PERIODIC ACID-SCHIFF-POSITIVE RETICULO-ENDOTHELIAL CELLS PRODUCING GLYCOPROTEIN

Functional Significance During Formation of Amyloid*

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The present study deals with investigations on functional stages of reticulo-endothelial cells characterized cytochemically by the presence in their cytoplasm of a polysaccharide-containing substance which is colored by the periodic acid-Schiff (PAS) technique.

In response to various noxious stimuli, a proportion of proliferating reticulo-endothelial cells (including plasma cells, reticulum cells, the Kupffer cells of the hepatic sinusoids, the endothelial cells of the renal glomerular tufts, endothelial and adventitial cells of vessels) were found to contain granular or globular PAS-positive material in the cytoplasm (PAS cells).

PAS cells, pyroninophilic cells, and intermediate cell types containing PAS-positive as well as pyroninophilic material in the cytoplasm are considered characteristic functional stages of reticulo-endothelial cells involved in the morphogenesis of a variety of morphologic lesions of mesenchymal tissue.

Evidence is presented that PAS cells are linked directly with the synthesis of amyloid and related substances. Apart from accounting for the local cellular secretion of polysaccharide-containing globulins during the formation of amyloid, which is known to be a glycoprotein, the findings may also provide a reasonable explanation for the associated abnormalities in the serum protein-bound polysaccharides.

ETIOLOGIC THEORIES ON THE CAUSATION OF AMYLOIDOSIS

Amyloidosis and paramyloidosis are characterized morphologically by deposition of a homogeneous, non-fibrillar, hyaline-like substance for which certain tinctorial methods are specific. Hass and his coworkers[1] found that amyloid is formed by two slightly different protein fractions and a sulfate-bearing polysaccharide in secondary amyloidosis, and showed that from 1 to 2 per cent of the amyloid molecule is of carbohydrate nature. It was suggested that the physicochemical properties of amyloid are not contributed by the

* Aided by grants from the Danish League against Rheumatism and King Christian X's Foundation.

Received for publication, November 4, 1955.

protein alone, and that components of the circulating fluid may be bound to this matrix. Amyloid of different species and from different sources may show variation in composition and staining properties. The basic etiologic theories have been reviewed recently.[2-4] Since amyloid is related chemically to the interfibrillary ground substance of connective tissue, it has been suggested that the excessive breakdown of connective tissue might release into the blood stream components of ground substance in soluble form, capable of precipitation elsewhere as amyloid. Several authors have considered amyloidosis an infiltration rather than a degeneration. Pirani[5] postulated that in conditions in which depolymerization of the ground substance occurs and in which raised glycoprotein levels are maintained for a sufficiently long period, glycoproteins will precipitate at the level of the reticulo-endothelial system. Faber,[6] who observed an increase in the serum glucosamine associated with suppurative infectious disease, suggested that the serum mucoid was deposited in the tissue as amyloid. It has been pointed out[7] that, like mucin, amyloid is a glycoprotein, and a relationship to the mucolysis that occurs in the joints in rheumatoid arthritis has been suggested.

An alternative hypothesis, put forward by me,[2] accounts for the association of amyloidosis, not only with rheumatoid arthritis, but with the other conditions in which it is frequently found. This theory is founded upon histologic and experimental studies of reaction of mesenchymal and reticulo-endothelial cells in various conditions, and the control of the protein-synthesizing function of the reticulo-endothelial system and its pyroninophilic derivatives, the plasma cells and their immediate precursors, by the actions of adrenal corticoids and ascorbic acid. The breakdown of this control in persistent antigenic stimulation or other form of stress results in the local formation of amyloid.

The studies on experimental amyloidosis showed evidence of inhibition of cellular proliferation in the reticulo-endothelial apparatus, probably associated with a disturbance of some enzyme system to account for amyloid formation. Injections of cortisone or corticotrophin induced or promoted amyloid deposition in mice treated with injections of sodium caseinate,[2] and under the same experimental conditions three injections of nitrogen mustard (NH_2)—each equivalent to from 2.5 to 5 mg. per kg. of body weight—induced an almost diffuse amyloid deposition in the spleen.[3] On the other hand, ascorbic acid maintained the pyroninophilic cellular reaction in the spleen of hyperimmunized rabbits.

The experimental results with cortisone and ACTH were confirmed by Latvalahti[8] (1953), and clinical observations of amyloidosis following treatment with cortisone (West and Newns,[9] 1952) or ACTH (Frenkel and Groen,[10] 1954) have been reported.

The active stage of mesenchymal diseases[11] and experimental conditions produced by repeated stimulation of immune mechanism[12] often were associated with a proliferation of plasma cells and pyroninophilia of the cytoplasm of proliferating reticulo-endothelial cells in the spleen, liver, renal glomeruli, adventitia of vessels, and capillary endothelium. Moreover, this active phase was characterized by elevated levels of serum γ-globulin. Treatment with cortisone exerted a marked depression of pyroninophilia and plasma cells and, under certain conditions, caused a local precipitation of a pale, homogeneous substance of a type conforming to some or all of the morphologic criteria of amyloid. Amyloid formation is associated with changes in the electrophoretic pattern of the serum proteins. This was studied in mice by Letterer[13] (1949) and by Bohle and co-workers[14] (1950); following from 12 to 20 injections of nucleic acid, they found unquestionably elevated levels of γ-globulin, followed by a fall in γ-globulin and a rise in the levels of α- and β-globulin. The animals which developed amyloidosis after 30 injections had lower levels of γ-globulin than those which remained unaffected.

Whereas plasma cells are concerned in the formation of antibodies in response to antigenic stimuli, the predominant rôle of plasma cells and other pyroninophilic mesenchymal cells in the acute stage of ascorbic acid deficiency[15] and in other conditions in which evidence of persistent antigenic stimulus is lacking is remarkable. Pirani and co-workers[16] observed marked amyloid deposition in guinea-pigs fed on a scorbutogenic diet for 8 weeks or longer. In post-mortem examinations comprising 28 cases of rheumatoid arthritis, Lindahl and I[17] found an exceedingly high incidence of amyloidosis *histologically* (17 of 28 cases); we considered amyloid deposition in the spleen, kidney, and other organs to be a characteristic histologic lesion in long-standing rheumatoid arthritis and to be much more frequent than suggested by approximately 100 cases (cf. Reece and Reynolds[18]) reported in the literature.

The association between rheumatoid arthritis and amyloidosis is of the greatest interest for the light it might throw on the pathogenesis of these two disorders. Altogether, the common essential factors involved in the pathogenesis of rheumatoid arthritis, amyloidosis, and general stress phenomena may account for the exceedingly high inci-

dence of amyloid lesions in rheumatic disease. Actually, amyloidosis and the preceding cellular reaction represent a characteristic phase in reticulo-endothelial cellular function following stress.[15,17] In this connection, attention may be drawn also to the observations of plasmacytosis in such conditions as atomic energy casualties (Liebow and co-workers,[19] 1949), chronic radium poisoning in rats (Thomas and Bruner,[20] 1933), and x-ray irradiation in dogs (Wohlwill and Jetter,[21] 1953). The studies of Lundin *et al.*[22] (1954) indicate that plasma cell proliferation is a general reaction promoted by some pituitary factor which is secreted in increased quantities during adaptation; subsequent studies from the same laboratory (Schelin *et al.*[23]) showed this to be the somatotrophic hormone.

PREVIOUS OBSERVATIONS OF PAS-POSITIVE SUBSTANCE IN RETICULO-ENDOTHELIAL CELLS

Among the oxidation methods, the periodic acid-Schiff technique (McManus,[24] 1946; Lillie,[25] 1947; and Hotchkiss,[26] 1948) has become extensively used in histochemical procedures. There seems to be fairly complete agreement that 1,2-glycols are demonstrated in tissue sections by the PAS technique, and that these are most numerous in material consisting of carbohydrate or containing a carbohydrate moiety. These methods have been used especially in various researches on intercellular substances in mesenchyme; but, apart from histochemical studies by Pearse[27] (1949), White[28] (1954), and Grundner-Culemann and Diezel[29] (1955) on the nature of Russell bodies and their relation to plasma cells, only slight attention has been paid to the significance of mesenchymally derived PAS-cells and their possible rôle in a variety of lesions examined in the present study.

Pearse[27] (1949) used the periodic acid-Schiff method of McManus and Hotchkiss, together with other techniques, to investigate the cytochemistry of Russell bodies in human plasma cells and of Kurloff bodies in lymphocytes of guinea-pigs, and presented evidence that both consist of mucoprotein. The substance of which the bodies are composed was found uninfluenced by ribonuclease, diastase, or hyaluronidase; it failed to bind methylene blue at pH 6, and was devoid of metachromatic properties. It also was demonstrated that the cytoplasm of some normal plasma cells contains mucoprotein, suggesting that these are polysaccharide-containing globulins (glycoglobulins, mucoglobulins). Pearse examined 6 cases of plasmacytoma, and found a PAS-positive reaction in a small proportion of plasma cells in 4, while in 2 cases a large proportion of the plasma cells and reticulum

cells showed a positive reaction. In tissue in which plasma cells containing Russell bodies were present in addition to the ordinary types, Pearse observed intermediate stages between the plasma cells with faintly PAS-positive cytoplasm and those containing the fully developed Russell bodies. Pearse considered the question whether the mucoprotein is secreted or absorbed by plasma cells, and adduced evidence in favor of secretion; this is in accord with my assumption[11] (1948) that plasma cells and other pyroninophilic reticulo-endothelial cells may produce amyloid and hyalin.

Cavallero[30] (1953), who made a morphologic study of the effect of somatotrophic hormone on the plasma cells, found that treatment of intact rats with this hormone resulted in an increasing number of plasma cells in the spleen, lymph nodes, and thymus. By the periodic acid-Schiff method abundant granular PAS-positive material was demonstrated in the immature plasma cells and in undifferentiated reticulum cells as well as in the interstitial spaces. Russell bodies frequently were seen in the lymph nodes.

Present Study

The periodic acid-leukofuchsin procedure of McManus and Hotchkiss was used to examine the cytologic changes in reticulo-endothelial and other cells derived from mesenchyme, which were previously found to be linked with the formation of amyloid and related substances.

Material and Methods

Five different groups, with a total of 80 female mice of C_3H strain, weighing from 15 to 25 gm., were fed ad libitum on an oatmeal diet during the experiment. The animals were given daily subcutaneous injections of 0.5 ml. of a 5 per cent casein solution in 0.25 per cent NaOH for periods varying from 28 to 36 days. In 25 animals the casein injections were followed by four daily subcutaneous injections of 0.3 mg. of cortisone, whereas 10 animals were treated with 0.5 mg. of ACTH in two daily doses. Another 16 mice received three injections of nitrogen mustard (NH_2), each equivalent to from 2.5 to 5 mg. per kg. of body weight.

A group of 48 rabbits were hyperimmunized with a formaldehyde-killed Pfeiffer bacillus culture, administered in intravenous injections three times a week for from 6 to 16 months (cf. the material previously used for studies on the effect of cortisone on plasma cell response[31] and in studies on the effect of cortisone on experimental glomerulonephritis.[12])

A group of 5 guinea-pigs were treated with daily subcutaneous injections of 2 ml. of a 5 per cent casein solution in 0.25 per cent NaOH for 5 weeks.

Specimens of various organs were fixed in 4 per cent formalin and examined histologically after staining with periodic and leukofuchsin —McManus (PAS) and Hotchkiss (PARS)—the Unna-Pappenheim methods, and with methyl violet and Congo red stain for amyloid.

DESCRIPTION OF FINDINGS

Experimental Amyloidosis in Mice

The earliest and most pronounced changes were observed in the *spleen*. Histologic examination showed increasing accumulations of plasma cells and highly pyroninophilic reticulum cells, especially in the red pulp; this also contained numerous giant cells with three or more nuclei in irregular arrangement.

Sections stained with the PAS technique showed, during treatment, an increasing number of proliferating reticulo-endothelial cells showing PAS-positive, often finely granulated material in the cytoplasm (PAS cells); also, after administration of casein for some weeks, the cytoplasm of giant cells showed globular PAS-positive inclusions. Comparison with sections stained by the Unna-Pappenheim method showed intermediate cell types containing a pyronine-positive zone of cytoplasm surrounding inclusions of non-pyroninophilic, PAS-positive substance. Such intermediate cell types were visible especially among the giant cells, presenting a decreasing amount of pyroninophilic material in the cytoplasm as the amount of PAS-positive substance increased. The pyroninophilia was removable by incubation with ribonuclease, whereas the PAS-positive substance remained uninfluenced by ribonuclease, diastase, or hyaluronidase, failed to bind methylene blue at pH 6, and was devoid of metachromatic properties.

Similar cytochemical changes were observed in relation to the cells of the splenic reticulum in the perifollicular zone and in endothelial and adventitial cells of vessels showing PAS-positive material.

In all cases it was evident that the PAS-positive reticulo-endothelial cells were directly linked with amyloid formation and deposition locally. This was especially pronounced in cases in which amyloid production was accelerated for a short period by additional administration of ACTH, cortisone, or nitrogen mustard. In this stage of intensive amyloid formation accompanied by gradual suppression of the cellular elements of the spleen, accumulations of large reticulum cells, showing bright red cytoplasm when stained with the PAS tech-

nique, in direct continuity with the amyloid precipitates were distinctive findings (Fig. 1). The observation in the border zone of large, hypertrophic PAS cells showing signs of secretion of a homogeneous material confluent with the amyloid precipitates (Fig. 2) showed strikingly the functional significance of reticulum cells in the secretion of glycoprotein during the local precipitation of amyloid. More scattered giant cells with PAS-positive cytoplasm also showed signs of a direct transformation into small isolated deposits of amyloid. Analogous changes in the splenic reticulum and vascular endothelium accounted for the amyloid deposition in relation to these structures. When the amyloid deposition was almost complete, the pyroninophilic cellular reaction was found to be suppressed, but a few reticulum cells with a strongly PAS-positive reaction of the cytoplasm often were found in the periphery of the amyloid deposits.

The *liver* was involved in the amyloid deposition later than the spleen, but the deposition could always be produced by further treatment with cortisone or nitrogen mustard after discontinuance of the casein injections. In early stages the Kupffer cells in the sinusoids were swollen, hypertrophic, and contained pyroninophilic cytoplasm, whereas the picture in the early amyloid stage was dominated by large, hypertrophic Kupffer cells with distinctly PAS-positive cytoplasm in direct connection with the amyloid deposits situated between the sinus endothelium and the liver cells. The amyloid appeared in the liver as a product of secretion by the PAS-positive Kupffer cells of the sinusoids (Fig 3) in harmony with the typical localization.

The *kidneys* showed analogous cellular reactions to account for amyloid changes in the glomeruli. Stages characterized by proliferating pyroninophilic endothelial cells in the glomerular tufts were followed by the appearance of PAS-positive material in the same cells, showing direct transition to confluent amyloid masses in the loops.

Hyperimmunized Rabbits

Rabbits immunized with a culture of formaldehyde-killed Pfeiffer bacillus given in intravenous injections three times a week for periods varying from 7 to 16 months showed widespread granulomatous and necrotic lesions in the spleen and lungs and more scattered lesions in the kidneys, liver, adrenal glands, myocardium, and other organs. In addition, especially the spleen and lungs showed a very pronounced proliferation of mature and immature plasma cells. Local amyloid lesions of varying degree and different types of glomerular lesions in the kidneys often were visible.

Besides the marked aggregations of pyronine-positive plasma cells, especially in the red pulp, sections from the *spleen* showed numerous morphologically identical cells, characterized by a bright red non-granular cytoplasm in sections stained with the PAS method. Such PAS-positive reticulo-endothelial and plasma cells were found either in large accumulations (Fig. 4) or scattered in the pulp among the dense aggregations of pyronine-positive plasma cells; numerous Russell bodies showing similar cytochemical properties also were present in the neighborhood. The cytoplasm of proliferating reticulo-endothelial cells in the perifollicular zone and that of some reticulum cells of the germinal centers were also PAS-positive. A direct transition from PAS cells to amyloid deposition was evident in many areas.

The *lungs* were involved in all cases of this group; in addition to dense aggregations of pyroninophilic plasma cells they presented a broadening of alveolar septa showing proliferating septal cells with faintly PAS-positive cytoplasm and pronounced accumulations of large reticulum cells with eccentric nuclei and containing globular inclusions of finely granular, bright red PAS-positive material (Fig. 5). The PAS-positive material often was surrounded by a pale cytoplasmatic zone, which proved to be pyronine-positive in sections stained with the Unna-Pappenheim method. A few of these cells were of the giant cell type with several peripheral nuclei surrounding the PAS-positive material. Among the PAS cells several Russell bodies were seen also. These were often especially numerous around the vessels.

In local areas with closely packed PAS cells, a gradual transition to epithelioid cells was remarkable, and numerous cells with pronounced PAS-positive inclusions in the cytoplasm were observed regularly in the epithelioid cell granulomas. The findings suggested that PAS cells represented precursor stages directly involved in the genesis of amyloid, hyalin, and also of epithelioid cell granulomas and necrotic granulomatous lesions.

Similar cellular changes were found in the other organs. In the liver the proliferating Kupffer cells with strongly PAS-positive cytoplasm often showed a transition to local amyloid depositions.

The findings in sections from the kidneys stained with the PAS technique were of interest with regard to the histogenesis of certain glomerular lesions that occur in specific disease. In animals showing the picture of acute glomerulonephritis, the proliferation of endothelial cells of the glomerular tufts showed very marked pyroninophilia as stated in the previous study, and the glomeruli appeared bright red in contrast to the surrounding parenchyma. However, in sections

stained with the PAS technique, globules of a finely granular PAS-positive material were visible regularly in the proliferating cells (Fig. 6). Transition from swollen glomerular cells containing PAS-positive material in the cytoplasm to deposits of homogeneous material in the tufts, such as amyloid, was a striking finding and indicated a *cellular* origin locally of such lesions.

Administration of cortisone or ACTH induced a marked increase in homogeneous precipitates and a decrease in cells containing pyroninophilic or PAS-positive substance. In some cases of subchronic glomerulonephritis the various cellular stages could be found in the same section.

Guinea-Pigs Treated with Sodium Caseinate Injections

The changes in guinea-pigs were exactly similar to the findings in experimental amyloidosis in mice. In the treated guinea-pigs, the spleen also showed incipient amyloid changes with typical PAS cells in the border zone of the amyloid deposits.

Control sections from all three groups of experiments were exposed to the action of diastase (saliva), hyaluronidase, or ribonuclease. The PAS reaction was still positive after treatment, and the material was devoid of metachromatic properties.

The findings taken together suggest that the cytoplasmic material in the cells investigated consisted wholly or partly of mucoprotein or glycoprotein.

Discussion

The widespread proliferation of reticulo-endothelial and other cells derived from mesenchyme, characterized cytochemically by the presence of a PAS-positive material in the cytoplasm, suggests a most important function of reticulo-endothelial cells in producing polysaccharide-containing globulins (mucoproteins or glycoproteins) in response to diverse stimuli or injuries.

It is evident that the inverse relationship between ribonucleic acid and mucoprotein, not only in plasma cells forming Russell bodies, as demonstrated by Pearse[27] (1949), but also in reticulum cells, Kupffer cells of the hepatic sinusoids, endothelial cells of the renal glomerular tufts, and in endothelial and adventitial cells of vessels, supports synthesis and not absorption. As stated earlier, various noxious stimuli may produce a proliferation of reticulo-endothelial cells showing pyronine-positive cytoplasm. The present study shows that, following over-stimulation, a varying proportion of these cells will show a decreasing amount of pyronine-positive material (ribonucleic acid)

and an increasing content of PAS-positive substance (mucoprotein).

Mesenchymal derivatives, especially reticulum cells colored by the PAS technique, were directly involved in the genesis of a variety of morphologic lesions of mesenchymal tissue, such as the formation of amyloid and hyalin, and also linked with the morphogenesis of epithelioid cell granulomas and granulomatous necrosis. The rôle of PAS cells during the formation of amyloid is of interest, as it may throw light on the pathogenesis of this disorder. The occurrence of proliferating, hypertrophic, strongly PAS-positive reticulum cells in the spleen, liver, glomeruli, and vascular walls in direct relation to the also partly PAS-positive amyloid precipitates gives direct evidence of a local cellular secretion of polysaccharide-containing globulins, and is inconsistent with the concept of a precipitation of amyloid from the blood, caused by the raised serum glycoprotein or serum mucoid levels, as suggested by several workers. The findings may account for the chemical composition of amyloid, which is known to be a glycoprotein, and will also provide a reasonable explanation for the associated abnormalities in the serum protein-bound polysaccharides and the serum protein pattern during amyloid formation. These questions will be the subject of further study.

Obviously, the proliferation of PAS cells can be considered a general adaptation reaction, closely related to the pyroninophilic cellular reaction.

Summary

In response to antigenic and diverse unspecific stimuli (experimental amyloidosis, immunization), various types of mesenchymal cellular derivatives, especially reticulo-endothelial cells, widely dispersed in the organs, showed a marked content of globular or finely granular periodic acid-Schiff-positive substance in the cytoplasm. This was considered to be mucoprotein or glycoprotein produced by the cells.

PAS cells, pyroninophilic cells, and intermediate cell types containing PAS-positive as well as pyroninophilic material in the cytoplasm are considered characteristic functional stages of various types of mesenchymal (especially reticulo-endothelial) cells, including reticulum cells and cells of morphologic plasma cell type.

The current study shows evidence that mucoprotein- or glycoprotein-producing PAS-positive cells are directly concerned in the formation of amyloid, hyalin, and related substances in, e.g., the spleen, liver, and the renal glomeruli, and are involved in the genesis of epithelioid cell granulomas and various other lesions. The cytochemical findings suggest a local secretion of polysaccharide-containing globulins during the stage of amyloid precipitation.

Apart from accounting for the pre-eminent distribution of amyloid in the tissues rich in reticulo-endothelial components, the findings also provide a reasonable explanation for the associated abnormalities in the serum protein-bound polysaccharides (mucoproteins, glycoproteins), and indicate that reticulo-endothelial cells of PAS cell type are one of the chief sources of the production of serum mucoprotein or glycoprotein.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

All sections are from formalin-fixed tissue, stained by the periodic acid-Schiff technique of Hotchkiss.

FIG. 1. Stage of intensive amyloid formation in the spleen. Mouse 43 treated with injections of sodium caseinate for 30 days followed by ACTH injections for 4 days. PAS-positive reticulum cells in direct continuity with amyloid precipitates in the spleen. × 460.

FIG. 2. Border zone of amyloid deposit in the spleen. Mouse 23 treated with injections of sodium caseinate for 30 days. Hypertrophic reticulum cells showing secretion of a homogeneous PAS-positive substance confluent with the amyloid precipitates. × 950.

FIG. 3. Hypertrophic PAS-positive Kupffer cells of hepatic sinusoids in stage of amyloid formation. Mouse 23 treated with sodium caseinate injections and also with three injections of nitrogen mustard. × 460.

FIG. 4. Aggregations of PAS-positive plasma cells in the splenic pulp. Hyperimmunized rabbit 2966. × 460.

FIG. 5. Section from the lung showing collections of large reticulum cells with eccentric nuclei and containing globular inclusions of finely granular PAS-positive material. The surrounding pale cytoplasmatic zone was proved to be pyronine-positive in sections stained with pyronine-methyl green. Hyperimmunized rabbit 2927. × 950.

FIG. 6. Proliferating endothelial cells of the renal glomeruli, showing globules of a finely granular PAS-positive material. In sections from the same block stained with pyronine-methyl green the substance was found to be surrounded by pyronine-positive material which was removable by hydrolysis with ribonuclease. Hyperimmunized rabbit 3785. × 950.

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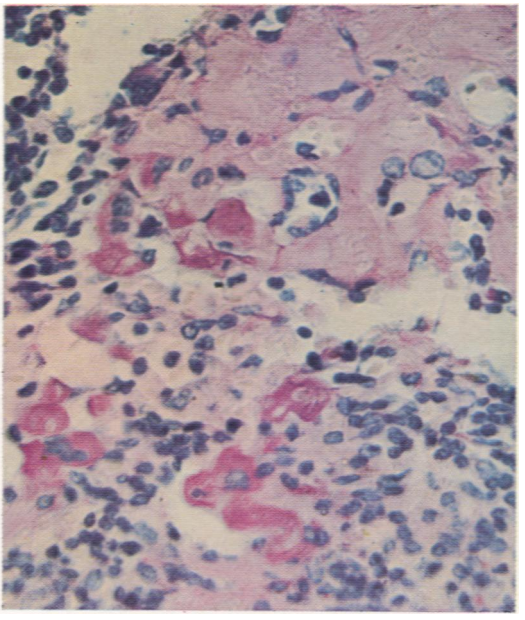

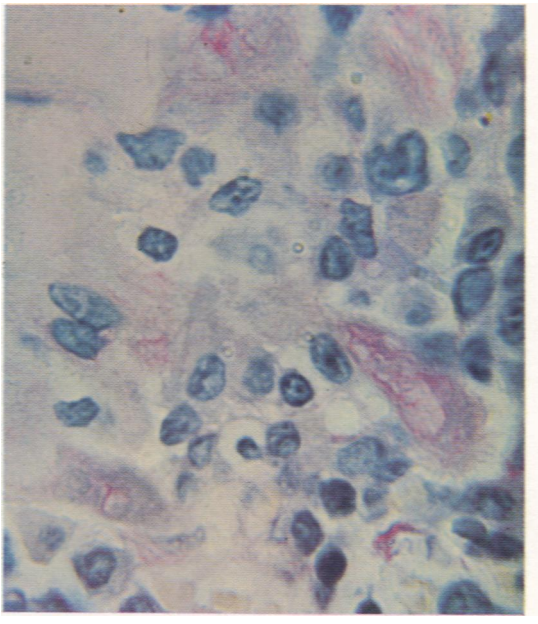

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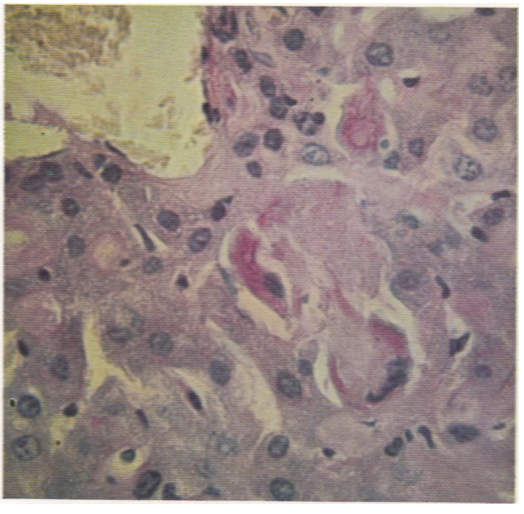

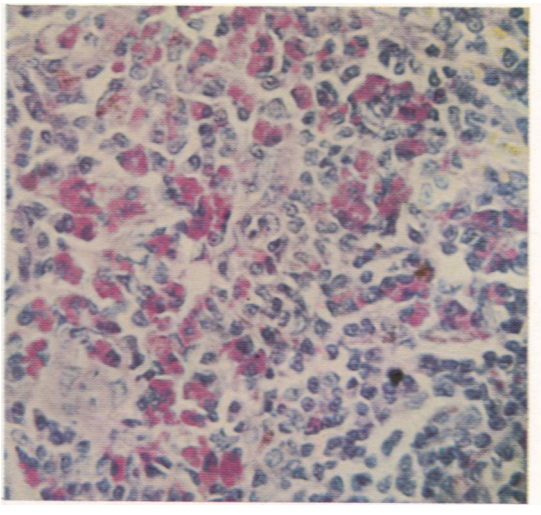

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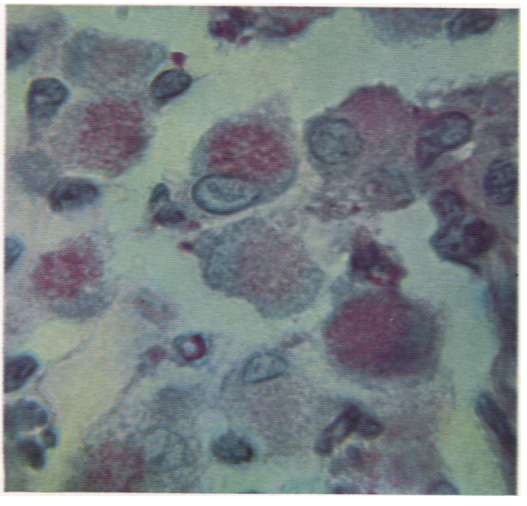

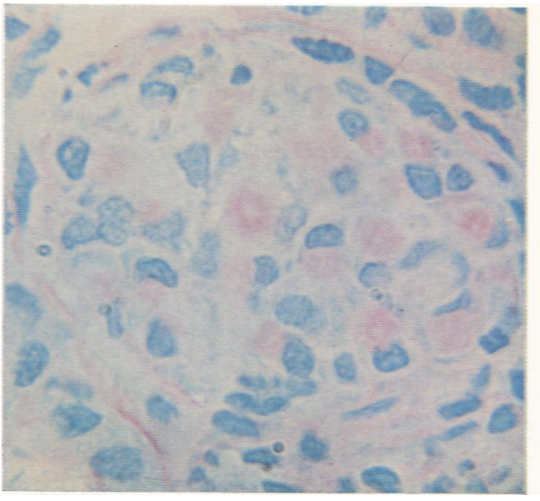

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