding increase of vacuoles and vesicular elements. The vesicles which appeared to have budded off from the cisternae were seen fusing to form electron dense structure with the morphology of lysosomes. Lysosomes were few in the early stages but became abundant if the macrophages persisted at the inflammatory site. The nature of contents of the lysosomes showed great variation. After inoculation the bacteria were seen in the interstitial tissue. Subsequently they were ingested by macrophages or heterophils.

Endocytic vacuoles containing bacteria (Fig. 52) or dextran sulphate particles as the case may be, were consistently found in the macrophages. In the case of dextran sulphate large endocytic vacuoles almost filling up the cytoplasm and pushing the nucleus in a cresentic form were occasionally noticed (Fig.53). Fusion of the profiles of phagosomes and lysosomes were occasionally found. There

Fig.50 Electronmicrograph - Macrophage - Mitochondria (M) showing degenerative changes. Rough surfaced endoplasmic reticulum dilated (ER). Lysosomes seen x 33000.

ER
ER
LYSOSOME
M
M

Fig.51 Electronmicrograph - Macrophage - well developed endoplasmic reticulum and numerous lysosomes. Portion of a lymphocyte also seen x 20000.

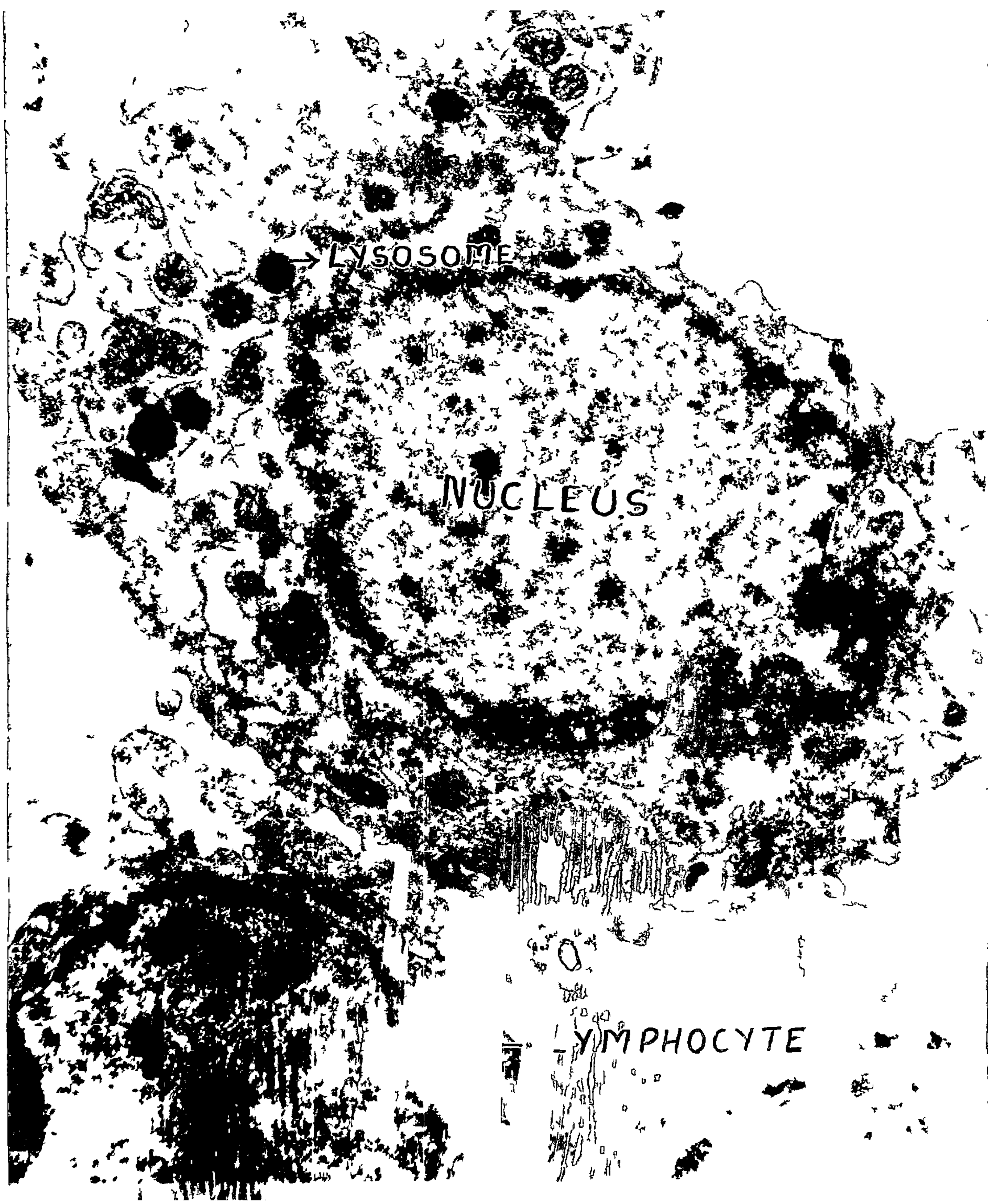

LYSOSOME
NUCLEUS
LYMPHOCYTE

Fig.32 Electronmicrograph - Macrophage - endocytic (S) vacuoles containing Staphylococcus x 10200.

S

Fig.53 Electronmicrograph - Macrophage - A large endocytic vacuole containing dextran sulphate. Nucleus crescent shaped and pushed to one side x 33000.

ENDOCYTIC
VACUOLE
NUCLEUS

were a few multivesicular bodies with rows of internal vesicles and an occasional coated vesicle. Mitochondria were numerous and some had intact cristae while some others had disoriented and broken up ones. Mitochondria with homogenous contents and with only remnants of mitochondrial outer membrane were also seen. Many cells had bizarre looking mitochondria with disoriented cristae (Fig.54).

Presence of heterophagolysosomes and autophagolysosomes was a constant feature of the mononuclear phagocytes seen after four days (Fig. 55). Remnants of cellular organelles and inclusions like mitochondria, endoplasmic reticulum ribosomes and glycogen particles were seen within the autophagolysosomes. Occasionally fusion of heterophagolysosomes and autophagolysosomes was also encountered. During the later stages there were many dense bodies or lamellated structures within the cytoplasm of many macrophages some of which had phagocytosed other degenerative cells like heterophils.

3.2.1. Giant cells

There were a few multinucleated giant cells in the lesions examined after the 5th day of the dextran sulphate injected ducks. The cell surface was irregular with

Fig.54 Electronmicrograph - Macrophage - Mitochondria (M) with bizarre and disoriented cristae x 15000.

M
M

numerous finger shaped protrusions. The cytoplasmic contents and organellar configurations were almost similar to those seen in a mature macrophage except for the nuclear numbers. In the giant cells also there was evidence of continued protein synthesis as shown by the prominent dictyosomes, well developed rough surfaced endoplasmic reticulum, polysomal configuration of ribosomes and presence of numerous electron dense granular structures. The predominant nature of the nucleus with euchromatin was also seen. Numerous dense bodies were seen irregularly distributed in the cytoplasm. Some of these giant cells had irregular strands of cytoplasmic filaments.

3.2.2. Epithelioid cells

After the eighth day, in the lesion induced by dextran sulphate, rounded or polygonal cells with irregular plasma membranes were seen amidst the typical macrophages (Fig.56). In the nucleus the relative content of euchromatin was more, compared to heterochromatin in the nucleus. Nucleolus with prominent nucleolonema, was more than one in these cells. Nuclear pores were also abundant. Mitochondria were moderate in numbers and a few profiles of dilated rough surfaced endoplasmic reticulum were present. Cytoplasmic filaments, vacuoles and pit like depressions

Fig.55 Electronmicrograph - Macrophage - Presence of numerous hetero and autophagosomes in the cytoplasm x 15000.

Fig.56 Electronmicrograph - Epithelioid cell - irregular plasma membrane and the nucleus showing more euchromatin. Phagolysosomes absent x 25000.

EUCHROMATIN

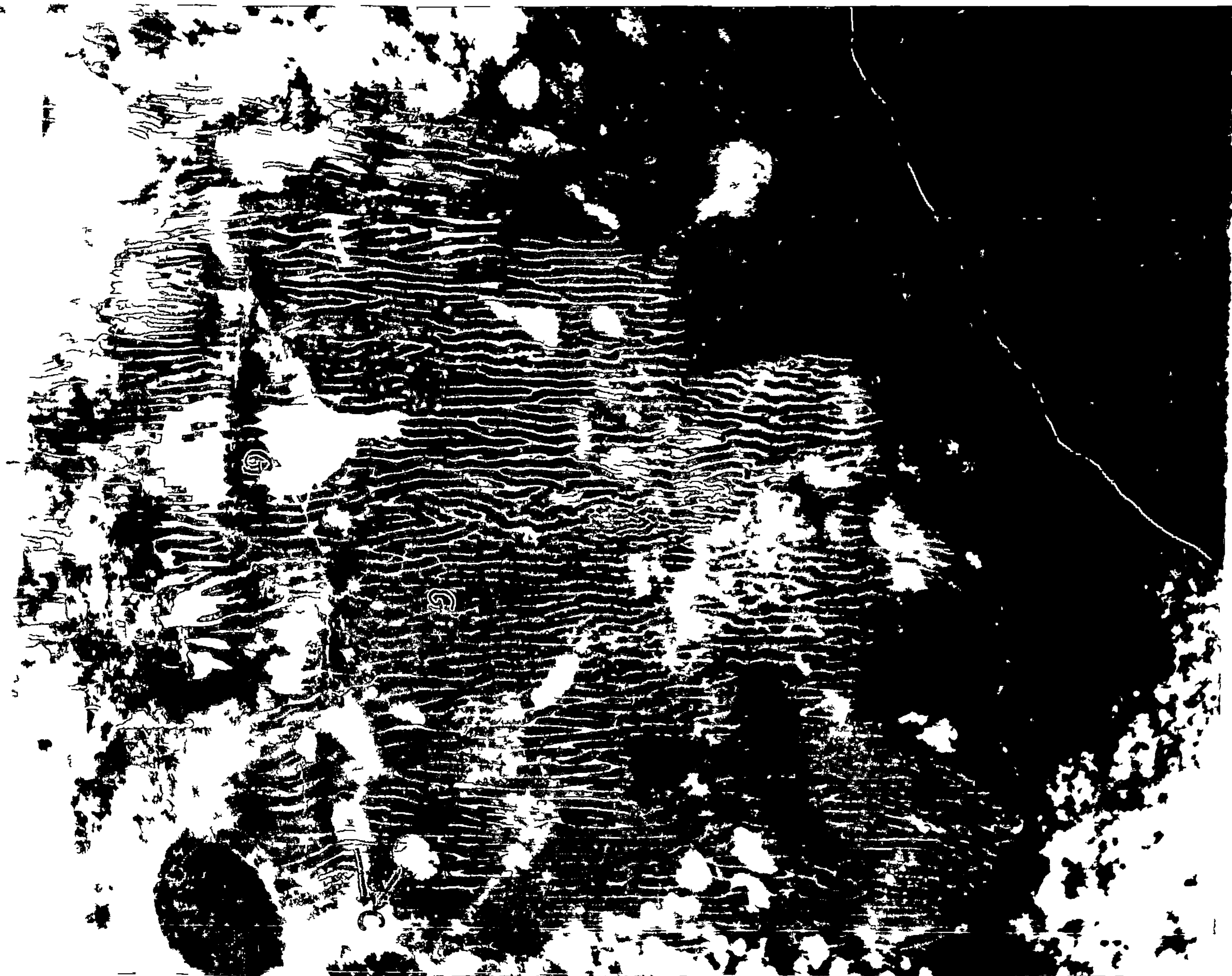
Fig.57 Electronmicrograph - Basophils - showing numerous oval to round granules. The membrane bound granule show particulate contents of varying density x 35000.

in the plasma membrane were also seen. These epithelioid cells did not show the presence of phagosomes or phagolysosomes even though some cells had an occasional dense body in the cytoplasm. Very advanced nuclear and cytoplasmic degenerative changes were encountered in some cells.

5.3. Basophils

Even though there was difficulty to discern the basophils, which had degranulated in light microscopy, this did not present considerable difficulty in tissues examined ultrastructurally. It was observed that the basophils tended to degranulate immediately after emigration from the small blood vessels. Partially degranulated and completely degranulated cells were seen in the perivascular location. Basophils were round cells with a slightly ruffled plasma membrane and contained numerous round or oval granules varying in size from 400 - 600 nm (Fig.57). The membrane bound granules had particulate matrix of varying electron density. While in some, the particulate grainy internal structures were loosely arranged, in others there were loosely structured granules with fine reticular content of less optical density. In some there were crescent a[illegible]s with a scooped up

Fig.58 Electronmicrograph - Basophil - showing granules some of which with thin reticular content of less optical density (G). Crescent shaped excavation can also be seen (C) x53000.



mixing up of the contents ultimately forming saccular structures which open through the plasma membrane were also evident in many cells. Many basophils had lost most of the granular contents presenting only remnants of perigranular membrane and granular co tents. In a few cells which had degranulated attempted formation of new granular structures was noticed in the region of the Golgi. Completely degranulated cells were also seen.

The nucleus was irregular with electron dense heterochromatin along the inner nuclear membranes. Coarse, lumpy chromatin was also irregularly scattered in the karyoplasm. Both granulated and filamentous euchromatin were seen. In mature basophils usually one nucleolus with a compact appearance and relatively little amount of nucleolonema was present. Cytoplasm was of moderate density with few strands of rough surfaced endoplasmic reticulum. Mitochondria were few in number and of different sizes. Golgi was moderately developed. Electron dense grainy structures identical to those in the basophilic granules were observed near to the degranulating cells.

It was observed that the tissue mast cells which were few in number also showed a tendency to degranulate when exposed to the irritants (Fig. 59).

Fig.59 Electronmicrograph - Mast cell showing partial (P) degranulation of granules x 33000.

P
NUCLEUS
P

3.4. Lymphocytes

The ultrastructural features of the lymphocytes showed a great diversity. While during the first two days, the cells were more or less uniform, later they varied in their size, nuclear configuration and organellar contents. The apparently smaller cells were round with a high nucleo-cytoplasmic ratio. (Fig.60). The nucleus was round or oval or some times irregular with heterochromatin and euchromatin in equal proportions. The heterochromatin occurred as dense granular structures and was present as aggregates on the inner nuclear membrane and as clumps elsewhere in the nucleus. The euchromatin occurred as uniformly distributed granular component. Nucleolus if present was not prominent. Both the nuclear membranes were clearly seen; the outer membrane was devoid of attached ribosomes and the perinuclear cisternae were devoid of any content.

The cytoplasm was moderately electrondense and there was a paucity of organelles. Mitochondria were small and few in number. They were oval or round, had dense matrix and few granules. Cristae were regularly arranged. Rough surfaced endoplasmic reticulum was absent and ribosomes were not abundant. Golgi complex was ill developed and the components were not very conspicuous. A few small electron dense granules were also observed.

Fig.60 Electronmicrograph - showing a lymphocyte and two cells with features of blast cells x [illegible],000.

BLAST CELLS
HETERO-
CHROMATIN
NUCLEOLUS
EUCHROMATIN
LYMPHOCYTE

The morphologically larger cells had more abundant cytoplasm. The nucleus appeared irregular and had lesser amount of heterochromatin than in the smaller cells. In addition the Golgi appeared slightly more prominent, with a few profiles of lamellae, vesicles and vacuoles. Few dispersed ribosomes were seen. Among these lymphocytes could be seen a population of cells which had the appearance of lymphoblasts. The nucleus was large and predominantly contained euchromatin and with plenty of nuclear pores. The two nuclear membranes were well separated and the outer membrane showed many irregularities. Nucleoli were well developed and the granular dense matrix had continuity with the nuclear chromatin. Inter-chromatinic granules were also present. Mitochondria numbered from six to eight and had the typical oval to elongate shape and prominent cristae. Ribosomes were seen either singly or as polysomal configuration Peroxisomes were not seen. Endoplasmic reticulum was very scanty. Golgi was well developed with prominent dictysomes. Small pinocytic vesicles were seen on the cell surface.

3.4.1. Plasma cells

Along with these lymphocytes, cells with ultrastructural features of mature and [illegible] cells were encountered

Fig.61 Electronmicrograph - Developing plasma cells (P) with prominent rough endoplasmic reticulum x 30000.

P
HETEROPHIL
P

characteristic feature of such a cell was a well developed rough surfaced endoplasmic reticulum with dilated cisternae almost filling up the cytoplasm. They were arranged as labrynthine structures containing electron dense or flocculent material. Ribosomes were numerous and arranged in polysomal configurations in the endoplasmic reticulum. Occasionally electron dense granular structures were seen within the dilated endoplasmic reticulum. Golgi complex was well developed with prominent lamellae, vacuoles and vesicles. The Golgi components were situated perinuclearly and electron dense vesicles were seen budding off from the lamellae which themselves contained electron dense material. Centrioles were seen occasionally.

The nucleus was usually eccentrically placed with heterochromatin arranged as blocks on the inner surface of the nuclear membrane. About 6 - 10 mitochondria were noticed.

Along with these mature plasma cells many immature transition cells of the plasma cell line were encountered. Immature cells had more free ribosomes, relatively less amount of endoplasmic reticulum and large nucleus which was prepond-erantly euchromatinic.

DISCUSSION

DISCUSSION

The basic cellular response of ducks due to inflammatory reaction induced by the various agents was more or less similar to that described in the fowl by Nair (1973). After the administration of the inciting agent, adherence of leukocytes to the walls of the vessels was noticed. Even though Illig (1961) reported that this sticking of leukocytes to the endothelium was confined to the venules, it was observed that in the duck both venules and arterioles were involved in this phenomenon. Janoff and Zweifach (1964) also made such an observation in mammals when employing cationic proteins.

Irrespective of the agents employed it was clear from this investigation that emigration of leukocytes was always concurrent even though there was quantitative difference in the number of different cell[illegible] have emigrated. The heterophils and mononuclear [illegible]eemed to emigrate at the same time from the same [illegible]. Although no temporal or topographic dissociation [illegible]igration of leukocytes was seen, it was obvious th[illegible]ophils were emigrating in sufficiently higher num[illegible]e mononuclear cells during the early phase of [illegible]n and thereafter the

latter cells appeared to emigrate in higher numbers than the former. At no stage was there evidence of emigration of mononuclears in the absence of emigrating heterophils. This observation was in agreement to that of Paz and Spector (1962) and Nair (1973) who studied the emigration of leukocytes following injections of a number of irritants in rats and fowl respectively. Even though there was no qualitative difference in the emigrating cells, it was evident that the onset and intensity of emigration of leukocytes varied with the irritant employed. For example, with turpentine emigration of heterophils was massive during the early phases while it was scanty and of delayed onset when talc was employed. Similarly when agents like Freund's complete adjuvant and dextran sulphate were used there was prolonged and continuous emigration of mononuclear cells. Nair (1973) had suggested that not only the nature of the irritant but also concentration can modify the onset and intensity of emigration.

The sequence of events involved an increase in the permeability of the capillaries, adherence of the heterophils to the capillary wall, migration of the heterophils through the endothelial gap of the vessel, and directed movement of the cell toward the bacteria of the species.

During the early phase of the inflammatory reaction there was a predominance of heterophils in the perivascular and intervascular areas. After about two days even though the heterophils were emigrating in larger numbers there appeared to be an increase in the mononuclears. Paz and Spector (1962) postulated that this increased predominance of mononuclear cells could be due to "migration of haematogenous mononuclears subsequent to that of polymorphs, delayed proliferation of tissue reticulo endothelial cells, and simultaneous migration of polymorphs and mononuclear cells, with differences in the rate of migration and subsequent fate of the two types of cells". Nair (1973) suggested that this predominance could be due to massive destruction of heterophils and local proliferation of haematogenous monocytoid cells.

It was seen from this study that heterophils were more sensitive to the irritants than the macrophages and that massive destruction of heterophils was seen especially when substances like turpentine were used. It was also seen that there has been a second wave of heterophilic emigration and this could be possibly due to local tissue destruction liberating mediators or due to the continued action of the irritant itself.

In the light microscopy it was found very difficult to

demonstrate the basophils probably because of their few numbers or because of their degranulation. Electron microscopy revealed a few basophils and mast cells which have degranulated or in the process of degranulation. The basophilic granules showed variation in the nature of their contents and density. Probably this is due to the differences in the maturity of the granules, state of preservation and the stage of degranulation. It is not quite evident from this investigation whether heparin liberated from the basophils and mast cells has a definite role in the arrest of movement of inflammatory cells as suggested by Nair (1973). Detailed cyto-chemical and experimental studies employing histamine, heparin and other mediators are necessary for assignment of specific roles for the basophils in the duck.

The early increase in vascular permeability is almost certainly due to the release of histamine from mast cells and basophils (Spector and Willoughby, 1965). But there is no consensus regarding the mediators of the delayed permeability response (Wilhelm, 1973). Certainly histamine does not appear to be involved since antihistamines were without effect on the delayed response inspite of pronounced suppression of the immediate response. Of the permeability factors described, the kinins seem to be the most likely mediator for the delayed

response (Wilhelm, 1973). Recently, attention has turned to the prostaglandins as mediators of inflammation (Zurier, 1974).

Once the heterophils adhered to the endothelium, they extended pseudopods that appeared to penetrate the endothelium at or near cell junctions. There is some good evidence in mammalian species that neutrophils release proteins that are chemotactic for other neutrophils. Zigmand and Hirsch (1973) described a protein derived from neutrophils incubated with aggregated gamma globulin that are strongly chemotatic for neutrophils. The rate and extent of phagocytosis depended on the particle to cell ratio.

In the present investigation the duck heterophil was found to be an efficient phagocyte as evidenced by its capacity to ingest Staphylococci and other particulate materials. No attempt was made in this study to evaluate the enzymatic changes associated with phagocytosis.

Ultrastructurally the duck heterophil was almost similar to the chicken heterophil. Nair (1973) described three types of granules in the fowl heterophil - the large dense granules which could be considered analogous to the azurophil granules, the light granules and the small dense granules.

In the present study only two types of granules could be discerned, the large dense granules with the electron dense core and the smaller granules with fibrillary contents. Since no histochemical studies were conducted it is not possible to definitely ascertain whether both these granules contain hydrolytic enzymes and therefore could be considered as lysosomes. The electron dense core of the larger granules was characteristic and it was evident that the electron density was acquired during the late stage of maturation of the granules because these areas were electron-lucent and pale in the immature cells. The formation of granules was associated with the rough surfaced endoplasmic reticulum and the Golgi complex. The vesicles which were liberated from the golgi fused to form larger granules. The phenominon of degranulation was noticed in the heterophils after endocytosis. Bainton (1974) suggested a definite sequence of degranulation with the specific granules degranulating before the azurophilic granules. From the present observation it was not possible to identify whether there existed any temporal or sequential factors in the degranulating process in the duck heterophil. During the process of degranulation there was fusion of the granule membrane with that of the endocytic vacuole. In the mature heterophil there were only few mitochondria. Rough surfaced endoplasmic

reticulum and golgi were not prominent indicating relative absence of new protein synthesis and granule formation.

The majority of the mononuclear cells which had emigrated during the first half hour onwards, was of the 'monocytoid' type and it was difficult to distinguish between typical monocytes and large or medium lymphocytes on histological preparations. These monocytoid mononuclear cells in the intervascular areas, at consecutive time intervals exhibited gradual morphological changes. These changes were characterised by an increase in size, and an 'open' pale staining nucleus. The presence of typical haematogenous monocytoid cells and other monocytoid cells in different stages of transformation into typical macrophages indicated that newly emigrated monocytoid cells have infiltrated along with those that have undergone morphological alterations and got fixed. During the early stages of the reaction the monocytes appeared to accumulate at the periphery of the bleb formed by the inoculum. Later they arranged themselves as neat rows and showed changes in size, shape and tinctorial property. Similar changes have been described by many earlier workers. The macrophages were found to be efficient phagocytes as revealed by the ingestion of varieties of particulate materials like dextran sulphate

and talc and of organisms like Staphylococcus aureus. The dynamics of changes and the transformation of mononuclear phagocyte appeared to be dependent on the nature of the irritant. Nair (1973) reported that the phagocytes exhibited a comparative increase in the acid phosphatase, arylsulphatase and B-glucoronidase content as the cells which have differentiated into macrophages in fowl during local inflammatory reaction which clearly indicated the metabolic alterations associated with increased phagocytic ability.

In the reaction induced by Freund's Complete adjuvant large numbers of pale staining cells with the morphology of epithelioid cells were seen. Epithelioid type cells were scanty when other irritants were employed. Nair (1973) had suggested that in fowl epithelioid cells probably developed when macrophages became immobilized at the site of inflammation without being called upon to undertake phagocytosis or when phagocytosis or pinocytosis resulted in complete elimination of the particle within a few days or when the irritant was digestible and not acutely toxic to the macrophages. The presence of well developed organelles, and an active nucleus in the epithelioid cells suggest that these cells are active functionally. Further work would be required to clarify the functional capabilities of the epithelioid cell.

Giant cells were noticed during the later stages of the inflammatory reaction. This was very pronounced when talc, Freund's Complete adjuvant and homologous erythrocytes were administered. Even though there are two possibilities regarding the histogenesis of giant cells in an inflammatory focus, Nair (1973) employing histochemical, autoradiographic and ultrastructural techniques conclusively proved that in fowl, giant cells are formed by the fusion of haematogenous macrophages rather than by mitotic division of pre-existing macrophages. Ultrastructural studies in this investigation support the above concept.

From the results of this investigation it can be concluded that the sequential ultrastructural changes of monocyte transformation to macrophages, giant cells and epithelioid cells in the duck were similar to that described earlier in mammals (Cohn et al., 1966; Sutton, 1967). The increase in euchromatin indicated heightened metabolic activity. There was relative increase in the ribosomes, endoplasmic reticulum and in the size of the golgi complex. The cell had increased in size and became endowed with large number of lysosomes which was very essential for foreign body degradation.

There was an increase in the size and number of mitochondria which may be due to the increased functional and

metabolic requirement of the macrophage. The bizarre looking mitochondria with altered cristae may indicate partially destroyed mitochondria which show an attempt for regeneration. It is also clear that there is a high turnover since small intact mitochondria were also encountered. Degenerated mitochondria were enclosed in lysosomal structures; these autophagolysosomes sometimes fused with heterophagolysosomes. The phagosomes showed partial or complete degradation. The phenomenon of bacterial degradation is not a mere physical process since other factors, mainly immunologic also have a significant role in these.

The requirement of macrophages for development of primary antibody responses to complex multi-determinant T-cell dependent antigens is now firmly established. The requirement for macrophages for development of secondary antibody responses to these antigens appears to be less than for primary responses. Macrophages do not appear to be required for development of antibody responses to so-called T-cell dependent antigens. Macrophages have alteast two crucial functions in the development of primary antibody responses to T cell analysis. One function is the presentation of antigen to the responding T cells and B-cells in a manner that efficiently stimulates these cells to cooperate in the development of an antibody

response. The second function is a viability promoting function in which T cells are made to survive and mediate their cooperative interactions with B cells (Gradebusch, 1979).

Nair (1973) reported that one consistant observation in the fowl was the presence of lymphoid foci in the inflammed tissue. Usually these were found as cuffs around blood vessels. He also reported that these lymphoid foci showed a tendency to infiltrate and spread. When immunogenic agents were used these lymphoid foci persisted with formation of germinal centres. In the present study emigration of lymphocytes and formation of noduler accumulations perivascularly were also noticed. But this is quantitatively of lesser intensity than reported in chicken. It is not clear from this investigation whether this represented a qualitative difference in the dynamics and circulatory pathway of lymphocytes. Nair (1973) had suggested that in the fowl because of the lack of organised lymphatic pathway, the movement of lymphocytes could be from the blood vessels to the tissue and back again into the blood vessels in contrast to that of mammals where passage to the blood vessels is via the lymph channel. Further sequential studies are needed to clarify this reaction in the duck.

In the present study it was seen that lymphocytes emigrated in large number after a time lapse of 4 - 6 hours and during that time the endothelial cells of these vessels appeared prominent and swollen. Such transformation of the endothelium of venules is remiscent of the changes in the post-capillary venules in the mammalian lymph nodes, which are considered, by some, to be the result of antigenic stimulation, while others believe that it is merely due to the increased traffic of small lymphocytes crossing the endothelium of post-capillary venules of lymph nodes draining areas where an immunogenic material has been deposited. In the fowl this transformation of the endothelium was related to the emigration of small lymphocytes and not to the immunogenic properties of the irritant. No attempt was made in this study to ascertain whether the emigration of lymphoid cells was between the endothelial or through the endothelial cells.

The emigration of small lymphocytes seen during the early phase of the reactions continued till lymphoid accumulations were seen. The emigration was not very marked when dextran sulphate was employed. In mammals it has been shown that hepari and other sulphated poly saccharides inhibited the emigration of small lymphocytes and delayed the onset of repair and fibrosis, while they had no effect on the emigration of

polymorphs and monocytes which continued to emigrate concurrently in what appeared to be in normal number, and the monocytoid mononuclears proceeded to transform into macrophages. Nair (1973) postulated that in the fowl the mucopolysaccharide content of basophils which was liberated during degranulation inhibited the spread of lymphocytes after emigration and the inhibited lymphocytes along with the proliferation of reticuloendothelial cells resulted in the formation of a replica of an anatomical and functional lymph nodule. A detailed investigation using graded doses of heparin and high molecular polysaccharides would throw more light on whether such an analogous situation develops in the duck. In addition secondary lymphoid nodules were seen forming in the lymphoid foci when antigenic substances like S. aureus, Freund's complete adjuvant and duck plague virus were injected. They were found to be morphologically similar to the lymphoid follicles present in the spleen of the duck and which represent the bursa-dependent lymphoid tissue. Ultrastructurally such foci revealed heterogenous collection of cells in various stages of transformation into the plasma cells. In addition to the mature lymphocytes with scanty cytoplasm and organelles and having a predominantly heterochromatic nucleus, there were numerous large cells which had blastoid features. The blast cells were identical to the immuno-blasts described by Movat and Fernando (1965). The relative role of

T lymphocytes in the inflammatory reaction, especially when viruses are involved, require further investigation. This would be possible when experiments are designed using thymectomised and/or bursectomised duck.

It was not possible from this study to assess the response of eosinophils in inflammatory reactions in the duck. In histological sections identification of eosinophil from the heterophil is difficult because of the similarity of appearance of the heterophilic and eosinophilic granules. In the limited electron microscopic observations, the involvement of eosinophils in the inflammatory response could not be ascertained with any degree of certaincy. Maxwell and Siller (1972) while describing the ultrastructural features of the duck eosinophil reported the presence of crystalline cores which would enable to differentiate with certainty the eosinophils from heterophils. To say definitely whether eosinophils in the ducks are the exact counterparts of mammalian eosinophils or not requires further detailed investigation.

In the local inflammatory reaction, repair was associated with active fibroplasia. When the irritant persisted at the site there was attempt to encapsulate the irritant and the reaction was identical to that in the mammals.

In order to study the response of the tissues of the duck to viral agents Ranikhet disease virus and Duck plague virus were employed. The Ranikhet disease virus is relatively non-pathogenic whereas the duck plague virus is a virulent host specific pathogen for the duck. The cellular response to RD virus infection was meagre from days five to nine. The tissue destruction following RD virus infection was negligible or absent. However, it did initiate a minimal initial heterophilic and later lymphocytic reaction. The adult duck therefore, can be considered as refractory to RD infection as observed by Iyer (1949) and Sriraman et al. (1980). But Sharma et al. (1977) and Sulochana et al. (1981) concluded from their studies that ducklings are susceptible. It would, therefore, appear that ducklings as they mature become immunologically competent. This is reflected in the mild cellular response and points to the fact that tissue destruction by the invading agent is a basic component of inflammatory response. Therefore, in an immunologically competent host, the cellular response perforce is meagre and tissue destruction is inapparent.

In contrast to this, in duck plague infection, to which the host is very susceptible the spectrum of inflammatory response was varied. There was basically virus induced tissue necrosis even on the fifth day and this had elicited

a cellular response characterised by heterophilic reaction. The virus caused multiple necrotic foci in various organs and this was followed by severe heterophilic reaction. It is pertinent to point out that irrespective of the pathogenic potential of the invading agent the primary response was heterophilic in nature. It would appear that the tissue destruction primarily induced by the virus was responsible for this reaction. This was followed by an immunologically mediated reaction characterised by lymphoid reaction which was seen to manifest as lymphoid nodules by day eight. The duck plague virus invasion and replication in the cells following experimental infection was massive and tissue destruction is bound to be extensive. This phase was heralded by heterophilic reaction and later there was an attempt at immunological compromise and this was marked by lymphoid response of a moderate to severe nature. When the infection was massive there was necrosis including necrosis of lymphoid cells.

SUMMARY

SUMMARY

A study of cellular dynamics of inflammatory reaction in the duck was made employing immunogenic and non-immunogenic agents. Turpentine, Dextran Sulphate, Talc, Freund's complete adjuvant, Homologous erythrocytes and Staphylococcus aureus were used to elicit local inflammatory reaction in the subcutaneous tissue, while Ranikhet disease virus and Duck plague virus were employed for studying the general reaction after intramuscular injection. The sequential involvement of the cellular components in the inflammatory response was studied and the development of the lesion was delineated when different agents were employed.

Irrespective of the agents employed to elicit inflammatory reaction, it was found that there was emigration of cells from the arterioles and venules after leukocyte adherence to the endothelium. The emigration of heterophils and monocytoid cells was always concurrent even though there was quantitative difference in the number of emigrating cells due to different etiologic agents. Initially there was heterophilic predominance and that was replaced later by a predominance of macrophages or lymphocytes. The heterophils and macrophages were found to be efficient phagocytes. Along with heterophils, few numbers of degranulating basophils

were noticed in the perivascular areas. The change in the cellular constituents of the inflammatory reaction appeared to result from difference in the rate of emigration and migration. It was not possible in this study to specifically assess the response of eosinophils. The emigrated lymphocytes accumulated around blood vessels and formed nodular structures. In such lymphoid nodules germinal centre formation was noticed when immunogenic agents were used. Immature and mature plasma cells were seen in the perivascular and intervascular areas from four days. The formation of an anatomical and functional replica of a lymph node during an inflammatory process could be considered a necessary factor for initiating and maintaining an immunologic reaction.

The monocytoid mononuclears were the haematogenous elements which transformed to macrophages, epithelioid cells and giant cells. Persistance of macrophages in the inflammatory zone could be due to continued emigration and multiplication at local site. Giant cells invariably formed part of the cellular exudate and their presence was frequently associated with necrotic debri and foreign bodies. The giant cells were formed from fusion of macrophages. Repair of inflammatory tissue was effected by removal of the

inciting agent, by fibroplasia and lysis and resorption of the infiltrating cells and exudate.

Ultrastructurally the duck heterophil possessed two types of granules, the large dense granule possessing one or more electron dense core. The other type - the light granule - was smaller with fibrillary matrix. Study of the development of the heterophils showed the association of golgi complex in the genesis of the granules. The process of endocytosis of the foreign body involved fusion of the phagosome with the granule forming the phagolysosome. The heterophil of the inflammatory exudate had a higher content of glycogen. Necrosis of the heterophil was associated with loss of organellar destruction and gradual lysis.

The monocytes after emigration from the blood vessels underwent structural alterations to form macrophages, epithelioid cells and giant cells. The transformation into macrophages was characterised by an increase in size and in the rough surfaced endoplasmic reticulum, and formation of well developed Golgi complex and lysosomes. The phagocytic and degrading capabilities of the macrophages were reflected in the numerous auto-phagolysosomes and hetero-phagolysosomes. Epithelioid cells were devoid of phagolysosomes and this indicated another function for these cells than phagocytosis. Transition forms between small lymphocytes, blast cells and

plasmacytic cells were encountered indicating that under antigenic stimulation, the lymphoid cells underwent structural alterations for the functional capability of antibody production. The blast cells had an active nucleus which was predominantly euchromatinic. The main characteristic feature of the plasmacytoid cells was the predominant rough surfaced endoplasmic reticulum with a polysomal configuration of ribosomes.

The basophils and mast cells showed degranulation liberating the mediators which altered the cellular response. It is possible that the arrest of lymphoid cells to form nodules could be due to the high molecular weight heparin liberated from the mast cell granules.

In order to study the systemic response of the tissues due to virus, Ranikhet disease virus and Duck plague virus were employed. The cellular response to Ranikhet disease virus was meagre and the tissue destruction was also minimal. In the Duck plague infection the spectrum of inflammatory reaction was severe and varied. There was necrosis of tissues and predominantly heterophilic infiltration and this was followed by lymphocytic reaction in different tissues. Lymphoid nodules with germinal centres were noticed in the visceral organs.

This study has brought out for the first time, the nature of cellular response in the duck caused by a variety of agents, immunogenic as well non-immunogenic. The ultrastructure of the inflammatory cells was also studied so as to clarify their functional attributes.

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THE CELLULAR RESPONSE IN INFLAMMATORY REACTION IN THE DUCK

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ABSTRACT

The cellular dynamics in ducks associated with inflammatory response induced by various immunogenic and non-immunogenic agents was studied for the first time. The inflammatory response was induced in the web of ducks using turpentine, dextran sulphate, talc, Staphylococcus aureus, homologus erythrocytes and Freund's complete adjuvant. The biopsy specimens were collected from half an hour upto 21 days at specific time intervals and examined to assess the cellular response and the chronology of cellular events taking place during the emigration process was depicted. The comparative features of cellular events taking place when different agents were employed were also clarified. The light microscopic studies were supported by electron microscopic studies. Irrespective of the agents employed to elicit the inflammatory reaction it was found that there was emigration of heterophils and monocytoid cells from the arterioles and venules concurrently even though there was quantitative difference in the number of emigrating cells due to different agents. Initially there was high predominance of heterophils in the exudate and later there was predominance of macrophages or lymphocytes. Participation of basophils was also evident

at the initial stages. Lymphoid foci formation with germinal centres particularly when antigenic stimulus was used was a characteristic feature. It was demonstrated that monocytoid mononuclear cells transformed into macrophages, epithelioid cells and giant cells.

The morphological features of the heterophilic granules were studied by electronmicroscopy. Large dense granules with one or two electron dense core and light granules with fibrillary matrix were seen. The process of endocytosis of the foreign body involved fusion of phagosomes. The active heterophil was demonstrated to contain more glycogen. The transformation of monocytoid cells into macrophages was demonstrated to be associated with increase in size and number of endoplasmic reticulum and formation of well developed golgi complex and lysosomes. Epithelioid cells were devoid of phagolysosomes. It was clarified that the lymphocytes underwent transformation into plasma cells under antigenic stimulation. The plasmacytoid transformation was evidenced by formation of rough surfaced endoplasmic reticulum.

The systemic response to Ranikhet disease virus and Duck plague virus was studied. The tissue destruction and cellular response to RD virus were meagre. Heterophilic and

monocytoid cell reaction was still the initial response. In duck plague infection there was progressive necrosis and this was associated with pronounced lymphoid reaction indicative of an immunologic reaction. In Ranikhet disease infection the lymphoid reaction was not as pronounced as in duck plague virus infection.