

CANCEROGENIC EFFECT OF VINYL CHLORIDE

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The demonstration of oncogenic activity of some chemical organic compounds employed for the preparation of "plastics" has been widely investigated and the limits and the effectiveness of their oncogenic power have been well defined. The present investigation demonstrates that it is possible to obtain for some experimental models a marked carcinogenic response to a monomeric precursor, i.e. vinyl chloride, of plastic polymers which follows a peculiar pattern for different tissues and organs of the rat.

Twenty-six Wistar (AR/IRE) male albino rats, of the average weight of 150 gr. were exposed to vapours of vinyl chloride 4 hours a day for 5 days a week during 12 months. The animals were kept in a plastic, air-tight container through which there was a constant flow of air containing 3% in weight equal to 30,000 p.p.m., of vinyl chloride. At the end of treatment the surviving animals were killed at twenty-day intervals, and the most important tissues and organs were histologically examined by conventional methods. Twenty-five rats of the same strain were kept aside as a control. During the period of exposure the animals were slightly soporific, but the first few months of treatment were well tolerated and the rats showed no change in body growth or behavior and no organic alterations visible macroscopically or by radiological means. 65% of the animals developed tumors of the skin and 26% of these showed tumors of the respiratory tract exclusively localized in the lungs. Very few animals developed tumors in the bone and in this case the tumors were localized in the metaphysical region of the femurs, and it is worthy to mention that the femurs of both posterior limbs showed the carcinogenic transformation.

Tumors of the skin

The skin transformation which developed more frequently in the para-aural region, has been the epidermoid carcinoma, but we have seen also papillomas and rarely mucoepidermoid carcinomas.

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Tumors of the respiratory tract

Even if as far as the percentage is concerned these occur in a smaller number, their morphologic appearance is mainly of an adenocarcinoma; only in one rat it has been observed a tumor of the epidermoid type. Sometimes the tumor showed an early developmental stage and consisted predominantly of cubic or columnar cells arranged as regular or irregular tubular and papillary elements supported by poorly developed fibrous stroma.

Tumors of the bones

In the metaphysical region of the four limbs a large proliferation of cartilaginous tissue arises outwardly to the periosteum and seems to derive directly out of the osseous cortical by the growth of its cells. The cartilaginous growth is dishomogeneous as it is shown by the extension of finger-like prolongations into the boundary of neoformed tissue. Beside the chondroblastic, chondrocytic and angiomatous areas, manifestation of noticeable growth power, there are cartilaginous zones with regressive features, as fibrosis and hyalinosis.

The results reported above indicated that vinyl chloride is an effective carcinogenic agent for the rat. As for many carcinogenesis studies, where multiple tumors arise at different sites, in different tissues and organs, the data obtained need to be evaluated considering a more prompt positive response according to the ideal concentration of the carcinogenic. It is noteworthy to emphasize that the cutaneous system represents an impressive pattern of susceptibility to the vinyl chloride. Another point to be considered is that all the cutaneous tumors developed in the same site, i.e., the region including the area in which submaxillary and parotid gland are located.

No implications to human pathology can be extrapolated from the experimental model reported in this paper.

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PATHOLOGY OF VINYL CHLORIDE

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During the last years a syndrome characterized by alterations of the skeleton, teguments, nervous system and hepatic function^{1, 2, 3, 4, 5, 6} has been observed among the workmen associated with vinyl chloride polymerisation process. An experimental programme has been performed in order to evaluate the toxic effect of vinyl chloride on the animal organism, and in order to try to reproduce in rats the acroosteolytic lesions observed in man.

MATERIALS AND METHODS

Twenty-five Wistar male albino rats, of average weight 150 gr., were exposed to vapours of vinyl chloride 4 hours a day for 5 days a week during 12 months. The animals were kept in a plastic air-tight container through which there was a constant flow of air containing 3% in weight, equal to 30,000 p.p.m. of vinyl chloride. At the end of treatment the surviving animals were killed at twenty-day intervals, and the paws, brain, liver, kidneys and thyroid were examined histologically after fixation, inclusion and colouration by the ematoxylin-eosine. Van Gieson, PAS, Weighert, Bielschowsky-Gomori methods. Twenty five rats of the same breed were set aside as a control.

RESULTS

During the period of exposure the animals were slightly soporific, but the first few months of treatment were well tolerated and the rats showed no change in body growth or behaviour and no organic alteration visible macroscopically or by radiological means. After ten months some of the animals began to show a decrease in weight, in aggressiveness, in their reaction to external stimuli, and often a disturbed equilibrium. Thirteen animals died from cardio-respiratory complications. Two animals died from hemato-peritoneum. Most of the animals showed pathological involvement

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of the brain, liver, kidneys, and thyroid. Six rats showed histopathological alterations of the skeleton.

The small metatarsal bones showed an extensive periosteal proliferation of cartilage-like material apparently originating by metaplasia directly from the bone. All around the newly formed cartilage the cellular components were compressed and arranged tangentially to the cartilage itself, forming a perichondral layer. The isogenous cartilagenous groups were irregularly arranged and their nuclei were of varying size and often number per cell. The edges of the cartilage were irregular, showing digital extroflexions resulting from a varying growth potential. There was also evidence of chondroid metaplasia in areas of mature compact bone, in which the cartilaginous elements were grouped around a central nucleus of bone, as in typical osteochondromatous processes. In the small bones the chondroid metaplasia was often very extensive and the bones seemed to be impregnated with a mucoid substance which altered the characteristic disposition of the bone tissue obliterating the cement lines. The skin of the paws showed areas of hyperkeratosis superficial thickening of the epidermis, vacuolization and degeneration of the basal layer, disappearance of the cutaneous adnexa and modest accentuation of the papillar layer of the derma. An almost constant state of edema was observed in the epidermic zone. The connective tissue showed dissociation of the collagen bundles and a diminution and fragmentation of the elastic reticulum. The small arterial vessels showed signs of endothelial fibrosis with a thickening and dissociation of the elastic membrane, often accompanied by a partial hyaline degeneration and homogenization of the walls and proliferation of the endothelium. Some of the vessels appeared completely blocked by a proliferation of the connective tissue in a formation not unlike that of onion skin.

The small nerve bundles were often surrounded and invaded by fibrotic processes. A microscopic examination of the brain revealed diffuse degenerative lesions of the grey and white matter. The external granular layer was often reduced and in some areas this was true also the medium sized and giant cells. Numerous examples were found of hypercoloured, atrophic neurons with disappearance of the nucleoli and sometimes also of the nucleus itself. At the level of the white matter there were zones of intense, reactive gliosis consisting for the most part of cytoplasmic and fibrillar astrocytes, some of which were arranged in a concentric disposition around the blood vessels. There was evidence of neuronal phagokaryosis with satellitosis and the deposition of neuroglial elements around the altered nerve cells.

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There was modest proliferation of microglial elements, especially in areas of more intense gliosis and zones of intense spongiosis showing an accumulation of astrocyte-type elements with basophilic cytoplasm and abounding in gemistocytic and microglial elements similar in appearance to "scavenger" cells. The cerebellum showed signs of atrophy of the granular layer which was often represented only by a few isolated nuclei. The layer of Purkinje cells was in some places profoundly modified, the cells appearing to be undergoing intense degeneration and all the various stages of this process were observed, from the whole cell to the small homogeneous bodies with indistinct outlines which represent the most damaged form of the cell. Except for small and limited areas, the molecular layer seemed to be well preserved.

The liver was often of increased volume, sometimes subicteric in colour, with a smooth surface and somewhat more brittle consistency than normal. A histological examination showed in the majority of cases signs of diffused interstitial hepatitis with numerous hepatic cells in the grip of regressive processes varying from torbid degeneration of the cytoplasm to necrosis with marked cytoplasmic and nuclear polymorphism. Some of the hepatocytes showed giant or double nuclei. In these areas of cellular necrosis a corpuscular exudation was always present consisting of round cellular elements of the lymphocyte or plasmacellular type. There was evidence of an abnormal proliferation of the Kupffer's cells, which were often hypertrophic. The triangular spaces appeared to be increased by the infiltration of round and polynucleated cells and eosinophiles showing marked polymorphism. The portal capillaries, centrolobular veins and sinusoids were often blocked by numerous centrally-located areas of partial necrosis, often accompanied by a diffuse steatosis in the form of small drops with clearly-defined areas of acidophile necrosis and the formation of elements similar to the round acidophile bodies of viral hepatitis and the "Councilman" bodies of yellow fever. In certain cases evidence was found of an intense fibrosclerotic reaction alongside the degenerative processes.

The kidneys did not appear to be excessively altered by the prolonged treatment with vinyl chloride, although they showed signs of tubulonephrosis, sometimes accompanied by chronic interstitial nephritis.

The thyroid often showed a colloid goitre and a marked increase in parafollicular cells.

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The control animals showed no histopathological alterations of the skeleton or of the various organs.

CONCLUSIONS

This research confirms that the animals are sensitive to the toxic action of vinyl chloride. The lesions of the bone and connective tissue are similar to those noticed in experimental osteo-latyrisms and to those described in a man affected with acroosteolysis of the hands (Marin). It should be pointed out that the roentgenological findings of skeleton in man rests on cartilaginous transformation of the bone tissue. Therefore according to recommendation made at XVI International Congress on Occupational Health of Tokyo (1969) I should like some precautions to be taken in the manufacturing plants polymerizing vinyl chloride, such as reduction of the threshold limit value of monomer, and substitution of manual cleaning of autoclaves by automatic devices.

SUMMARY

The results of this investigation show that animals exposed to vapours of vinyl chloride may show degeneration of the skeleton and connective tissue with histopathological pictures similar to those observed in human acroosteolysis of the hands. The bones in fact undergo processes of intense periosteum growth and diffuse, chondroid metaplasia. The connective tissue is dissociated into collagen bundles with a reduced number of cells, the elastic reticulum is markedly reduced and fragmentary, the small vessels of the deep layers of the derma show hypertrophy of the walls with a diminution and sometimes obliteration of the lumen, the nerve endings are surrounded and infiltrated by fibrous tissue. Degenerative processes of the parenchyma were observed in the brain, in the liver and in the kidneys.

NOTE: Figures on available copy were not suitable for reproduction. They are as follows:

Fig. 1 -- Periosteal proliferation of metatarsal bone of a rat 16th months old, exposed to vinyl chloride vapours for 12 months

Fig. 2 -- Periosteal proliferation of metatarsal bone of a rat, 16th months old, exposed to vinyl chloride vapours for 12 months

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Fig. 3 -- Periosteal proliferation of metatarsal bone of a rat, 16th months old, exposed to vinyl chloride vapours for 12 months.

Fig. 4 -- Chondroid metaplasia in mature bone of metatarsus of a rat, 16th months old, exposed to vinyl chloride vapours for 12 months

Fig. 5 -- Chondroid metaplasia in mature bone of metatarsus of a rat, 16th months old, exposed to vinyl chloride vapours for 12 months

Fig. 6 -- Chondroid metaplasia in mature bone of metatarsus of a rat, 16th months old, exposed to vinyl chloride vapours for 12 months

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Oncogenic Response of Rat Skin, Lungs, and Bones to Vinyl Chloride¹

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SUMMARY

Rats (Ar/IRE Wistar strain) exposed for 12 months to vapors of vinyl chloride developed tumors of the skin, lungs, and bones. The cutaneous tumors, which always appeared in the area in which submaxillary and parotid glands are located, have been histologically recognized as epidermoid carcinomas, papillomas, and mucoepidermoid carcinomas. The morphological characteristics of lung tumors, which occurred in a lower percentage, were mainly of the adenocarcinoma type, with the exception of a single epidermoid tumor originating from the epithelial covering cells. In a minor number of rats, a large proliferation of cartilaginous tissue diagnosed as osteochondroma developed in the metacarpal and metatarsal regions of the four limbs.

INTRODUCTION

The oncogenic properties of some chemical organic compounds used for the preparation of "plastics" have been widely investigated, and their limits and effectiveness have been well established. Detailed information on this subject may be found in the literature (5, 6, 8); however, all information and references are exclusively related to highly polymerized compounds of roughly the same size as used in various industries.

Oncogenic polymers produce sarcomatous tumors, with the exception of polyurethans which, as reported by Hueper (6), also induce adenocarcinomas. Recently, it has been demonstrated that sarcomatous tumors (1-4, 7) are transplantable, and it has been suggested that they may originate from the tissues of the capsule that gradually covers the plastic film. Thus, Brand *et al.* (2-4) have observed premalignant areas made of poorly-differentiated fibroblasts which were firmly attached to the plastic film up to the moment of malignant transformation.

This investigation demonstrates that even the monomer vinyl chloride possesses oncogenic properties when used with an appropriate model different from the models previously reported by several authors (9-12). The carcinogenic response

to vinyl chloride follows a singular pattern for the different tissues and organs of the rat.

MATERIALS AND METHODS

The experiments were performed with vinyl chloride ($\text{CH}_2=\text{CHCl}$, the monohalogenate derivative of ethylene) of a commercial grade (99% purity) assumed to contain insignificant amounts of various noncarcinogenic contaminants.

Three-month-old Wistar (Ar/IRE) male albino rats (about 150 g body weight) were exposed to vinyl chloride vapors for 4 hr a day, 5 days a week, for 12 months. The animals were kept in metal or plastic, air-tight cages in which a constant flow of air, containing 3% v/v (equal to 30,000 ppm) of vinyl chloride, was introduced. Twenty-five rats of the same strain were the control group. At the end of the treatment, the surviving animals were killed at 20-day intervals, and the most important tissues and organs were examined histologically by standard methods. During the period of exposure, the animals were slightly soporific; however, the first few months of treatment were well tolerated and no changes in growth or behavior were noticed. After 10 months of treatment, some animals began to show a hard mass in the parauricular region which became progressively larger until it reached the size of a walnut or slightly larger. In most cases, the swelling was unilateral; it was bilateral in only a few animals. After 1 or 2 months, the growing masses became ulcerated and discharged necrotic debris, while a certain amount of tumorous tissue began to form on their surfaces. In addition, we observed that the masses were an integral part of the parauricular region and could not be distinguished from the local tissues. Caseous necrotic zones were found. Pleura and pericardium often showed diffuse inflammation of a fibrous nature and, in many cases, the lungs were covered with a number of white formations as large as grains of rice or even larger and harder than the lungs themselves. In 2 cases, the lungs were hemorrhagic with milky, thick fluid in the pleural cavities. The liver was sometimes increased in size and very fragile. The animals were subjected to X-ray analyses at different intervals from the beginning of treatment in order to control the state of the skeletal bones.

All the animals that inhaled vinyl chloride showed a series of parenchymal lesions. Among these, most prominent were the disappearance of granular and Purkinje cells, degeneration

¹ A preliminary report of the results reported here was given at the Tenth International Cancer Congress, Houston, Texas, May 22-24, 1970.

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of the cerebellum, severe chronic hepatitis, interstitial pneumonia, and moderate swelling of the kidney parenchyma, often assuming the pattern of tubulonephrosis.

RESULTS

The rates of survival and the main findings are summarized in Table 1. Almost all the animals developed tumors of the skin and lungs. Very few animals developed bone tumors; in these cases, the tumors were localized in the metacarpal and metatarsal bones of all 4 extremities. Skin tumors were by far the most frequent, amounting to 65 or 70%.

Skin Tumors. The tumor that developed most frequently in the paraauricular region was the epidermoid carcinoma, but we also observed papillomas and rarely mucoepidermoid carcinomas. In all cases, the neoplasms were of epithelial nature. The 3 patterns noticed cannot be compared to various histotypes but rather to a transition of one type into the other or, and which is more likely, to different stages of the same proliferative type.

Warty subauricular growths occurred in some rats. The histological picture showed papillar epithelial proliferation, with progressive increase in the thickness of the epidermis (Fig. 1).

The papillary warts of exophytic type had a fibrous vascular stroma and various degrees of inflammatory lymphocytic infiltration, marked hyperkeratosis, parakeratosis, acanthosis, and areas of individual dyskeratosis or pearl-like horny formations.

The epithelial cells of the penetrating columns (Fig. 2) were irregularly arranged and were frequently accompanied by an inflammatory infiltration of the dermis. The typical features of the Malpighian layer and of the stratum corneum of the prickle cells and of germinal layers were easily recognized (Fig. 3). The horny layer was composed largely of cell nests, which appeared at certain points within the epithelial masses and assumed the well-known appearance of "horny pearls" (Fig. 3, arrows); they were made of flattened and compressed prickle cells without nuclei and were located around a central core of keratin.

Table 1
Oncogenic effects of inhaled vinyl chloride as a function of time
The animals were exposed to vinyl chloride vapors for 4 hr a day, 5 days a week, for a total of 12 months, in a constant flow of air containing 3% v/v vinyl chloride.

Rat	Survival rate	Tumors		
		Skin	Lungs	Bones
1	300	Mucoepidermoid carcinoma	Adenoacanthoma	Osteochondroma
2, 3	280-300	Epidermoid carcinoma, keratinizing type	No tumor	No tumor
4	310	Epidermoid carcinoma, keratinizing type	Adenocarcinoma	No tumor
5	333	Papilloma, keratotic type	No tumor	Osteochondroma
6	337	Epidermoid carcinoma	No tumor	Osteochondroma
7	347	Epidermoid carcinoma	No tumor	No tumor
8	347	Mucoepidermoid carcinoma	No tumor	Osteochondroma
14	354	Epidermoid carcinoma	No tumor	No tumor
16, 17	359	Epidermoid carcinoma	Adenocarcinoma	No tumor
21	380	Epidermoid carcinoma	No tumor	No tumor
22	380	Epidermoid carcinoma	Adenocarcinoma	Osteochondroma
23	380	Epidermoid carcinoma	No tumor	No tumor
24	380	Epidermoid carcinoma	Mucus-producing adenocarcinoma (alveolar cell carcinoma?)	No tumor
25	380	Epidermoid carcinoma	No tumor	No tumor
26	380	Epidermoid carcinoma	Squamous cell carcinoma	No tumor

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Keratinization was irregular and often parakeratosis was observed in the areas of the tumor in which the cells were loosely aggregated and undergoing an individual rather than a collective type of keratinization (Fig. 4). In dyskeratotic areas, a few epithelial cells tended to form pearls. The tumor seldom showed an undifferentiated growth with cellular pleomorphism and several mitotic figures (Fig. 5). A few tumors showed little nests of isolated pale cells (Figs. 6 and 7) of 3 types: mucin-producing cells (originating from the duct epithelium of sweat glands or salivary glands); squamous cells; and intermediate cells with minor tendencies towards differentiation.

Respiratory Tract. These tumors, although occurring rarely, were mainly adenocarcinomatous. Only in 1 rat did we observe an epidermoid tumor originating from the epithelium-covering cells. Sometimes, the tumors were seen in their early development and consisted predominantly of cubic or columnar cells arranged as regular or irregular tubular and papillary elements (Fig. 8) and supported by poorly developed fibrous stroma (Fig. 9).

In other cases, glandular structures were often imperfectly formed and appeared as sheet-like proliferations of undifferentiated or pleomorphic character. The cells showed a tendency to extend into the pulmonary parenchyma, thus simulating the microscopic features of the so-called alveolar cell carcinoma (Fig. 10). Sometimes, this was the prevailing pattern. The pulmonary air sacs were limited by 1 or more layers of cubic, columnar, or polyhedral cells with abundant cytoplasm that was faintly eosinophilic (Fig. 11), frequently, adenopapillary excrescences spreading into the alveolar spaces were present.

In some areas, foci of cellular polymorphism with hyperchromatic nuclei were noticed; mucin was produced in varying amounts and secreted into the lumen of the tubules and acini. There were small pools of mucin in which signet ring cells occurred, either alone or in small clusters (Fig. 12). The alveolar walls were often quite thick and, at times, showed an inflammatory infiltration.

As mentioned before, a single tumor showed squamous structures and appeared to have been formed mainly by spindle and oval undifferentiated cells (Fig. 12).

Bones. In the metacarpal and metatarsal regions of the 4 limbs, a large proliferation of cartilaginous tissues arose outward from the periosteum and, from the appearance of its cells, seemed to derive directly from the cortical bone (Fig. 14). The periosteum also grew and in some areas spread as finger-like prolongations into the newly formed cartilage (Fig. 15). In these places, a gradual transition between fibrous cartilage, periosteum, and bone could be noticed.

The newly formed cartilage appeared irregular with atypical areas; the cells, which had nuclei larger than the normal chondrocytes, lay in well-formed, capsulated lacunae. The cells occurred singly, in pairs, or in tetrads and, although of different size and shape, they usually contained a single, darkly stained nucleus. The cartilaginous growth was not homogeneous, as shown by the extension of the finger-like prolongations into the boundary of newly formed tissue. Besides the chondroblastic, chondrocytic, and angiomatous areas which indicated a rapid growth, there were also

cartilaginous zones possessing regressive features, such as fibrosis and hyalinosis. The perichondrium appeared often as a compressed and structurally altered fibrous tissue.

In some cases, the tumor growth was related to the stage of the endochondral ossification occurring below the "epiphysal plate." Ossification was irregular, so that osseous trabeculae varied greatly in thickness and contours. In other cases, foci of active cellular proliferation, cartilaginous areas, chondroid developmental nests, and calcified bones occurred in the deeper portions of the trabeculae (Fig. 18).

The new formation appeared to be an osteochondroma and consisted essentially of a bony protuberance capped by cartilage and a fibrous layer, which represented the perichondrium.

The fibrous layer was continuous with the periosteum of the adjacent cortical bone and extended inward to form septa separating and enclosing lobules of cartilage.

Control Animals. The control rats were kept under the same conditions as the experimental rats and at the appropriate time were subjected to a constant flow of air without vinyl chloride. None of these animals developed tumors or the types of parenchymal lesions developed by the rats that inhaled vinyl chloride. In a very few of the control rats, there was a swelling of the liver and kidneys.

DISCUSSION

As in many carcinogenesis studies in which multiple tumors arise in different tissues and organs, the data must be evaluated in terms of a more prompt positive response to be obtained with the ideal concentration of the carcinogenic compound. The cutaneous system is the most susceptible to the oncogenic effects of vinyl chloride.

Our experimental data do not explain why the cutaneous tumors developed in the same site, i.e., the region including the area in which the submaxillary and parotid glands are located. It is possible that the salivary glands may be involved in the concentration or excretion of vinyl chloride or some of its active decomposition products. This hypothesis is strongly supported by morphological findings showing the specific tendency of the developed tumor to appear as a mucoepidermoid carcinoma, which indicates the active contribution of the mucus glandular cells to the tumorous growth. Such kinds of histotypes are often related to some glandular aggregates, and, therefore, the histogenesis of human mucoepidermoid carcinoma has been restricted to the cells of intercalated ducts. This hypothetical interpretation must be confirmed by future experiments in which the action of the vinyl chloride should be restricted to the salivary system. At the concentrations used, saturation and wetting of the fur might well be expected. Under these circumstances, the natural cleansing habit of the rat might add a significant ingestion problem with subsequent concentration of vinyl chloride in the salivary glands. The local extreme concentration could be the result of the difficulty of a complete cleansing by the rat.

The neoplastic response of the lower respiratory tract, although of lesser magnitude, is of relevant interest, since some

of the tumors were morphologically similar to those described for the skin. The hypothesis that mucus-producing cells may be capable of retaining vinyl chloride or its decomposition products seems to be relevant once more. Obviously, a different interpretation is required for the pathogenesis of the bone tumors and for their simultaneous incidence at the level of all 4 limbs.

REFERENCES

1. Banerjee, M., and Bates, R. Prevalence of Heteroploidy in Plastic Film-induced Primary Sarcomas. *Brit. J. Cancer*, 20: 555-563, 1966.
2. Brand, G., and Buoen, L. Polymer Tumorigenesis: Multiple Preneoplastic Clones in Priority Order with Clonal Inhibition. *Proc. Soc. Exptl. Biol. Med.*, 128: 1153-1157, 1968.
3. Brand, G., Buoen, L., and Brand, I. Malignant Transformation and Maturation in Non-dividing Cells during Polymer Tumorigenesis. *Proc. Soc. Exptl. Biol. Med.*, 124: 675-678, 1967.
4. Brand, G., Buoen, L., and Brand, I. Carcinogenesis from Polymer Implants: New Aspects from Chromosomal and Transplantation Studies during Premalignancy. *J. Natl. Cancer Inst.*, 39: 663-679, 1970.
5. Druckrey, H. Experimentelle Beiträge zum Mechanismus der Carcinogenen Wirkung. *Arzneimittel-Forsch.*, 1: 383-395, 1951.
6. Hueper, W. C. Cancer Induction by Polyurethan and Polysilicone Plastics. *J. Natl. Cancer Inst.*, 33: 1005-1027, 1964.
7. Johnson, K., Buoen, L., Brand, I., and Brand, G. Polymer Tumorigenesis: Clonal Determination of Histopathological Characteristics during Early Preneoplasia: Relationships to Karyotype, Mouse Strain, and Sex. *J. Natl. Cancer Inst.*, 44: 783-793, 1970.
8. Kegan, A. Experimental Blastomogenesis Caused by Synthetic Polymers (Plastics). *Meditsina, Moscow*, 1: 215-237, 1965.
9. Mastromatteo, E., Fisher, A. M., Christi, H., and Danziger, D. Acute Inhalation Toxicity of Vinyl Chloride to Laboratory Animals. *Am. Ind. Hyg. Assoc. J.*, 21: 394-397, 1960.
10. Torkerson, T. R., Oyen, F., and Rowe, V. K. The Toxicity of Vinyl Chloride as Determined by Repeated Exposure of Laboratory Animals. *Ind. Hyg. J.*, 354-361, October 1961.
11. Van Duren, B. L., Sivak, A., Goldschmidt, B. M., Katz, C., and Melchionne, S. Carcinogenicity of Halo-Ethers. *J. Natl. Cancer Inst.*, 43: 481-486, 1969.
12. Viola, P. L. Pathology of Vinyl Chloride. 16th International Congress of Occupational Health. (Communication No. 38), Tokyo, 1969.

Photomicrographs of the original article are not being distributed with this copy because of the poor quality of their reproduction.

- Figs. 1 to 18. All sections were stained with H & E.
- Fig. 1. Squamous cell papilloma of the skin. X 25.
- Fig. 2. Transitional stage from papilloma to infiltrative type of cancer. X 80.
- Fig. 3. Finger-like trabeculae branch to form secondary processes showing horny pearl formation (arrows). X 25.
- Fig. 4. Dyskeratotic area. Few epithelial cells with a tendency to form whorls. Little capacity to develop into horny pearls. X 250.
- Fig. 5. Atypical cells partly lacking prickles and showing considerable variation in size, shape and many mitotic figures. X 250.
- Fig. 6. Mucoepidermoid skin tumor. Occurrence of hydropic epidermoid cells, basal cells, columnar cells, and oxyphilic cells in various proportions. X 100.
- Fig. 7. Mucoepidermoid skin tumor, showing differentiation of masses of squamous epithelium from columnar cells, lining and proliferating into distended tubular spaces. X 100.
- Fig. 8. Micronodular adenocarcinoma of the lung arising from a segmental bronchus. X 25.
- Fig. 9. Detail of Fig. 8 showing tubular aggregates made up by cells with hyperchromatic nuclei.
- Fig. 10. Nodular bronchiolar alveolar mucus-secreting adenocarcinoma. X 100.
- Fig. 11. Detail of Fig. 10, showing an alveologenic pattern. X 250.
- Fig. 12. Mucus-producing cells with hyperchromatic and pleomorphic nuclei and occurrence of signet ring cells. X 250.
- Fig. 13. Squamous cell carcinoma of the lung. X 100.
- Fig. 14. Osteochondroma developing as a protuberance from the small bones of metacarpus and metatarsus. X 25.
- Fig. 15. Magnification of Fig. 14. The protuberance consists of bone trabeculae, capped by cartilage, and a fibrous layer functioning as perichondrium. X 100.
- Fig. 16. Osteochondroma. Cartilaginous growth characterized by finger-like proliferation. X 100.
- Fig. 17. Osteochondroma. Area of endochondral ossification. X 100.
- Fig. 18. Cellular area of an osteochondroma showing the transition from cartilage to osteoid. X 100.

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