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MANUFACTURING CHEMISTS ASSOCIATION

1825 CONNECTICUT AVENUE, N.W. WASHINGTON, D.C. 20009 (202) 483-6128

March 21, 1975

TO: Technical Task Group on Vinyl Chloride Research

SUBJECT: Items related to Vinyl Chloride

Attached for your information are the following:

1. A copy of the paper presented by Dr. Gordon of Industrial BIO-TEST Laboratories at the March 5 meeting of the International Academy of Pathology in New Orleans entitled "Comparison of the Morphologic Features of Hepatic Angiosarcoma in Man and Rodents Following Prolonged Exposure to Vinyl Chloride."

2. A news release on the availability of a new book entitled "The Determination of Vinyl Chloride - A Plant Manual," published by the Chemical Industries Association Limited, Alembic House, 93 Albert Embankment, London SE1 7TU

Sincerely,

Milton Freifeld

Milton Freifeld, Project Manager

MF/etv

Attachments _

Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

TOXICOLOGY
ENVIRONMENTAL SCIENCES
CHEMISTRY
PLANT SCIENCES
MEDICAL SCIENCES

AREA CODE 312
TELEPHONE 272-3030

March 11, 1975

Mr. Milton Freifield
Manufacturing Chemists Association
1825 Connecticut Avenue, N. W.
Washington, D. C. 20009

Dear Mr. Freifield:

Enclosed is a copy of the full text of the paper which I presented on March 5, 1975, at the International Academy of Pathology meeting in New Orleans. As I indicated, this particular scientific session was arranged in cooperation with the Society of Pharmacological and Environmental Pathologists (SPEP) and the proffered papers were submitted and presented by members of this organization. Most of the papers dealt with chemically-induced carcinogens, their morphologic features and diagnosis.

I am also enclosing a copy of the abstract of my paper as it appeared in the program (Abstracts of Papers) and also appeared in Laboratory Investigation, Vol. 32, No. 3, 1975.

The paper was well received. Dr. Squire, Head of the Tumor Pathology Branch at the National Cancer Institute in Bethesda, Maryland, informed me that Dr. Maltoni plans to visit their facility in early April to present his pathologic findings to date. I have been invited and plan to attend unless MCA has some objections.

Sincerely,

D. E. Gordon

Donovan E. Gordon, D. V. M., Ph. D.
Section Head, Pathology

DEG:gz

cc: Dr. J. C. Calandra
Dr. M. L. Keplinger

MANUSCRIPT FOR PRESENTATION AT THE INTERNATIONAL ACADEMY OF PATHOLOGY MEETING ON MARCH 5, 1975, IN NEW ORLEANS, ENTITLED "COMPARISON OF THE MORPHOLOGIC FEATURES OF HEPATIC ANGIOSARCOMA IN MAN AND RODENTS FOLLOWING PROLONGED EXPOSURE TO VINYL CHLORIDE." D. E. Gordon, L. B. Thomas, J. C. Calandra, H. Popper and G. Kent - Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois; National Cancer Institute, Bethesda, Maryland; Northwestern University Medical School, Chicago, Illinois; Mount Sinai School of Medicine, New York, New York; and Northwestern Memorial Hospital, Chicago, Illinois.

Ladies and Gentlemen:

Vinyl chloride monomer (VCM) is a gas at ambient temperature and atmospheric pressure. It is made by several processes that involve the chlorination of ethylene or acetylene. VCM is used primarily in the manufacture of polyvinyl chloride (PVC), a resin which serves as the parent compound for a number of plastics.

In May of 1970, Dr. Viola, from Italy, presented a paper at the 10th International Cancer Congress in Houston, Texas, on the carcinogenic effects of vinyl chloride (VC) in rats. He reported tumors in the skin (para-aural carcinomas), lung (epidermoid and adenocarcinomas) and bone (osteochondromas) of Wistar rats that had been exposed to 30,000 ppm of VC vapor for 4 hours a day, 5 days a week, for 10 months. This was probably the earliest report of a carcinogenic effect of VC in experimental animals. His study was originally designed in an attempt to develop an animal model for acroosteolytis, a bone disease that was reported in the early 1960's among VCM and PVC workers.

In view of Dr. Viola's findings, inhalation studies in rats were subsequently initiated in Bologna, Italy, by Dr. Cesare Maltoni in which vapor concentrations of 50 to 10,000 ppm were investigated. Early in 1973, it was disclosed that angiosarcomas of the liver and tumors in other organs (Zymbal's gland tumor of the ear canal and Nephroblastomas of the kidney) had been seen in these animals. At that time, liver tumors had not been reported in rats at exposures of 50 ppm of VC. In view of these findings by Drs. Viola and Maltoni, a vapor inhalation study with VCM in mice, rats and hamsters was started in September of 1973 at Industrial BIO-TEST Laboratories, at the voluntary request of the Manufacturing Chemists Association (MCA) which represents a group of 31 companies in the United States involved in the production of VCM or PVC. The study was designed, by this group, as a life-span study in each of these species to complement Dr. Maltoni's study. The exposures selected were 50, 200 and 2,500 ppm of VC vapor with exposures 7 hours a day, 5 days a week. Two hundred of each species and each exposure level were used, for a total of 600 animals per exposure level. Preliminary tumor data from animals on this study were reported at the end of the first 8 months when liver angiosarcomas appeared in mice that had died at the 50 ppm and higher levels. This preliminary data was presented on April 15, 1974, to representatives of the Environmental Protection Agency (EPA), Occupational Safety and Health Administration (OSHA) and the National Institute of Occupational Safety and Health (NIOSH).

Meanwhile, the first 3 fatal cases of liver angiosarcomas among vinyl chloride workers in the United States had been reported in January of 1974. With the identification of this rare tumor in humans and experimental animals, there rapidly resulted a national interest in this problem. Dr. Louis Thomas, at the National Cancer Institute in Bethesda, Maryland, and Dr. Hans Popper, who was at that time a Fogarty Scholar at the National Institute of Health, were asked to review all the angiosarcoma cases among workers in the vinyl chloride industry. In September of 1974, I met with Drs. Thomas and Popper to review slides from both the human and mouse angiosarcoma cases. At that time, Dr. Thomas had tumor material from 14 or 15 industrial workers. Slides of human angiosarcoma cases of unknown etiology from Dr. Godfrey Kent at Northwestern Memorial Hospital in Chicago were subsequently reviewed.

Slide #1.

Gaseous Vinyl Chloride-Induced Hepatic Angiosarcomas in
- 15 workers in Polymerization Plants
- Rodent Experiments

Does Similar Morphologic Evaluation in Man and Mice Enforce Causal Relation and Permit Differentiation From Angiosarcomas of Unknown Etiology?

Within 8 Months of Exposure - Hepatic Angiosarcomas Seen in
- 2/200 Mice at 50 ppm
- 11/200 Mice at 200 ppm
- 28/200 Mice at 2,500 ppm

The purpose, then of this presentation is to present some of the comparative,

early developmental and morphologic features of the human and rodent angiosarcomas that have been studied thus far. In this slide, preliminary tumor data at 8 months from our mouse study revealed an exposure-related relationship in the incidence of grossly visible liver tumors that were later verified histologically as angiosarcomas. The first hepatic angiosarcoma appeared at 6 months in 1 animal at the 2,500 ppm level that had died.

The gross appearance of these tumors in man and animals was similar. The livers were frequently enlarged and contained solitary to multiple dark red circumscribed or nodular foci. The tumor foci in mice varied in size from 1 mm to 3 cm in diameter and the cut surface contained blood-filled cystic spaces associated with necrotic foci. The tumors usually extended up to and involved the capsular surface of the liver. In most of the animals with liver tumors, there was blood present in the peritoneal cavity that resulted from hemorrhages at the capsular surface of these tumors. Internal hemorrhage was one of the major causes of death in these animals.

Slide #2.

This is a section of human liver in which there was an angiosarcoma in another portion. The earliest lesion that Drs. Thomas and Popper have been able to detect, and have reported, is this irregular focal sinusoidal dilatation without necrosis of hepatocytes.

Slide #3.

This is just a higher magnification of the previous field to show a few enlarged sinusoidal lining cells.

Slide #4.

In the human cases, there is a characteristic, progressive portal tract and capsular fibrosis present throughout the liver that can be seen in this Aniline Blue-stained section in which collagen and reticulin fibers stain blue. This feature has not been observed in liver sections from our VC-exposed animals. However, the liver of rodents (rats, mice, hamsters) normally contain less connective tissue within the portal tracts and lobules when compared to the liver of man.

Slide #5.

This is the earliest lesion in the mouse which again is focal sinusoidal dilatation.

Slide #6.

At higher magnification, some of the lining cells appear to be slightly increased in size. There is also focal hypertrophy of hepatocytes in these areas.

Slide #7.

There is also a focal hypertrophy of hepatocytes in areas without sinusoidal dilatation and some of these hepatocytes are binucleated.

Slide #8.

The next stage in progression of both the mouse and human lesions is the formation of small to large blood-filled cavities or areas of peliosis. This section is from a mouse. The larger lesions are usually located just beneath the capsule and, microscopically, they are frequently early or advanced lesions of angiosarcoma.

Slide #9.

At higher magnification of such a peliotic focus, the neoplastic process can be seen extending up to the capsule. Rupture of the capsule at these sites may result in fatal internal hemorrhage. In addition to fresh blood, these peliotic foci contain large fibrin thrombi which are not present in this field.

Slide #10.

A slightly higher magnification of the angiosarcoma reveals papillary projections of liver cords extending into the peliotic space which are enveloped by neoplastic lining cells. In some areas of these tumors, there is slight widening of the Space of Disse. However, this feature is not as prominent nor as frequent as seen in man. There is also hypertrophy of the entrapped hepatocytes which later undergo necrosis.

Slide #11.

In other areas of the tumor, there are solid sheets of neoplastic cells that have replaced the liver cords.

Slide #12.

This is the counterpart of the above liver angiosarcoma in man where we again see a papillary projection of liver cells extending into a blood-filled space that is covered by neoplastic lining cells. There is a prominent separation of the Space of Disse which contains a non-neoplastic proliferation of different types of cells including macrophages, lipocytes and fibroblasts.

Slide #13.

This is another area of the same tumor to show the presence of intracannicular bile thrombi in hepatocytes due to stasis of bile flow.

Slide #14.

This Aniline Blue-stained section shows an increase in perisinusoidal connective tissue. Eventually, with destruction of the entrapped hepatocytes, there is extensive fibrosis in the area of the angiosarcoma.

Now, I would like to briefly show you a series of slides of the developmental lesion and angiosarcoma in rats.

Slide #15.

Again, this is the initial lesion, an irregular focal sinusoidal dilatation without necrosis of hepatocytes. This lesion seems to occur more frequently in the subcapsular region of the liver lobes.

Slide #16.

At higher magnification, there is hypertrophy of a few endothelial cells and some nuclei contain mitotic figures.

Slide #17.

We now see hyperplasia and hypertrophy of the lining cells which have enveloped most of the liver cords. Some of these cells are clearly neoplastic.

Slide #18.

The hepatocytes in the immediate vicinity of the tumor have now been replaced by these sarcomatous lining cells.

Slide #19.

A few large pleomorphic tumor cells can be seen in the lumen of one vessel as well as in and along the margin of the tumor.

Slide #20.

In this trichrome-stained section, there is an absence of collagenous connective tissue in the stroma of the tumor.

Slide #21.

In this section, tumor cells can be seen within a portal tract vessel adjacent to a bile duct.

Last Slide.

Common Features of Human and Mouse Angiosarcomas Support Etiology and Epidemiology:

1. Multiple, Focal, Sinusoidal Dilatation
2. Blood-filled Sinusoidal Cysts (Peliosis)
3. Proliferated Hepatocytes Enveloped by Sarcoma Cells
4. Progression to Papillary Pattern
5. Vasoformative and Anaplastic Nodules are Rare

Dissimilarities:
in Mice and Rats

1. Less Fibrosis (in Tumor and Parenchyma)
2. Often Uniform Sarcoma Cells vs. Multipotential Cells in Widened Tissue Space of Disse in Man

In summary, there are a number of common features between the human, mouse and rat lesions from exposure to vinyl chloride. These common or similar features are: multiple, focal areas of sinusoidal dilatation. We do not believe that these are angiosarcomas at this stage. We have no way of knowing at just what step, histologically, the angiosarcomas start and then progressively develop into a clinically important tumor. The second point of similarity is that of the peliotic lesions (blood-filled sinusoidal cysts). Then, there is, at the early stage of angiosarcoma, focal to multifocal hypertrophy and proliferations of hepatocytes, enveloped by sarcoma cells. This progresses to a papillary pattern and, finally, there may be solid anaplastic areas within the angiosarcoma. There are a couple of points of difference: in the mice and rats, we see very little or no increase in connective tissue in the neoplastic and unaffected portions of the liver when compared with man. Finally, there seems to be a somewhat more uniform population of tumor cells in the animal tumors than in the human. In man, there is a possibility of not only the endothelial lining cells but some of the other cells in the tissue space of Disse being involved in the neoplastic process.

Thank you.

opposed or exaggerated estrogenic stimulation. Most of these cellular characteristics, with the exception of intracytoplasmic microfilaments, are comparatively reduced in severe adenomatous hyperplasia (carcinoma *in situ*) and well differentiated adenocarcinoma, and are absent in neoplasms of lesser differentiation. The gradual failure to express estrogen-dependent cellular alterations in these lesions seems to be related to neoplastic dedifferentiation, and to be independent of the estradiol receptor content. The subcellular effect of progesterone or synthetic progestins on hyperplastic and well differentiated neoplastic endometria is that of epithelial regression and includes secretory conversion, lysosomal hydrolytic activity, a decrease in the length of microvilli, and lack of ciliogenesis. These observations support the concept that progestogens act upon endometrial hyperplasia and neoplasia by a direct, nonimmunologic, cellular effect by promoting secretion and inhibiting development of estrogen-influenced cellular specialization.

The Pathogenesis of Simian Hemorrhagic Fever: Hematologic and Histopathologic Studies

W. E. GIDDENS, JR., AND D. JESSUP, H. E. BRANSON, L. A. MAYER, D. R. BENJAMIN, AND W. T. LONDON. Departments of Pathology and Laboratory Medicine, the Regional Primate Research Center, University of Washington, Seattle, Washington, 98195, and the National Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, Maryland 20014.

Simian hemorrhagic fever (SHF) is an acute viral disease of monkeys of the genus Macaca. It has caused several epizootics in primate colonies around the world, resulting in the loss of thousands of monkeys. To understand more about the pathogenesis of this disease, we gave five rhesus monkeys intravenous injections of a 10^{-4} dilution of serum containing the Corbel strain of SHF virus. Blood was taken before inoculation and at selected intervals postinoculation (PI) for hematologic, ultrastructural, and coagulation studies. Monkeys were subjected to euthanasia at 3, 5, and 7 days PI; one monkey died spontaneously at 7 days PI.

Disseminated intravascular coagulation was a prominent feature of the disease and was characterized hematologically by markedly decreased plasma fibrinogen levels and increased degradation products of fibrinogen and fibrin. Platelet counts were reduced to about one-half of the preinoculation values. Clinically, there was hyperthemia, anorexia, weakness, epistaxis, and dysentery. At necropsy the blood often failed to clot, and there was hemorrhagic enterocolitis, lymphadenopathy, splenomegaly, and widespread petechiae and ecchymoses. On histopathologic examination, there was necrosis of lymphocytes in the centers of the Malpighian corpuscles of the spleen and in the germinal centers of the lymph nodes and peribronchial lymphoid follicles, in the Peyer's patches and the lamina propria of small and large intestine, and in the thymus. A consistent lesion was engorgement of splenic sinusoids with fibrin, and fibrin thrombi were present in many small arterioles and venules in the intestinal mucosa, lung, and other organs. Nonsuppurative meningoencephalitis was present throughout the brain, especially in the pons, cerebellar peduncles, and thalamus. SHF appears to be an excellent primate model for virus-induced disseminated intravascular coagulation and other human diseases.

Ultrastructural Changes in Endometrium of Women Bearing Copper Intrauterine Devices

AMADOR GONZALEZ-ANGULO AND RAMON AZNAR-RAMOS. Departamento de Investigacion Cientifica, Centro Medico Nacional, Instituto Mexicano del Seguro Social, Mexico, D. F., Mexico.

Clinical studies have confirmed that the addition of metallic copper to inert polyethylene intrauterine devices increases significantly their antifertility action. The continuous release of copper by the device has been postulated as one of the mechanisms whereby contraception is attained. The aim of the present study was to investigate epithelial and stromal changes possibly related to copper deposition. Endometrial biopsies were obtained from 12 young women bearing TCu-200 intrauterine contraceptive devices for more than 6 months. Six biopsies were taken at day 10 and six at day 20 of the menstrual cycle. The main changes were located in the cell organelles at day 10 of the cycle. The mitochondria disclosed vacuolation of the matrix and myelin figure formation in 70 to 80 per cent of the epithelial cells of four patients. There were also increased numbers of lysosomes and hyperplasia of Golgi structures. Stromal capillaries showed myelin figures in endothelial cells at day 10. Only in one case of secretory endometrium were similar alterations present. Preliminary studies by energy-dispersive x-ray analysis (Kevex) failed to reveal copper in various cell organelles. The above observations seem to indicate that there is a definite alteration of mitochondria of epithelial cells which may result in impairment of respiratory mechanisms and energy production, rendering the endometrial environment inhospitable for the fertilized egg.

Comparison of the Morphologic Features of Hepatic Angiosarcoma in Man and Rodents following Prolonged Exposure to Vinyl Chloride

D. E. GORDON, L. B. THOMAS, J. C. CALANDRA, H. POPPER, AND G. KENT. Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois, National Cancer Institute, Laboratory of Pathology, Bethesda, Maryland, Northwestern University Medical School, Chicago, Illinois, Mount Sinai School of Medicine, New York, New York, and Northwestern Memorial Hospital, Chicago, Illinois.

A causal relationship has been established between the development of hepatic angiosarcomas among workers engaged in the polymerization of vinyl chloride. The histologic features of similar hepatic tumors that developed in rodents (mice and rats) within 1 year after exposures of 2500, 200, and 50 p.p.m. of vinyl chloride for 7 hours a day, 5 days a week, were compared with 14 cases of those that have occurred in man. To date, the incidence of hepatic angiosarcoma in rodents has been related to the level of exposure to vinyl chloride. Despite dissimilarities between rodent and human lesions, such as minimal fibrosis in mice, the basic evolu-

tion was the same. The earliest lesion was focal dilation of sinusoids in which the lining cells were increased in number, piled up, and exhibited hyperchromatic and bizarre nuclei. Subsequently, sarcomatous lining cells enveloped plates of hepatocytes which were hypertrophic and hyperplastic. They become cords surrounded by proliferated lining cells of different types in papillary arrangement associated with blood-filled cavities resulting from sinusoidal ectasia. Eventually, nodules of anaplastic sarcomatous cells with vascular spaces developed with hemorrhage and necrosis. This characteristic evolution common to animal and man indicates a specific and identifiable effect of vinyl chloride or its metabolites upon hepatic mesenchymal and epithelial cells. It enforces the causal relation of vinyl chloride exposure to angiosarcoma formation, but also assists in the exclusion of vinyl chloride as the cause of some angiosarcomas of different appearance.

Ultrastructural Changes in Pulmonary Bleomycin Toxicity

F. Gyorkey, I. Daskal, and P. Gyorkey. Veterans Administration Hospital and Baylor College of Medicine, Houston, Texas 77031.

Ultrastructural studies of the nuclei of type I and type II pulmonary epithelial cells and of alveolar septal cells from patients with diffuse pulmonary fibrosis following bleomycin treatment revealed that the drug had a differential effect on the fine morphology of these cells.

The most striking alterations were observed in the type I alveolar cells; their number was markedly decreased, with formation of nucleolar fibrillar centers and a large increase in nuclear bodies. These nuclear bodies ranged in size from 0.3 to 0.7 μm. in diameter and were of three main varieties: membranous, beaded, and granular. Some nuclei contained up to 12 nuclear bodies. There was an abundance of nuclear bodies in all of the type I cells, which contained nucleoli with well defined fibrillar centers. The nuclei of type II alveolar cells (granular pneumonocytes) contained compact nucleoli with ill defined fibrillar centers and few, if any, nuclear bodies. When present, the nuclear bodies were of the membranous variety. The nuclei of the alveolar cells were similar in appearance to those of type I cells but did not contain nuclear bodies of the granular variety. We postulate that the above described ultrastructural changes, in particular the appearance of fibrillar centers and nucleolus-derived nuclear bodies, were induced by bleomycin.

A Macroscopic Method for Evaluating Succinic Acid Dehydrogenase in the Placenta

T. D. Hall, B. A. Woodling, N. E. Warner, S. B. Furukawa, and H. W. Puffer. Department of Pathology, University of Southern California School of Medicine, Los Angeles, California.

A histochemical assay for succinic acid dehydrogenase described for evaluating respiratory enzyme in myocardium (Nachlas, M. M., and Schnitka, T. K. *Am. J. Pathol.* 42: 379, 1963) was adapted and modified for the study of 106 placentas from normal and complicated pregnancies. The method entails incubation of the placenta with nitro-blue tetrazolium solution, an oxidation-reduction indicator which yields purple formazan pigment at sites of dehydrogenase activity. Areas of dehydrogenase deficiency had a white to yellow-white appearance and were readily differentiated from adjacent purple-stained tissue in which formazan pigment was deposited. Succinic acid dehydrogenase activity was present in the placentas from uncomplicated pregnancies, whereas placentas from complicated pregnancies showed slight to marked decrease in formazan pigment. In these placentas, addition of substrate did not enhance the intensity of formazan pigmentation. Placentas of infants with low Apgar scores and placentas associated with neonatal morbidity and mortality consistently demonstrated reduced enzyme activity. The clinical diagnosis of uteroplacental insufficiency was also associated with absence or marked decrease of succinic acid dehydrogenase activity. (Supported by Professional Staff Association, The Los Angeles County-University of Southern California Medical Center, Project 2-155-0-C.)

Acquisition of Columnar (Barrett Type) Epithelium in the Distal Esophagus after Partial Esophagogastrectomy

Stanley R. Hamilton and John J. Yardley. Department of Pathology, The Johns Hopkins University School of Medicine and Hospital, Baltimore, Maryland 21205.

The origin of the columnar cells in the columnar epithelium-lined distal esophagus (Barrett esophagus) is unknown. It has been proposed, however, that the columnar epithelium may be: (1) congenital, resulting from incomplete replacement of embryonic columnar epithelium; or (2) acquired, being a response to chronic gastric reflux.

We have studied at autopsy a 74-year-old male who had undergone partial esophagogastrectomy for squamous carcinoma of the distal esophagus 6 years earlier. The body of the stomach, with fundic mucosa at the margin, had been anastomosed to the remaining normal esophagus. Postoperatively there was persistent gastric reflux. Following death from cardiopulmonary disease, a mild stricture and a 0.5- to 1-cm. circumferential zone of tan mucosa was found in the distal esophagus just above the suture line. There was no tumor in the area of resection. The circumferential zone resembled mucosa of a Barrett esophagus, being composed of columnar, goblet, and endocrine cells. The columnar cells of the surface and glands showed periodic acid-Schiff (PAS)-positive and Alcian Blue (AB)-negative mucin. Scattered goblet cells stained with both PAS and AB. Numerous argyrophil cells and scattered argentaffin cells were demonstrated.

Findings in this case strongly indicated that the columnar epithelium in the distal esophagus was acquired, perhaps stimulated by gastric reflux. It has been suggested that Barrett epithelium may arise from cardiac mucosa. This case also indicated, therefore, that the presence of cardiac mucosa is not necessary for formation of columnar epithelium-lined esophagus, although its origin remained unclear. Metaplasia of squamous