Keratinization was irregular and often parakeratosis was observed in the areas of the tumor in which the cells were loosely aggregated and underwent 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 compressed and structurally altered fibrous tissue.

In some cases, the tumor growth was related to the stage of the endochondral ossification occurring below the "epiphyseal 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 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 tumor growth. Such kinds of histotypes are often related to the glandular aggregates, and, therefore, the histogenesis of this 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 vinyl chloride should be restricted to the salivary system. In the concentrations used, saturation and wetting of the [illegible] might well be expected. Under the [illegible] natural cleansing habit of the rat might add a slight ingestion problem with subsequent concentration of vinyl chloride in the salivary glands. This local concentration could be the result of the difficulty to complete cleansing by the rat.

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

the tumors were morphologically similar to those described in 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.

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Figs. 1 to 18. All sections were stained with H & E.

Fig. 1. Squamous cell papilloma of the skin. × 25.

Fig. 2. Transitional stage from papilloma to infiltrative type of cancer. × 80.

Fig. 3. Finger-like trabeculae branch to form secondary processes showing horny pearl formation (arrows). × 25.

Fig. 4. Dyskeratotic area. Few epithelial cells with a tendency to form whorls. Little capacity to develop into horny pearls. × 250.

Fig. 5. Atypical cells partly lacking prickles and showing considerable variation in size, shape and many mitotic figures. × 250.

Fig. 6. Mucoepidermoid skin tumor. Occurrence of hydropic epidermoid cells, basal cells, columnar cells, and eosinophilic cells in various proportions. × 100.

Fig. 7. Mucoepidermoid skin tumor, showing differentiation of masses of squamous epithelium from columnar cells, lining and proliferating into dilated tubular spaces. × 100.

Fig. 8. Microadenocarcinoma of the lung arising from a segmental bronchus. × 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. × 100.

Fig. 11. Detail of Fig. 10, showing an alveolopapillomatic pattern. × 250.

Fig. 12. Mucus-producing cells with hyperchromatic and pleomorphic nuclei and occurrence of signet ring cells. × 250.

Fig. 13. Squamous cell carcinoma of the lung. × 100.

Fig. 14. Osteochondroma developing as a protuberance from the small bones of metacarpus and metatarsus. × 25.

Fig. 15. Ossification of Fig. 14. The protuberance consists of bone trabeculae, capped by cartilage, and a fibrous layer functioning as perichondrium. × 100.

Fig. 16. Osteochondroma. Cartilaginous growth characterized by finger like proliferation. × 100.

Fig. 17. Osteochondroma. Area of endochondral ossification. × 100.

Fig. 18. Cellular area of an osteochondroma showing the transition from cartilage to osteoid. × 100.