Immunobiological cytobiology

To characterize cellular mechanisms in immunological processes, three fundamental points of view must be pointed out first:

1. Like any synthesis, the formation of specific antibodies (immunoglobulins) is a purely cellular process.
2. Immunizing cells i.e. those able to synthetise antibodies are unpolar cells, which have kept the pluripotency of mesenchymal cells in morphological and functional respect. Only this pluripotency makes possible to «work» «unknown» metabolic problems as constituted by antigens. Antigens are substances for the metabolization of which the organism lacks the enzymatic outfit.
3. The morphological condition of a cell depends on its function. According to the function, therefore, cells of equal origin form various morphological and functional = biochemical variants with flowing changes. The rigid

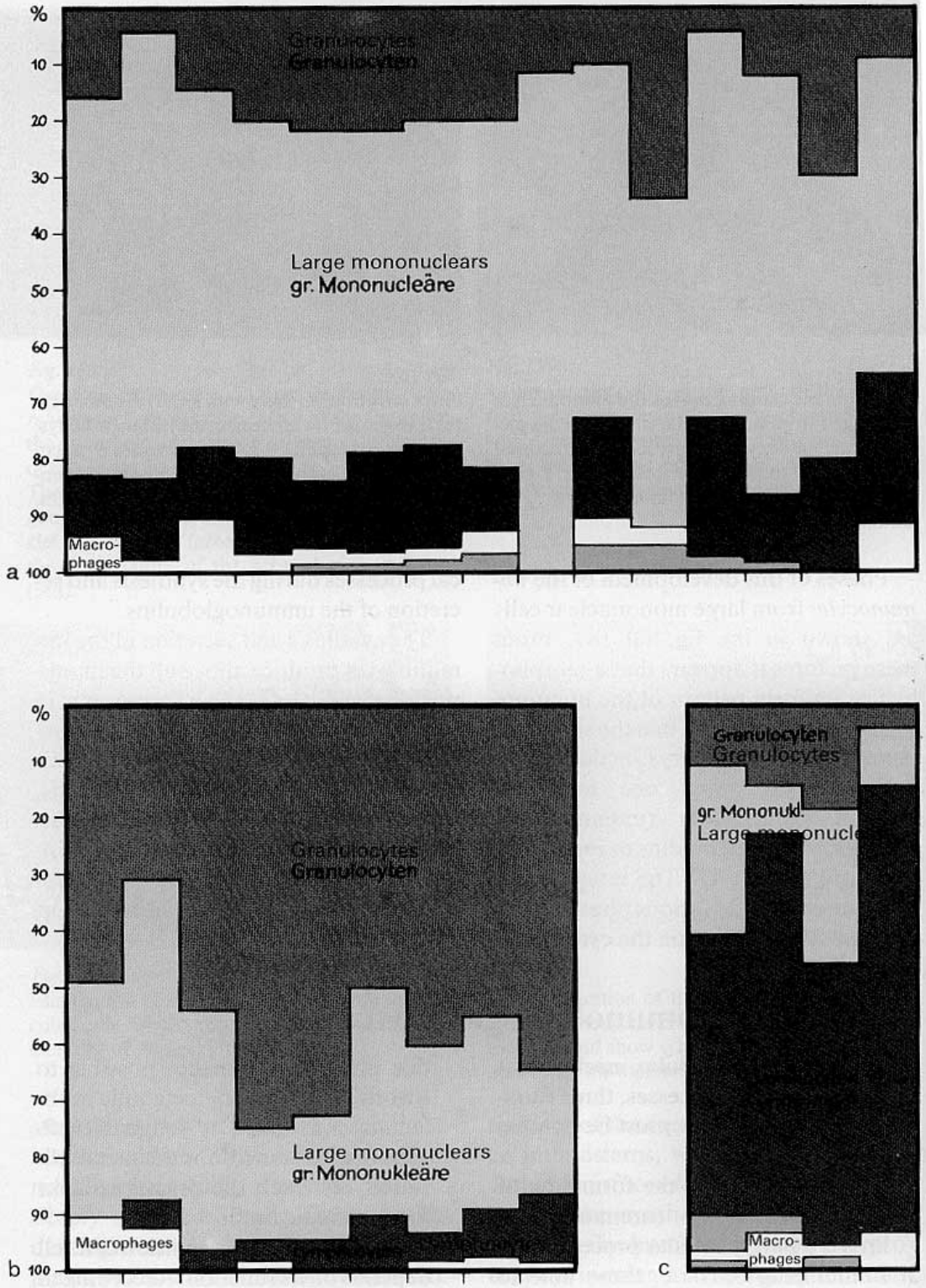


Fig. 164:
Differences of the cell-differential picture of the peritoneal exudate of guinea-pigs (a) after BCG sensitisation, (b) in non-sensitised controls and (c) in animals infected with virulent Mycobacterium tuberculosis; predominance of the mononuclears in the immunized animals.

division after indirect methods, as resulting from the conception of B-cells and T-cells, has didactic advantages but inhibits the development of cognition.

The immunologically competent cells form a deeply echeloned system, which covers the entire organism and has closely linked functions.

As an example, the course of the immunological change at the first contact with antigen can be demonstrated with the functional interplay of mesothelial and lymphatic tissues in the abdominal cavity, which constitutes the largest reservoir of immuno-competent cells. The production of cell material by puncture permits longitudinal observations in the living organism. The author (SCHMID F., 1955–1963; HAGGE W.) obtained the following results when provoking non-specific and specific stimulations in guinea-pigs and mice.

By evoking a non-specific stimulation with paraffin-glycerin, the cellular response in the peritoneal exudate will be different. Differentiations of 200–500 cells, 48 hours after injections of paraffin, revealed:

in healthy animals:
50% granulocytes,
35% monocytes,
6% lymphocytes

in tuberculin-positive, BCG-sensitised animals:
18% granulocytes,
62% monocytes,
17% lymphocytes,
2% fibroplasts

in guinea-pigs with generalized tuberculosis (infected with human mycobacteria):
13% granulocytes,
23% monocytes,
62% lymphocytes,
2% fibroblasts.

This means that the cellular response to a non-specific stimulation depends on the biological starting situation of the organism.

Immunocytes

The antibodies are formed by cellular derivatives of the reticulo-histiocytary system, which show distinctive morphological changes parallel to the functional stage.

The term « plasma cell» goes back to WALDEYER (1895), the formal characterization to UNNA (1891). The development has been described in detailed, more modern studies by GOWANS and MCGREGOR, FAGRAEUS, BRAUNSTEINER, BESSIS (1972), the cytochemical findings were treated by F. SCHMID (1963, 1966); KRÜPE discussed the biological importance.

The difficulties with the morphological classification of transitional forms, on the one hand, and the understanding that «other» mononuclear cells e. g. the lymphocytes play a part in the formation and transmission of antibodies, on the other hand, have called in question the monopoly of the plasma cells to form antibodies. These difficulties are essentially due to the fact that defined functions are ascribed to defined forms. The supposition that the form is virtually patterned by the function clarifies the interrelations as the same kind of cells can show different morphological properties in different functional phases.

Controversy has been carried on for decades. MAXIMOW (1928, 1932) as well as DOWNEY and STASNEY (1936) considered the plasma cell as a functional form of a lymphoid cell. Others, as e. g. UNNA

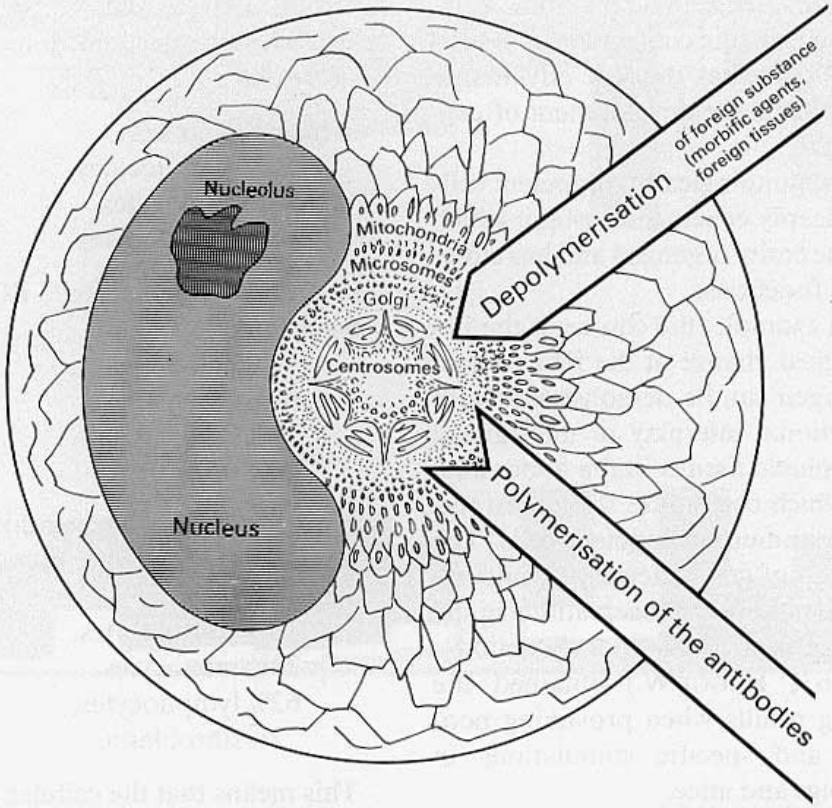


Fig. 165:
Principle of the immunological change of mononuclear cells: the depolymerisation of foreign substances (microbes, proteins) is followed by the polymerisation of new compounds (antibodies).

(1891), DOMENICI (1920), ROHR (1940) and BESSIS and SCEBAT (1946) see direct transitions from reticulum cells and «perithelial» cells to plasma cells.

As the *functional* ability to form antibodies is much more specific for the «plasma cells» than for the form, F. SCHMID suggested in 1963 the name «immunocytes» ; the same term was favoured by DAMESCHEK (1964).

Morphologically, plasma cells are described as medium-sized to large (9–20 microns in diameter) mononuclear cells with the following characteristics:

a) marginal or eccentric, oval to flattened nucleus. The spoke-like form of the nucleus is an electron-optical phenomenon of the dispersion of chromatin;
b) dark to deeply basophile cytoplasm by panchromatic staining;
c) marked endoplasmatic reticulum.

This description relates only to «ripe» plasma cells. Functionally, the immunocytes (plasma cells) are characterised by the biological capability of

d) reacting to stimulations from antigens;
e) proliferating through these stimulations;
f) synthetising antibodies;
g) keeping a memory of previous contacts with antigens.

Extensive studies conducted from 1950–1966 on peritoneal exudate cells of the guinea-pig after sensitization with various antigens – BCG bacteria, human gamma-globulin, human Mycobycteri-

um tuberculosis, coli and proteus – gave the following summary. The course depends on the form and dose of antigens, the principle remains the same. The tests in the peritoneal space allows a dynamic observation by current differentiations of cells (cells obtained by puncture) instead of the mostly static findings in sections of thymus, spleen or lymph-nodes. The following phases respond regularly to stimulations by antigens:

Activation of microphages

Every stimulation by antigens is followed first by a multiplication of the polynuclear leukocytes. They perform the initial phagocytosis but later fall victims of phagocytosis by macrophages (within 24 to 48 hours) when their membranes have been loaded with antigenic substances (fig. 119–124).

The stage of mobilization

of the mononuclears is initiated few hours after the antigenic stimulation. The cells eliminated from the reticulohistiocytary and mesothelial tissues round off into monocytoid forms (fig. 159) with loosely structured nucleus and neutral cytoplasm. Mitotic and amitotic divisions increase the cellular potential (fig. 166–169).

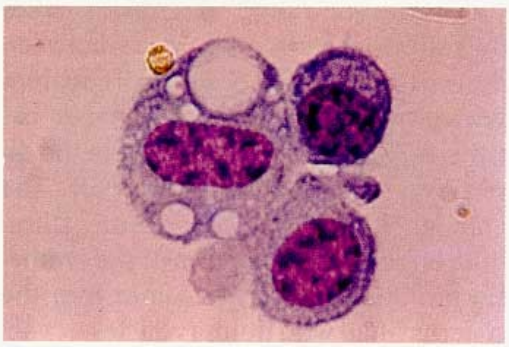


Fig. 166:
Mononuclear cells (monocytes, lymphoid cells) from the peritoneal exudate of guinea-pigs; 116th day after immunization with human gamma-globulin.

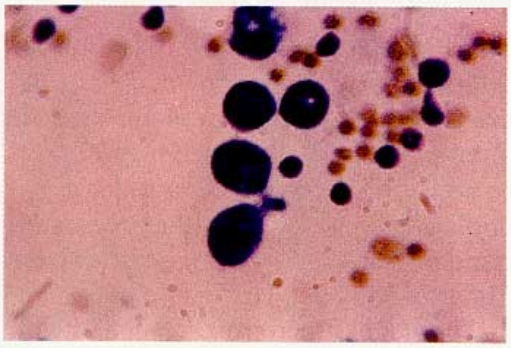


Fig. 167:
Mitosis of plasma cells in the peritoneal exudate after BCG-immunization. The cellular potential is increased by mitosis in the free exudate.

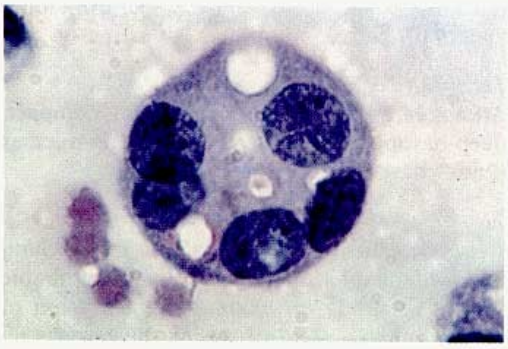


Fig. 168:
Amitotic processes cause formation of giant cells (peritoneal exudate of guinea-pigs after BCG-sensitisation).

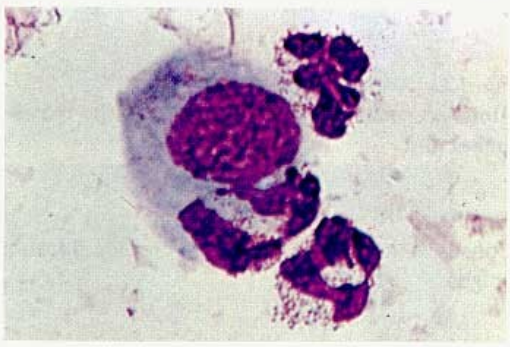


Fig. 169:
Direct elimination of polynuclear granulocytes from mesothelial cells of the peritoneal exudate increases on acute stimuli the potential of microphages.

Stage of transformation

The transformation of the mononuclear cells begins on the second or third day after the stimulation by antigens. The volume of the cytoplasm of the mononuclears grows. The cytoplasm becomes basophilous from the middle of the cell, the structure becomes more compact.

Electron-optically, these processes are characterized by the formation of dense structures in the middle of the cells (fig. 188) with abundant numbers of mitochondria, extended Golgifield and a growing endoplasmatic reticulum (fig. 186, 187).

The stage of maturity

is dominated by deeply basophilous cytoplasm (fig. 173), which covers the marginal, comparatively small, nucleus often to such an extent that it can barely be seen. Electron-optically, a dense endoplasmatic reticulum interlaces substantial areas of the cytoplasmatic space (fig. 186–189).

The stage of maturity is reached with the first contact with antigens, between the 7th and 10th days after the supply of antigens, according to the latter's quantity and quality

The stage of secretion

sets in already on the summit of the stage of maturity and lasts for 2–3 weeks (from the 8th to the 25th day). Light-optically, the homogenous-basophilous cytoplasm is interrupted by small «vacuoles» or vesicles filled with fluid; these proliferate and grow as the secretion goes on till at the end of the phase of secretion a large cell results, dominated by a system of vacuoles (fig. 29, 128, 176, 177, 178, 179, 181, 189).

Electron-optically, this process takes the following course: the spaces between the tubes of the endoplasmatic reticulum grow wider (fig. 187). The spaces between the lamellae, turned first in the direction of the lamellae, round off. The enlargement, probably pressure filtration, effects the evacuation of the synthesis products of the ribosomes into the cisterns filled with fluids (vesicles = vacuoles).

Fig. 170:
Monocytes of the non-sensitised animal.

Fig. 171:
On the 4th day after *sensitisation:* increased cytoplasmic space (in proportion to the size of nucleus), basophil consolidation in the Golgi-area of the cell.

Fig. 172:
Emission phase of a plasma cell from the mesothelial unit.

Fig. 173:
Mature plasma cell (immunocyte) with compact, deep-basophil cytoplasm, marginal nucleus (2nd-3rd week after sensitisation).

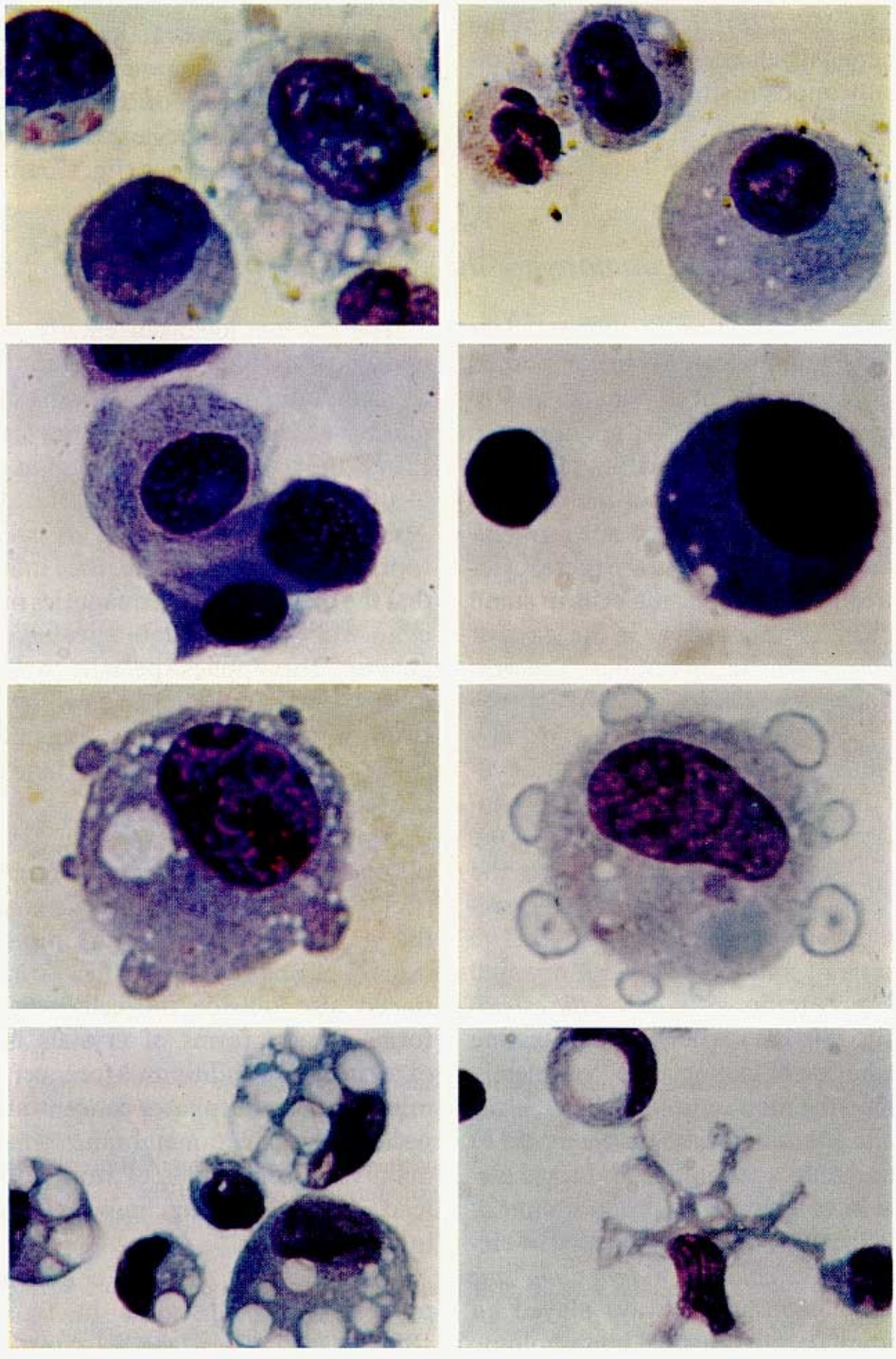
Fig. 174:
Consolidation of *basophil products* in membrane bulges.

Fig. 175:
Evacuated membrane bulges with direct channels into the interior of cytoplasm, the probable way of antibody extrusion. After extrusion of the basophil (= acid) material, the cell has readopted the character of monocytes.

Fig. 176:
Ageing secretory plasma cell (immunocyte) speckled with cisterns («vacuoles»).

Fig. 177:
Old immunocyte with large cisterns (vacuoles) and rigid cytoplasmatic structures, flattened nucleus, 3 months after sensitisation.

Fig. 170–181:
Maturation and ageing of immunocytes (plasma cells); characteristic examples of a longitudinal study of 3 months (peritoneal-exudate cells of guinea-pigs after sensitisation with xenogenous protein; 0.5 ml of human gamma-globulin or bovine serum).



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These intracellular ecological spaces follow a centripetal course and eliminate the synthesis products (including the antibodies) into the humoral system.

The elimination of the acid synthesis products seems to follow geometrical laws (fig. 174, 175); in the interest of the biochemical balance, it takes place at diagonally opposite sites.

The redifferentiation

occurs apparently in contrary direction. After the elimination of the antibodies and metabolites accompanying the synthesis, the cytoplasm decreases, takes a neutral colour, the nucleus gets mellow and less eccentric. Direct transitions to monocytes or reticular cells can be observed. Striking are the groupings of small lymphocytes on the membranes of excreting immunocytes; they seem to have transport functions (fig. 177).

Processes accompanying the synthesis of immune bodies

The transformation of mononuclear cells into immunocytes is accompanied by a concentration of *nucleotides* and *ribonucleic acid* (fig. 178) in the cytoplasm. The degree of polymerization of the RNA rises in the course of the first two weeks after stimulation of antigens. Lipoids and carbohydrates are there, cytochemically, in ripe plasma cells in small concentration but appear in the stage of secretion by higher quantities. The *acid mucopolysaccharides* (fig. 179) concentrate in the cytoplasm from the 3rd day after the sensitization and appear as interior lining in the cistern of the immunocytes, extracellularly, from the 7th to the 12th days; a net-like connection of the mucopolysaccharides to the cell membrane can nearly always be proved. The synthesis of antibodies is accompanied by a concentration of *non-specific esterases* (fig. 180, 181) whereas the alkaline phosphatase of leukocytes is completely absent in the mononuclear cells.

Little attention has so far been paid to the inorganic quantitative and trace elements in connection with the immunological change though particularly elements as e. g. *calcium, magnesium, zinc* and *aluminium* have always played an important part in practical immunology.

The *calcium* localized chiefly in the nucleus (fig. 182, 183) concentrates in the cytoplasm during the ripening of the plasma cells and can be demonstrated extracellularly in substantial quantities in the second week after sensitization. Amorphous, crystalline and crystallised formations are found here; they indicate that the cells lose ample quantities of calcium while the immunological process goes on. This applies specially to the cytolysis caused by antigens, which explains also the therapeutic effect of the calcium in conditions of allergy and anaphylactic shock.

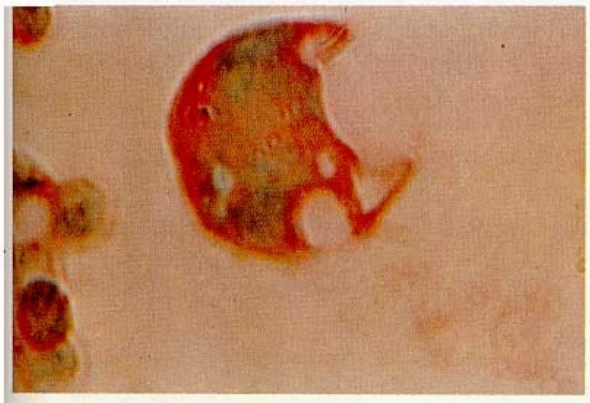
Magnesium is concentrated in the plasma cell, chiefly in the cell membrane (fig. 184, 185) and along the cisterns. In the stage of secretion of the immunocytes, substantial quantities can be traced also outside the cells where it forms bizarre forms of crystals under experimental conditions. Moreover, aluminium and phosphates concentrate especially in the cell membranes whereas mainly concentrations of iron, copper, lead, zinc and sulphur compounds occur in the cytoplasm.

Examples of these concomitant processes are illustrated in fig. 166–185.

Kinetics of the immunoglobulin synthesis

Measured on titres of antibodies after the first contact with antigens, 4 phases can be defined (BRANDIS):

1. No antibodies can be traced in a *latent phase* of 2–3 days;
2. During an *exponential phase* (from the 3rd to the 7th day), the antibody titre increases rapidly up to a maximum;
3. From the 6th–7th day the titre remains on the same level for 2–4 weeks (= 2nd–5th week after the sensitization): *stationary phase;*
4. During months to years, the antibody titre will slowly decrease: *phase of reduction.*



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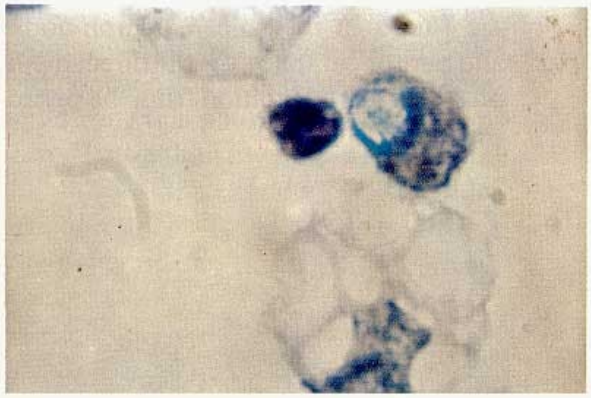

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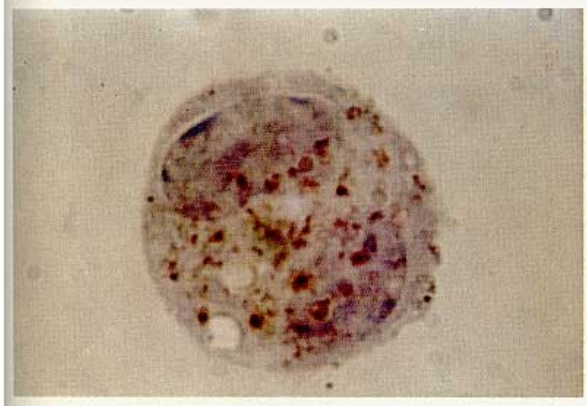

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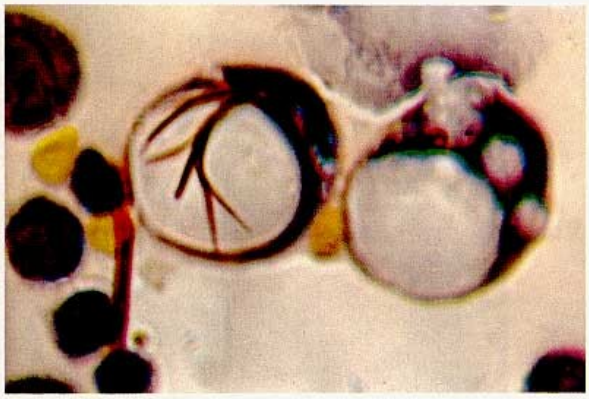

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Fig. 178:
RNA concentration in the cytoplasm of an immunocyte at the beginning of the secretion (pyronin-positive cell). The synthesis of immunoglobulin depends on an increasing concentration of RNA. Methyl-green-pyronin staining; RNA = red, DNA = green.

Fig. 179:
Acid mucopolysaccharides on the cellular membranes of immunocytes in the stage of secretion. Turquoise staining with alcian blue.

Fig. 180:
Non-specific esterases take up mitochondria in the synthesis stage of the immunocytes. Esterase staining after LÖFFLER, esterase brick-red.

Fig. 181:
After the synthesis of immunoglobulin, the *esterase* is found chiefly in the cisterns, often in the form of crystals. Secretion stage of an immunocyte, esterase staining.

The cellular antibodies (IgM) attain a maximum late on the third day or on the fourth day, humoral antibodies appear on the fifth day. While the IgG level rises rapidly, the IgM level drops.

Latency-phase

The *phase of latency* corresponds to the transformation phase of the immunocytes. Transitional forms from mononuclears to «plasma cells» (often referred to as plasmablasts, fig. 171, 186) are found in the spleen, lymph-nodes and peritoneal space. As the direct method of cell differentiation from the peritoneal exudate provides more exact kinetic aspects than autoradiography with 3 H – thymidin or the arrest of mitosis with colchicine, the phase of latency can be subdivided in a polynuclear episode of about 24 hours and a mononuclear phase, provable as from the second day after the exposition of antigens.

The indications of the rebuilding to the protein synthesis appear cytochemically and electron-optically. The cytoplasm becomes gradually basophile, pyronin-positive (= takes up RNA), the Golgi-apparatus grows, the mitochondria multiply, the endoplasmatic reticulum takes shape.

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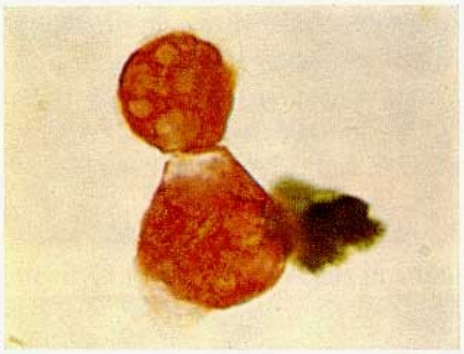

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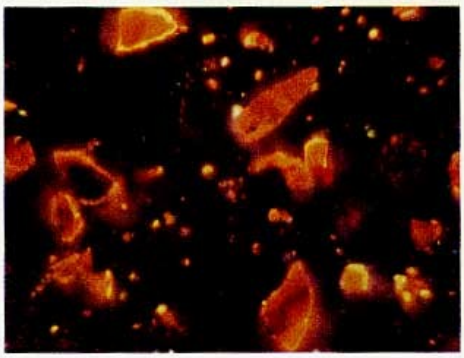

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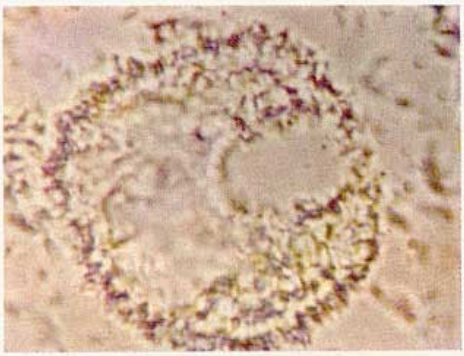

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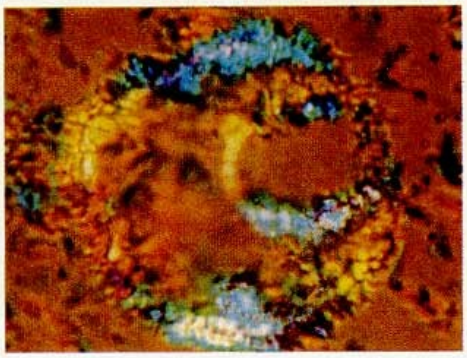


Fig. 182:
Concentration and extrusion of *calcium* from monocytes: cells of peritoneal exudate 2 weeks after sensitisation. Calcium-red coloration.

Fig. 183:
Extracellular calcium deposits in the peritoneal exudate, 3rd week after sensitisation.

Fig. 184:
Concentrations of *magnesium* on the cellular membrane of an immunocyte in the secretory stage and along the membrane of a cistern (vacuole); peritoneal exudate, magneson staining.

Fig. 185:
Cell as in fig. 184: under crossed nicols of the polarization microscope, the accumulations of magnesium membranes appear in a *crystaloid arrangement*.

The exponential phase

results from the quick rise of the titre of antibodies in the serum. The rapid division (fig. 167) of the mononuclears changed into immunocytes makes the antibody-producing cell potential go up rapidly till the 4th–6th day after the exposition of antigens. The numbers of cells and the titre of antibodies double in this phase within 6–12 hours (BRANDIS).

The exponential rise of the titre reflects the IgM production. Two cellular processes act together: the multiplication and ripening of the cells. The interference of the cell multiplication with a doubling rate within 9–12 hours and the doubling of the synthesis rate of the single cells every 9 hours make the production of antibodies double within 4.5–6 hours.

Cytochemically, basophilia and the expansion of the volume of cytoplasm as well as the pyronin positivity (= high concentration of RNA) characterize this phase; electron-optically, a dense ergastoplasm rich in ribosomes prevails (fig. 163, 173, 186–188).

Stationary phase

From the 6th to 7th day after the antigen stimulation, the titre of the antibodies remains steady for 2–4 weeks. This level is maintained within the classes of antibodies as the IgG values rise and the IgM concentrations decrease. The secretion during this phase could not be supported better than by the rise of humoral antibodies at the expense of the cellular antibodies. The IgG titres keep in this way rising till the end of the 3rd week,

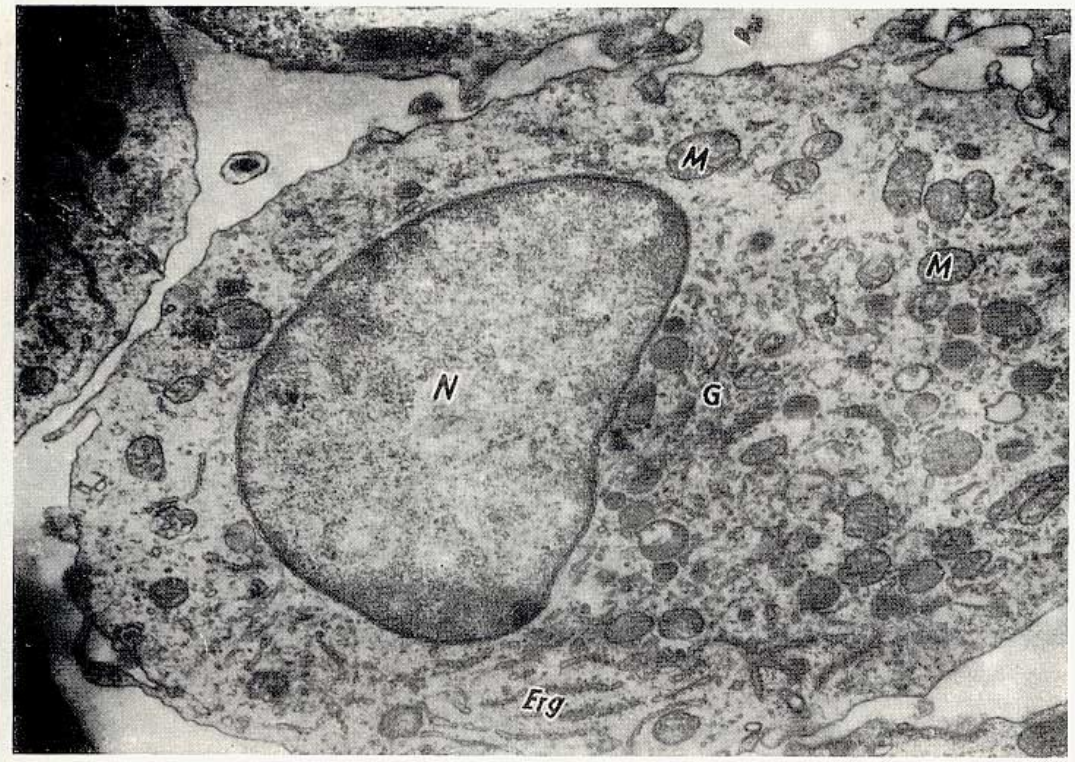


Fig. 186:
Early stage of plasma-cell (problasmoblast). Still oval but already eccentric nucleus (N), consolidation of the Golgifield (G), beginning formation of ergastoplasm (Erg). Abundance in mitochondria (M). Electron-opt. 1:15,000.

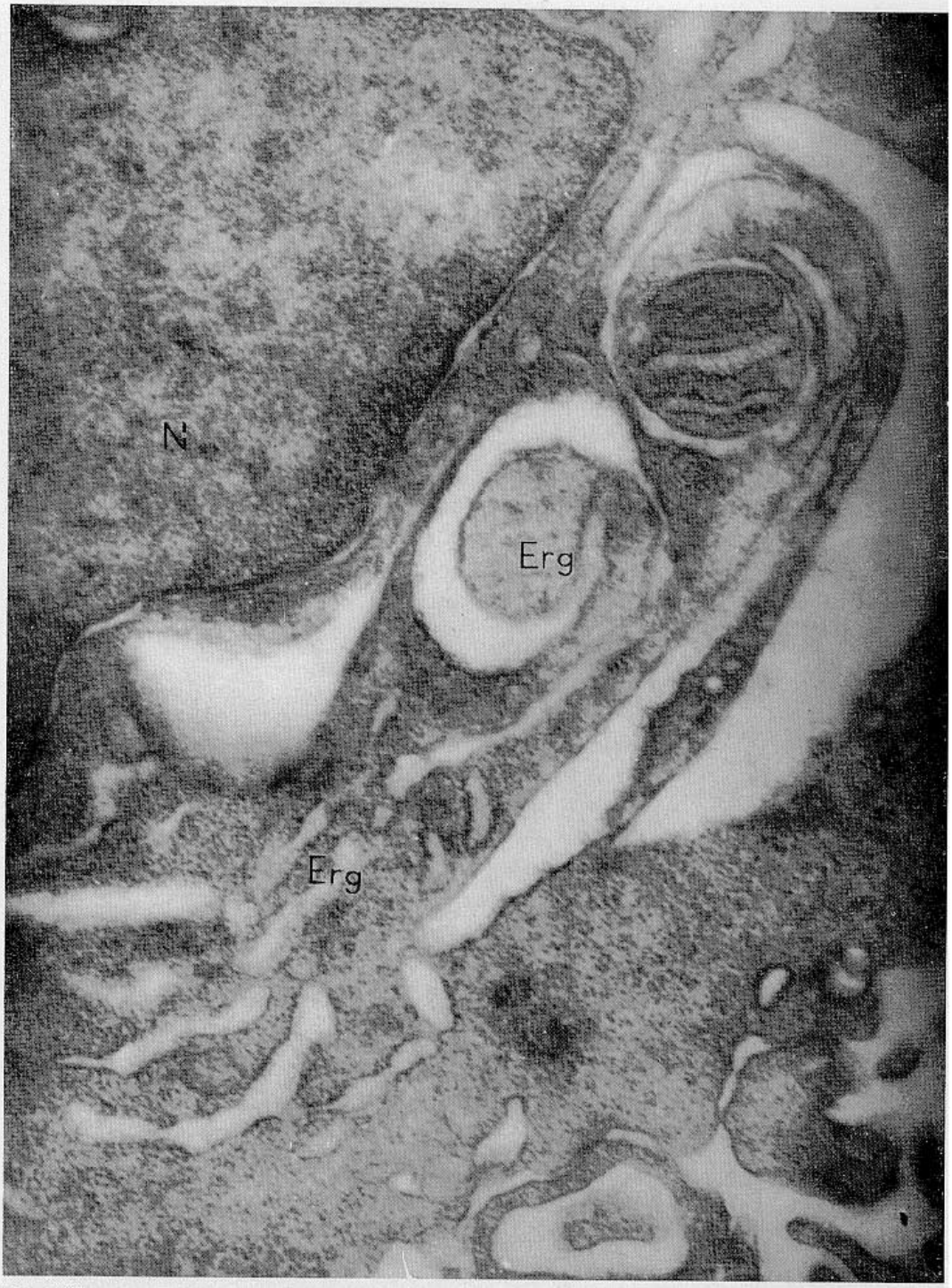


Fig. 187:
Formation of the endoplasmatic reticulum in an immunocyte on the 17th day after sensitisation; Erg = endoplasmatic reticulum; N = nucleus, 1:40,000.

Fig. 189: ▶
Plasma cell in the *stage of secretion*. Large vacuoles (V), into which the synthesis products of the cytoplasm organelles are emptied by pressure filtration (arrows). Note the consolidations of cytoplasm at the edge of the vacuoles. Electron-opt. 1:20,000.

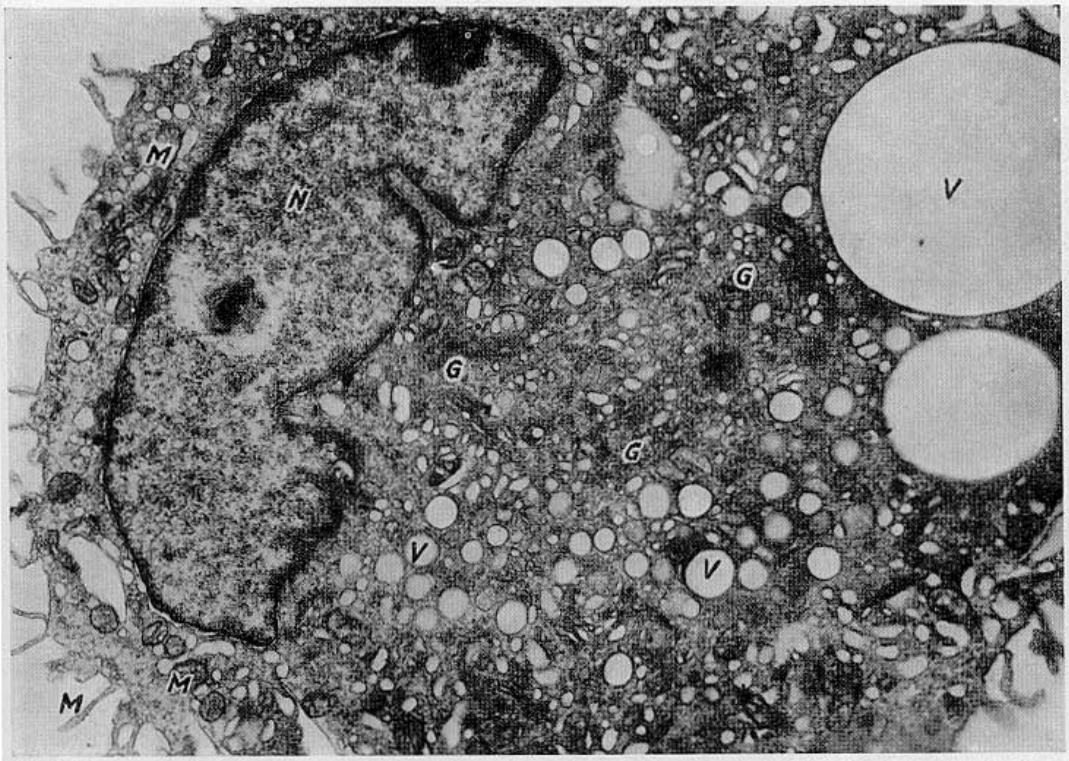
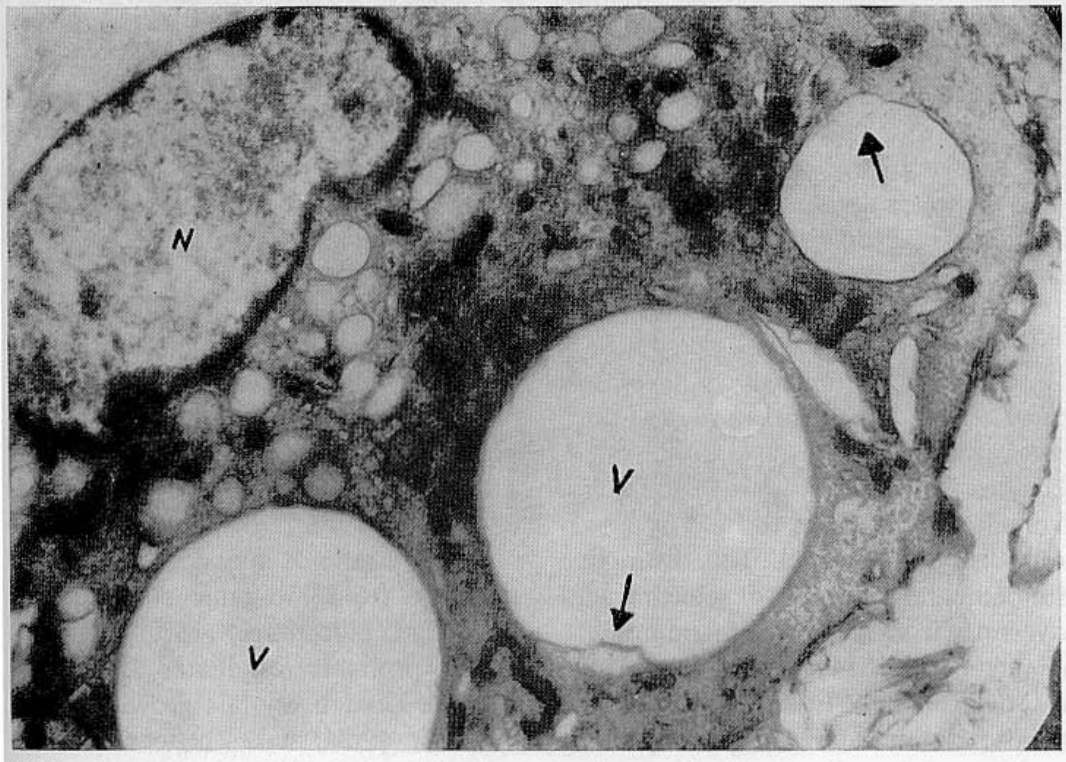


Fig. 188:
Mature plasma cell of the loose connective tissue. Marginal flattened nucleus (N), extended Golgi-area (G-G), consolidated, irregular cytoplasm; small to medium vacuoles (V) surrounding the Golgi-field, small mitochondria (M). Electron-opt. 1:12,000.



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followed by a real stationary condition of about 2 weeks, during which the production of IgG and the disintegration of the immunoglobulin are equal.

The morphological equivalent of the stationary phase is secretory plasma cells with increasingly more vesicles and cisterns (fig. 174–177, 178, 181, 184, 189).

Phase of reduction

As the cellular potential producing the antibodies diminishes, the level of antibodies drops for months or years according to the stimulations by antigens. For this time, and longer, cells (memory cells) resuming after another contact with the same antigen the production of antibodies faster than at the first contact (secondary response) will remain.

Within wide outlines, this principle equals the immunokinetics. The spaces of time change according to the quantities of antigens. The more antigens, the faster and more intense the synthesis of antibodies will take place.

Eosinophils

An essential part in the defense mechanism of the body is ascribed to the eosinophilous leukocytes with a polymorphous nucleus. This kind of blood cells known since 1879, whose multiplication in diseases so different as asthma, parasitosis and in the monocytary-eosinophilous phase of overcoming after infections concealed the biological common denominator, have virtually been clarified as to their form and function. They are no longer classified under the collective name «leukocytes», at least since it has been known that more eosinophils than neutrophils can be found in the bronchial secretion of patients suffering from allergic asthma.

Morphology

Eosinophilous leukocytes have a diameter of 12–17 mi m and are found mostly in the area of the large-sized leukocytes. The main volume of the membrane-enclosed cell is formed by 2 (–3) roundish-ovoid nuclei. The distinctive fundamental capability of selecting acid dyestuffs from a panchromatic mixture (PAPPENHEIM, MAY-GRÜNWALD, GIESMA) detected by P. EHRLICH accounts for the separation from the neutrophilous and basophilous leukocytes. The space of cytoplasm contains several, usually twenty, roundish, oval or elliptical granules; their refractive power is due to the axis crystals, sometimes referred to as «cores». The basic protein of the granule accounts for the acidophilia; it contains an unusually high percentage of arginin (KOENIG, GLEICH, etc.). The granules are covered by a simple cytomembrane. The densities of the axis-crystalloids and matrix in a cell are considerably different (ZUCKER-FRANKIN, 1978).

The electron-optical fixation methods define the optical picture of the granules. Whereas the fixation of osmium makes appear the crystalloids denser (darker) than the matrix (fig. 190), phosphorus-molybdane or hypertonic fixation methods produce a negative picture showing the matrix black and the crystalloid as a bright, rectangular space, with ground-off ends. The elements of the basic structure of the crystalloid have a periodicity of 40 Å (fig. 191).

The crystalloids may derive their importance from inorganic substituents. The high percentage (15%) of zinc, which probably originates from the Charcot-Leyden crystals growing from the eosinophils, makes believe that the crystalloid axes of the granules owe their

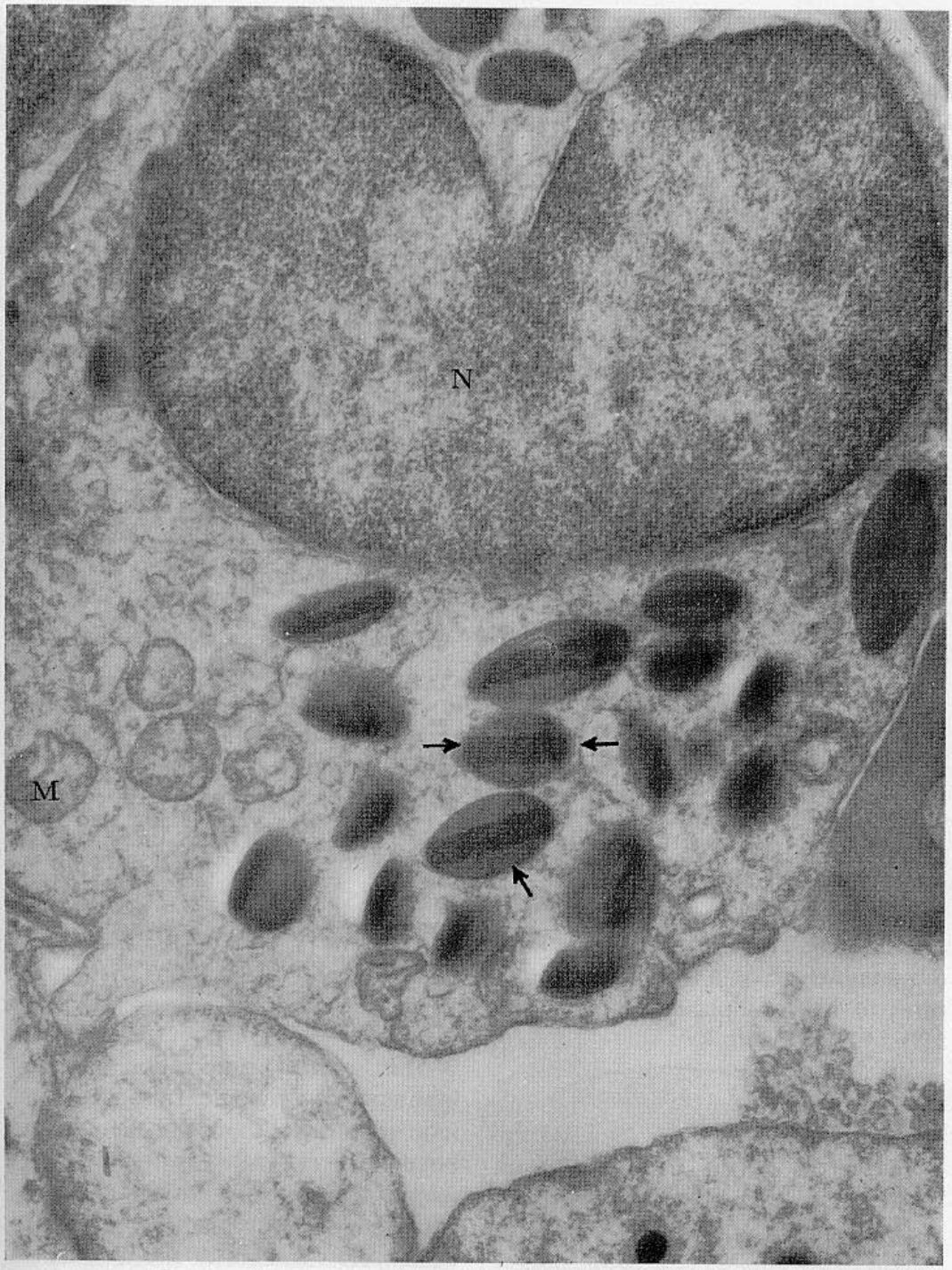

Fig. 190:
Eosinophilous granulocyte. In the (more compact) granules the axis crystalloids (arrows). N = nucleus; M = mitochondria 1:20,000.

crystalline form to a zinc complex compound, more so because zinc (Zn^{++}) inclines to form tetraedic complexes. For the inorganic order of crystals speaks the fact that the crystalloids are sparingly soluble in biological media and resistant to mechanical, osmotic and enzymatic influences. Even during the foudroyant destruction in digestive vacuoles of macrophages, the crystalloids resist the longest.

The *Charcot-Leyden crystals* (CHARCOT: 1853 for leukocytemia; LEYDEN 1872 for bronchial asthma) are found in the neighbourhood of accumulations of eosinophils. They can be produced in vitro by destruction of eosinophils (by detergents, Na-dihexyl-sulphosuccinate) (AYRES). The granules contain zinc, iron, copper, ubiquinon. Of the metals, zinc is likely to form the nucleus of the crystallization; in the crystal occur proteins and amino-acids, specially arginin, tyrosin and tryptophane.

The cytoplasm of the eosinophils contains more mitochondria, abundant quantities of small to medium, often grouped, vesicles, abundant numbers of ribosomes and a well marked Golgi-apparatus (fig. 15–18). «Microgranules» are dumb-bell-like or ring-shaped structures of the endoplasmatic reticulum, 100–150 Å in length.

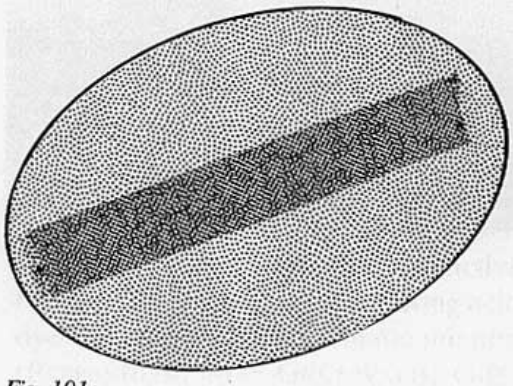

Fig. 191:
Scheme of the eosinophilous granules with the axillary crystalloids and the matrix surrounded by the membrane (see text).

Biochemistry

Histochemically, most of the lysosomal enzymes were traced in the eosinophils. The quantity of peroxidase is larger than in the granules of the neutrophils and moreover seems to function in a somewhat different way (ZUCKER-FRANKIN). The myeloperoxidase has a highly bactericide potency, the peroxidase of the eosinophils evidently not. Regarding the eosinophil granules there is the remarkable fact that Zn^{++} (and $Fe^{++,+++}$) take part in the formation of peroxide from plasmalogenes in the tissue (OHNISHI, F.).

The arylsulphatase B, too, is more concentrated in eosinophils than in neutrophils. This enzyme is said to have a function inactivating the «slow reacting substance» of the anaphylaxy (SRS-A). Another two substances are of importance though the site of their origin has so far not been located: bradykinin neutralises histamin; a factor denoted as EDI (inhibitor of histamin release) prevents the release of histamin from tissue mast-cells and basophilous leukocytes. In tissue mast-cells, histamin is bound to heparin in the form of a ternary complex (KERP G. and STEINHAUESER G.).

Origin

The general supposition that all granulocytes originate from the common mother cells (myeloblasts) of the bone-marrow, conflicts with certain objective observations. Patients suffering from agranulocytosis can have increased eosinophil numbers in the absence of ripe neutrophils (ZUCKER-FRANKIN D.). In congenital neutropenia, the myeloblasts do not ripen fully, the development of the leukocytes with polymorphous nuclei is arrested on the step to the myelocyte; then ripe eosinophils cannot only be normal but even multiply. From

mother cells of the human blood originate in agar cultures either neutrophils or eosinophils, no mixed populations.

The formation of eosinophils in the thymus of rats has been proved safely (YOKE M. S. and SAINTE-MARIE G., 1965). As for the human thymus, J. SCHAFFER described the occurrence of eosinophils already in 1891.

Eosinophils circulate in the Ductus thoracicus lymphaticus where neutrophils are rare (ZUCKER-FRANKIN D.). The narrow relations between the thymus and the production of eosinophils have been proved repeatedly by the lack of eosinophils in thymic insufficiency of various species of animals. On the other hand, individuals suffering from agammaglobulinaemia of the Swiss type can show considerable increases in eosinophils in dysplasia of the thymus.

Function

Clinical and experimental findings have substantiated the supposition that the eosinophils play an important part in the immune-defense. Studies on parasite eggs of schistosoma (MACKENZIE et al.; MCLAREN et al.; KÖNIG W.) have shown that eosinophils effect a membrane adherence with pseudopodia after «palpating» the parasite membrane. By accumulating enzymes in the neighbourhood of the site of contact, the active ingredients –specially peroxidase– injuring the parasite membrane are produced; they start the destruction of the parasites as the usual way via the phagocytosis is not feasible owing to the dimensions.

The following factors account for this no doubt most important function:

The eosinophil chemotactic factor (EFC) is a peptide favouring (besides other powers) the contact of the eosinophils with immune complexes; it is released from lymphocytes and neutrophils (KÖNIG). Another factor of lymphocytary origin, the eosinophil stimulating factor (ESP) controls the migration and does not depend on immune complexes.

Eosinophils are chemotactically attracted by immune complexes (specially after complementary activation) and bacteria.

Anaphylactic reactions are always associated with eosinophils, ruptured mast-cells are surrounded by eosinophils. Histamin seems to have a special «affinity» to eosinophils. Owing to the nearly regular correlation between IgE and the multiplication of eosinophils, a direct relation between the two components is postulated.

Basophils

Basophilous granulocytes and tissue mast-cells have as a common characteristic basophilous, or rather, metachromatic granules. Metachromatic means that toluidine-blue or giemsa does not stain the granules blue but in gradations of violet. *Basophilia* represents highly acid structures taking up greedily the basic constituents from a colour mixture.

The blood of healthy persons contains 25–50 basophils/mm^3. There are no regular quantitative relations between the blood basophils and the tissue mast-cells. In the bone-marrow, the basophils make about 0.33%, in the tissue, the tissue mast-cells make 0.001–0.02 (BESSIS, 1972; BRAUNSTEINER and ZUCKER-FRANKIN, 1962; WOLF-JURGENSEN, 1968; FREDERICKS and MOLONEY, 1959).

The basophils multiply in hypothyreosis, after splenectomy, in cirrhosis of

the liver; they decrease after an anaphylactic shock, in thyreotoxicosis, in rheumatic arthritis.

Morphology

In granulocytopoiesis, the first metachromatic granules appear in the stage of the promyelocytes; they become more distinct in the myelocytes. The granules originate in the Golgi-apparatus and unite gradually to form larger structures.

The (metachromatic) basophilous granulocyte is the smallest granulocyte offshot (∅ 10–14 microns). The granules, 0.1–1.0 (up to 1.7) microns in size, are roundish, oval or angular; frequently, they are placed in cisterns (vacuoles). The granules, covered by a simple membrane, include a marginal matrix, and a parallel layered or hexagonally structured compact interior mass (see fig. 193); DVORAK, 1978; ZUCKER-FRANKIN, 1967. The particles are not separated sharply from the matrix and vary between 113 and 260 Å in diameter. The granules can comprise particles of varying size and membrane structures. Smaller peroxidase-negative granules are found in the vicinity of the nucleus.

The cytoplasm is pale, grey to reddish in the ground tone, contains abundant quantities of vesicles and of glycogen deposits. A small Golgi-apparatus, few mitochondria, few ribosomes and scarce rough endoplasmatic reticulum, microtubuli and microfilaments have been detected. The nucleus is «S» or «J»-shaped, less segmented than in the segment-nuclear cells or eosinophils and has compact chromatin structures.

Cytochemistry

Basophilous granulocytes and tissue mast-cells contain many bioactive, bio-

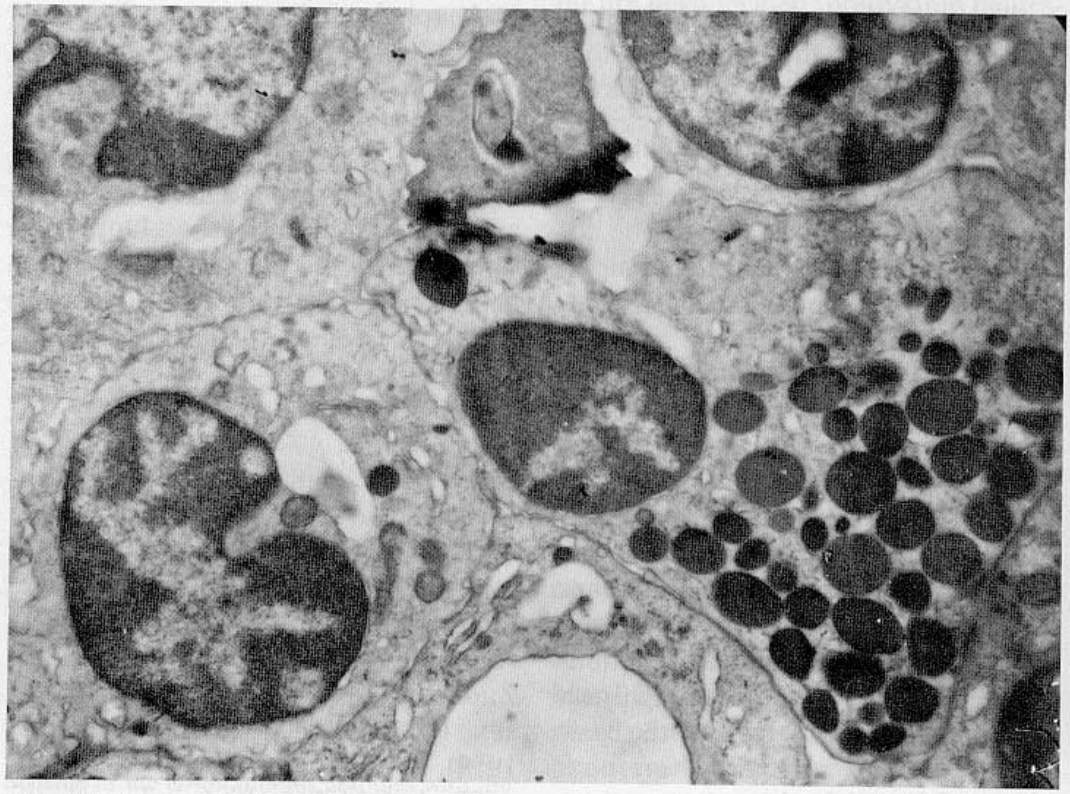

Fig. 192:
Basophilous granulocyte from the peritoneal exudate of the guinea-pig; 1:10,000.

chemical substrates and enzymes (ACKERMANN, 1963).

Acid mucopolysaccharides

The phenotypical basic property – metachromasia, basophilia – is due to acid mucopolysaccharides of the granules. Blue dyestuffs like toluidine blue, methylene blue, methylene violet provoke colours in red (positive metachromasia), red dyestuffs (neutral red, safranin, pyronin) yellow to yellowish-greenish shades (negative metachromasia). ORENSTEIN et al. found 85% of chondroitin sulphate and dermatan sulphate, 15% were heparan sulphate (not heparin, as supposed earlier).

Histamin

Whereas in various animals only part of the body-histamin occurs in basophils / mast-cell reservoir, the entire depot of histamin seems to be located in the basophilous cells in man. The human basophils include 1–2 pg of histamin, mast-cells of the peritoneal exudate of rats up to 20 pg (DVORAK). Histamin is bound to the granular fraction.

The synthesis takes place through a cytoplasm enzyme, the histidin-decarboxylase. In tissular cultures of guinea-pig basophils tagged with 3 H histidin, the increase of histamin was registered after 1 hour already, the maximum of production is attained after 24 hours.

Platelet-activation factor

The accumulation of blood-platelets around degranulated basophils suggested a connection. The aggregation-promoting factor (PAF) is found especially in «sensitized» basophils and anti-IgE antibodies. PAF is a low-molecular protein (molecular weight = 1,100), which forms rapidly compounds with albumin and cell membranes. It does not cause contractions of unstriated muscles but improves the permeability of vessels and cannot be influenced by antihistamins or antiserotonins.

Further substances

In contrast to the sparingly soluble crystalloid axes of the eosinophils, the basophilous granules dissolve readily in water and glycerin; this makes the cytochemical analysis more difficult. Nevertheless, quite a series of further substances has been detected.

The positive PAS-reaction proves the

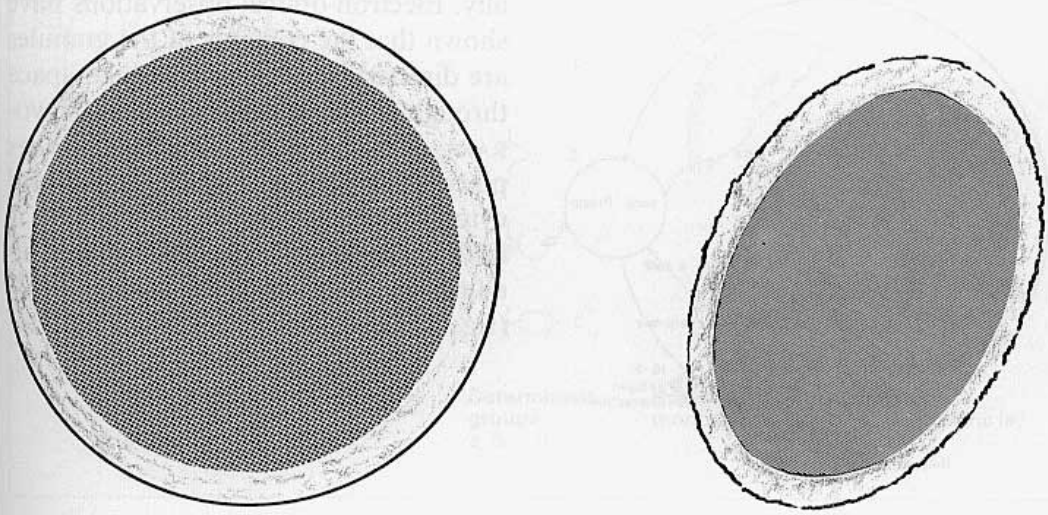

Fig. 193:
Scheme of the basophilous granules (see text).

presence of *glycogen (or glycoprotein) aggregates* located outside the granules, with particles up to 300 Å in diameter. The presence of *lipids* has been demonstrated with Sudan-black-B.

The enzyme pattern of the basophils is not quite clear. The most important enzyme seems to be the *histidin decarboxylase,* which transforms histidin to histamin. Neutral *esterase-proteases* have been found. Oxydative enzymes such as *diaphorases* and various *dehydrogenases* are ascribed to the mitochondria fraction of the basophils. The *activity of the peroxidase* seems to differ in various species. There are some deviations from the normal lysosomal enzymes as e.g. the *absence of the lactate-dehydrogenase.* Phosphatases, aminopeptidases, phosphorylases seems to be absent. The *non-specific esterases* –unlike the mast-cells– have not been proved in blood basophils. A *plasminogen-activating factor* has been detected in the membrane of basophils, not however in the purified granular fractions (summaries by DVORAK, 1978; BESSIS, 1972; ACKERMANN, 1963).

Human mast-cells and basophils of leukemic patients contain the *eosinophilous chemotactic factor (EFC-A)* and the *slow-reacting substance (SRS-A).*

After the influence of microradiations or distilled water, needle-shaped crystals will grow in the granules.

Function

Basophils move like amoebae, their phagocytic power is inferior to that of other species of leukocytes; they can phagocytose complexes of antigens and antibodies as well as sensitized erythrocytes. Their main function, possibly, is to act as a histamin reservoir of the body. In spite of certain differences in granule-chemistry, the blood basophils and the tissular mast-cells constitute a functional unit, with the basophils representing the transport system and the tissular mast-cells the depot system.

One of their most important properties is the degranulation. Whereas, physiologically, the degranulation can eliminate the granular contents when required, the evacuation of the granules takes place in the form of explosion under immuno-pathological conditions. After the elimination of the compact contents, vesicles rimmed with membranes will remain and disappear gradually. Electron-optical observations have shown that the contents of the granules are directed into the extracellular space through a «cytoplasma canal» (DVORAK), or that a partial evacuation takes place after fusion of the granular and cytoplasmic membranes. This process is initiated by immunity reactions mediated through cellular membranes and IgE (fig. 192, 194, 204).

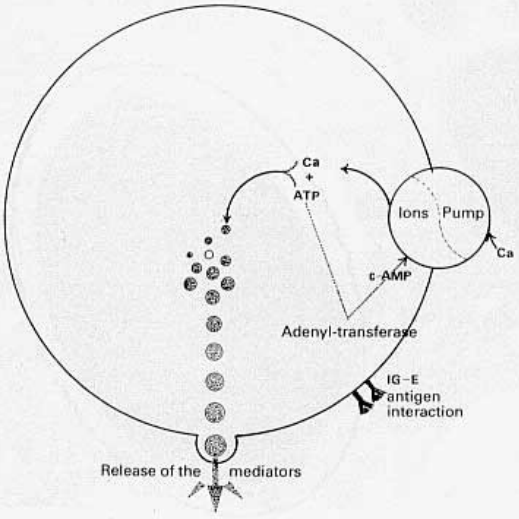

Fig. 194:
Degranulation of a basophils.