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GENESIS OF ELEMENTS HAVING THE APPEARANCE OF TISSULAR MAST CELLS

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Introduction

The advances achieved during the past two decades in the knowledge of the bio- and histochemistry of the metachromatic substance contained in mast cells have still not clarified the genesis of these cells. A survey of the pertaining literature reveals that the histiocytic origin, suggested by Maximov in 1904 is the most commonly accepted concept, which has recently been reinforced by the embryological investigations carried out by Rijih [15] and by the reticular-histioblastic evolutional aspects reported by Bessis [2]. On the other hand, the fibroblastic origin is maintained in a number of works such as those published by Hissard et al. [7, 8] and by Fawcett [5]. The hypothesis of Zavarzin and Elisseev on a multiple origin has been resumed in a recent work by Danilova [3], who maintains that mast cells arise from various cells belonging to connective tissue. Other investigators, such as Dégos and Cottenott [4] suggest an autonomous origin, on the basis of amitosis patterns and other observations made in tissue cultures.

The administration of polysaccharides with a view to enhancing the genesis of mast cells by experimental means has hitherto brought no particular clarification. Consequent upon the intraperitoneal injection of heparin, Frick [6] noted a neoformation of mast cells as a result of the injected substance being phagocyted by the non-differentiated adventitial cells of the peritoneum which display a histiocytic appearance. This author found no transformation of fibroblasts into mast cells. After injecting heparin and tissues containing a large amount of hyaluronic acid Hissard et al. [7] noticed a direct and progressive transformation of fibroblasts into mast cells, without any transition through the histiocytic form.

This brief presentation leads to the conclusion that the genesis of tissular mast cells from mesenchymal cells is nowadays admitted. The transformation of the latter elements into mast cells is supposed to take place in varied evolutional stages corresponding to particular microscopic patterns designated

as non-differentiated adventitial cell, polyblast, clasmatocyte, reticular cell, histioblasts, histiocyte, fibroblast, etc. The process of the conversion of mesenchymal cells into tissular mast cells in the adult period has hardly been studied. There is no available information as to the nature of the factors which determine the accumulation of polysaccharides within the cell and we are far from knowing the details of the onset and evolution of this accumulation.

Methods

Our own observations in sections and prints of lymph nodes, lung, skin, spleen, uterus, collected both from human subjects and from laboratory animals, enabled us to find microscopic patterns which might be interpreted as processes related to the genesis of tissular mast cells. They seem to be extremely frequent in adult age under certain experimental and morbid circumstances, when the presence is noted of many cells with a more or less important polysaccharide content.

Our experimental material revealed that the most frequent patterns that might be interpreted as processes of mast cell genesis are to be met with in the lungs in which biological determinations disclose the presence of an increased amount of histamine. This has made us to suggest the hypothesis of the existence of a mast cell genesis induced by histamine and to carry out a systematic study on this subject.

Our necropsy material revealed that the most frequent patterns that could be interpreted as processes of mast cell genesis were to be found in lungs and tracheobronchial lymph nodes affected by anthracosis. This has made us to suggest the hypothesis of the existence of a mast cell genesis determined by anthracosis.

Finally, in order to set up a comparative experimental model permitting a follow-up in the same pulmonary cells of the stages of polysaccharide accumulation and the possible participation of reticulo-endothelial cells in the intracellular metabolism of these substances, we performed intratracheal instillations of heparin and noted the onset and evolution of mast cell genesis induced by heparin.

We shall now proceed to a successive presentation of our observations on the experimental model of heparin-induced mast cell genesis, on histamin-induced mast cell genesis and. finally, on mast cell genesis determined by anthracosis.

Personal observations

The prints and sections of normal lungs, both human and collected from laboratory animals, display, after toluidine blue staining, three types of cells exhibiting a metachromatic reaction.

Firstly, there are the mucus-secreting cells in the epithelium of the air passages and appending glands. In sections they display a particular arrangement. In prints they can be easily identified by their elongated shape, the triangular and tachychromatic appearance of the nucleus and the absence of phagocyted particles ; all these peculiarities prevent their presence from being a misleading factor in the interpretation of the process of intrapulmonary mast cell genesis.

Secondly, there are the mast cells. In human subjects, they mostly assume the appearance of tissular cells and less frequently that of basophile granulocytes. In the guinea-pig, which was the animal used in our experimental investigations, all the cells bearing the appearance of mast cells observed in the normal lung were basophile polymorphonuclear cells. In pneumograms

they are present in a proportion of 0,2 per cent. In some of these cells the excessive amount of stored metachromatic substance covering even the nucleus, makes the cell appear compact to resemble a human tissular mast cell. After the polysaccharides have been removed from prints by means of hydrolysis at 60° C in normal hydrochloric acid and re-staining with May Grünwald—Giemsa stain, the polymorphous nucleus of the basophile granulocyte becomes clearly apparent, but of 50 elements photographed successively before and after acid hydrolysis, none was noticed to display the overlacy or round shaped nucleus of the tissular mast cell. This is why basophile polymorphonuclear cells are very often liable to be considered as mast cells whenever the above-mentioned technique is not adequately applied in view of the accurate identification of the nature of the cell containing metachromatic material. In sections, the difference is even more difficult to establish than in prints.

Thirdly, there are the reticulohistiocytic cells consisting mainly of pulmonary macrophages whose nucleus or protoplasm contains diffuse or granular metachromatic substances of alpha, beta or gamma intensity, according to PEARSE's classification [14] and which appear in pneumograms in a proportion of 0,5—2 per cent. We considered their quantitative variations to be an indication concerning the existence of a more or less extensive process of tissular mast cell genesis. We could not interpret these metachromatic substances as resulting from the phagocytosis of cells in mastocytoclasia, as we noticed no such processes in the neighbourhood of macrophages containing metachromatic particles. The considerably increased percentage of such macrophages under certain experimental and morbid conditions was an important argument showing that this was no process of phagocytosis.

Proceeding from the above findings, we made the following observations in prints and paraffin sections stained according to the techniques that will be mentioned for each particular investigation.

Heparinic mast cell genesis

Working method. Intratracheal drip-instillations of 0,5 ml of an aqueous solution of heparin (Heparin "RICHTER", Budapest) were performed in 60 adult guinea-pigs under chloral hydrate anaesthesia. Each instillation lasted 15—30 minutes. In the 0,5 ml solution each animal in the first group of guinea-pigs received 625 I. U. heparin : in the second group the animals received 1250 I. U. and in the third group 2500 I. U. The animals were sacrificed after an interval ranging from 1 hour to 9 days. No evident local change was noticed on gross examination after the penetration of heparin into the air passages, nor were any evident alterations observed to occur as regards blood co-agulability.

Evolutional features. After staining of the prints for 1—3 minutes with a 0,5 per cent aqueous solution of toluidine blue, our findings were as follows.

1. The penetration of heparin into the cells was the more rapid, the less units were contained in the 0,5 ml solution and the lower was its viscosity. This penetration is evident and diffuse 24 hours after the instillation.

2. At the end of 1—3 days, practically all the cells in the respiratory parenchyma are loaded with heparin and thus become metachromatic (Fig. 1*a*). However, the intensity of metachromasia varies with each individual cell,

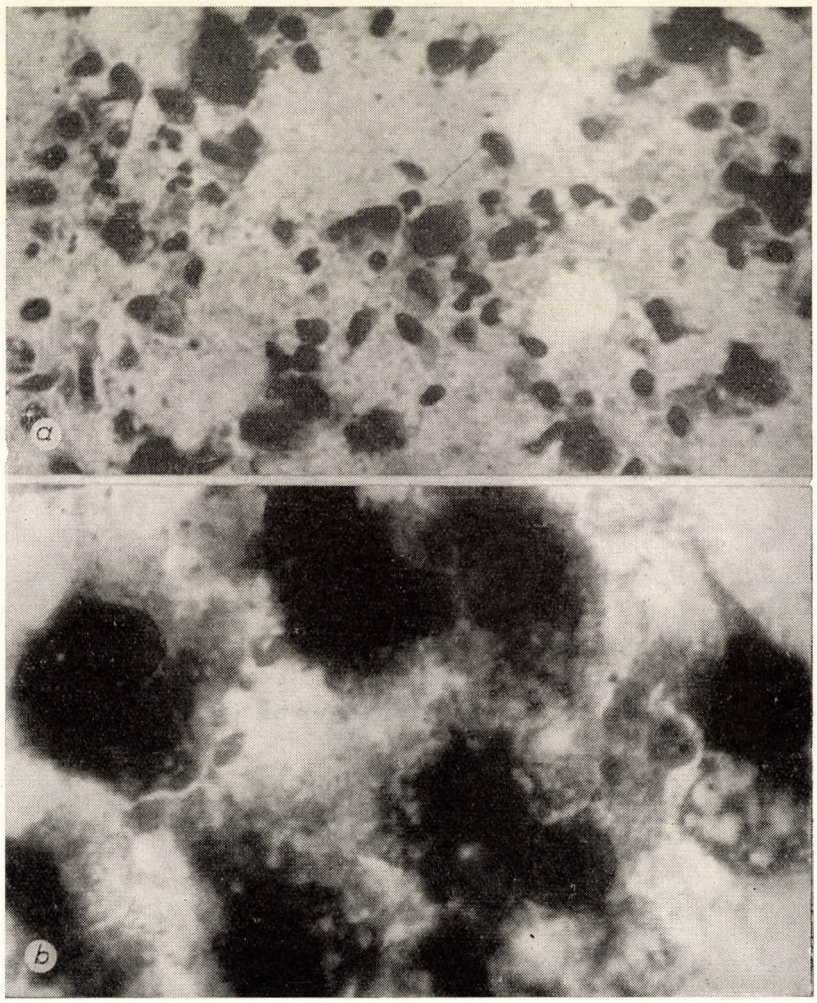

Fig. 1. Heparinic mast cell genesis. (Lung prints from guinea-pigs subjected to instillations of heparin.) *a)* 48 hours after instillation. Practically all the cells in the respiratory parenchyma have accumulated heparin and became metachromatic. (Toluidine blue staining, ×520.) *b)* The same preparation at a greater enlargement shows the various degrees of loading in each cell. Diffuse intracellular heparin storage is dominant. (Toluidine blue staining, ×1500.)

according to the spread of intracellular penetration, to the amount of accumulated substance and to the metabolic capacity of the cell (Fig. 1*b*).

3. Heparin is stored in the first place in pulmonary macrophages, apparently irrespective of the number of existing protoplasmic inclusions.

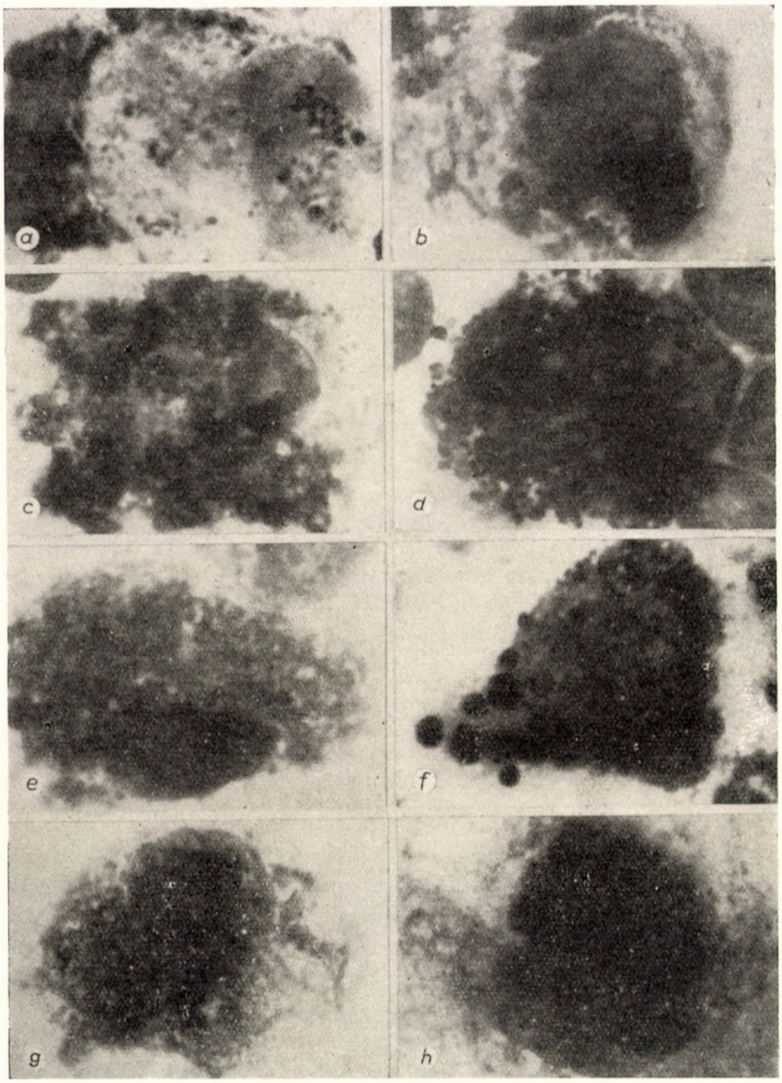

Fig. 2. Heparinic mast cell genesis. (Lung prints from guinea-pigs subjected to instillations of heparin.) *a)* Obvious tendency towards intranuclear accumulation of more diluted solutions, in the shape of small granules. These granules are situated in vacuoles. Compact agglomeration of heparin at the periphery of the cell (toluidine blue staining, × 2200). *b)* Obvious tendency towards intranuclear accumulation of more concentrated solutions in the shape of vesicles (toluidine blue staining, × 2200). *c)* Prevalent heparin concentration at the periphery of the protoplasm of macrophages in the shape of large sized particles (toluidine blue staining, × 2200). *d)* Prevalent heparin concentration around the perinuclear area of the cytoplasm, in the shape of granules of the mast cell type (toluidine blue staining, × 2200). *e)* Diffuse intracytoplasmic spreading of granules of the mast cell type. The nucleus is seen to be intensely metachromatic (toluidine blue staining, × 2000). *f)* Intracytoplasmic conversion of accumulated heparin in the shape of spherical granules of variable size (toluidine blue staining, × 2000). *g)* Intracytoplasmic and intranuclear agglutination of the granules (toluidine blue staining, × 2000). *h)* Intracellular concentration of heparin in the shape of intensely metachromatic, diffuse nucleocytoplasmic masses (toluidine blue staining, × 2200)

It also appears to be present in the cells forming reticular syncytia, in histiocytes, monocytes, endothelial cells, neutrophile granulocytes as well as in elements having a lymphoreticular appearance. None of the cells in the upper and lower air passages displayed the appearance of heparin acculumation.

4. A particularly striking fact is the considerable speed heparin accumulation in the cell nuclei, in varied shapes, ranging from small isolate metachromatic granules (Fig. 2*a*) to large compact vesicles (Fig. 2*b*). This peculiarity will be more extensively discussed below.

5. The intracytoplasmic accumulations are successively dominated by a tendency towards concentration at the periphery of the cell (Fig. 2*c*) and then, by a tendency towards perinuclear concentration (Fig. 2*d*). The protoplasm of cells in the pulmonary connective interstices and especially that of macrophages seems to possess the capacity of concentrating dilute solutions of heparin and of splitting the large vesicles from solutions of high concentrations. This process results in the appearance of cells resembling human tissular mast cells, which are evident especially between the 3rd and 5th day after the instillation. Their protoplasm contains small granules of about the same size (Fig. 2*e*), or of variable size (Fig. 2*f*), agglutinated granules forming large corpuscles (Fig. 2*g*) or homogeneous compact metachromatic material (Fig. 2*h*). Transition stages between all these patterns may be seen to exist and some of the microscopic fields display a certain prevailing type in the distribution of the instilled substance (Fig. 1*b*).

Pneumograms reveal that, 24 hours after the instillation, more than 50 per cent of the pulmonary macrophages contain accumulated metachromatic material : at the end of 48 hours this proportion attains 82 per cent. 20 per cent of these cells display the typical appearance of a mast cell and they can no longer be differentiated by toluidine blue and May Grünwald—Giemsa staining from the genuine mast cells of the lung. This great variety of patterns of intracellular penetration of heparin is followed at the end of 5—9 days after the instillations, by equally varied aspects, betraying the elimination of the instilled substance from the cell. At the end of the experimental period, we found that 10 per cent of the macrophages maintained the typical appearance of a mast cell for a period which we have as yet been unable to ascertain.

6. Consequent upon the penetration of heparin into the cells of the respiratory parenchyma, the appearance is noticed of structures suggestive of tissular mast cells, due to the reticular cells, macrophages, histiocytes and monocytes being loaded with metachromatic substance. The appearance is likewise noticed of structures suggestive of vascular mast cells determined by heparin accumulation within endothelial cells and of basophile granulocytes, resulting from the penetration of metachromatic substances into neutrophile polymorphonuclear cells. All these cells display dynamic patterns of assimilation, loading, accumulation, conversion and elimination of heparin

permitting the identification of clear successive stages of experimental mast cells genesis, from the appearance of the earliest metachromatic granules and up to their maximal intracellular accumulation.

Morphofunctional peculiarities. The foremost place among these peculiarities is held by the intranuclear accumulation of the instilled substance which was quite obvious and constant in all the cells under investigation. It is followed by some histochemical changes, among which we will mention the following.

1. Gradual insolubilization of the heparin which has penetrated into the nucleus. This process is quite obvious, beginning with the 3rd and 5th day after instillation, provided the same group of cells is followed up in a print first stained with toluidine blue, then discoloured by prolonged washing with water and restained with the same solution. As the experiment progresses in time, the number of nuclei from which heparin as no longer dissolved upon washing and which re-display intense metachromatic staining, gradually increases (Fig. 3*a* and *a'*).

2. The accumulation of desoxyribonucleic acid in areas displaying a maximum concentration of heparin. This process can be rendered evident by following up in prints the same cells stained with May Grünwald—Giemsa stain, subjected to normal hydrochloric acid hydrolysis at 60°C, and restained by the same technique (Fig. 3*b* and *b'*). The same intranuclear accumulations, which can be restained after removal of the instilled metachromatic substance and of ribonucleic acid, stain reddish-violet upon restaining according to the Feulgen-method, and green upon methyl green-pyronine staining. At the same time, as has been pointed out in a former study [19], ribonucleic acid is eliminated from the nucleus into the cytoplasm and accumulates in the areas in which a maximum concentration of heparin has been attained.

3. Some of the intranuclear heparin gradually becomes orthochromatic, another portion is converted in a faintly metachromatic substance which diffuses into the protoplasm amongst the intensely metachromatic granules of apparently unmodified heparin. This intergranular substance which in prints fixed in absolute alcohol is quite evident 5 days after instillation, does not disappear on washing, but it does so after treatment with testicular hyaluronidase (Luronase "BAYER"). Inactivated hyaluronidase leaves it unchanged. It probably results from the conversion of heparin into muccopolysaccharides of the type of hyaluronic acid or chondroitin sulphuric acids (Fig. 3).

These experimental observations demonstrate that the cells of the respiratory parenchyma, which display the microscopic appearance of mast cells' genesis consequent upon the gradual accumulation of heparin, are at the same time altering this substance. It becomes more stable by being bound to the acid protein structures in the nucleus and protoplasm. It is then probably subjected to desulphatation and depolymerization processes and converted

into other polysaccharides which are extracted by hyaluronidase. The process of experimental mast cell genesis determined by heparin has therefore the value of an obvious proof of the capacity of pulmonary reticulo-endothelial cells to take an active part in the metabolism of polysaccharides.

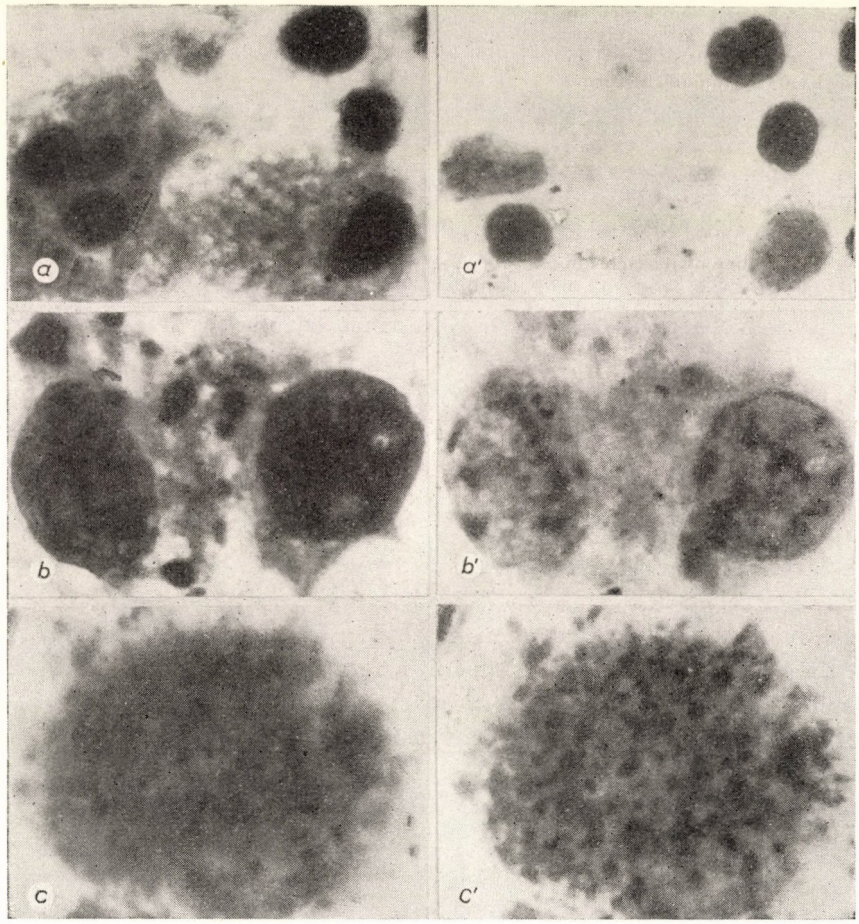

Fig. 3. Heparinic mast cell genesis. (Lung prints from guinea-pigs subjected to instillations of heparin.) *a* and *a'* : Same group of cells stained with toluidine blue *(a)*, discoloured with distilled water and restained with toluidine blue *(a')*, showing total disappearance of metachromasia in the protoplasm and in two nuclei only. In the remaining three nuclei, heparin had become insoluble and was not swept off upon clearing, the nuclei recovering their metachromasia (× 2000). *b* and *b'* : Same two cells stained with May Grünwald—Giemsa stain *(b)*, discoloured with hydrochloric alcohol, subjected to 60° hydrolysis in normal hydrochloric acid and restained with May Grünwald—Giemsa stain *(b')*. After removal of heparin and ribonucleic acids, a more intensely stained material appears, consisting of desoxyribonucleic acids and accumulating in the areas with the highest concentration of heparin (× 2000). *c* and *c'* : Same cell afterfixation in absolute alcohol, stained with toluidine blue *(c)*, discoloured with hydrochloric alcohol, subjected to hyaluronidase extraction and restained with toluidine blue *(c')*. The action of hyaluronidase results in the disappearance of the intergranular metachromatic substance (× 3000)

Histaminic mast cell genesis

Working method. These investigations were based on the previous observation that an increased amount of metachromatic substances appeared in pulmonary macrophages under the influence of certain pneumokoniotic factors which determine a simultaneous intrapulmonary increase of histamine [13]. Four hundred guinea-pigs were used in the following experiments.

1. Inhalations of tobacco smoke by the application on the animals' muzzle of a rubber tube provided with a lighted cigarette which was kept burning by the very act of respiration. After the daily smoking of 1/2 cigarette by each animal, the biological determinations of histamine by its action on the terminal intestine of the guinea-pig, performed by C. RACOVEANU, yielded the following figures :

— in normal animals : 30 μg per 1 g of fresh lung tissue ;
— after 30 inhalations : 48 . 40 μg per 1 g of fresh lung tissue ;
— after 60 inhalations : 66 . 40 μg per 1 g of fresh lung tissue ;
— after 90 inhalations : 104 . 48 μg per 1 g of fresh lung tissue ;

These figures express mean values obtained in groups of 5—10 guinea-pigs.

2. Inhalations of pure silicium dioxide in a concentration of 15 mg per 1 ml in 24 hours with a prevalent size of particles of 1—3 μ. According to USPENSKI (16) as well as to our own investigations, such inhalations are followed by an increase in the pulmonary histamine content, resembling that obtained under the influence of tobacco smoke.

3. Intratracheal instillations of exogenous histamine (Histamine hydrochloride "CIF" Bucharest). Ten μg of the drug were applied three times at 4 days' intervals. As has been pointed out in other studies by USPENSKI [17] as well as by our own biological determinations [10], this method causes a considerable and progressive increase of pulmonary histamine content, similar to the one obtained with inhalations.

4. Electrical stimulation of the cervical vagus nerve for 3 minutes with an induction current of 52/sec. frequency and 5 volts in the primary circuit of the bobbin. This technique, which has been devised by one of us [20], causes an intrapulmonary accumulation of endogeneous histaminic substances, attaining values of 70—90 μg during the first 5 days after stimulation of the vagus nerve.

5. Parallel investigations were carried out in groups of animals which received an intraperitoneal injection of 0,001 mg of promethazine, a synthetic antihistaminic drug (Phenergan "SPECIA" Paris), 30 minutes before the inhalation of tobacco smoke and before the instillation of histamine. Biological determinations revealed that these injections maintain the amount of pulmonary histamine at subnormal levels (between 13.80 and 19.20 μg per g of fresh lung tissue) [11].

Evolutional features. After counting 500 elements in the pneumograms of normal guinea-pigs, we found 0,5 to 2 per cent of the macrophages to contain metachromatic particles. After the first 3 days of the experiment, their number increased, attaining

— 18 per cent after inhalations of tobacco smoke
— 10 per cent after inhalations of silicium dioxide
— 7 per cent after instillations of histamine
— 8 per cent after stimulation of the vagus nerve.

In animals treated with injections of the antihistaminic drug, the percentage of macrophages containing metachromatic material was within normal limits.

After the first 5 days of the experiment, the increase continued, attaining

— 19—20 per cent after inhalations of tobacco smoke
— 14 per cent after inhalations of silicium dioxide
— 12 per cent after instillations of histamine
— 9 per cent after stimulation of the vagus nerve.

In animals receiving injections of antihistaminic drugs the percentage of macrophages containing metachromatic material was still kept within normal limits.

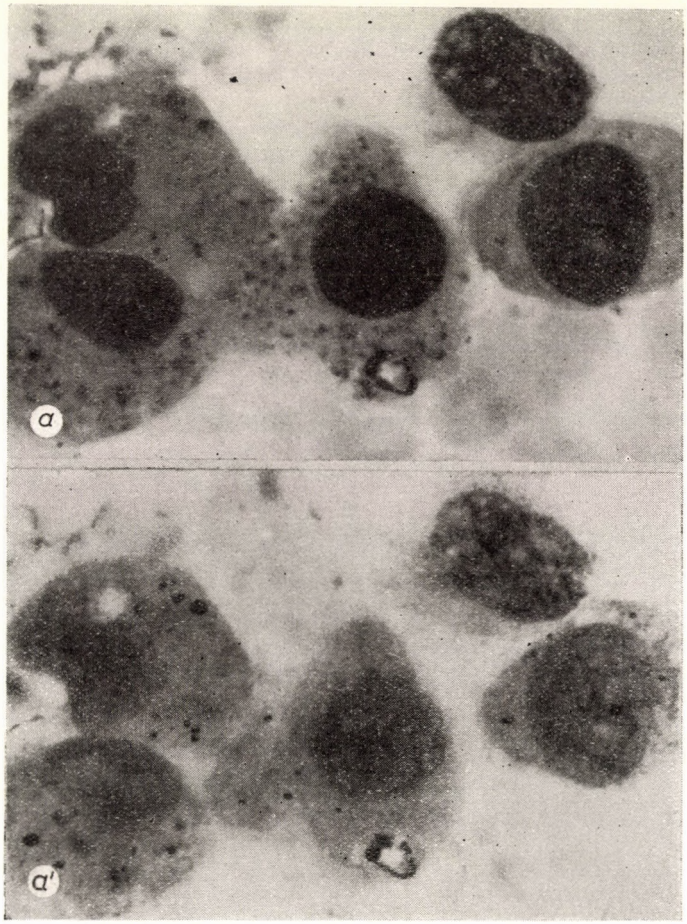

Fig. 4. Histaminic mast cell genesis. (Lung prints from guinea-pigs subjected to instillations of histamine.) *a* and *a'*: A group of macrophages 5 days after intratracheal instillation of histamine. Toluidine blue staining reveals metachromatic nuclei, while in the protoplasm the appearance is noticed of a variable number of granules of different metachromatic intensities *(a)*. After discolouring in hydrochloric alcohol and restaining of the same cells with haemalum-eosin, the metachromatic material no longer appears. Only mineral particles are visible *(a')* (× 2000)

While the experiment is continued, the progressive increase in the number of cells containing metachromatic material is gradually replaced by a tendency towards a progressive intracellular accumulation of these substances.

As regards the peculiarities of the microscopic appearance of the intrapulmonary accumulations of metachromatic material, they may be summed up in the following points.

1. The appearance of groups of macrophages in which, along with the mineral cell inclusions, toluidine blue staining begins to reveal the first polysaccharide granules displaying a metachromatic reaction, varying in intensity between alpha and beta, according to the classification of PEARSE [14]. Hydrochloric alcohol discolouring of the prints and restaining with haemalum-eosin allows of a comparative determination to be made, in the same groups of cells, of the amount of intracellular space occupied by polysaccharides (Figs. 4*a* and *a'*).

2. Some of the macrophages initially exhibit more obvious accumulations of metachromatic substance within their nuclei. The material appears in the shape of compact blocks disposed on the chromatin network. It is not pyroninophile, nor is it more intensely stained than the remainder of the nucleus with the Feulgen-method (Fig. 5*a*). It generally shows a higher metachromatic intensity than the granules in the protoplasm. For this matter, in some of the macrophages, the concentration of polysaccharides seems to take place at first only inside the nucleus. In other cells, the intensity of nuclear metachromasia increases from beta towards gamma. Consequently, the intranuclear polysaccharide accumulations are far better outlined and show a tendency to become arranged around the nucleoli and nuclear vacuoles (Fig. 5*b*). Some of the macrophages exhibit a considerable increase of polysaccharide particles both in the nucleus and in the protoplasm. The intranuclear ones maintain the characteristic perivacuolar arrangement, whereas the protoplasmic ones accumulate in groups without any apparent agglutination (Fig. 5*c*).

Consequent upon their continuous accumulation, the metachromatic substance undergoes a quantitative increase both in the nucleus and in the protoplasm along with the appearance of coalescent granular patterns and metachromasia of variable intensity (between alpha and gamma) (Fig. 5*d*). The macrophages having stored an even greater amount of polysaccharides, begin to resemble in appearance the tissular mast cell; their protoplasm is full of granules of a smaller (Fig. 5*e*) or larger (Fig. 5*f*) size, similar to the structures developing in the same pulmonary macrophages 3—5 days after the intratracheal instillation of heparin (see Figs. 2*d*, 2*e* and 2*f*). Along with the gradual accumulation of granules, apart from their tendency towards agglutination, metachromatic reactions of a lower intensity may be observed in the intergranular protoplasm as well (Fig. 5*g*). At the final stage of this evolutional process, the intergranular substance and the granules form an intensely metachromatic compact mass wherein no structure can any longer be distinguished and the cells display the typical appearance of tissular mast

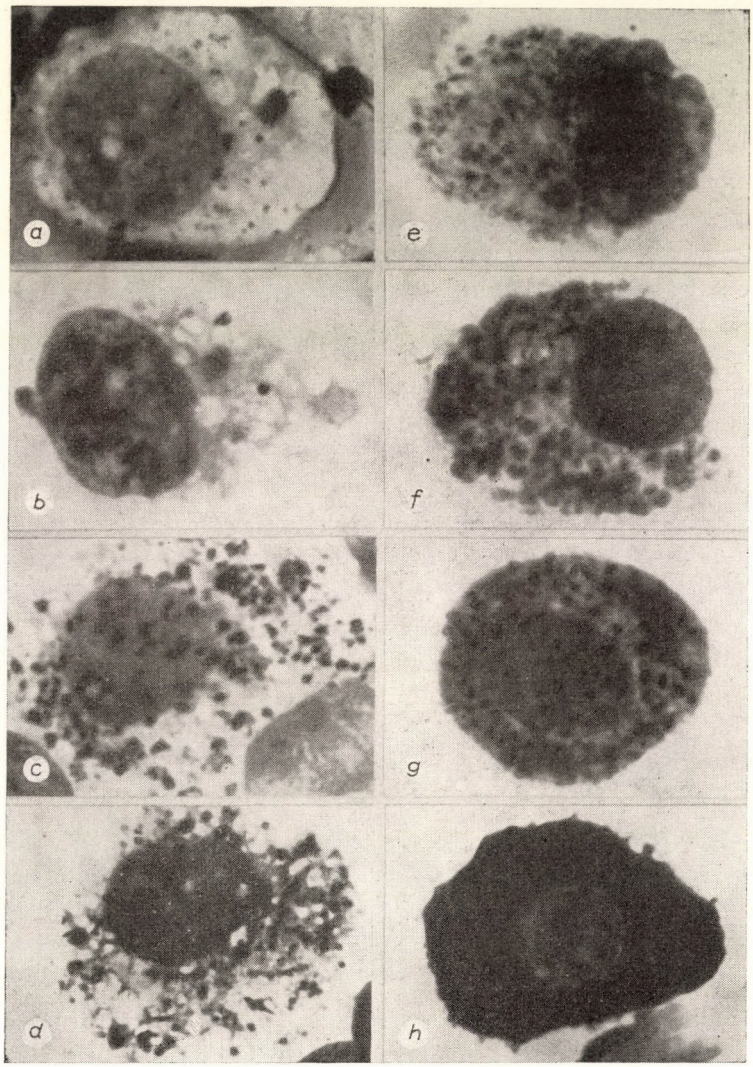

Fig. 5. Histaminic mast cell genesis (Lung prints from guinea-pigs subjected to inhalations of tobacco smoke.) Evolutional aspects of intracellular polysaccharide accumulation. *a)* Appearance of intranuclear metachromatic masses and of slightly metachromatic granules in protoplasm (toluidine blue staining, × 2200). *b)* Increased amount and intensity of the metachromasia of intranuclear polysaccharide masses (toluidine blue staining, × 2200). *c)* Increased number of metachromatic granules in the nuclei and protoplasm. The nuclear granules are located around vacuoles (toluidine blue staining, × 2200). *d)* Granular coalescence in the nucleus and protoplasm, with intense metachromasia (toluidine blue staining, × 2200). *e)* Massive accumulation of small-sized granules (toluidine blue staining, × 2200). *f)* Massive accumulation of large-sized granules (toluidine blue staining, × 2200). *g)* Intergranular metachromatic reaction of the protoplasm (toluidine blue staining, × 2200). *h)* Typical appearance of a tissular mast cell (toluidine blue staining, × 2200)

cells (Fig. 5*h*). We observed a similar appearance after intratracheal instillations of heparin (see Figs. 1*b* and 2*h*).

3. This evolutional process takes place during the first 2 months of the experiment and results in the conversion of some of the pulmonary macrophages into cells of the appearance of a tissular mast cell, owing to a progressive intracellular accumulation of polysaccharides. It may be assumed that not all the cells which eventually develop metachromatic substances complete the entire evolution and attain the appearance of a mast cell and that many of them do not go beyond certain transitional stages such as those featured in Figs. 4 and 5. In the group of animals treated with antihistaminic drug, we did not observe the occurrence of this process of mast cell genesis attaining the appearance of tissular mast cells, but only a few macrophages loaded with metachromatic granules resembling the cells featured in Fig. 4*a*.

Topographically, the cells containing metachromatic material are septal macrophages or mobilized macrophages in the alveolar lumen. Their distribution within the respiratory parenchyma is not uniform. Besides macrophages identified on the basis of the inclusions which they contain, we observed the presence of metachromatic particles in reticular cells, histiocytes and monocytes.

4. Consequent upon the intraprotoplasmatic accumulation of metachromatic material, the appearance is noticed of pericellular haloes due to the passage of these substances from the cells into the connective interstice of the lung. The passage of polysaccharides from macrophages into the interstice is particularly obvious towards the end of the second month of the experiment, when it gives rise to an accumulation of polysaccharides within the alveolar walls. These accumulations are supposed to play a definite part in the subsequent development of the process of sclerosis [12].

Morphofunctional peculiarities. Among these peculiarities we will mention the following.

1. Histochemically, the metachromatic substances that accumulate within pulmonary macrophages are intensely PAS positive upon Hotchkiss—McManus staining (Figs. 6*a* and *a'*). Preliminary acetylation of the sections prevents this reaction, and treatment with dilute potassium hydroxide determines its reappearance. Best's carmine staining, as well as the Millon test were negative. The metachromatic granules are not more evident than the remainder of the protoplasm or of the nucleus when stained by methyl green-pyronine or by the Feulgen-technique.

2. Testicular hyaluronidase extraction (Luronase "BAYER") yielded no obvious results except in the second month of the experiment and only in the cells having the appearance of a tissular mast cell, *i. e.* those in which considerable polysaccharide accumulation had taken place. In some of these cells, the pericellular metachromatic halo disappears after extraction, whereas

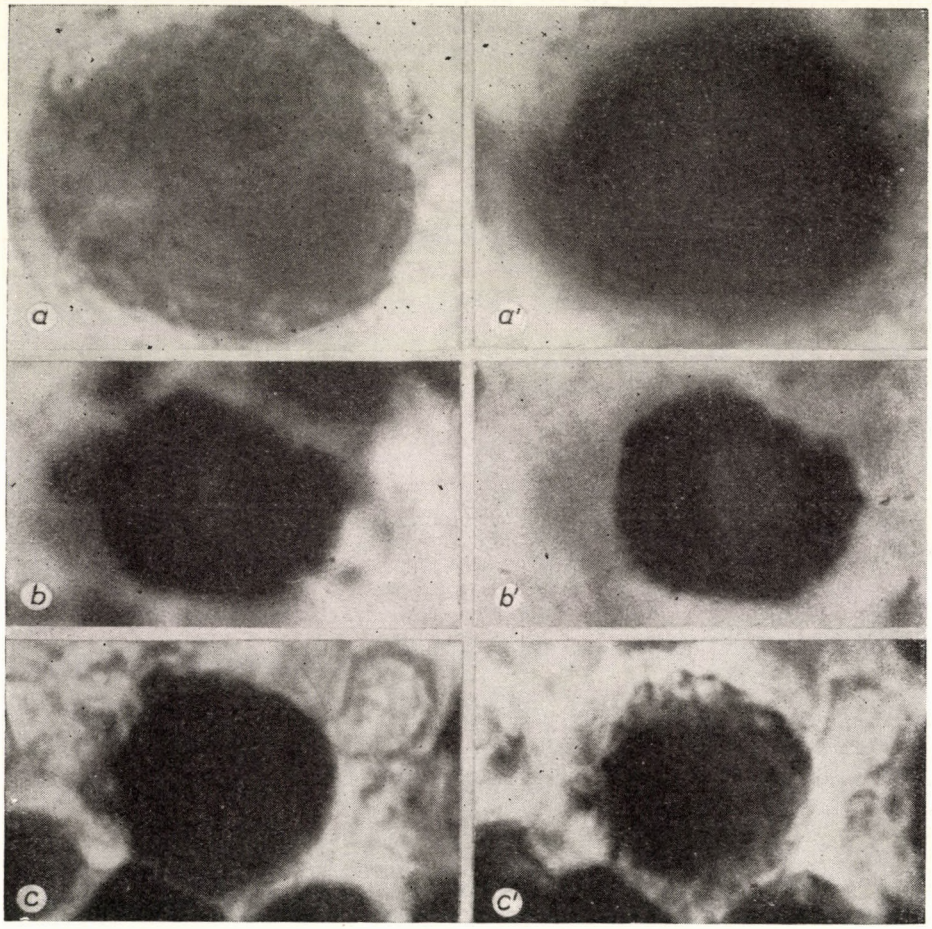

Fig. 6. Histaminic mast cell genesis (Lung prints from guinea-pigs subjected to inhalations of tobacco smoke.) *(a* and *a'):* Upon toluidine blue staining, the nucleus appears to be diffusely and intensely metachromatic, while the protoplasm displays a less marked metachromasia *(a)*. After discolouring in hydrochloric alcohol and restaining according to the Hotchkiss—McManus technique, the same cell is shown to be intensely PAS positive in its entirety *(a')* (× 3000). *(b* and *b') :* A polysaccharide-loaded cell, displaying upon toluidine blue staining a less metachromatic diffuse pericellular halo *(b)*. After discolouring in hydrochloric alcohol, hyaluronidase extraction and restaining with toluidine blue, the pericellular halo disappears, while the protoplasm and the nucleus maintain the same intensity of the metachromatic reaction *(b')* (× 2000). *(c* and *c'):* A polysaccharide-loaded cell, displaying, after fixation in absolute alcohol, an intense and diffuse metachromatic reaction upon toluidine blue staining *(c)*. The same cell, after discolouring in hydrochloric alcohol, hyaluronidase extraction and toluidine blue staining, displays partial disappearance of protoplasmic polysaccharides, particularly of intergranular ones, while the nucleus recovers its intense metachromasia *(c')* (× 2000)

the protoplasm and the nucleus remain unchanged (Figs. 6*a* and *b'*). In other cells the enzymatic action is followed by the partial disappearance of the metachromatic contents of the protoplasm and particularly of the intergranular substance (Figs. 6*c* and *c'*). These results are similar to those obtained after hyaluronidase extraction in animals sacrified 5 days after the instillation of heparin, in which the intergranular metachromatic substance in the protoplasm was also noticed to disappear (see Figs. 3*c* and *c'*).

3. After removal of the metachromatic material and of ribonucleic acid by means of acid hydrolysis in normal hydrochloric acid at 60° C, the Feulgen test sometimes results in a reddish-violet staining which is more intense than in the remainder of the nucleus, located in the perinuclear areas and in the proximity of the nuclear membrane, *i. e.* the former sites of polysaccharide accumulation. These patterns resemble those obtained after the intratracheal instillation of heparin, when we found an increased amount of desoxyribonucleic acid in the areas in which heparin attained the highest concentration (see Figs. 3*b* and *b'*). Another consequence of hydrolysis is the disappearance, upon May Grünwald—Giemsa restaining, of the azurophile material concentrated in the same regions of the protoplasm as the polysaccharide particles.

Anthracosic mast cell genesis

Working method. The investigations were carried out in the lungs and tracheobronchial lymph nodes of 50 subjects, displaying a certain degree of anthracosis. Specimens exhibiting tuberculous lesions or other considerable changes determined by prolonged toxic or infectious onditions were discarded.

Evolutional features. Intermediate stages between the macrophage and the tissular mast cell resembling those obtained in mast cell genesis determined by heparin and histamine, are mainly encountered in the tracheobronchial lymph nodes. A single microscopic field may contain 2—3 cells displaying either similar or very different degrees of polysaccharide accumulation, ranging from a few granules to a maximum loading (Fig. 7*a* and *b*).

A follow-up of this evolutional process in prints and photographs of the same cells successively stained with toluidine blue and haemalum-eosin revealed the following facts.

1. In some of the macrophages loaded with small coal particles, the appearance of small amounts of metachromatic material within the nucleus and the protoplasm is noticed. They are mainly deposited on the mineral inclusions, but they also appear as isolated granules (Figs. 8*a* and *a'*). As they are accumulating in larger amounts, they partly dissimulate the mineral

inclusions and gradually come to prevail within the cell (Fig. 8*b* and *b'*). At a later stage, they undergo fusion resulting in larger corpuscles (Figs. 8*c* and *c'*) or multiplication as isolated granules which fill up the whole cytoplasm, thereby causing the macrophage to assume the appearance of a tissular mast cell

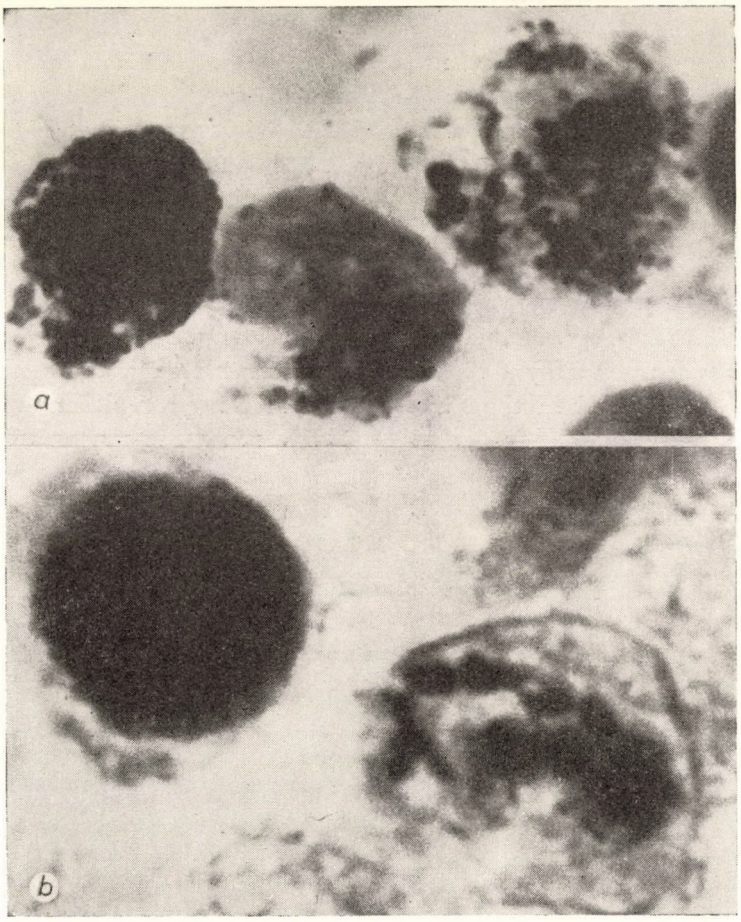

Fig. 7. Anthracosic mast cell genesis (sections of human tracheobronchial lymph nodes). *a)* Varied aspects of polysaccharide loading in 3 cells (toluidine blue staining, × 2000). *b)* A cell displaying diffuse polysaccharide loading next to a cell which displays but a few granulations (toluidine blue staining, × 3000)

(Figs. 6*d* and *d'*) similar to that obtained 3—5 days after the instillation of heparin (see Figs. 2*c*, *d*, *e* and *f*) and at the end of the first month's gradual increase of histamine in the lung (see Figs. 5*e*, *f* and *g*). The above-mentioned patterns are noticed both in the lung and in the tracheobronchial lymph nodes.

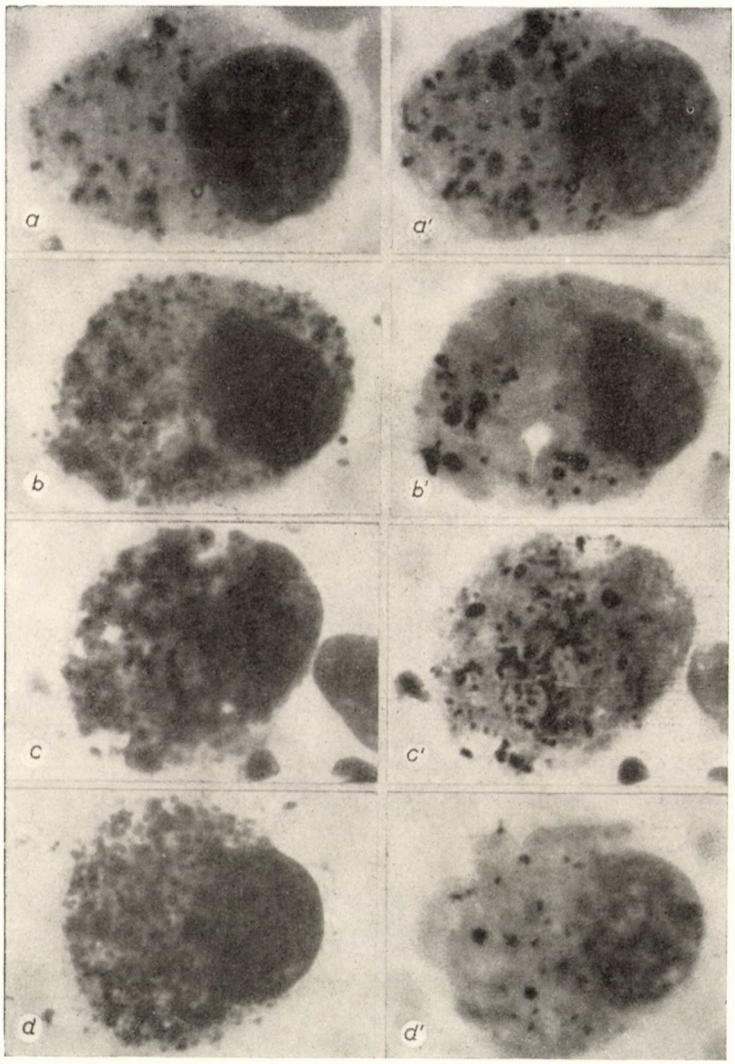

Fig. 8. Anthracosic mast cell genesis (prints of human lung). Evolutional aspects of intracellular polysaccharide accumulation. *(a* and *a') :* Upon toluidine blue staining, the mineral inclusions are less clearly visible, as they display a slightly metachromatic halo *(a)*. After discolouring in hydrochloric alcohol and restaining by haemalum-eosin, the same macrophages exhibit sharply outlined patterns of cell inclusions *(a')* (× 2200). *b* and *b' :* While polysaccharides are gradually accumulated, toluidine blue staining shows them partly covering the mineral inclusions and being deposited in the spaces between these inclusions *(b)*. After discolouring in hydrochloric alcohol and restaining with haemalum-eosin, the same macrophage no longer exhibits granules amongst the coal particles *(b')*. (× 2200). *(c* and *c'):* A tendency towards agglutination of accumulated metachromatic particles causing the cell to assume the appearance of a mast cell upon toluidine blue staining *(c)*. After discolouring in hydrochloric alcohol and restaining with haemalum-eosin, the same macrophage appears to be deprived of metachromatic substance, but it contains a large amount of mineral inclusions *(c')*. (× 2200). *(d* and *d'):* Massive agglomeration of metachromatic material, causing the cell to assume the appearance of a mast cell upon toluidine blue staining *(d)*. Discolouring in hydrochloric alcohol and restaining with haemalum-eosin shows that the major part of the protoplasm was covered by metachromatic material *(d')*. (× 2200)

The human lung affected by anthracosis displays areas wherein 35—82 per cent of the pulmonary macrophages contain mucopolysaccharide substances. Of these cells, $^1/_3$ has the appearance of a tissular mast cell and contains mineral inclusions. In some of the lymph nodes, there are areas wherein photographs of 100 cells displaying the typical appearance of mast cells revealed that 72 of them contained mineral inclusions grouped around the nucleus, scattered throughout the cell, placed at the periphery of the protoplasm. Some of these inclusions were stained dark blue with Prussian blue, the test used for the detection of iron.

Morphofunctional peculiarities. Among the morphofunctional peculiarities of these cells, let us mention the following.

1. In those cells which contain polysaccharide films around the mineral inclusions, enzymatic extraction generally failed to modify these aspects.

2. In some of the mast cells, originating from the cells which contained no mineral inclusions, after May Grünwald—Giemsa staining of the prints, their discolouring with hydrochloric alcohol and restaining with toluidine blue, the metachromatic material was swept off at discoloration, whereas the granules which were present on the nuclear surface, reappeared with intense metachromasia (Figs. 9*a* and *a'*). This might prove that human mast cells also contain intranuclear metachromatic substances and the above-mentioned figure features patterns which might be interpreted as stages in the transition of these granules from the nucleus towards the protoplasm.

3. Extraction by means of testicular hyaluronidase is followed in some cells by the partial disappearance of the protoplasmic metachromatic material, whereas the intranuclear and perinuclear polysaccharides do not seem to have undergone any change as they restain with the same intensity with toluidine blue (Figs. 9*b* and *b'*). In other cells, hyaluronidase extracts the whole protoplasmic content, leaving only the intranuclear polysaccharides unchanged, while in others, the metachromatic content of the whole cell is apt to disappear (Figs. 9*c* and *c'*). These patterns are another argument in favour of the intranuclear presence of metachromatic substances in human mast cells. They are comparable to some of the results obtained in processes of experimental cell genesis. In a special study carried out on this subject [18] by the examination of 200 mast cells in human tracheobronchial lymph nodes, photographed successively before and after hyaluronidase extraction, we found that about 10 per cent are totally and 15 per cent partly deprived of their metachromasia under the influence of hyaluronidase. When hyaluronidase extraction is preceded by pepsin digestion, the proportion of elements which are totally deprived of their metachromasia is increased to 20 per cent, while partial depletion occurs in 50 per cent. In so far as such changes did not occur after inactivated hyaluronidase treatment, we considered them to be a direct consequence of the enzymatic action.

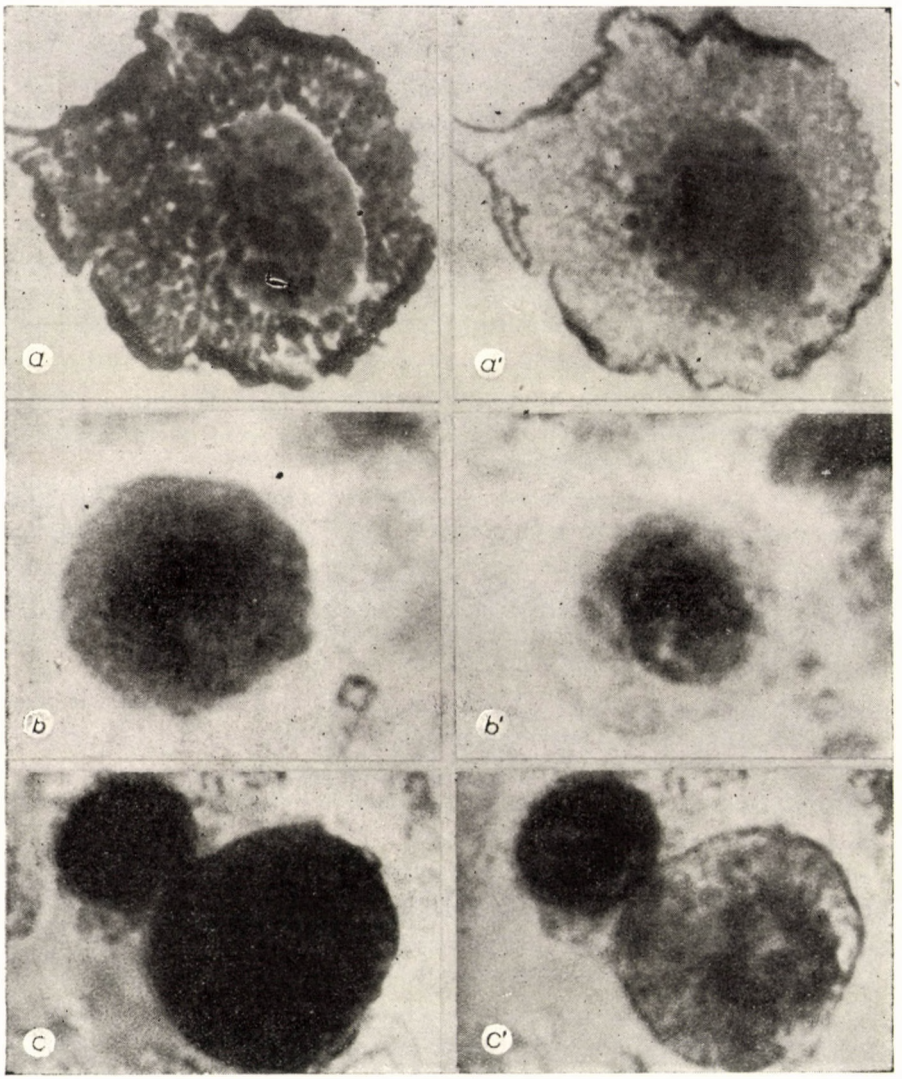

Fig. 9. Anthracosic mast cell genesis (prints and sections of human tracheobronchial lymph node). *(a* and *a') :* A cell which contains no mineral inclusions, displaying the appearance of a mast cell, in a print according to May Grünwald—Giemsa stained *(a)*. Discolouring in hydrochloric alcohol and restaining with toluidine blue is followed by the disappearance of the whole amount of metachromatic material from the protoplasm, while the polysaccharide granules in the nuclei again become metachromatic *(a')* (× 2200). *b* and *b' :* A cell which contains no mineral inclusions, displaying the appearance of a mast cell, in a section subjected to formalin fixation and toluidine blue staining *(b)*. Discolouring in hydrochloric alcohol, hyaluronidase extraction and restaining with toluidine blue determine the disappearance of the metachromatic material from the protoplasm, except in the perinuclear area. The polysaccharides within the nucleus recover intense metachromasia *(b')* (× 2000). *(c* and *c'):* A cell which contains no mineral inclusions, displaying the appearance of a mast cell in a section subjected to formalin fixation and toluidine blue staining *(c)*. Discolouring in hydrochloric alcohol, hyaluronidase extraction and restaining with toluidine blue determine the disappearance of the metachromatic material both from the nucleus and from the protoplasm *(c')* (× 2000)

Discussion

Our observations on experimental and necropsy specimens show that the intratracheal instillation of heparin, as well as the progressive increase of polysaccharide accumulation within the pulmonary reticulohistiocytic cells, consequent upon an increase of intrapulmonary histamine and the irritative action of certain phagocyted particles, results in the appearance of many similar microscopic patterns, indicating the transformation of certain cells of the respiratory parenchyma and tracheobronchial lymph nodes into cells belonging to the type of tissular mast cells. In necropsy specimens the progress of these transformations had a course to be reconstructed. However, this reconstruction was based on a comparison with the evolutional features induced by the intracellular penetration of heparin and by the gradual numerical increase under the influence of histamine of the elements which store increasing amounts of polysaccharides. We therefore consider that certain analogies may be established between these three aspects. Consequently, a more general concept of the genesis of cells displaying the appearance of a tissular mast cell may be justified.

We must begin by stating that it is as yet impossible to ascertain whether the metachromatic substances, whose gradual accumulation is quite apparent, are the result of a cell secretion or of the uptake of endogeneous polysaccharides from interstitial fluids. We insisted upon the tendency towards intranuclear accumulation of heparin, upon the appearance of metachromatic material within the nuclei of pulmonary macrophages under the influence of histamine, as well as upon the presence of metachromatic substances in the nuclei of human mast cells. However, none of these observations have enabled us to prove that the substances in question result from nuclear secretion. In the available literature, LEUCHTENBERGER and SCHRADER [8] are the only authors who observed that polysaccharides are synthetised in the salivary glands from substances resulting from the metabolism of desoxyribonucleic acid. Could there be established a similarity with the changes observed after intratracheal instillations of heparin, particularly in animals that received the least concentrated solution, we should rather maintain that intranuclear accumulation of endogenous polysaccharides is a result of the progressive concentration at this level of substances which invade the protoplasm in amounts that cannot be demonstrated by means of the present histochemical methods.

Whereas in the matter of the origin of the intranuclear metachromatic material, these assumptions remain to be verified in the future, the participation of the nucleus in the metabolism of this material has been clearly demonstrated by the investigations presented in this study. Exogenous heparin is gradually rendered insoluble in water, then partly transformed into ortho-

chromatic material and partly into desulphated polysaccharides, presumably of the type of hyaluronic and chondroitin sulphuric acids, which are extracted by testicular hyaluronidase. These experimental results verify ASBOE—HANSEN's hypothesis [1], according to which heparin might be considered as a precursor of hyaluronic acid. The intranuclear metabolism of exogenous heparin is associated with quantitative and topographic changes in nucleic acids. In the process of mast cell genesis, determined by histamine, hyaluronidase extractions likewise demonstrate the existence of intranuclear polysaccharides resisting the action of the enzyme, whereas the polysaccharides contained in the protoplasm disappear after hyaluronidase extraction. In these cases it might likewise be maintained that some of the cells displaying the appearance of a tissular mast cell contain polysaccharides of the heparin type in their nucleus and of the hyaluronic and chondroitin sulphuric acid type in their protoplasm. Similar observations were made in human specimens which show this process to be of more general nature. We encountered many patterns wherein the nuclear concentration of metachromatic substances was far more evident than the protoplasmic one and which displayed patterns that might be interpreted as a passage of granules from the nucleus towards the protoplasm.

All these observations raise new problems related to the morphofunctional structure of mast cells. The metachromatic material is probably first accumulated and metabolized within the nucleus, being subsequently eliminated into the protoplasm. This point of view has to be substantiated by further investigations, the more so as it is opposed to current hypothesis maintaining that phagocytosis and engulfing of polysaccharides from the extracellular spaces occurs exclusively at the level of the cytoplasm. We presented the process of mast cell genesis under the influence of histamine and anthracotic particles, owing to the obvious appearance of transitional forms from pulmonary macrophages towards the tissular mast cell. This process may also be encountered in non-anthracotic lungs, which are affected by certain inflammatory processes, as well as in other organs such as the skin, myocardium, spleen, uterus and particularly the lymph nodes. The determining factors seem therefore to be very numerous and, in a more general level, this process might be looked upon as one of the adaptation reactions of reticulo-histiocytic cells to the irritative action of certain harmful factors. According to their physicochemical and biological properties, to the reactivity of the body in general and to the reactivity of cells of the macrophage type in particular, these factors determine the appearance of what we suggest should be called "states of polysaccharide loading".

This is a metabolic process rather than an actual cell genesis and it might be analogically compared to the lipoid loading of the same cells. Not all of the cells which develop fat particles evolue to the adipocytic stage. According

to the degree of their loading and to their enzymatic equipment, the remainder stop at various reversible intermediate stages.

At present there is no longer any doubt as regards the origin of basophile granulocytes from the myeloid series, whence they go through a series of well-known stages consisting of cells with definite morphofunctional peculiarities. The cells displaying the appearance of tissular mast cells seem to result from an intracellular accumulation of polysaccharides which is liable to take place in various mesenchymal cells. This is no process of genesis in the usual meaning of the word, but rather a particular metabolic activity. Thus considered, the differences between the basophile granulocyte and the cell displaying the appearance of a tissular mast cell, become even more evident as only the former is an autonomous cell. From the same point of view, certain notions related to the pathology of mast cells should perhaps also be taken into account. Certain benign mastocytomes might be considered as equivalent, as regards carbohydrate metabolism, to lipomata in lipoid metabolism, while certain forms of mastocytosis might be interpreted as accumulative reticuloses, namely polysaccharide thesaurismoses, rather than as leukaemic conditions.

Summary

The conversion of mesenchymal cells into cells displaying the appearance of tissular mast cells has been investigated by studying mast cell genesis caused by heparin, histamine and anthracosis, respectively. It has been found that the cells displaying the appearance of mast cells result from an excessive accumulation of polysaccharides under the influence of irritating factors in various reticulohistiocytic cells and particularly in those which are to become macrophages. The suggestion is made that the reversible stages of this accumulation be called "states of polysaccharide loading".

The nucleus of macrophages takes an active part in the process of intracellular accumulation and transformation of metachromatic substances, displaying varied nuclear and protoplasmic metabolic patterns. In discussing the results of this study, the cells displaying the appearance of tissular mast cells are not considered autonomous. They are supposed to represent the morphological expression of special activities in the carbohydrate metabolism of various mesenchymal cells.

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ГЕНЕЗ ТКАНЕВЫХ ТУЧНЫХ КЛЕТОК

Ц. ВЕЛИКАН и Д. ВЕЛИКАН

Авторы исследовали преобразование клеток мезенхимы в тканевые тучные клетки и изучали вызванный гепарином или же гистамином и антракозом генез тучных клеток. Они установили, что те клетки, из которых можно отводить генез тучных клеток, возникают путем повышенного накопления полисахаридов на действие возбуждающих факторов из различных ретикулогистоцитов, особенно таких, из которых образуются макрофаги. Авторы предлагают называть обратимое состояние этой аккумуляции «состоянием полисахаридной насыщенности».

Ядра макрофагов принимают активное участие в аккумуляции и преобразовании метахроматических веществ, на основе различного метаболизма ядра и плазы. При обсуждении полученных результатов авторы не рассматривают появления тканевых тучных клеток, как автономное появление, а предполагают, что тучные клетки представляют из себя морфологическое выражение специальной активности, проявляющейся в углеводном обмене веществ различных клеток мезенхимы.

GENESE DER GEWEBSMASTZELLEN

C. VELICAN und D. VELICAN

Es wurde die Umwandlung der Mesenchymzellen zu Gewebsmastzellen, ferner die durch Heparin, bzw. durch Histamin und Anthrakose verursachte Genese der Mastzellen untersucht und festgestellt, dass die Zellen, denen die Mastzellen entstammen, im Wege einer Akkumulation von Polysacchariden auf Wirkung von Reizfaktoren aus verschiedenen Retikulo-Histocyten entstehen, besonders aus Zellen aus welchen die Makrophagen hervorgehen. Verfasser schlagen vor, dass der reversible Zustand dieser Akkumulation "Zustand der Polysaccharidensättigung" genannt werde.

Die Kerne der Makrophagen beteiligen sich aktiv an der Akkumulation und Transformation von intrazellulären metachromatischen Stoffen, und zwar auf Grund der metabolischen Verschiedenheit von Kern und Plasma. Das Erscheinen der Gewebsmastzellen wird nicht als ein autonomer Prozess aufgefasst. Es wird angenommen, dass die in dem Kohlenhydratstoffwechsel der verschiedenen mesenchymalen Zellen erscheinende spezielle Aktivität ihren morphologischen Ausdruck in den Mastzellen findet.

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