

MCA

MANUFACTURING CHEMISTS ASSOCIATION

1825 CONNECTICUT AVENUE, N.W., WASHINGTON, D.C. 20009

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Subject to Protective Order in
Ross v. Conoco, Inc., No. 89-4237
14th Judicial District Court
Calcasieu Parish, Louisiana

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July 31, 1978

TO: Vinyl Chloride Technical Panel
Vinyl Chloride Research Coordinators
Vinyl Chloride Medical Subcommittee

221-2227

Gentlemen:

As reported in Section 6.0 of the Record of the University of Louisville Meeting on June 14, 1978, Drs. Tamburro and O'Connell promised to send the Project Manager complete abstracts of all research reviews presented on all component studies. We are pleased to report that they have exceeded their promise by sending us complete transcripts of the actual research reviews covering Dr. Tamburro's "Overview of the University of Louisville Multi-Disciplinary Approach to the Study of Chemical Carcinogenesis" and the reports on the component research projects as well. Included in the enclosed collection of papers are copies of all slides which were presented during the June 14th meeting.

We trust you will find these research summaries helpful and, for the benefit of those of you who were unable to attend, they afford an excellent insight into the overall value of this research program.

Sincerely,



J. T. Seawell
Project Manager
Vinyl Chloride Research

JTS:ep
Enclosure

CMA 002212

THE WRITER'S DIRECT DIAL NUMBER IS (202) 328-4258

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Subject to Protective Order in
Ross v. Conero, Inc., No. 90-4807
14th Judicial District Court
Calcasieu Parish, Louisiana

APPENDIX

CMA 002213

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Subject to Protective Order in
Ross v. Coraco, Inc., No. 90-4357
14th Judicial District Court
Calcasieu Parish, Louisiana

A B S T R A C T

LYMPHOCYTE RESPONSE TO ANTIGENS OF LIVER ANGIOSARCOMA IN
VINYL CHLORIDE WORKERS.

H. Philip Fortwengler, Michael E. Dever, Carlo H. Tamburro,
and Enrique Espinosa, University of Louisville School of
Medicine, Louisville, Kentucky 40232.

Angiosarcomatous liver tissue of vinyl chloride workers was shown by immunodiffusion and immunofluorescent procedures to include a tumor-associated antigen, antigens shared with normal liver and other tissues and tumor-bound immunoglobulin G. In addition, evidence has been presented for the existence of a tissue or plasma antigen induced by, or conjugated with vinyl chloride or a vinyl chloride metabolite. Possible lymphocyte sensitization to such antigens in 79 vinyl chloride workers including 26 with liver abnormalities was assayed by the lymphocyte transformation test. Antigen extracts used were prepared from the angiosarcomatous tumor and normal liver tissues. Twenty normal individuals having no exposure to vinyl chloride served as controls. Stimulation indices were calculated from cellular incorporation of tritiated thymidine. Mean stimulation indices in the normal individuals for antigens of angiosarcoma and normal liver tissues were $4.7 (SE \pm 1.7)$ and $3.8 (\pm 0.9)$ and in vinyl chloride workers, $1.8 (\pm 0.2)$ and $2.5 (\pm 0.3)$ respectively. Mean stimulation indices for PHA and concanavalin A in the normal individuals were 235 (± 35) and 209 (± 30) and in vinyl chloride workers 201 (± 23) and 180 (± 19) respectively. Thus, these results suggest that vinyl chloride workers have a decreased lymphocyte response to antigens of liver angiosarcoma and normal liver tissues rather than the hypothesized increased reactivity. This appears to be due to a lower overall lymphocyte responsiveness in these chemical workers in view of their decreased reactivity to PHA and concanavalin A. (Supported in part by the Manufacturing Chemists Association)

Federation Proceedings 37: 362, 1978

CMA 002214

MARCH 1, 1979 • VOLUME 38, NUMBER 3

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Ross v. Conoco, Inc., No. 90-4837
14th Judicial District Court
Calcasieu Parish, Louisiana

PART III
of Three Parts

Federation Proceedings

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Subject to
Ross v.
14th
Calcasieu

Abstracts

63rd Annual Meeting
Dallas, Texas
April 1-10, 1979

Society Grouping II—APS, AAP, and AAI (April 6-10)

FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY

ENDOTHELIUM AND SMOOTH MUSCLE (4061-4066)

999

CMA 002215

4062

PATHOLOGY

FACTOR VIII CONTENT AS EVIDENCE FOR ENDOTHELIAL ORIGIN OF VINYL CHLORIDE ASSOCIATED LIVER ANGIOSARCOMA (VCA). H. Philip Fortwengler*, Douglas Jones*, Carlo H. Tamburro* and Enrique Espinosa (SPON: G. Randolph Schrodt). Univ. of Louisville School of Medicine, Louisville, KY. 40232

To ascertain the endothelial cell origin of VCA we have looked for Factor VIII in the tumor since this factor appears to be specific for such cells (Moyer et al., 1973). Frozen sections of three VCA and one idiopathic case were examined for presence of Factor VIII by indirect immunofluorescence. Sections of angiosarcoma demonstrated a strikingly increased specific fluorescence which lined enlarged sinusoids. This intense fluorescence was easily seen on low power (100x) as an irregular or blotchy pattern. Occasional striations of linear fluorescence which did not follow hepatic cords were also present. A similar pattern of staining was also given by the idiopathic angiosarcoma but was never seen in sinusoids of normal liver. These observations were further supported by the finding that absorption of Factor VIII antiserum with experimental angiosarcoma tissue resulted in complete inhibition of immunofluorescence. These findings demonstrate that VCA and idiopathic angiosarcoma include proliferating cells containing Factor VIII and therefore strongly support an endothelial cell origin of this tumor. (Supported in part by the Manufacturing Chemists Association.)

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Subject to Protective Order in
Ross v. Conoco, Inc., No. 80-4837
 14th Judicial District Court
 Calcasieu Parish, Louisiana

DECREASED GLUCOSE-6-PHOSPHATASE ACTIVITY IN LIVER IN VINYL CHLORIDE EXPOSED RATS. J.T. Du* and C.H. Tamburro* (SPON: M. Fonda) Dig. Dis. & Nutr. Sect., Dept. Med., Cancer Center, Univ. of Louisville Med. Sch., Lou., Ky. 40201.

Increases in key glycolytic enzymes paralleling hepatoma tumor growth (Heinrich, et al., FEBS Letters, 42:145, 1974) and decreases in key gluconeogenic enzymes prior to and with the development of hepatomas (Isok, et al., Voprosy. Med. Khim 19:568, 1973) have been shown. We exposed adult Sprague-Dawley rats to 10,000-20,000 ppm of vinyl chloride (VC), 4-8 hrs./day, 5 days/wk. for 3-4 wks. (40-140 hrs. exposure) to induce liver injury and angiosarcoma formation. Glucose-6-phosphatase, a key gluconeogenic enzyme in the liver microsomal fraction, decreased 25% over control ($P < 0.05$ from combined data). Other microsomal proteins and enzymes related to VC metabolism, i.e., P450, NADPH-cytochrome c reductase and mixed function oxidase were unchanged in the same microsomal fraction with no differences in either mitochondrial cytochrome oxidase or blood transaminases. The decrease in glucose-6-phosphatase is similar to the lower gluconeogenic enzyme findings in hepatomas. This may reflect an increase in glycolysis and ribose-5-phosphate production associated with de novo purine biosynthesis prior to tumor development, inhibition in enzyme synthesis, or increased breakdown due to VC exposure. Work is under way to determine the mechanism. The decrease in glucose-6-phosphatase may be an early biochemical lesion usable as an indicator of liver injury associated with the subsequent development of angiosarcoma.

Fed. Proc., 35, 1422 (1976).

CMA 002216

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Subject to Protective Order in
Ross v. Conoco, Inc., No. 90-4337
14th Judicial District Court
Calcasieu Parish, Louisiana

ABSTRACT

INCREASED CAPABILITY OF DETOXIFICATION BY ELEVATION OF GLUTATHIONE-S-TRANS-
FERASE AND GLUTATHIONE IN RAT LIVER EXPOSED TO VINYL CHLORIDE.

Du, J.T.; and Tamburro, C.H. Digestive Diseases and Nutrition Section,
Department of Medicine, Cancer Center, University of Louisville Medical
School, Louisville, Kentucky 40201.

Vinyl Chloride (VC) is believed to be metabolized in the liver to mutagenic compounds chloroethylene oxide and chloroacetaldehyde and detoxified by glutathione transferase leading to the formation of mercapturic acid. Adult male rats were exposed to 28,000 ppm VC, 7 hrs/day, 5 days/week for 2, 4, and 6 weeks. During the 6 weeks, there was no evidence of abnormality by clinical liver function tests in blood. However, the activity of glutathione epoxide-S-transferase (GEST, 1,2-epoxy-3-(p-nitrophenoxy) propane as substrate) and glutathione aralkyl-S-transferase (GAST, p-nitrobenzyl chloride as substrate) in liver were elevated significantly over controls after 4 and 6 weeks respectively. GEST increased 17%, 28% and 42% and GAST increased 24%, 28% and 35% over controls at 2, 4 and 6 weeks suggesting enzyme induction by VC exposure. The content of reduced glutathione (GSH) was also elevated in the VC treated group (21%, 45% and 49% over control at 2, 4 and 6 weeks of exposure), and the activity of glutathione reductase, the enzyme to regenerate GSH from its oxidized form was also increased (50% to 86% after 2-6 weeks of exposure). The content of P-450, in the meantime was reduced in the exposed group. These results suggest that VC exposed rats had increased capacity of detoxification by induction of glutathione-S-transferase and elevation of glutathione.

CMA 002217

Type Abstract in Space Below

TISSUE AND URINARY GLYCOSAMINGLYCANS IN TRANSPLANTABLE HEPATOMAS. C. E. Kupchella, K. L. Curran, E. Drake, J. Kennedy, and C. H. Tamburro. Cancer Center, and Division of Digestive Diseases and Nutrition, University of Louisville, School of Medicine, Louisville, Kentucky.

The purpose of this investigation was to evaluate: a) the glycosaminoglycans (GAGs) in different behavioral/histological types of intermuscularly transplanted hepatomas, b) GAG patterns in tumor tissue in relationship to degrees of fibrosis and necrosis, c) the GAG changes in the livers of tumor-bearing animals, and d) urinary GAG excretion as a function of tumor growth. Three types of Morris hepatomas, 7777, 5123tc, and 9613A, which differ in rates of growth, metastatic potential, fibrosis, and necrosis, were studied. Urinary and tissue GAGs were extracted as cetylpyridinium complexes and measured as uronic acid. Tissue GAGs were also evaluated histochemically using alcian blue staining with and without enzyme pretreatment. Tumor tissue exhibited four- to six-fold greater GAG levels in the hyaluronic acid (90 ± 10 , 91 ± 12 , and 111 ± 9 vs. 25 ± 3 μ g uronic acid/g dry liver, respectively) and chondroitin sulfate (261 ± 29 , 217 ± 51 , and 208 ± 22 vs. 47 ± 7 μ g uronic acid/g, respectively) fractions than normal liver; the heparin fractions did not differ significantly (31 ± 7 , 17 ± 4 and 67 ± 11 vs. 39 ± 10). The livers of tumor-bearing animals exhibited slightly greater hyaluronic acid levels than normal livers. Moreover, increased urinary GAG excretion was evident after two weeks in animals bearing fast-growing tumors. The GAG tissue levels in fast vs. slow-growing tumors were not significantly different. This further supports our previously reported studies of urinary GAG excretion in human hepatic angiosarcoma (Curran, K. L., et al., Cancer 40 (6): 3050-3053) in suggesting that urinary GAG analyses may be useful in the detection, screening and diagnosis of hepatic cancer.

Gastroenterology, 1979, in press

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Subject to Protective Order in
Ross v. Conoco, Inc., No. 90-4837,
14th Judicial District Court
Calcasieu Parish, Louisiana

GLYCOSAMINOGLYCAN CHANGES ASSOCIATED WITH HEPATIC TUMORS: THE CONTRIBUTIONS OF REGENERATION AND NECROSIS. C. E. Kupchella, E. M. Secskas,* J. S. Kennedy,* and E. Espinosa*. Cancer Center and Department of Pathology, University of Louisville, School of Medicine, Louisville, Kentucky.

Although glycosaminoglycans (GAGs) have been shown to be elevated in many types of animal and human tumors including hepatic tumors, the cause and significance of these changes in neoplasia are still open questions. Regeneration and necrosis are operative in hepatic cancer and the purpose of this investigation was to evaluate the GAG changes associated with hepatic regeneration and hepatic necrosis. Regeneration was induced in male Sprague Dawley rats by partial hepatectomy and hepatic GAGs were evaluated at 4, 8 and 12 days post operatively. Necrosis was induced by: a) ligating the medium lobe, b) by resecting and placing median lobes in the peritoneal cavity and c) by resecting median lobes and incubating them in vitro in sterile saline. Analyses were carried out after 5 days of treatment. While regenerating livers exhibited GAG levels that were not statistically different from sham operated controls or non-operated controls, in vivo necrosis was accompanied by 3-4 fold increases in tissue GAGs. These data suggest that necrosis may make a substantial contribution to the elevated GAG levels found in some tumors.

Clinical Reports 1979, in press

CMA 002219

AMERICAN PUBLIC HEALTH ASSOCIATION
DECEMBER 1978 SAN DIEGO

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Subject to Protective Order in
Ross v. Conoco, Inc., No. 80-1357
- 14th Judicial District Court
in Calcasieu Parish, Louisiana

3097

Early Detection of Disease in Individuals Exposed to Vinyl Chloride.

Richard Greenberg, MD, and Carlo Tamburro, MD, University of Louisville, Louisville, Kentucky

This is a collaborative comparison study of the effectiveness of ultrasound, nail bed capillary study and urinary glycosaminoglycan excretions in vinyl chloride workers with biochemical dysfunction or histopathologically documented injury.

It represents an effort to detect early morbidity in vinyl chloride workers. Included is Dr. Maricq's method of investigating capillaries of the middle and distal phalanges of the fingers, including the nailfold, as a screening test for early changes from vinyl chloride exposure. Another new method is Dr. Taylor's "grey scale" ultrasonographic method which has not yet been widely adopted for diagnosis of liver disease. The University of Louisville group reported that the first indicator of liver changes occurred in the vascular sinusoids and was reflected by the appearance of mucopolysaccharides in the urine.

The results of the massive clinical study in Louisville should soon be able to indicate whether more sensitive criteria of early disease may be forthcoming, especially from the liver function testing. The special value of the capillary and ultrasound method remain doubtful at this time for early detection of the effects of exposure to vinyl chloride.

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Subject to Protective Order in
Ross v. Conoco, Inc., No. 83-4337.
14th Judicial District Court
Calcasieu Parish, Louisiana

Type Abstract in Space Below

URINARY GLYCOSAMINOGLYCAN PATTERNS IN HUMAN HEPATIC ANGIO-SARCOMA, HEPATOMA, AND IN WORKERS AT RISK FOR ANGIOSARCOMA. K. L. Curran, C. E. Kupchella, J. Sandoz, and C. H. Tamburro. Cancer Center and Division of Digestive Diseases and Nutrition, University of Louisville, School of Medicine Louisville, Kentucky.

A previous study reported an aberration in glycosaminoglycans (GAGs) eluted from anion-exchange columns with 1.25 and 1.5 M NaCl in the urine of patients with hepatic angiosarcoma. A controlled pilot study examined urinary GAG patterns in workers at risk for angiosarcoma. Six individuals with a history of high vinyl-chloride exposure and documented liver disease were paired with individuals with a high exposure index to vinyl-chloride but no clinical liver disease. Similarly six persons with low exposure but abnormal liver function were paired with low exposure/normal liver function individuals. A 24-hour urine was collected from each individual and the GAGs analyzed by anion-exchange chromatography. Individuals with clinically active liver disease at the time of this study were found to have urinary GAG excretion patterns which were similar to those described in angiosarcoma. No significant differences were found between high vs. low vinyl-chloride exposure or between those with inactive liver disease and those with normal liver function. GAG excretion was also studied in an additional hepatic angiosarcoma and human hepatoma and confirm the reported urinary changes. These findings support the concept that urinary GAGs are increased only in active hepatic disease and may be useful in evaluating the degree of activity at the various stages of liver disease in humans.

Gastroenterology

CMA 002221

PROCEEDINGS

SIXTH ANNUAL MEETING SOUTHEASTERN CANCER RESEARCH

KIAWAH ISLAND
SOUTH CAROLINA
NOVEMBER 15-17, 1978

24

TISSUE AND URINARY GLYCOSAMINOGLYCANS IN TRANSPLANTABLE HEPATOMAS OF DIFFERING

GROWTH RATES. Kupchella, C.E., Curran, K.L.,
Drake, E., Kennedy, J., and Tamburro, C.H.
Cancer Center and Department of Medicine,
University of Louisville, Louisville, KY. 40232.

The purpose of this investigation was to evaluate the glycosaminoglycans (GAGs) in different behavioral/histological types of intermuscularly transplanted hepatomas and in the livers and urine of animals bearing these tumors. Fast (7777), medium (5123tc), and slow (9618A) growing Morris Hepatomas were studied. Urinary and tissue GAGs were extracted as cetylpyridinium complexes and measured as uronic acid. Tissue GAGs were also evaluated histochemically. Tissue from fast, medium, and slow growing tumors exhibited 4 to 6 fold greater GAG levels than normal liver in the hyaluronic acid (90 ± 10 , 91 ± 12 , and 111 ± 9 vs. 25 ± 3 μ g uronic acid/g dry liver, respectively) and chondroitin sulfate/heparan sulfate (261 ± 29 , 217 ± 51 , and 208 ± 22 vs. 47 ± 7 μ g uronic acid/g, respectively) fractions; the heparin fractions did not differ significantly. The livers of tumor-bearing animals exhibited slightly greater hyaluronic acid levels than normal livers. Increased urinary GAG excretion was evident after two weeks in animals bearing fast-growing tumors. These data together with those of our previously reported studies of GAG excretion in human hepatic angiosarcoma (Curran, K.L., *et al.*, Cancer 40(6): 3050-3053) suggest that urinary GAG analyses may be useful in the detection and diagnosis of hepatic cancer.

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Ross v. Conoso, Inc., No. 90-4837
14th Judicial District Court
E. Calcasieu Parish, Louisiana

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PROCEEDINGS

SOUTHEASTERN CANCER RESEARCH ASSOCIATION

SIXTH ANNUAL MEETING

NOVEMBER 15-17, 1978

CONFIDENTIAL

Subject to Protective Order in
Ross v. Conoco, Inc., No. 93-4337
14th Judicial District, 66-18
Calcasieu Parish, Louisiana

25 GLYCOSAMINOGLYCAN CHANGES ASSOCIATED
WITH HEPATIC TUMORS: THE CONTRIBUTIONS OF
REGENERATION AND NECROSIS. Kupchella, Charles
E., Secskas, Elaine, Kennedy, Jeffery, and
Espinosa, Enrique, Cancer Center and Department
of Pathology, University of Louisville, School
of Medicine, Louisville, KY. 40232.

Although glycosaminoglycans (GAGs) have been
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with hepatic regeneration and hepatic necrosis.

Regeneration was induced in male Sprague
Dawley rats by partial hepatectomy and hepatic
GAGs were evaluated at 4, 8 and 12 days post
operatively. Necrosis was induced by: a)
ligating the median lobe, b) by resecting and
placing median lobes in the peritoneal cavity
and c) by resecting median lobes and incubating
them in vitro in sterile saline. Analyses were
carried out after 5 days of treatment.

While regenerating livers exhibited GAG
levels that were not statistically different
from sham operated controls or non-operated
controls, in vivo necrosis was accompanied by
3-4 fold increases in tissue GAGs. These data
suggest that necrosis is a substantial contri-
butor to the elevated GAG levels found in some
tumors.

KIAWAH ISLAND
SOUTH CAROLINA

CMA 002223

Histologically these tumors consisted primarily of fusiform nevus cells with prominent eosinophilic cytoplasm embedded in a dense connective tissue stroma. Junctional activity, theque formation, and pigment were uncommon features. As a result desmoplastic nevi may be confused with reticulohistiocytoma, atypical fibroxanthoma, dermatofibroma, and even necrobiotic granulomas. Well defined intranuclear invaginations of cytoplasm occurred in 12 cases, and this was an important feature differentiating desmoplastic nevi from these fibrohistiocytic lesions. Desmoplastic malignant melanoma must also be considered in the microscopic differential diagnosis, but distinguishing features of desmoplastic melanoma include identification of preexisting malignant lentigo or acral lentiginous melanoma, atypical mitotic figures, and foci of necrosis. Clinically most desmoplastic nevi were dome-shaped nodules. The age range was from 7 to 53 years (mean 28); duration from 2 months to 15 years; male to female ratio, 6/8; and four patients gave a positive history of preceding trauma. Five lesions were located on the thigh or leg; and the rest, with the exception of sparing the palms and soles, were widely distributed. Desmoplastic nevi were compared to the ordinary variants of mixed spindle cell and epithelioid cell nevi in an attempt to define etiologic factors explaining a desmoplastic reaction. Initially, it was thought that patient age or preceding trauma might be important. No satisfactory explanation could be found since, with the exception of a predilection for occurrence on the lower extremities, the other clinical variables were not statistically different.

Computer-Assisted Morphologic Quantitation of Collagen in Human Liver Biopsies

G. H. BARROWS, M. J. JOYCE, G. R. SCHRODT, R. GREENBERG, AND C. H. TAMBURRO. Departments of Pathology, Epidemiology and Biostatistics, and Medicine, University of Louisville, Louisville, Kentucky 40202.

Because unfixed liver is rarely available for analytical studies, appraisal of the extent of collagen must be made from appropriately stained sections of liver biopsy material leading to judgemental as well as sampling error. We have developed a computer-assisted morphologic technique to evaluate the distribution of collagen in liver biopsy material. We examined multiple biopsy samples from patients dying suddenly with no history of liver disease to establish normal values. A Hewlett-Packard 9864-A digitizer and 9815-A microcomputer were used to quantitate areas of trichrome stainable collagen within the biopsy samples. Random area selection and statistical analysis proved to be important considerations in determining the experimental model and these factors were readily incorporated into the calculator program. Stainable collagen estimates in normal liver varied from 0 to 6.1 per cent (mean = 1.25 per cent) in patients with no evidence of liver disease and showed considerable variation even in different biopsies from the same patient. As anticipated, subcapsular biopsies had more collagen than deep biopsy; however, the specimens from several deep biopsies often had significant variation in collagen content. Differences in central, mid-zonal, and portal collagen could be obtained using this method. The computer-

assisted morphologic technique for evaluating hepatic collagen appears to be readily adaptable to clinical studies. This study of normal liver implies that considerable care must be taken before the clinical diagnosis of significant fibrosis can be made.

Uptake and Nuclear Transfer of 17-Fluorescein-Labeled Estradiol in Normal and Abnormal Human Endometrium

G. H. BARROWS, J. D. RIEHM, AND M. RILEY. Department of Pathology, University of Louisville School of Medicine, P.O. Box 35260, Louisville, Kentucky 40232.

We have developed a 17-fluorescein-labeled estradiol which is specifically transferred to nuclei of target tissue (breast, endometrium) and inhibited by synthetic and natural estrogens. By *in vitro* incubation, patterns of temperature-dependent nuclear uptake may be observed. Marked cyclic variation is observed in normal human endometrium. With proliferative and early secretory endometrium, temperature-dependent nuclear labeling is present and appears to peak at late proliferative stages. Mid and late secretory endometrium, while having specific cytoplasmic labeling, do not exhibit temperature dependent nuclear labeling. This suggests that estradiol transport during mid and late secretory phases is inhibited or minimal. Abnormal endometrial samples exhibit a variety of patterns with the fluorescein-labeled estradiol. Endometrium with cystic hyperplasia reveals very little nuclear glandular staining, whereas in adenomatous hyperplasia strong nuclear uptake of the fluorescein-labeled compound is observed. Some cases of well differentiated endometrial carcinoma have strong nuclear uptake of the fluorescein-labeled estradiol whereas poorly differentiated endometrial carcinoma has little or no nuclear uptake. The method provides a good means of determining the endocrinologic behavior of glandular *versus* stromal elements of endometrium and suggests that in abnormal endometrium either variation in steroid uptake mechanisms or hormonal milieu may be important in the development of the lesion.

Aortic Infarction following Dissecting Aortic Aneurysm

SANFORD H. BARSKY. Departments of Pathology, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts 02215.

Aortic infarction was observed in 21 of 34 cases of dissecting aortic aneurysm. This lesion occurred as a central zone of necrosis, sparing media adjacent to the true and false lumina. This infarct followed rather than preceded dissection, took approximately 48 hours after dissection to develop, and did not organize with time. Lesions months to years in duration were similar histologically to those only several days old, and although there was some tendency to calcify in the older lesions, inflammation was consistently absent. The lesion occurred exclusively in the thoracic aorta. Histologically, aortic infarction was completely distinct from medial cystic necrosis (MCN). Aortic infarction was characterized by preserved elastic laminae with no increase in mucopolysaccharides, in contrast to MCN which was characterized by elastica disruption and increased mucopolysaccharides. When both MCN and aortic infarc-

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Abstracts of the Annual Meeting of the American Society for Microbiology 1978

H 128 Chloroacetaldehyde-induced Damage to *Bacillus subtilis*. A.D. LAURBACH, U.N. STREIPS* and J.L. WONG. Bureau Foods, FDA and U. Louisville, Louisville, KY.

Chloroacetaldehyde (CAA), a proposed metabolite of vinyl chloride metabolism, has been shown to be mutagenic in the *Salmonella* assay system, and to specifically inhibit the growth of a recombination-deficient mutant of *Bacillus subtilis* (Ellmore, et al BBA, 442: 405, 1976).

We have examined further the biological activity of this compound. First, CAA caused a significant increase in induced mutations to streptomycin resistance. Using *B. subtilis* 169, the relative mutation frequency (treated 3mM CAA/control) was 13.6, while using an *her* mutant of *B. subtilis* the frequency was 14.2. Secondly, a survey of the available repair-deficient mutants of *B. subtilis* revealed, that the only strains sensitive to CAA were of the *rec*⁻ genotype. Furthermore, the several *rec*⁻ mutants, show three types of response to CAA. Strong killing by CAA (inhibition of growth of 10 mm or more) was exhibited on the *recA*, *recB*, *rec-4*(GSY1616), and *recD27*(GSY1627) strains. Intermediate killing (3mm-8mm) was shown with strains bearing the *recE*, *recF*, and *rec-13*(BD246) loci. All other strains examined (*recC*, *recH*, *rec-41*, *uvr*, *her*, *polA*, as well as several repair-proficient cultures) grew in the presence of 0.1M CAA.

These studies are part of an ongoing program sponsored by the MCA to determine the molecular basis for vinyl-chloride induced carcinogenesis.

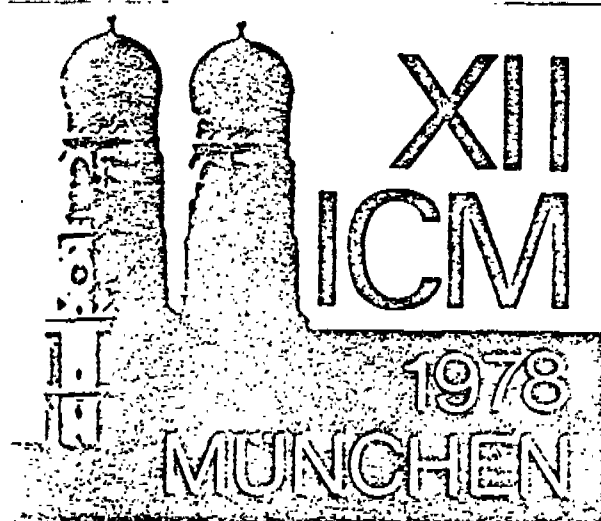
H 131 Influence of Mutagens on the Nucleoid Structure of the Chromosome in *Escherichia coli*. K.KLEPPE* and I. LOSSIUS, Department of Biochemistry, University of Bergen, Bergen, Norway.

The chromosome in a bacterium exists in a tightly supercoiled structure termed the nucleoid. We have investigated the effect of two mutagenic compounds, methyl methane sulfonate and mitomycin C, on the nucleoid structure of the chromosome in *Escherichia coli* K-12. Treatment of cell envelope-free nucleoids with the mutagens caused no changes in the structure as measured by their sedimentation coefficients. The nucleoids from mutagen treated cells, however, were found to be partially unfolded, having sedimentation coefficients of approximately 800S. In some mutants, defective in repair or recombination, even greater changes in the nucleoid structure were seen. The altered sedimentation behaviour was shown to be due to relaxation of supercoils in the chromosome caused by nicks in the DNA strands. During the repair period the intact nucleoid structure was partially restored. The mutagens also caused considerable changes in the structure of the cell envelope-associated nucleoid.

CMA 002225

INTERNATIONAL ASSOCIATION OF MICROBIOLOGY SOCIETIES

Abstracts of the XII International Congress of Microbiology BACTERIOLOGY SECTION INTERSECTIONAL MEETING MYCOLOGY SECTION



K.6

Intrinsic Resistance to Penicillin in *Staphylococcus aureus*: A Problem of Cytoplasmic Membrane Structure. M. KERN, H. KOPPEL, J. GRIFFIN, H. STUTZMANN. Pharmakologisches Institut, Universität Köln, D-5000 Köln 41, Federal Republic of Germany

In gram-negative bacteria the enzyme-independent type of penicillin resistance is assumed to be based on a permeability barrier in the outer membrane. In gram-positives, however, a similar function of the cell wall could not be established until now. From earlier studies we received some evidence for a possible role of the cytoplasmic membrane in this resistance mechanism. We therefore studied the binding of 14 C-benzylpenicillin (14 C-BP) to the membranes of resistant and sensitive *Staphylococci*.

First whole cells were incubated in buffer with increasing concentrations of 14 C-BP at 37°C for 30 min and then cytoplasmic membranes were prepared following the method of H. A. FASACK (Meth. Enzymol. XXII, 99-120 (1971)). In membranes of sensitive *Staphylococci* the binding increases sharply even at low penicillin-concentrations and levels out above 0.3 nmoles 14 C-BP/nl. In contrast, the binding to the resistant cells is reduced to a high degree. It reaches only 1-2% at 0.3 nmoles/nl. If the binding constants are calculated it can be seen that the number of binding sites is the same both in the resistant and in the sensitive membranes (20 nmoles BP/g membrane protein). However with the resistant membranes the affinity to penicillin is decreased by a factor of 250 to 700.

To exclude a permeability barrier in the cell wall the binding of penicillin to isolated membranes was examined. The incubation of the membranes with 14 C-BP was carried out under the same conditions as already mentioned. In this case, too, the binding to the resistant membranes was greatly reduced. This is also due to a high loss of affinity by approx. the same factor as above.

From these results a permeability barrier in the cell wall can be excluded. The cause for this type of resistance must thus be located in the membrane itself.

Therefore the membrane lipids were analysed. Differences were observed in the phospholipid fraction. The resistant membranes contain 40-50% more cardiolipin and 10-15% more phosphatidylglycerol than the sensitive ones. Additionally a cardiolipin derivative is found with the hydroxyl group of the central glycerol molecule acylated. The other lipids are unchanged in quantity and in fatty acid composition.

From this it can be concluded that the intrinsic resistance of *Staphylococci* is based on an altered membrane structure caused by increased lipophilicity.

K.5

Chloroacetaldehyde-induced Damage to *Bacillus subtilis*. A. D. LAIBINIS, E. N. BIKELIS, and J. L. MONG. Bureau Foods, FDA, and University of Louisville, School of Medicine, Health Sciences Center, Louisville, Kentucky 40232 (USA).

Chloroacetaldehyde (CAA), a proposed metabolite of vinyl chloride metabolism, has been shown to be mutagenic in the *Salmonella* assay system, and to specifically inhibit the growth of a recombination-deficient mutant of *Bacillus subtilis* (Ellmore, et al., BBA, 452: 405, 1976).

We have examined further the biological activity of this compound. First, CAA caused a significant increase in induced mutations to streptomycin resistance. Using *B. subtilis* 168, the relative mutation frequency (treated 5 mM CAA/control) was 13.6, while using an *hcr* mutant of *B. subtilis* the frequency was 14.2. Secondly, a survey of the available repair-deficient mutants of *B. subtilis* revealed, that the only strains sensitive to CAA were of the *rec*⁻ genotype. Furthermore, the several *rec*⁻ mutants, show three types of response to CAA. Strong killing by CAA (inhibition of growth of 10 mM or more) was exhibited on the *recA*, *recB*, *rec-4* (CSY1616), and *recD27* (CSY1677) strains. Intermediate killing (3-10 mM) was shown with strains bearing the *recE*, *recF*, and *rec-13* (SD246) loci. All other strains examined (*recC*, *recH*, *rec-41*, *uvr*, *hcr*, *polA*, as well as several repair-proficient cultures) grew in the presence of 0.1M CAA. The effects of CAA on the biological activity of DNA molecules *in vitro* are being studied.

These studies are part of an ongoing project sponsored by the Manufacturing Chemists Association to determine the molecular basis for vinyl-chloride induced carcinogenesis.

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Abstracts

63rd Annual Meeting
Dallas, Texas
April 1-10, 1979

Society Grouping II—APS, AAP, and AAI (April 6-10)

SATURDAY, PM

TUMOR BIOLOGY IV: ROLE OF ANTIGENS (4449-4453)

1069

4449

PATHOLOGY

TWO LIVER ANTIGENS UNDETECTABLE IN A FAST GROWING LINE OF TRANSPLANTED HEPATOMA (MORRIS HEPATOMA 7777). Enrique Espinosa, Sam Caple*, Charles Kupchella* and Sue Chia*. Dept. Pathology and Cancer Center, Univ. of Louisville School of Medicine, Louisville, KY 40232

During investigations of antigenic changes in liver tumors we examined fast (7777), medium (5123tc) and slow (9618A) growing Morris hepatomas for presence of liver antigens. Analyses of normal liver and hepatoma tissue saline extracts were performed by immunodiffusion methods using rabbit antiserum to rat liver extract absorbed with pooled normal rat plasma. Hepatoma 5123tc and 9618A gave immunoelectrophoretic patterns similar to normal liver. In contrast, hepatoma 7777 showed 2 arcs of precipitation missing. This was confirmed by the finding that after absorption of antiserum with hepatoma 7777 two lines of precipitation given by normal liver extract persisted whereas absorption with extract of normal liver, hepatomas 5123tc or 9618A abolished all lines of precipitation. The liver antigens found to be absent in hepatoma 7777 were characterized as proteins relatively unstable to heating and acid pH and unrelated to liver-specific F antigen. In Sephadex G-200 and Bio-Gel A-5m columns these antigens eluted as proteins of approximately 51,000 and 240,000 daltons respectively. The fact that 7777 is the fastest growing and least differentiated of the tumors studied suggests a possible functional relationship between the absent antigen and these properties. (Supported in part by the Manufacturing Chemists Association)

4450

IMMUNOLOGY

LIVER-SPECIFIC F ANTIGEN IN TRANSPLANTABLE HEPATOMAS HAVING DIFFERENT GROWTH RATES. Enrique Espinosa, Sue Chia*, Sam Caple* and Charles Kupchella*. Dept. Pathology and Cancer Center, Univ. of Louisville School of Medicine, Louisville, KY 40232

In the course of studies of antigenic changes associated with liver neoplasia we measured the relative concentration of liver-specific F antigen in fast (7777), slow (9618A) and medium (5123tc) growing Morris hepatomas in comparison with its concentration in normal adult rat liver. F antigen was solubilized from each tissue by aqueous homogenization. The supernatant containing the antigen was then tested by a double diffusion dilution assay capable of detecting a minimum of 1-2% the concentration of antigen present in normal rat liver. The F antigen antiserum used was prepared according to Fravi and Lindemann by immunizing CSA mice with BALB/c mouse liver extract. F antigen was undetectable in the fast growing hepatoma and ranged from less than 2% to 10% of the normal liver concentration in the slow growing one. The medium growing hepatoma showed similar or higher concentration than normal rat liver. Thus, the level of F antigen in these tumors does not appear to correlate with their rates of growth. (Supported in part by the Manufacturing Chemists Association)

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30TH ANNUAL MEETING OF THE TISSUE CULTURE ASSOCIATION, INC.

Properties of 14 Week Maintenance Cultures of PLC/PRF/5 Cells. P.B.
JOHNSTON*, E. Espinosa, S. Chia and S. Caple. Univ. of Louisville,
Louisville, Ky. 40232

This established cell line was originally described as being short lived with in a given passage, with considerable cell loss by day 6 to 8. However, after 80 passages, we found that culturing in glass roller bottles at 0.25 RPM allowed maintenance for at least 14 weeks with cells exhibiting excellent morphology without sloughing. After treating monolayers with Hoechst 33258 DNA stain, intense cytoplasmic fluorescence was seen in a proportion of the cells, possibly a function of the subviral hepatitis B genome. HBsAg of culture supernates was monitored and solid phase RIA assay revealed 31 to 47 ratio-units/0.2 ml after 2 to 14 weeks in medium with 10% fetal calf serum. The 14 week old cultures were extremely well adapted to maintenance in serum free medium for 14 additional days when supplementing with 100 ng/ml of insulin, in contrast to 1 week cultures which survived well for only 5 days without serum. The HBsAg titers of supernates from these 1 week or 14 week cultures, were very similar to the previous harvests in 10% serum. In addition, both young and 14 week cultures were similar in their rate of acid production; and in that both elaborated serum albumin, fibrinogen, transferrin and alpha-2 macroglobulin, the proteins tested to date.

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