Virchows Arch. A Path. Anat. and Histol. 362, 115—128 (1974)

Storage of Vinylpyrrolidone-Vinylacetate (VP-VA) in Rats Following Endotracheal and Subcutaneous Injection

M. H. Blessing

Pathologisches Institut der Universität Düsseldorf
(Direktor: Prof. Dr. H. Meessen)

Received April 12, 1973

Summary. Vinylpyrrolidone-vinylacetate (VP-VA) the major component of many commercial hair-sprays was injected endotracheally in female Wistar rats. The material is stored in large macrophages which often fill the alveolar spaces completely. There is no inflammation associated with the process. The alveoli, however, contain a large amount myelin structures some lamellar, mostly however in a lattice-like form. It is suggested that this material is associated with the surfactant. Some of the VP-VA is obviously eliminated from the lung via interstitial macrophages and the lymphatics, most of it, however, over the mucociliary pathway.

Following subcutaneous injection of VP-VA most of the material is stored in the spleen, which considerably enlarges, and some in other organs.

The excretion is via the urinary system.

Even 12 months following the last endotracheal or subcutaneous injection there is no evidence of tumor or systemic disease.

Key words: Vinylpyrrolidone-Vinylacetate — Lung Macrophages — Surfactant — Spleen — Excretion.

Vinylpyrrolidone-vinylacetate (VP-VA) is a high molecular copolymer closely related to polyvinylpyrrolidone (PVP) as Kollidon, Periston and Periston N used in medicine.

Since VP-VA is contained in many cosmetic hair-sprays, sometimes a thesaurosis is thought of in differential diagnosis when a lung lesion is found or suspected. Since in the literature there are only a few and also contradictionary remarks (Bergmann *et al.*, 1958, 1962; Draize *et al.*, 1959; Schepers, 1962; Brunner *et al.*, 1963; Ludwig, 1964; Edelston, 1969) as to the effects of hair-sprays especially VP-VA containing ones on the lung tissue we studied experimentally the incorporation of this substance.

The exposure of guinea pig, rabbits and rats to VP-VA containing hair-sprays showed results which are difficult to reproduce. This we mainly attribute to particles of different size which are not respirable for all animals. Therefore we injected pure VP-VA into rats via the trachea and also subcutaneously. This also eliminated side effects due to propellants, solvents, parfumes and softener.

Materials and Methods

We used vinylpyrrolidone-vinylacetate (VP-VA) made by BASF Ludwigshafen with the tradename of Luviskol VA 64. The relation of VP: VA is 60:40 and the K-value 31 ± 4. The residual monomeres are below 0.8%, the nitrogen content near 7.6%, the solids over 95% and the amount of water below 5%. In all experiments we used VP-VA in a concentration

of 10 g in 15 ml of physiological sodium chloride solution. The water-clear highly viscosic solution was injected at body-temperature.

As experimental animals we used female Wistar rats with a body weight of about 200 g. The animals were kept in a separate climatized room and had access to water and standard feed ad libitum. In ether narcosis 20 animals were given endotracheally in single or repeated doses 0.5 ml of the standard solution. 30 animals received in daily doses up to seven times 2 ml of the standard solution under the skin of the back. In this way between 1.1 and 45.0 g VP-VA were injected per kg of body weight. 15 controls received physiological NaCl-solution endotracheally in similar doses. The animals were killed by exsanguination in ether narcosis between one and 365 days following the application of the VP-VA-solution. Some of the animals were perfused via the pulmonary vein with glutaraldehyde.

Tissues were studied in frozen and in paraffin sections and stained with HE, EvG, PAS, PTAH, HS, congored, chlorazol fast pink and congored according to Freiman and Gall (1955). Tissues for electron microscopy were embedded in Araldite and stained with lead and studied in a Siemens electron microscope.

Results

A. Endotracheal Injection

a) Light Microscopy

One to two days after the injection in all of the lobes the alveoli are closely packed with macrophages. These cells are large, more voluminous than in the typical foam-cell pneumonia of rats. The cytoplasm contains vacuoles and only sometimes the nucleus appears to lay in an empty space surrounded by a cell membrane. The nuclei are round to oval and show a fine chromatin pattern. The vacuoles exhibit neither sudanophilic nor PAS-positive materials. Sometimes we could achieve a slight staining with chlorazol fast pink. The storage cells are dispersed in the alveoli without any associated reaction. There is no concomittant pneumonia or a bronchitis or bronchiolitis.

Four to six days after the first endotracheal injection there are numerous large macrophages to be found in the interstitium of the lung especially in the peribronchial and perivascular lymphatics. These structures are often densely packed with vacuolated cells and considerably enlarged. The associated hilar lymphnodes and those in the region of the trachea show sometimes a few storage cells and these could be stained with congored according to Freiman and Gall (1955).

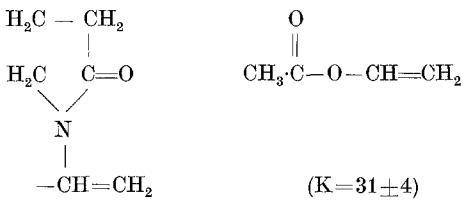
Four to six months after the last injection there is still—depending on the amount of the injected material—VP-VA to be found in the lung. If some weeks and months have elapsed macrophages are predominantly found in alveoli close to bronchi and vessels as well as near fibrous septae. There are always single or in groups of two to four. Animals killed one year after the last injection do not show any more storage cells in the lung.

Other organs like liver, kidneys and bone marrow show no deposition of VP-VA after endotracheal injections. Only in the spleen there are after repeated injections a few storage cells, some solitary, some arranged in groups. There was no inflammatory reaction.

The lung and other organs of the control animals did not show any signs of inflammation or other abnormalities.

VP — VA

Vinylpyrrolidone — Vinylacetate



copolymerisat (60% : 40%, Luviskol VA 64 BASF)

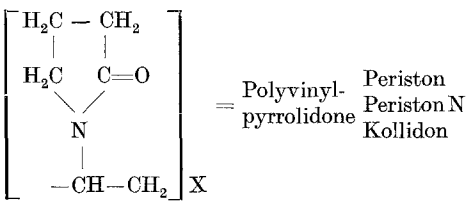


Fig. 1. Formula of vinylpyrrolidone-vinylacetate (VP-VA)

b) Electron Microscopy

The submicroscopic investigation of the lungs shows in most cases the capillaries deeply embedded into the septae and only a small portion of the circumference is protruding into the alveolar spaces. The basement membranes of the capillaries are inconspicuous. The endothelial cells often show numerous pinocytotic vesicles, especially there where the adjacent interstitium contains many vacuolated macrophages. Venules and arterioles are without abnormalities.

On scanning the alveoli are three cell types to be recognized: next to the lining quite flat epithelial cells (type I) the granular pneumocytes (type II) which are considerably larger and more protruding than in control animals, as well as numerous large intraalveolar macrophages. The alveolar lining is intact and the cells are resting on a thin continuous basement membrane.

The type I-cells contain the usual organelles, many pinocytotic and only a few larger vesicles. The granular pneumocytes appear to be more numerous than in controls. On their free surface there are many microvilli. Their cytoplasm, compared to controls, contains more rough endoplasmatic reticulum and there are large vesicles with densely packed lamellar structures. Besides there are, similar to the type I-cells, a few larger empty appearing vesicles which are not noted in controls.

Alveolar macrophages which are first seen two days after the endotracheal injection of VP-VA, show cytoplasmatic structures similar to the type II epithelial cells. Besides the nucleus they contain cell organelles like Golgi apparatus, endoplasmatic reticulum, mitochondria and ribosomes. These macrophages are very large and show mainly sizable cytosomes which are surrounded by a singular membrane and contain lamellar myelin structures. These are mostly dense and

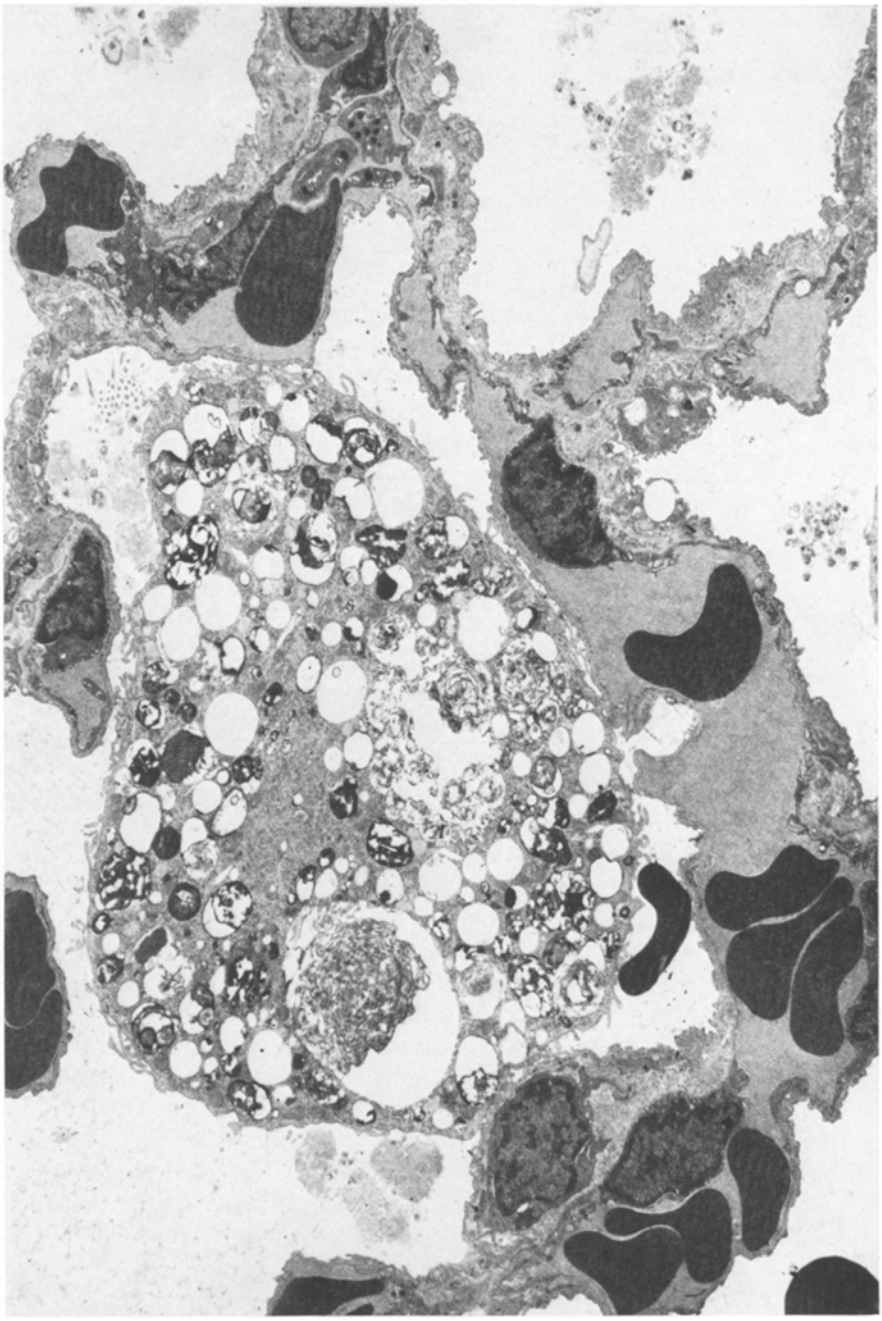


Fig. 2. Intraalveolar macrophage with empty appearing vesicles and such with lamellar structures. In the alveoli lattice-like material three days after VP-VA instillation (El. × 5700)

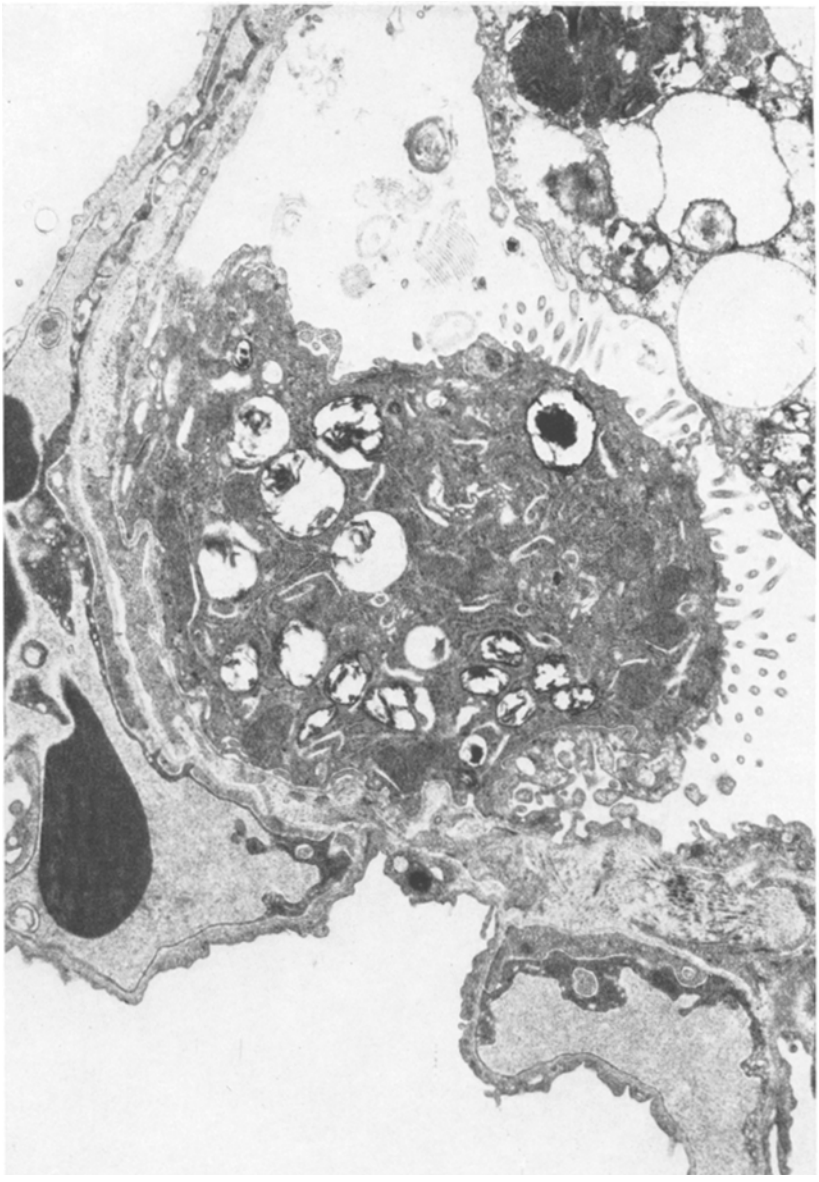


Fig. 3. Granular pneumocyte (type II-cell) with different vesicles. Portion of a macrophage at the right upper corner (El. × 15200)

thightly packed, sometimes, however, they show a delicate network. The nucleus is often intended by cytosomes, sometimes pushed to periphery of the cell, exhibiting a pericentrally aggregated chromatin. Occasionally the macrophages are so large as to fill almost completely a single alveolus, mostly however several

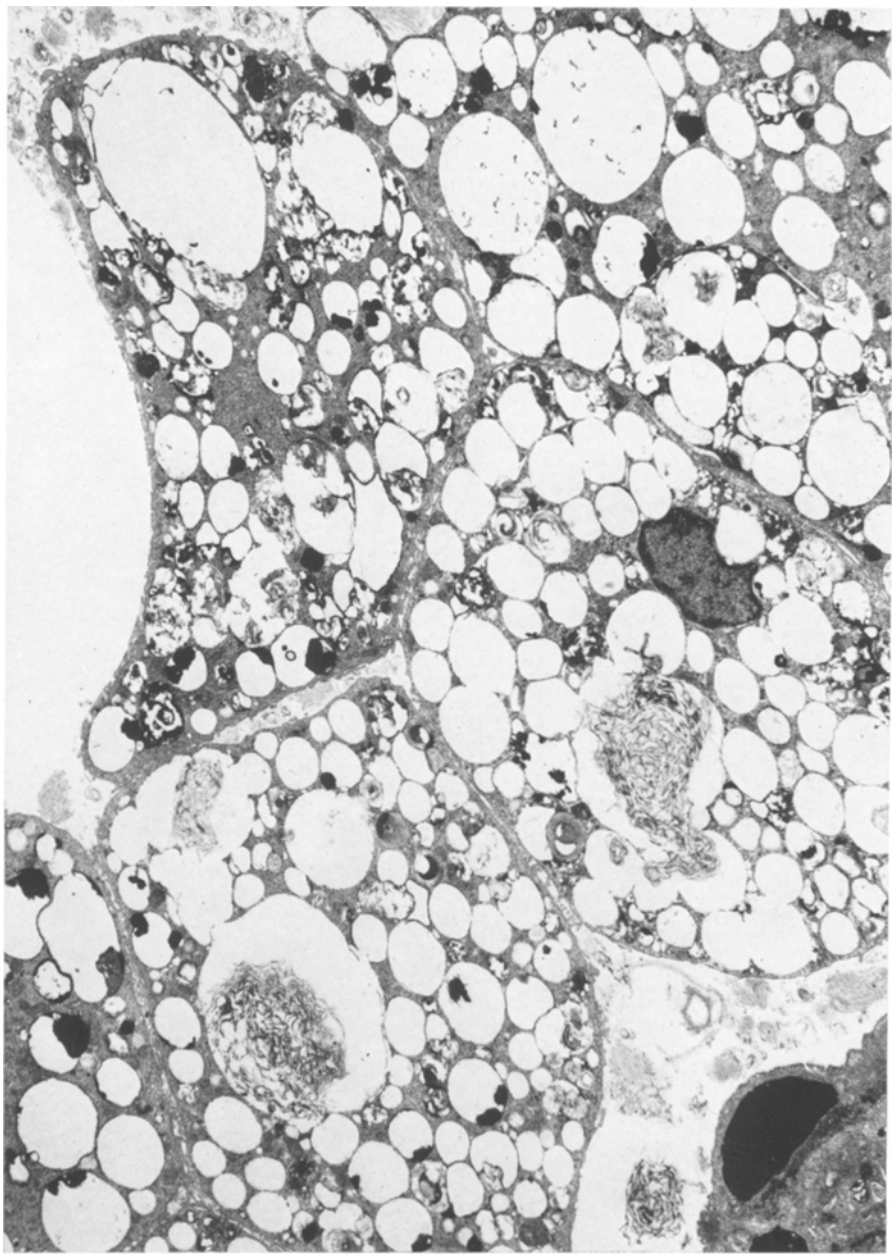


Fig. 4. Densely packed intraalveolar macrophages with different cytosomes surrounded by free lattice-like material. (El. × 4800)

of them are closely packed together. While in the macrophages cytosomes with lamellar inclusions predominate there are also some which either appear to be empty or which show on their surrounding membrane electron dense material

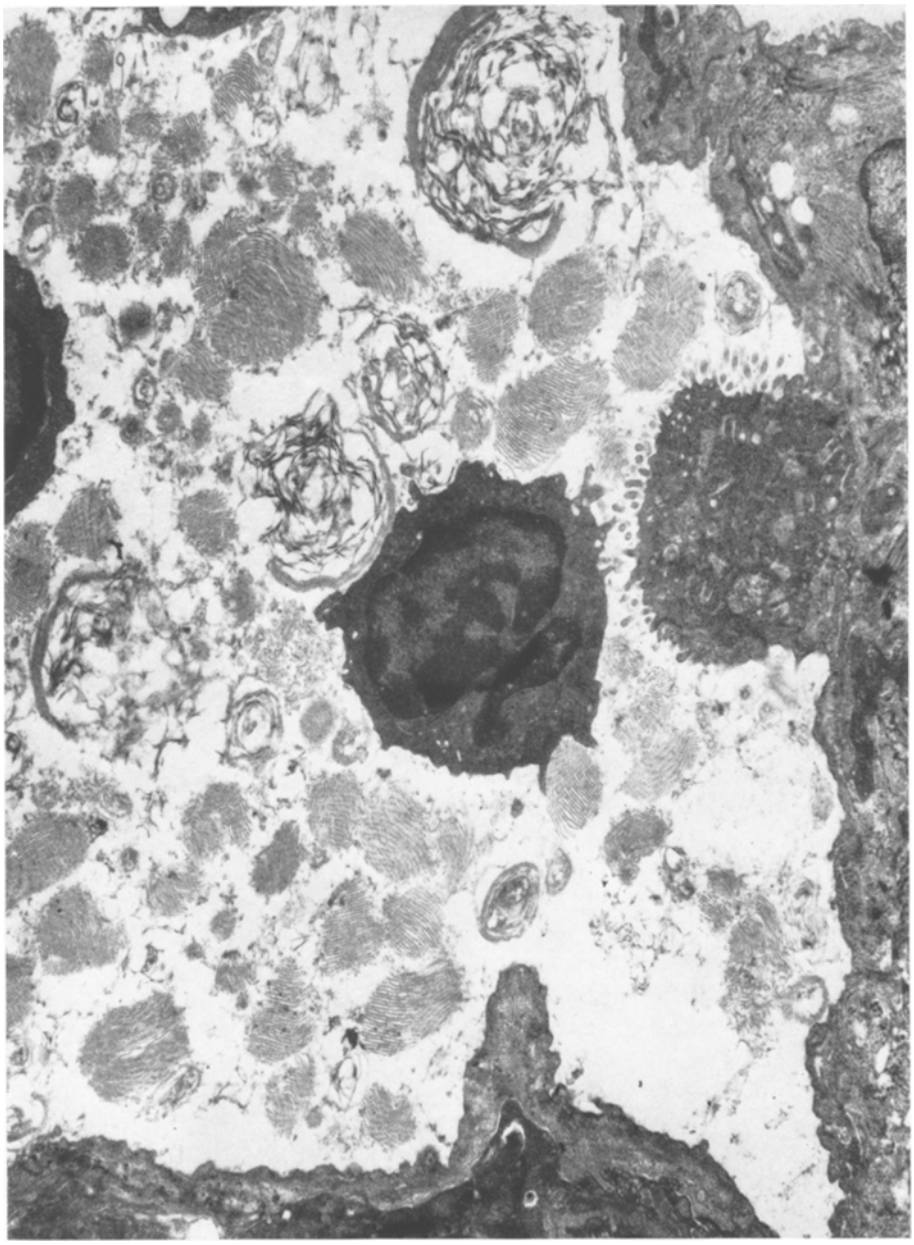


Fig. 5. Part of an alveolus with a large amount of free lamellar substances and lattice-like material two days after endotracheal VP-VA administration (El. ×4000)

which project into the lumina. A very few alveolar macrophages are lacking completely vesicles with lamellar structures. Even in serial sections they do not reveal such structures nor peripherally arranged dense particles.

One day after the endotracheal injection of VP-VA and even before the appearance of macrophages one finds in the alveolar spaces a large amount of free extracellular lamellar structures, similar to intracellular myelin figures as well as net- or lattice-like sometimes interwoven structures. The latter materials show a gap of 450 Å from one electron dense point or line to another. The alveoli are frequently, especially a few days after the last injection densely packed with extracellular materials predominantly of the net-like variety. These substances are occasionally closely but irregularly associated with the surface of macrophages. Sometimes there is a distinct transition from centrally lamellar in peripherally net- or lattice-like structures.

The interstices of the lung contain occasionally macrophages which are aggregated in groups. They are characterized by large empty appearing membrane bound vesicles which contain only a few peripherally situated electron dense particles besides a nucleus and the usual cell organelles. We did not observe lamellar inclusions in these cells.

B. Subcutaneous Injection

a) Light Microscopy

After subcutaneous injection most of the VP-VA is stored in the spleen. After repeated injections the organs increase up to 80% in weight compared to controls of equal body weight and age. Two to three days after the injection large reticular cells are found in the subcapsular region of the spleen which transform into vacuolated cells. The flattened nucleus is usually pressed against the periphery of the cells. After approximately six days the red pulp is completely filled with large storing cells which sometimes are also seen in the germinal centers of follicles. These changes in the spleen last from one to six months after that the cells appear to be smaller and the contents seem to become more dense. They decrease in number and frequently show a positive iron-reaction and appear to be incrusted. Even after a year there are still some of these cellular elements found in various parts of the spleen.

After repeated injections there are a few groups of large vacuolated cells in the portal regions of lobuli of the liver. The kidneys show a deposition of VP-VA in the podocytes of the glomerula which are sometimes transformed into large aggregations of foam-cells. There is also evidence of storing activity in the epithelial cells of the tubuli contorti I as well as in the interstitial tissue and in the lymphatics. We never noted any inflammatory changes.

Reticular cells of bone marrow and lymphnodes reveal very infrequently signs of storing VP-VA.

Following the subcutaneous injection of VP-VA there are sometimes large macrophages in the interstitial tissue of the lung. They are always solitary and show no specificity in their topographic localization.

b) Electron Microscopy

The large cellular elements of the spleen contain almost only membrane bound vesicles of different size. Ruptures of intervesicular membranes are frequent. Often these vesicles contain on their internal surface bead like electron dense

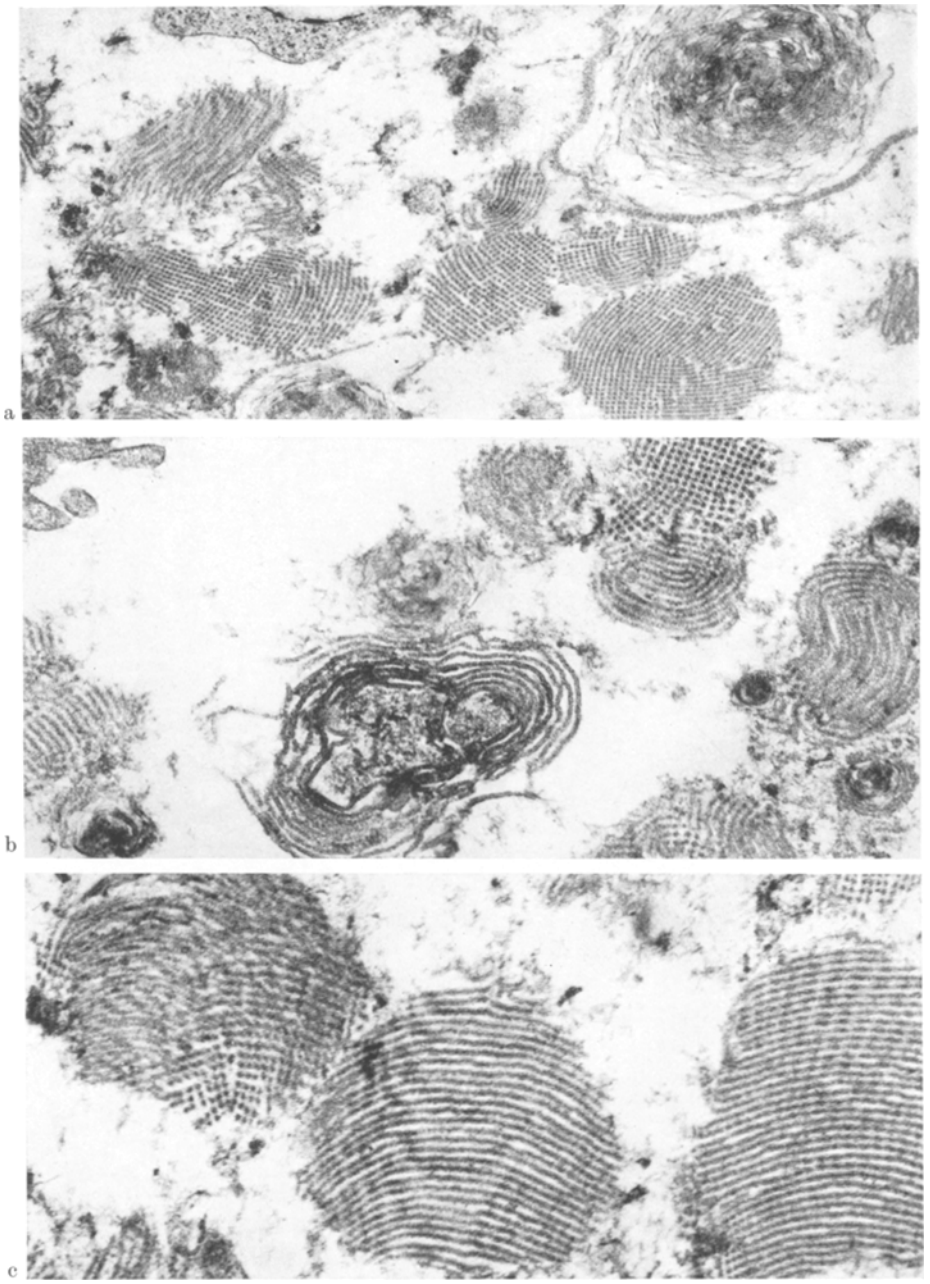


Fig. 6a—c. Free intraalveolar lattice-like (a and b) and tubular myelin figures. In a and b centrally located lamellar structures (El.) a × 6800, b × 13800, c × 13300

aggregations with a fine granular structure next to sometimes centrally located lighter material. Nucleus and the usual cell organelles appear to be pushed to the periphery.

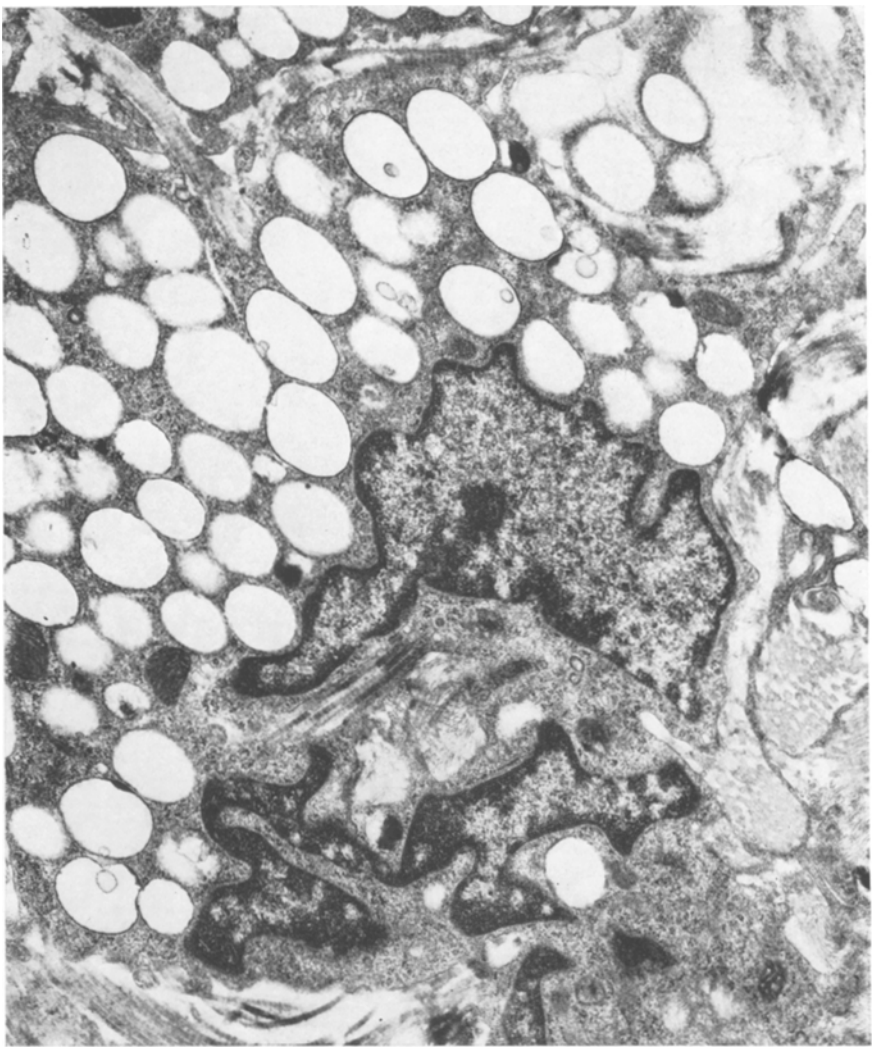


Fig. 7. Macrophages in the interstities of the lung following endotracheal administration of VP-VA (El. $\times$ 22500)

C. Excretion

After a single subcutaneous dose of 2 ml of the standard solution one can already after half an hour demonstrate VP-VA in the urine using a colorimetric method employing a KJ_3-solution. The maximum of the excretion is reached after $1^1/_2$ hours decreasing after that continuously.

Discussion

Vinylpyrrolidone-vinylacetate (VP-VA) the main component of many of the commercial hair-sprays is incorporated by rats after endotracheal and sub-

cutaneous injection over a certain period of time. One has, however, to be reminded of the fact that the polymer itself is not visible in light or electron microscopic preparations. It is highly soluble in water and alcohol and is removed in preparing sections. Only occasionally one is able to stain the material with the congored reaction according to Freiman and Gall (1955) which is also used for the demonstration of other synthetic materials. Studying the problem of incorporation of VP-VA a number of changes in cell structures are suggestive of a storing process compared to control animals. Especially intracellular vesicular structures will have to be considered.

Following endotracheal injections of VP-VA there is no inflammatory change in the lung tissue quite contrast to the instillation of silicon oil (Blessing and Lenz, 1973) which results in inflammation, necrosis or subsequent scarring. The only light microscopic changes already evident one day after the injection are numerous intraalveolar macrophages which after some days often completely fill the alveolar spaces. Over a short period there are closely packed macrophages in the pulmonary interstitium especially in perivascular and peribronchial lymphatics. There are only a few storage cells found in the regional lymphnodes and the spleen. The other organs show no signs of storing in contrast to observation following subcutaneous injections.

Electron microscopically one notes in the alveolar spaces of the lung predominantly macrophages which contain besides a few empty appearing vesicles many membrane bound inclusions of a lamellar structure. The composition of these cells shows a distinct similarity to type II-cells which by themselves appear to be increased in size and number when compared to controls and seem to show more lamellar inclusions. On their surface these macrophages reveal a large number of microvilli. There are also a few macrophages characterized by a lack of lamellar inclusions and which contain only empty appearing vesicles. Storage cells of the interstices are also marked by empty vesicles and a failure of showing lamellar structures.

These morphological aspects of the macrophages following VP-VA administration are quite noteworthy even so we do not want to draw conclusions as to the origin of these cells. These problems have been extensively dealt with by Bowden (1971). Especially the observations of Shorter *et al.* (1964, 1966); Bertalanffy (1964), Simnett and Heppleston (1966), Bowden *et al.* (1969) seem to point not to a epithelial but an interstitial aborigin of alveolar macrophages.

The quite abundantly found net- or lattice-like structures were never seen in any close connection to the alveolar surface. Sometimes one gained the impression that it evolved from the periphery of concentrically arranged or amorphous lamellar materials. Within macrophages (Schaffner *et al.*, 1967) or in type II-cells we never found morphologically equivalent substances. Similar tubular structures were seen by Schulz (1959) after instillation of quartz dust intra- and extracellular and by Harrison and Weibel (1968) following the inhalation of pure oxygen. Since in the lamellar bodies of the type II-cells and in the intraalveolar net-like structures no saturated fatty acids were found, Adamson and Bowden (1970) assume, that these materials are not identical with the alveolar surfactant (Groniowski and Byczyskowa, 1964, 1968; Finley *et al.*, 1968). It was repeatly discussed (Gil and Weibel, 1969/70, 1971) that they represent an artefact, probably

a precipitated protein. However, recent studies suggest (Harrison and Weibel, 1968; Schaffner *et al.*, 1967; Gil and Weibel, 1969/70, 1971) a direct connection between surfactant and these myelin structures. Kilburn (1968) assumes that the lattice-like substances are the result of an condensation of surface material (surfactant) and the liquide hypophases.

The remarkable accumulation of lattice-like structures in the alveoli may well be the result of the VP-VA causing an alteration of the surfactant. But it seems to be more probable that this effect is due to an increased production of these materials judging how the large amount of lamellar material contained in the type II-cells. Studies of Askin and Kuhn (1971) have clearly shown that surfactant is produced by the type II-cells and not as it was sometimes suggested by the so called Claracells.

We might assume that VP-VA is probably an inducing factor for the releasing of lattice-like structures due to its specific physico-chemical composition since also an other long-chained polymer as i.g. silicon oil (Blessing and Lenz, 1973) causes similar material to appear in the alveoli in large amounts. In no instance there were any changes in the distention of the alveoli and especially indications for a collaps.

One has to assume that the lung is mainly cleared of VP-VA over the mucociliary pathway as it happens with other polymers (Mohn, 1960). In this case most of it will reach the alimentary tract (Hilding, 1963) via the oesophagus. Since over the lymphatics only a very small amount comes into the circulation—in contrast to the subcutaneous injection—only little of the material is stored in the spleen and no accumulation in the kidney was observed. Since the polymer chaines of VP-VA are of different size it is most likely that especially the smaller parts travers the alveolar epithelium and reach the interstitial tissues (Schneeberger-Keeley and Karnovsky, 1968) and are stored in this way in interstitial macrophages.

After several weeks intraalveolar macrophages are usually only seen near fibrous septa, bronchi and vessels. This is probably due to the fact that the respiratory movements of the lung are necessary for the transport of macrophages to the ciliated epithelium (Kilburn, 1968) and that portions of the lung which are moved to a lesser degree appear to be predisposed to an accumulation of particles (Irmscher und Schulz, 1961).

VP-VA can not be visualized when injected into a glutaraldehyde perfused or unfixed and subsequent perfused lung. In these cases there were also no increase of intra- or extraepithelial myelin structures.

The inclusion of VP-VA in lysosoms (Fetzer, 1967) within the alveolar macrophages is similar to the behavior of polyvinylpyridine-N-oxid (PVNO) (Grundmann und Schlipköter, 1969; Grundmann, 1967). After subcutaneous injection of VP-VA one finds only a few macrophage in the interstitium of the lung but never to such an extent as after PVNO administration. Intracellular storing of VP-VA is probably also depending on the length of the polymer chain (Fresen and Weese, 1952).

Following subcutaneous injection of VP-VA the storing of the material takes predominantly place in the spleen. The organ increases in size and weight and the changes have even after one year not completely subsided. Principally shows the

storage of VP-VA in the spleen as to the location and duration a distinct similarity to other macromolecular substances (Bargmann, 1947; Fresen and Weese, 1952; Hübner, 1962) as e.g. Mohn (1960) could show for PVP and Grundmann (1967) for PVNO. The reticular cells of the spleen that have taken up VP-VA show electron microscopically membrane bound, empty appearing inclusions with granular dense aggregates on the inner surface.

In none of our experiments did we find during the one year period of observation any other morphological changes, especially no systemic diseases or tumors.

Literatur

Adamson, Y. R., Bowden, D. H.: The surface complexes of the lung. Amer. J. Path. **61**, 359–376 (1970)

Askin, F. B., Kuhn, C.: The cellular origin of pulmonary surfactant. Lab. Invest. **25**, 260–268 (1971)

Bargmann, W.: Über Milzveränderungen nach Zufuhr des Blutflüssigkeitsersatzes Periston. Virchows Arch. path. Anat. **314**, 162–166 (1947)

Bergmann, M., Flance, I. J., Blumenthal, H. T.: Thesaurosis following inhalation of hair spray. A clinical and experimental study. New Engl. J. Med. **258**, 471–476 (1958)

Bergmann, M., Flance, I. J., Cruz, P. T., Klam, N., Aronson, P. R., Joshi, R. A., Blumenthal, H. T.: Thesaurosis due to inhalation of hair spray. Report of twelve new cases, including three autopsies. New Engl. J. Med. **266**, 750–755 (1962)

Bertalanffy, F. D.: Respiratory tissue: Structure, histophysiology, cytodinamics — Part I, review and basic cytomorphology. Int. Rev. Cytol. **16**, 233–328 (1964)

Blessing, M. H., Lenz, W.: Pathologisch-anatomische Veränderungen der Rattenlunge nach endotrachealer Injektion von Methylsilikonöl. Int. Arch. Arbeitsmed. **31**, 277–293 (1973)

Bowden, D. H.: The alveolar macrophage. Curr. Top. Path. **55**, 1–36 (1971)

Bowden, D. H., Adamson, I. Y. R., Granzham, W. G., Wyatt, J. P.: Origin of the lung macrophage: evidence derived from radiation injury. Arch. Path. **88**, 540–546 (1969)

Brunner, M. J., Giovacchini, R. P., Wyatt, J. P., Dunlap, F. E., Calandra, J. C.: Pulmonary disease and hair-spray polymers: A disputed relationship. J. Amer. med. Ass. **184**, 851–857 (1963)

Draize, J. H., Nelson, A. A., Newberger, S. H., Kelley, E. A.: Inhalation toxicity studies of six types of aerosol hair-sprays. Proc. Sci. Sect. TGA No **31**, 28 (1959)

Edelston, B. G.: Thesaurosis following inhalation of hair spray. Lancet **1969II**, 112–113

Fetzer, J.: Speicherung von Polyvinylpyridin-N-oxid in Lysosomen. Naturwissenschaften **54**, 518–519 (1967)

Finley, T. N., Pratt, A. J., Ladman, A. J., Brewer, L., McKay, M. B.: Morphological and lipid analysis of the alveolar lining material in dog lung. J. Lipid Res. **9**, 357–365 (1968)

Freiman, D. G., Gall, E. A.: A staining method for the detection of polyvinylpyrrolidone (PVP) in tissue sections. Amer. J. clin. Path. **25**, 1427–1429 (1955)

Fresen, O., Weese, H.: Das gewebliche Bild nach Infusion verschiedener Kollidon-Fraktionen (Periston N, Periston, hochvisköses Periston) beim Tier. Beitr. path. Anat. **112**, 44–62 (1952)

Gil, J., Weibel, E. R.: Improvements in demonstration of lining layer of lung alveoli by electron microscopy. Resp. Physiol. **8**, 13–36 (1969/70)

Gil, J., Weibel, E. R.: Extracellular lining of bronchioles after perfusion-fixation of rat lungs for electron microscopy. Anat. Rec. **169**, 185–200 (1971)

Groniowski, J., Biczyskowa, W.: Structure of the alveolar lining film of the lungs. Nature (Lond.) **204**, 745–747 (1964)

Groniowski, J., Biczyskowa, W.: Surface acide mucopolysaccharides of the lung alveoli. IV. European Regional Conference on Electron Microscopy, Vol. II, ed. by D. S. Boccia-relli, p. 509–510. Rome: Tipografia Poliglota Vaticana 1968

Grundmann, E.: Experimentelle Untersuchungen über die zelluläre Speicherung des Polyvinylpyridin-N-oxids. Fortschritte der Staublungenforsch. (Hrsg. H. Reploh und H. J. Einbrodt), Bd. 2, S. 223–229. Münster 1967

Grundmann, E., Schlipköter, H.-W.: Über die Rückbildung experimenteller silikotischer Granulome unter Polyvinylpyridin-N-oxid. Verh. dtsch. Ges. Path. **53**, 367–370 (1969)

Harrison, G. A., Weibel, J.: The membranous component of alveolar exsudate. J. Ultrastruct. Res. **24**, 334–342 (1968)

Hilding, A. C.: Phagocytosis, mucous flow, and ciliary action. Arch. environm. Hlth **6**, 61–73 (1963)

Hübner, G.: Elektronenmikroskopische Untersuchungen zur Pinocytose der Leber nach Injektionen makromolekularer Substanzen. Frankfurt. Z. Path. **71**, 498–510 (1962)

Irmscher, G., Schulz, G.: Experimentelle Silicose bei Ratten nach einseitiger Phrenicus exhairese. Arch. Gewerbepath. **18**, 422–441 (1961)

Kilburn, K. H.: A hypothesis for pulmonary clearance and its implications. Amer. Rev. resp. Dis. **98**, 449–463 (1968)

Ludwig, E.: Zur Frage der Gesundheitsschädigung durch sogenannte Haarsprays. Parfümerie und Kosmetik **45**, 5–8 (1964)

Mohn, G.: Die Speicherung von Polyvinylpyrrolidon im Rattenorganismus, dargestellt durch den direkten fluoreszenzmikroskopischen Nachweis. Acta histochem. (Jena) **9**, 76–96 (1960)

Schaffner, F., Felig, P., Trachtenberg, E.: Structure of rat lung after protracted oxygen breathing. Arch. Path. **83**, 99–107 (1967)

Schepers, G. W. H.: Thesaurosis versus sarcoidosis. J. Amer. med. Ass. **181**, 635–637 (1962)

Schneeberger-Keeley, E. E., Karnovsky, M. J.: The ultrastructural basis of alveolar-capillary membrane permeability to peroxidase used as a tracer. J. Cell Biol. **37**, 781–793 (1968)

Schulz, H.: The submicroscopic anatomy and pathology of the lung. Berlin-Göttingen-Heidelberg: Springer 1959

Shorter, R. G., Titus, J. L., Divertie, M. B.: Cell turnover in the respiratory tract. Dis. Chest. **46**, 138–142 (1964)

Shorter, R. G., Titus, J. L., Divertie, M. B.: Cytodynamics in the respiratory tract of the rat. Thorax **21**, 32–37 (1966)

Simnett, J. D., Heppleston, A. G.: Cell renewal in the mouse lung. The influence of sex, strain and age. Lab. Invest. **15**, 1792–1801 (1966)

Prof. Dr. med. Manfred H. Blessing
Pathologisches Institut
der Universität Düsseldorf
D-4000 Düsseldorf
Moorenstraße 5
Federal Republic of Germany