

Competitive Inhibition of Organic Acid Transport in Kidney and Choroid Plexus by DDA and 2,4-D. JOHN B. PRITCHARD, *Laboratory of Pharmacology, Marine Pharmacology and Biomedicine Section, NIEHS, Research Triangle Park, North Carolina 27709 and Mt. Desert Island Biological Laboratory, Salisbury Cove, Maine 04672*

Initial *in vivo* studies showed that the polar DDT metabolite, DDA (2, 2-bis-*p*-chlorophenyl acetic acid), was distributed very differently from DDT in the winter flounder, *Pseudopleuronectes Americanus*. In particular, its urinary excretion was nearly 250 times DDT excretion. Its structure and polarity suggested that transport of DDA on the renal organic acid system might explain these results. Using isolated flounder renal tubules as a test system, extensive accumulation of DDA was shown. Tissue-to-medium ratios reached 15–20. This accumulation was inhibited by metabolic inhibitors and by other organic acids. Autoradiography demonstrated that the bulk of this uptake was intracellular and showed directly that cyanide and organic acids reduced intracellular DDA. Kinetic studies demonstrated that DDA competitively inhibited renal *p*-aminohippurate uptake. Its potency as an inhibitor was more than half that of probenecid, a potent inhibitor of this system. On the basis of these results a model of renal DDA secretion was proposed. This model predicted that DDA and chemically similar compounds such as 2,4-dichlorophenoxy acetic acid (2,4-D) should also inhibit organic acid transport at other sites. A particularly crucial site might be the choroid plexus where acidic metabolites of serotonin and dopamine are secreted from the cerebrospinal fluid into the blood for subsequent elimination elsewhere. Both DDA and 2,4-D were shown to inhibit rabbit choroid plexus transport of the serotonin metabolite, 5-hydroxy-3-indole acetic acid (HIAA). Related compounds which are not organic anions such as the parent amine, serotonin, or the parent pesticide, DDT, were not inhibitory. Similarly, dieldrin did not inhibit HIAA transport, although it is reported to alter HIAA levels *in vivo*. Probenecid and other organic acids were all inhibitory. Kinetic techniques showed competition between the transported anion, HIAA, and DDA or 2,4-D. Thus, as in flounder kidney, DDA inhibits through its interaction with the organic acid carrier.

In Vitro Studies of Blue Crab Gill Na⁺, K⁺-ATPase and its Response to DDT. G. NEUFELD AND J. PRITCHARD, *Marine Pharmacology and Biomedicine Section, Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709*

Cellular transport mechanisms are susceptible to disruption by a variety of organochlorine pesticides. In particular, the Na⁺, K⁺-ATPase, or "sodium pump" of cell membranes has been shown to be inhibited by several organochlorine compounds. The Na⁺, K⁺-ATPase in the gill of the blue crab, *Callinectes sapidus*, plays an important role in the adaptation to changing environmental salinity. The blue crab exhibits sensitivity to DDT and other organochlorines, particularly at low environmental salinity. It may thus provide a model system for assessing the involvement of Na⁺, K⁺-ATPase in the toxicity of DDT and the likelihood that Na⁺, K⁺-ATPase inhibition may induce failure of physiological regulatory mechanisms.

The activity of Na⁺, K⁺-ATPase in homogenates of gill tissue from adult blue crabs was measured by a modification of the technique of Miller et al. [Am. J. Physiol. 231: 370 (1976)].

Enzyme activity was much higher in the posterior gills, particularly gill pairs numbers six and seven. Female crabs accli-

ated to full strength seawater exhibited nearly twice the enzyme activity in these gills than comparable males. Adaptation to 50% seawater (i.e., osmotic stress) caused approximately an 80% increase in enzyme activity in these posterior gills in males. No comparable increase occurred in females.

When DDT was present *in vitro*, there was a significant inhibition of the Na⁺, K⁺-ATPase at concentrations as low as 2.86 μM (1.0 ppm). Near maximal inhibition was achieved at a DDT concentration of 25 μM (9.0 ppm). Further increase to 100 μM (35 ppm) resulted in only a small additional increase in inhibition.

Thus, blue crab gill Na⁺, K⁺-ATPase is biochemically sensitive to DDT when presented *in vitro*. It remains to be shown, however, whether such ATPase inhibition will lead to impaired osmoregulatory ability in the living animal.

Xenobiotic Metabolism in Marine Species Exposed to Hydrocarbons. M. O. JAMES, E. R. BOWEN, R. P. WEATHERBY, AND J. R. BEND, *Marine Pharmacology and Biomedicine Section, Laboratory of Pharmacology, NIEHS Laboratory at C. V. Whitney Marine Laboratory, St. Augustine, Florida 32084*

Several investigators have demonstrated that most fish species bioconcentrate pollutants, including hydrocarbons, from their aqueous environments. Thus food fish caught near the site of an oil spill could constitute a human health hazard, especially if the more toxic and carcinogenic polycyclic aromatic hydrocarbons are present in the fish. Hydrocarbons are slowly metabolized by fish, and excreted in urine and bile. The initial metabolic product is often an alkene or arene oxide which is usually more toxic than the parent hydrocarbon, and which may be further metabolized by epoxide hydase or glutathione S-transferase activity. We have studied the effect of hydrocarbon pretreatment of some fish species on the enzymes involved in hydrocarbon metabolism, paying particular attention to the polycyclic aromatic hydrocarbons. Repeated injection of 3-methylcholanthrene (3-MC) or 1,2,3,4-dibenzanthracene into flounder, skates, or sheepshead, but not stingrays, caused a 10- to 35-fold increase of aromatic hydrocarbon hydroxylase (AHH) activity in hepatic microsomes. No induction of epoxide hydase or glutathione S-transferase activity was observed in any species. Hepatic AHH activity in sheepshead remained elevated for at least 4 months after a single dose of 3-MC, but epoxide-metabolizing enzymes were not induced at any of the time points or doses studied.

Investigations in progress include the *in vivo* metabolism of selected components of crude oil in representative marine species, and further elucidation of the role of induction in the metabolism and toxicity of xenobiotics.

Dose-Dependent Metabolism of ¹⁴C-Styrene Oxide in the Isolated Perfused Rat Liver (IPL). J. VAN ANDA, J. R. BEND, AND J. R. FOUTS, *Marine Pharmacology and Biomedicine Section, Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709*

The metabolism of ¹⁴C-styrene oxide (SO; 50, 100, and 500 μmole/liver) was examined in the isolated perfused rat liver (IPL). After 60 min of perfusion, the metabolic profile, histopathological alterations, and the amount of radioactivity covalently bound to liver protein were determined at each dosage. In this time, the bile contained 1.5–8 μmole radioactivity at the lowest, 6–8 μmole at the intermediate, and 2.5–10 μmole at the

highest dose, respectively. The predominant biliary metabolite was the thioether conjugate of SO with glutathione. At each dose, the rates of SO metabolism by the glutathione S-transferase and epoxide hydrase pathways were approximately equal. At a SO dose of 500 μ mole/liver covalent binding to liver protein was 15-fold greater than at the lowest dose. Hepatotoxicity was assessed histologically and by measuring glutathione S-transferase activity appearing in the perfusate. Liver samples showed extensive centrilobular damage at a dose of 500 μ mole SO/liver. At 100 μ mole SO/liver, some livers showed centrilobular necrosis while others appeared normal. There were no detectable centrilobular changes at a dose of 50 μ mole SO/liver.

[This paper presented at the Fall ASPET Meeting and to be published (PHARMACOLOGIST, 1977)].

Separation of Two Forms of Cytochrome P-450 From Hepatic Microsomes of 1,2,3,4-Dibenzanthracene (DBA)-Pretreated Little Skates (*Raja erinacea*). T. H. ELMAMLOUK, R. M. PHILPOT, AND J. R. BEND, *Marine Pharmacology and Biomedicine Section, Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709, and Mt. Desert Island Biological Laboratory, Salsbury Cove, Maine 04672*

Cytochrome P-450 was solubilized and partially purified from hepatic microsomes of DBA-dosed skates by treatment with sodium cholate and chromatography on DEAE-cellulose by using Emulgen 913. Two separate cytochrome fractions were obtained which had different dithionite reduced-CO spectra. One form had its maximum absorption peak at 448 nm and was purified 5- to 6-fold, whereas the other form was eluted at higher ionic strength, had its maximum absorption peak at 451 nm, and was purified only 2-fold. The relative content of cytochrome P-448 to P-451 in the column eluates ranged from 1:1 to 1:1.6. The two partially purified forms of cytochrome P-450 obtained were free of cytochrome P-420, cytochrome B₅ and NADPH-cytochrome c reductase activity. By the same procedure, two separate peaks of epoxide hydrase activity with both benzo[a]pyrene 4,5-oxide and styrene 7,8-oxide were obtained. Liver microsomal NADPH-cytochrome c reductase was purified 6-fold by elution from the microsomal sample on the same column with a linear gradient of 0-0.5M KCl. The reductase was contaminated with cytochrome B₅.

[This paper presented at the Fall ASPET Meeting, and to be published (PHARMACOLOGIST, 19: 1977)].

Partial Purification of Rabbit Hepatic and Pulmonary Glutathione S-Transferases—Alkene and Arene Oxide Metabolism. J. H. MAGUIRE, J. R. FOUTS, AND J. R. BEND, *Marine Pharmacology and Biomedicine Section, Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709*

Microsomal supernatant fractions of Dutch belted rabbit liver and lung were fractionated by procedures similar to those reported by Habig et al. [J. Biol. Chem. 249: 7130 (1974)]. Chromatography of liver and lung supernatant fractions on DEAE-cellulose columns was monitored by elution of glutathione S-transferase (GS-T) activity toward 1-chloro-2,4-dinitrobenzene (DNCB) or 1,2-dichloro-4-nitrobenzene. Elu-

tion from a CM cellulose (CMC) column with a KCl gradient gave qualitatively similar patterns for liver and lung. Five peaks of GS-T activity toward DNCB were observed. Of the GS-T applied to the column, one peak in the liver CMC eluate accounted for virtually all (> 95%) of the activity with styrene 7,8-oxide (SO) as substrate. This peak also accounted for 40% of the GS-T activity with benzo[a]pyrene 4,5-oxide (BPO) as substrate. The remainder of the GS-T activity toward BPO was distributed in the four other GS-T peaks, which contained very little or no activity toward SO. In contrast, two major peaks of GS-T activity toward SO were present in CMC eluates from pulmonary preparations. The majority (90%) of the pulmonary GS-T activities toward BPO was bound to the DEAE column; however, at least five GS-T activity peaks toward BPO were observed upon KCl gradient elution from a CMC column.

Supported in part by NIH Fellowship No. ESO2281 (NIEHS). This abstract was originally published in *FED. PROC.* 36: 962 (1977).

Rabbit Pulmonary Mixed-Function Oxidase System: Purification of Two Forms of Cytochrome P-450 and Reconstitution of Activity. M. SZUTOWSKI, C. R. WOLF, AND R. M. PHILPOT, *Toxication-Detoxication Section, Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709*

We have reported the purification of a form of pulmonary cytochrome P-450 with a λ_{max} at 450 nm [J. Biol. Chem. 251: 3213 (1976)]. We have now isolated a pulmonary cytochrome with a λ_{max} at 452 nm. The cytochromes and NADPH cytochrome c reductase are separated by chromatography of cholate-solubilized microsomes on DEAE cellulose. Cytochrome I (λ_{max} 452 nm) is eluted with 0.2% emulgen and cytochrome II and the reductase are eluted with KCl in the presence of 0.2% emulgen. Cytochrome I is further purified to 8-10 nmole/mg protein by chromatography on hydroxylapatite, calcium phosphate gel, and DEAE cellulose. Cytochrome II has been purified to 7 nmole/mg protein by the present procedure. Reductase preparations containing about 2500 units/mg protein are obtained following chromatography on hydroxylapatite. The O-deethylation of 7-ethoxycoumarin using pulmonary enzymes requires phospholipid, neutral lipids, and cholate for maximum activity.

This work presented at the 1977 Fall ASPET Meeting.

Enhancement of Glutathione S-Transferase (GST) Activity and Microsomal Cytochrome P-450 Content in Rat Ovaries During Pregnancy. H. MUKHTAR, J. H. MAGUIRE, R. M. PHILPOT, AND J. R. BEND, *Toxication-Detoxication Section, Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709*

A time-dependent increase in GST activity was observed in the ovaries of pregnant rats with styrene 7,8-oxide (SO) and benzo[a]pyrene 4,5-oxide as substrates. No concomitant increase was observed in adrenal, liver or serum GST activity during pregnancy. This increase in ovarian GST activity was not a function of the estrous cycle. GST specific activity (SA, nmole/min-mg protein) of virgin female ovaries was relatively high (97) with SO. Enzyme activity increased during early pregnancy (day 7, SA 109), reached a maximum in mid-pregnancy

(day 11, SA 176), remained relatively constant until late pregnancy (day 19, SA 179), but increased again during lactation (day 4, SA 249). The specific content of cytochromes P-450 (P-450 SC, nmole/mg protein) and b_5 (b_5 SC) in microsomes prepared from rat ovaries was 3.7- to 4.7-fold higher during mid- (P-450 SC, 0.11; b_5 SC, 0.11) and late pregnancy (P-450 0.14, b_5 0.10) than in nonpregnant animals (P-450 0.03, b_5 0.04); no alteration in mitochondrial P-450 or b_5 occurred. Similarly, pregnancy had no effect on ovarian microsomal aryl hydrocarbon hydroxylase or epoxide hydrolase activity. Enhanced GST activity in ovary during pregnancy and lactation may protect against the toxic effects of circulating electrophiles.

This paper presented at the 1977 Fall ASPET Meeting.

Metabolism of Benzo[a]pyrene and Benzo[a]pyrene 4,5-Oxide by the Isolated Perfused Rabbit Lung. BRIAN R. SMITH AND JOHN R. BEND, *Marine Pharmacology and Biomedicine Section, Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709*

The isolated perfused rabbit lung was found to metabolize benzo[a]pyrene (BP) (20 μ mole) at a rate of 5.9 ± 1.8 nmole/min-g lung, comparable to mixed-function oxidation rates for this hydrocarbon in pulmonary microsomes. Phenols, dihydrodiols, and glutathione conjugates were found as metabolites. A lag time of 11.9 ± 0.8 min in the appearance of water-soluble products in the perfusion medium was observed followed by a linear phase where water-soluble materials appeared at rates of 0.18 to 0.93 nmole/min-g lung. The lag time may be related to the accumulation of arene oxide(s) to concentrations sufficient to support the conjugation reaction. BP was covalently bound to lung tissue to an extent of 3.1 ± 1.3 nmole/g lung. Pretreatment of the animals with 3-methylcholanthrene did not significantly increase the overall metabolic rate and did not affect covalent binding (7.5 ± 4.9 nmole/g lung). Benzo[a]pyrene 4,5-oxide (BPO) (5 μ mole) was metabolized principally to benzo[a]pyrene 4,5-dihydrodiol (BP-DHD) and to the corresponding glutathione conjugate. Thioether conjugates were formed at rates markedly lower than those observed with *in vitro* lung preparations. BPO bound covalently to lung protein, but not to DNA. The isolated perfused lung is an excellent preparation for hydrocarbon biotransformation studies, as the integrated enzymatic systems responsible for their metabolism remain intact.

This abstract originally appeared in Fed. Proc. 36: 999 (1977).

Spectral Characterization of Purified Hepatic Cytochrome P-450 From 3-Methylcholanthrene-Treated and Untreated Rabbits. R. M. PHILPOT AND C. J. SERABJIT-SINGH, *Toxication-Detoxication Section, Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709*

The procedure used to purify hepatic cytochrome P-448 from 3-methylcholanthrene-treated rabbits was used to obtain cytochrome P-448 from untreated rabbits. The specific content of cytochrome from both sources was 14-16 nmole/mg of protein. The estimated monomeric molecular weight of the cytochrome by SDS polyacrylamide gradient gel electrophoresis was 53,500 daltons. The UV-visible absorbance spectra of the cytochromes were identical, including Soret maxima for the oxidized forms at 394 nm and 418 nm. Caffeine or butanol added to the oxidized cytochrome resulted in a shift in absorbance from 394 nm to 418

nm. After dialysis to remove caffeine or butanol the 394 nm peak was restored even after the cytochrome was extensively dialyzed in the presence of butanol prior to removal of butanol. The correspondence between the absorption at 394 nm and a high-spin form of the cytochrome as well as the effect of butanol on it was confirmed by EPR spectrometry. Therefore, if the high-spin heme of rabbit liver cytochrome P-448 is due to an endogenous type I compound, it must be bound in such a way as to be displaceable from the heme but not from the cytochrome molecule.

This report was presented at the 1977 FASEB Meeting in Chicago.

Xenobiotic Metabolism in Skin of Hairless Mice Exposed to Ultraviolet Radiation, Aroclor 1260, or Chlordane. R. J. POHL AND J. R. FOUTS, *Toxication-Detoxication Section, Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709*

7-Ethoxycoumarin deethylase (7-EC) activity measured in chopped skin from the backs of female hairless mice, Hrs/J strain, was increased after exposure to shortwave (254 nm) or sunlamp (280-750 nm). 7-EC activity was not elevated in mouse skin 24 hr after topical application of 2 mg Aroclor 1260, but was increased 40% by application of 0.1 ml of vehicle (DMSO or acetone). 7-EC activity in skin from mice exposed to 254 nm or sunlamp 24 hr after Aroclor 1260 was not different from controls exposed to ultraviolet after vehicle. Skin 7-EC and 7-ethoxyresorufin deethylase (7-ERF) may have been synergistically increased when 254 nm UV exposure was repeated 2,5 and 6 days after Aroclor 1260 application; UDP-glucuronosyltransferase (4-methylumbelliferone) activity (activated by freezing and thawing) in skin pieces was not changed by UV and/or Aroclor 1260 treatments. IP and topical treatment of mice with chlordane or its persistent metabolite, oxychlordane, at doses which increase hepatic 7-EC activity, inhibited 7-EC activity in skin pieces. 7-ERF activity was unchanged by topical application of oxychlordane to skin. 7-EC activity was inhibited in mouse skin treated, *in vitro*, with chlordane or oxychlordane.

This report presented at the 1977 Fall ASPET Meeting.

Differences in Phenobarbital- (PB) or 3-Methylcholanthrene- (3-MC) Induced Alterations in Intestinal and Hepatic Drug-Metabolizing Enzymes of Various Animal Species. C. L. MIRANDA AND R. S. CHHABRA, *Toxication-Detoxication Section, Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709*

A previous report from this laboratory has demonstrated mainly quantitative differences in activities of intestinal microsomal drug-metabolizing enzymes (DME) among various laboratory animal species [Drug Metab. Disp. 2: 443 (1974)]. The present study was undertaken to determine if there were differences in inducibility between intestinal and hepatic microsomal DME by PB or 3-MC given PO or IP in mouse, rat, guinea pig, and rabbit. The microsomal DME studied were ethylmorphine demethylase (EMD), aniline hydroxylase (ANH), aryl hydrocarbon hydroxylase (AHH), 7-ethoxycoumarin deethylase (ECD), and cytochrome P-450 content. Doses of PB and 3-MC varied with the route of administration and animal species. Differences in the inducibility of EMD, AHH, and ECD due to the route of administration of PB were

observed among guinea pig, mice, and rat small intestines. There was no difference between PB (po) and PB (ip) in the induction of hepatic DME but differential effects of po- and ip-administered 3-MC on hepatic AHH and cytochrome P-450 were noted in rat and guinea pig. The induction of intestinal DME varied with the animal species and the type of drug substrate used. None of the rabbit intestinal DME were induced by PB or 3-MC even in rabbits fed semipurified diet. However, the rabbit intestinal DME were not totally resistant to environmental insults since starvation significantly reduced their activities. All hepatic DME studied were induced by PB except AHH in rat. 3-MC induced hepatic ECD in mouse and rat but inhibited it in rabbit. These results suggest that there are quantitative and qualitative differences between small intestine and liver. Unlike in liver, the inducibility of intestinal DME activities by 3-MC or PB depended on the type of drug substrate and animal species used.

Presented at the 1977 Society of Toxicology Meeting, Toronto, Canada.

Effects of Environmental Pollutants on the Intestinal Absorption of Nutrients. L. M. BALL AND R. S. CHHABRA, *Toxication-Detoxication Section, Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709*

The *in vitro* everted sac and *in situ* tied-off intestinal loop techniques were used to investigate the effects of various environmental pollutants on the absorption of nutrients from rat small intestine. Adult rats treated with lead acetate (0.02% w/v) in their drinking water for 3 months showed a decrease in absorption of ¹⁴C-glucose (Glu) compared to controls given distilled water. Uptake of ¹⁴C-leucine (Leu) was similar to that seen in controls. Young rats exposed for 1 week *in utero* via their mothers' drinking water, and subsequently via their own, initially suffered slight impairment in both Glu and Leu absorption, which returned to control levels by the age of 12 weeks. To investigate the effects of different forms of lead, adult male rats were given acute doses of various lead salts (up to 65 mg/kg-day po or ip for 3 successive days). Glu uptake was reduced slightly by lead monoxide and also by triphenyllead acetate (TPLA). TPLA considerably decreased Leu transport.

Following treatment of adult male rats with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at toxic levels (100 µg/kg po) Glu uptake was reduced in the first few hours, increased above control levels between 1 and 2 weeks later, then declined again after 3 weeks. Untreated animals pair-fed to match the decreased food intake observed in treated rats showed an even greater stimulation of Glu uptake after 1 week. Leu absorption was depressed throughout.

Thus the adult rat intestine is relatively insensitive to lead, which is present at low levels throughout the environment, while an acutely toxic but infrequent contaminant such as TCDD exerts a more drastic influence on absorption, which may in fact be secondary to its other deleterious metabolic effects.

Studies on the Isolation of Foreign Compound-Metabolizing Cells from the Lung. T. R. DEVEREUX, GARY E. R. HOOK, AND J. R. FOUTS, *Toxication-Detoxication Section, Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709*

Viable cells have been released from the rabbit lung by digestion with 0.1% pronase in Krebs-Ringer bicarbonate (KRBC)

instilled into the trachea. These cells have been separated into five fractions on the basis of size using the elutriator centrifuge. The five fractions have been compared on the basis of drug metabolism activity and cytochrome P-450 content and by electron microscopy and light microscopy, and several differences have been observed. Fraction 1, which contains mostly debris, shows the highest P-450 content and the highest drug-metabolizing activity (on a per-milligram protein basis) if NADPH is added. However, fractions 4 and 5 have the most activity when metabolism is measured in whole cells without the addition of NADPH. Major efforts now are focused on improving viable cell yields and getting a higher proportion of the drug metabolism activity in viable cells. Also, some cultures have been started from different fractions. Cells will be cloned from these cultures and individual cell populations will be examined for drug metabolism activity.

Soluble Proteins of the Lung Acellular Lining Layer. DIANNE Y. BELL AND GARY E. R. HOOK, *Molecular Pharmacology and Biochemistry Section, Laboratory of Pharmacology, NIEHS, Research Triangle Park, and the Department of Medicine, Duke University, Durham, North Carolina*

Soluble proteins of the lung acellular lining layer obtained by segmental lavage of volunteers and soluble protein components of lung lavage effluents from patients with pulmonary alveolar proteinosis (PAP) were compared by two-dimensional polyacrylamide gel electrophoresis. In addition, serum proteins from normal individuals and from patients with PAP were compared with the corresponding lavage effluent soluble proteins, and with each other. On the basis of similar electrophoretic mobility in one dimension and similar molecular weight, it appears that many, but not all, serum proteins are found in the soluble fraction of lung lavage effluent. The apparent absence of certain major serum proteins such as α₂-macroglobulin and the haptoglobins, however, precludes the origin of the soluble lining layer proteins by simple transudation from serum, both under normal conditions and in the case of PAP. The soluble fraction of the lavage effluent from patients with PAP contains two proteins not found in that of normal volunteers, nor in serum from individuals in either group. These preliminary analyses indicate that the processes giving rise to the soluble protein components of the lung acellular lining layer are more complex than simple transudation of serum proteins. The presence of unique proteins in the lavage effluent of patients with PAP may be of significance in diagnosing the disease, and their identification useful in determining its etiology.

This work was presented at the NIEHS Science Seminar, June 2-3, 1977.

Evidence for Phosphatidylcholine Exchange Protein in Mammalian Lung. J. W. SPALDING AND GARY E. R. HOOK, *Molecular Pharmacology and Biochemistry Section, Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709*

Very little is known about control mechanisms that regulate phospholipid metabolism. It is currently accepted that phospholipids are transported from their site of synthesis in the endoplasmic reticulum to other membrane organelles of the cell—e.g., mitochondria, nucleus, plasma membrane, lyso-

somes. There is recent and convincing evidence that the transport of phosphatidylcholine (PC) and phosphatidylinositol (PI) between endoplasmic reticulum and other membrane-containing organelles is mediated by specific phospholipid-exchange proteins. Dipalmitoyl-phosphatidylcholine (DPPC) is the major component of surfactant which is synthesized and secreted by lung alveolar Type II cells. DPPC is accumulated and sequestered in lamellar bodies and the latter are subsequently secreted into the alveolar space of the lung. The best evidence to date indicates that 1-palmitoyl-2-acylphosphatidylcholine is synthesized in the endoplasmic reticulum and transported to the lamellar bodies where it is modified to the DPPC form. We have demonstrated the presence of a PC-exchange protein in preparations from the 105,000g supernatant of rat and rabbit lung homogenates. The phospholipid exchange proteins of rat and rabbit lung can mediate PC exchange between liver and lung mitochondria and microsomes. Exchange of labeled PC also readily occurs between mitochondria and liposomes composed of egg PC. The exchange reaction is temperature sensitive and linear with time up to 30 min. The degree of exchange is proportional to the amount of phospholipid exchange protein in the reaction system. The possible role of phospholipid exchange protein in lung PC transport and DPPC synthesis is being investigated.

Alkaline Phosphatase Isoenzymes in Lavage Effluents From Normal and Diseased Lungs. D. NADEAU, M. J. REASOR, AND GARY E. R. HOOK, *Molecular Pharmacology and Biochemistry Section, Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709*

Alveolar proteinosis (AP) is a human disease of unknown etiology characterized by the accumulation of large amounts of proteinaceous material in the acini of the lungs. Following sedimentation of particulate material (178,000g_{45'}/60 min) from pulmonary lavage effluents of patients with AP, a soluble phase was isolated which possessed high alkaline phosphatase (AKP) activity (14.7 ± 1.5 nmole *p*-nitrophenylphosphate hydrolyzed/min-mg protein, mean $\bar{X} \pm SE$, $n = 6$). This soluble AKP was exclusively present (> 90%) as an unusually high apparent molecular weight complex (MW > 20×10^6 daltons) as judged by its exclusion from Sepharose-4B (SP). Treatment of the complex with *n*-butanol resulted in the release of relatively low MW AKP (227,000–295,000) with isoelectric points (pI) at 4.6, 5.2, and 5.5–5.9. After homogenization of normal autopsy lung tissue (AL), 65% of the total soluble AKP activity recovered from SP-4B was retarded through the column, while the remaining was eluted at the void volume. Some differences in relative electrophoretic mobilities and apparent MW were observed between the butanol-treated AKP from AP and AL. However, there were similarities in sensitivity to heat, apparent K_m values, amino acid inhibition, and pI values. Comparative studies on fiber optic bronchoscopy lavage effluents from human volunteers showed that AKP isoenzymes with an apparent MW of 125,000 were present at the extracellular lung lining, and were different than the isoenzymes from AP and AL. The AKP found in lavage effluents from AP patients has been partially characterized and purified, and may potentially be useful as a monitor of the disease.

This abstract has been previously published in part in *Am. Rev. Resp. Dis.* 113: 208 (1976).

Particulate Components of Lavage Effluents from the Lungs of Patients with Pulmonary Alveolar

Proteinosis. GARY E. R. HOOK, LINDA B. GILMORE, DENIS NADEAU, AND DIANNE Y. BELL, *Molecular Pharmacology and Biochemistry Section, Laboratory of Pharmacology, NIEHS, Research Triangle Park, and the Department of Medicine, Duke University, Durham, North Carolina*

Pulmonary alveolar proteinosis (PAP) is a disease of unknown etiology characterized by the accumulation of lipid-rich proteinaceous material in the acini of the lungs. The confinement of disease symptoms to the lungs has suggested that the disease may be environmentally related. The nature of the accumulated materials is obscure and its origins have not been established. We have examined the particulate components of pulmonary lavage effluents from patients with PAP by electron microscopy: both cellular and noncellular materials are present. The cellular constituents consist of lymphocytes, alveolar macrophages of unusual appearance, degenerate cells and cells in the process of disintegration. The noncellular particulate components are different from those present in lavage effluents from normal human lungs. Much of the material from diseased lungs resembles cell debris although the major components are bizarre myelinlike structures which are never seen in normal lungs. These results indicate that the materials which accumulate in the lungs of patients with PAP arise at least partially from cellular disintegration and partially from a process involving the extracellular formation of myelinlike structures.

This work was presented at the NIEHS Science Seminar, June 2-3, 1977.

The Formation of a Large Molecular Weight Form of ACTH by Chemically-Induced Rat and Mouse Lung Tumors. M. N. KHAN, A. GHOSH, I. LINNOILA, P. NETTESHEIM, AND R. P. DIAUGUSTINE, *Molecular Pharmacology and Biochemistry Section, Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709*

The formation of polypeptide hormones by human nonendocrine tumors is well known. Patients with lung squamous cell carcinomas have been previously reported to produce a large molecular weight form of adrenocorticotrophic hormone (ACTH) which has negligible bioactivity [*Am. Rev. Resp. Dis.* 111: 279 (1975)]. In the present study, squamous cell carcinomas induced in rat and mouse lungs or tracheal explants with 3-methylcholanthrene or benzo[a]pyrene were examined for the presence of ACTH immunoreactivity. Tumors grown subcutaneously were minced, homogenized in 4M urea containing $2 \times 10^{-3}M$ DTT and $5 \times 10^{-4}M$ phenylmethylsulfonyl fluoride, heated at 50°C for 15 min, and then centrifuged at 150,000g for 30 min. ACTH measured in the supernatant fractions ranged from 7 to 19 ng/g wet weight for the tumors. When an extract of one of the rat tumors was applied to a column of Sephadex G-150 equilibrated with 4M urea and 0.05% sodium azide in 0.1M phosphate buffer, pH 7.2, two peaks of immunoreactivity were observed. One eluted after bovine serum albumin (MW 67,000 daltons) but before carbonic anhydrase (MW 29,000 daltons). The other peak eluted with human ¹²⁵I-(1-39)ACTH. Chromatography of the extract, dialyzed to remove urea, on QAE-Sephadex using a gradient from pH 6.4 to 4.0 yielded immunoreactivity in the void (V₀) region that co-eluted with the labeled ACTH and another peak that eluted at about pH 6.2. Immunoreactive forms of ACTH more acidic and of a larger molecular weight than (1-39) ACTH were also identified in ex-

tracts of mammalian pituitary glands and might be precursors of the bioactive hormone. These data indicate that the "ectopic" synthesis of polypeptide hormones or prohormones might be frequently present in carcinogen-induced mammalian lung squamous cell carcinomas.

Distribution and Excretion of Halogenated Xenobiotics (PCB, PBB, and Kepone). H. B. MATTHEWS, N. M. MORALES, S. KATO, J. D. MCKINNEY, AND D. B. TUEY, *Pharmacokinetics Section, Laboratory of Pharmacology and the Branches of Biometry and Environmental Chemistry and Biology, NIEHS, Research Triangle Park, North Carolina 27709*

Polychlorinated biphenyls (PCBs), 2,4,5,2',4',5'-hexabromo-biphenyl (PBB), and Kepone were readily absorbed from the intestine, rapidly removed from the blood, and initially stored in the liver and muscle. At later time points the less chlorinated PCBs were metabolized and excreted while the highly chlorinated PCBs and the PBB were translocated from the liver and muscle to the skin and adipose tissue which were the sites of long-term storage. The degree and rate of metabolism and ultimate biological half-life of PCBs was determined by the degree and position of chlorination. PCB metabolism tended to decrease as the chlorine content increased, and the effect of increasing chlorine content was most pronounced when the chlorine atoms were arranged so that the biphenyl molecule did not have two adjacent unsubstituted carbon atoms.

The PBB and Kepone are highly halogenated compounds which do not have two adjacent unsubstituted carbon atoms. Thus, as would have been predicted from the experience gained with the PCBs, the PBB and Kepone were not subject to appreciable metabolism. Kepone was stored primarily in the liver, excreted in the bile at a rate proportional to the liver concentration, and had a half-life of approximately 18 days in the rat. On the other hand, the PBB was stored primarily in adipose tissue, subject to very little excretion, and had an infinite half-life in the rat.

Conjugation of 15-Keto Prostaglandins (PGs) and Glutathione. A. CHAUDHARI, T. E. ELING, M. W. ANDERSON, AND L. G. HART, *Pharmacokinetics Section, Laboratory of Pharmacology and Biometry Branch, NIEHS, Research Triangle Park, North Carolina 27709*

Incubation of ^3H -15-keto prostaglandin F_{2a} with glutathione (GSH) produced metabolite(s) of 15-keto PGF_{2a} which was not extractable from aqueous solution and thus termed "water-soluble metabolite(s)." The addition of 100,000g supernatant of guinea pig liver to incubation mixture increases the formation of water-soluble metabolites of 15-keto PGF_{2a} 3-fold. The conversion of ^3H -keto PGF_{2a} to water-soluble metabolite(s) in both the presence and absence of enzyme was linear in 10 min of incubation and required 2.5 mM GSH for maximal activity. Liver and kidney 100,000g supernatant possess about 70 and 25 times as much enzymatic activity compared to lung. Formation of PGA_1 -GSH conjugate by soluble enzymes, GSH S-transferases, has been suggested by Cagen et al. [Biochem. Biophys. Acta 398: 205 (1975)]. The addition of PGA_1 to incubation mixture of ^3H -keto PGF_{2a} completely abolished the formation of water-soluble metabolite(s) of ^3H -keto PGF_{2a} . Presumably other

15-keto PGs are also converted to GSH conjugates by GSH S-transferases. This indicates that 15-keto metabolites produced by prostaglandin dehydrogenase may be further metabolized to GSH conjugates.

This report was presented at the 1977 FASEB Meeting in Chicago.

Covalent Binding of Intermediate(s) in Prostaglandin Biosynthesis to Tissue Protein. A. G. E. WILSON, T. E. ELING, A. CHAUDHARI, A. KUNG, D. J. CRUTCHLEY, H. HAWKINS, AND M. ANDERSON, *Pharmacokinetics Section, Laboratory of Pharmacology and Biometry Branch, NIEHS, Research Triangle Park, North Carolina 27709*

We have investigated the possible covalent binding of intermediates in prostaglandin (PG) biosynthesis to tissue macromolecules following incubation of arachidonic acid- $1\text{-}^{14}\text{C}$ (AA) with guinea pig lung or bovine and ram seminal vesicle microsomes. Radioactivity was associated with the microsomal protein which was not dissociated from the protein by exhaustive solvent extraction. The radioactivity associated with the protein was not dissociated by filtration through Sephadex G-25. These results suggest covalent binding of AA metabolites to protein. The covalent binding of AA metabolites was inhibited by indomethacin and N-0164. The addition of glutathione to the incubation mixture also inhibited the covalent binding. Oxygen consumption and covalent binding was correlated when AA was metabolized by ram seminal vesicle microsomes. Radioactivity was not associated with the protein when ^3H - PGE_2 , ^3H - PGF_{2a} , or ^3H -thromboxane B_2 were incubated with microsomal protein. Our studies suggest that PGG_2 or an early intermediate in the synthesis of PGG_2 covalently binds to tissue protein. This covalent binding may be of physiological and pathological significance.

Co-Oxygenation of Chemicals (Benzopyrene) During Prostaglandin Biosynthesis. K. SIVARAJAH, M. ANDERSON, AND T. ELING, *Pharmacokinetics Section, Laboratory of Pharmacology and Biometry Branch, NIEHS, Research Triangle Park, North Carolina 27709*

It has been reported that during the formation of prostaglandins (PG) from arachidonic acid (AA) in ram seminal vesicles, various chemicals are cooxygenated [J. Biol. Chem. 250: 8510 (1975)]. We have examined the oxygenation of the carcinogen, benzopyrene (BP) during the formation of PG in guinea pig lung (GPL) and ram seminal vesicles (RSV). In the presence of AA, guinea pig lung microsomal incubation systems metabolize BP to materials that migrate on thin-layer systems as quinones. Monohydroxy and dihydrodiol BP metabolites were formed but in small amounts. Total BP metabolites produced by GPL microsomes in the presence of AA were 300 pmole/2 mg protein in 5 min. In the presence of NADPH, large amounts of dihydrodiol and monohydroxy BP metabolites were formed, with smaller amounts of quinones and non-mobile metabolites detected. Total amount of BP metabolites formed by GPL microsomes in the presence of NADPH was approximately 800 pmole/2 mg/5 min. RSV also metabolizes BP in the presence of AA to material which migrated as quinones in thin layer. Since RSV did not contain cytochrome P-450, the addition of NADPH to the incubated system did not result in increased formation of BP metabo-

lites. Although RSV produce approximately 100 times more PG from AA, the amount of BP metabolized by RSV (approximately 1200 pmole/2 mg/5 min) was only four times that observed in GPL. The amount of BP which became covalently linked to GPL microsomal protein during incubation varied from 5% of the total BP metabolized in the presence of NADPH to 17% of the BP metabolized in the presence of AA. Thus it appears that BP is oxygenated during the formation of PG to reactive metabolites which covalently link to tissue macromolecules. The significance of this finding in relation to the carcinogenesis of BP was discussed.

The Comparison of the Effect of PCBs and the Tumor Promoter Phorbol Ester on Phosphatidylcholine Turnover in Human Lung Fibroblasts *in vitro*. J. W. SPALDING AND E. FORD, *Molecular Pharmacology and Biochemistry, Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709*

The tumor promoter, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), is known to stimulate the incorporation of ¹⁴C-choline into phosphatidylcholine (PC) in mouse skin epidermis *in vivo* and in cells growing *in vitro*. We have previously demonstrated that the organochlorine agents, DDT and 4-chlorobiphenyl (4-Cl-BP), selectively stimulate choline incorporation into PC of mouse L5178Y lymphoma cells [Biochem. Pharmacol. 25: 2051 (1976)]. These studies indicate that the above agents increase the rate of PC turnover.

In the work reported here, we have compared the effects of TPA and 4-Cl-BP on ¹⁴C-choline uptake and subsequent incorporation into the PC of normal human lung fibroblasts (WI-38 strain). TPA (10⁻⁷M) increases ¹⁴C-choline uptake into confluent WI-38 fibroblasts by 30-40%, while 4-Cl-BP (10⁻⁴M) has no significant effect. Both TPA and 4-Cl-BP elicited a 4- to 6-fold increase in ¹⁴C-choline incorporation into PC. This stimulation of PC biosynthesis was independent of any effect by these agents on ¹⁴C-choline uptake by the cells. The Aroclor mixtures, 1221, 1242, 1248, 1016, and 1254, at concentrations equivalent to 4-Cl-BP (10⁻⁴M) also stimulated PC biosynthesis. The effect of TPA and 4-Cl-BP on ³²PO₄ incorporation into the four major phospholipid species of WI-38 cells was also compared. The incorporation of ³²PO₄ into PC was stimulated 5- to 7-fold. Incorporation of ³²PO₄ into the other major phospholipid species was either inhibited or only slightly increased. The similarity of the pattern of ¹⁴C-choline and ³²PO₄ incorporation into PC of treated WI-38 cells suggests that the stimulation of PC biosynthesis occurs via the cytidinediphosphocholine pathway. Furthermore, these results indicate that TPA and 4-Cl-BP stimulate PC turnover by the same molecular mechanism. Since there is evidence that PCBs and other organochlorine agents have both tumorigenic and tumor-promoting activity in animals, a comparison of the biological activity of TPA and PCBs at the molecular level is a subject that requires further investigation.

Part of this work was previously reported in Proc. Amer. Assoc. Cancer Research (1976).

Microsomal Activation of Aflatoxin B₁ and G₁ Metabolites Mutagenic in *Neurospora crassa*. TONG-MAN ONG, *Laboratory of Environmental Mutagenesis, NIEHS, Research Triangle Park, North Carolina 27709*

Aflatoxin is one of the most common mycotoxins found in agricultural commodities and food products. Aflatoxin B₁, the most abundant species of aflatoxins, is known to be one of the most potent chemical carcinogens in the environment and is suspected to be responsible for the high incidence of hepatoma in several areas in the world.

Our previous studies have shown that aflatoxin B₁ and G₁, *per se*, are not mutagenic in the wild type strain of *Neurospora*. These compounds, however, can be converted by hamster and mouse liver homogenates to mutagenic metabolites. Further studies on the relationship between carcinogenesis and mutagenesis and the mechanism of mutagenesis were conducted by using the *ad-3* test system of *Neurospora crassa*. Conidia from a ultraviolet sensitive strain of *N. crassa* were treated with aflatoxin B₁ and G₁ and organ homogenates from different animal species. Studies were carried out under different treatment conditions. The results show that: (1) the liver homogenates from hamsters and mice can all convert aflatoxin B₁ and/or G₁ to metabolites mutagenic in the *uvs-2* strain of *Neurospora*; (2) the liver or kidney homogenates from either male or female rats can all convert aflatoxin B₁ and G₁ to mutagenic metabolites; (3) aflatoxin B₁ is much more mutagenic than aflatoxin G₁ in an *in vitro* activation system using either liver or kidney homogenate; (4) an NADPH generating system does not appear to be necessary for the conversion of aflatoxin B₁ to mutagenic metabolites; (5) pretreatment of liver homogenates with heat at 60°C for 10 min does not seem to have a significant effect on the conversion of aflatoxin B₁ to mutagenic metabolites; (6) pretreatment of rats or hamsters with phenobarbital does not enhance the mutagenic activity of aflatoxin B₁ in an *in vitro* activation test system. Our studies also show that aflatoxin B₁ *per se*, is a weak mutagen in the ultraviolet repair deficient strain *uvs-2* of *Neurospora*. It appears, therefore, that aflatoxin B₁ causes certain genetic damages which can be repaired by the wild type but not by the ultraviolet-sensitive strain.

Inactivation and Mutation Induction in Doubly Repair-Deficient Strains of *Neurospora crassa*. R. C. HARVEY, H. INOUE, D. F. CALLEN, AND F. J. DE SERRES, *Laboratory of Environmental Mutagenesis, NIEHS, Research Triangle Park, North Carolina 27709*

Several loci which control radiation sensitivity in *Neurospora crassa* have been identified in earlier studies and the DNA repair processes characterized to some extent on the bases of responses of mutant strains to radiations and chemical agents. In order to understand the complex biological processes involved in DNA repair and mutagenesis, it is useful to consider the interactions of particular gene products in the various repair pathways available in response to prelethal and premutagenic lesions.

The interactions of mutant alleles that individually confer radiation sensitivity in *Neurospora crassa* are being studied in several homokaryotic double-mutant strains with regard to the effects on inactivation and forward-mutation induction at the *ad-3* loci by ultraviolet and x-ray irradiation.

The two excision-repair deficient alleles, *upr-1* and *uvs-2*, interact epistatically with respect to the lethal and mutagenic effects of ultraviolet irradiation. However, they show an additive type of interaction for both inactivation and mutagenesis by ionizing irradiation. The *upr-1, uvs-3* double mutant is more sensitive than either single mutant strain to inactivation by x-rays and ultraviolet irradiation [J. Bacteriol. 112: 632 (1972)]. Like the *uvs-3* strain, this double mutant is not subject to significant increases in forward-mutation frequency by exposure to either type of irradiation. The *uvs-2, uvs-6* combination results in an

increased sensitivity of the strain to inactivation by both types of radiations, but does not increase its sensitivity to mutation induction. The combination of the *uvs-3* and *uvs-6* mutant alleles is shown by tetrad analysis to be nonviable.

The lethal and mutagenic effects of ultraviolet and x-ray irradiation in these double-mutant strains are interpreted in terms of the alternative repair systems in *Neurospora*.

Chemical Mutagenesis in UV-Sensitive Strains of *Neurospora crassa*. HIROKAZU INOUE, TONGMAN ONG, AND F. J. DE SERRES, NIEHS, Research Triangle Park, North Carolina 27709

To elucidate repair mechanisms of DNA damage induced by chemical mutagens, we have studied inactivation of conidia and mutation induction by three well-known chemical mutagens 4-nitroquinoline-1-oxide (4-NQO), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), and ICR-170 in the wild type and five different ultraviolet-sensitive strains (*upr-1*, *uvs-2*, *uvs-3*, *uvs-5* and *uvs-6*) of *Neurospora crassa*. Conidia from each strain were treated with different concentrations of chemicals. Treated and untreated conidia were assayed for survival and for frequency of *ad-3* mutations.

The results of survival tests showed that the order of sensitivity to 4NQO treatment is: *uvs-2* > *uvs-3*, *upr-1* > *uvs-6* > wild type, *uvs-5*. *Uvs-2* is about 10 times more sensitive, and *uvs-3* and *upr-1* are 3 times more sensitive than wild type. The order of sensitivity to MNNG treatment is: *uvs-5*, *uvs-6* > *uvs-3* > *uvs-2* > *upr-1* > wild type. *Uvs-5* is about 10 times more sensitive and *uvs-2* is 3 times more sensitive than wild type. The order of sensitivity to ICR-170 treatment: *uvs-2*, *uvs-3*, *uvs-6* > *uvs-5* > *upr-1* > wild type. *Uvs-2*, *uvs-3* and *uvs-6* are about 3 times more sensitive than wild type. The results of mutation induction experiments indicated that *upr-1* and *uvs-2* are more sensitive than wild type to 4-NQO and MNNG treatments. In the treatment with ICR-170, *upr-1* is similar to wild type, but *uvs-2* is 3 times less mutable than wild type. Both *uvs-3* and *uvs-5* are less mutable. ICR-170 and 4-NQO seem to be nonmutagenic in *uvs-3*. Mutation induction in *uvs-6* is usually similar to that of wild type.

Based on the data reported here, it is suggested that the *uvs-3* mutant is similar to the *recA* mutant of *Escherichia coli* and the *uvs-5* mutant is similar to the *rev* mutant of yeast.

Description and Calibration of a Forward Mutation System in *Salmonella*. CARMEN PUEYO, Laboratory of Environmental Mutagenesis, NIEHS, Research Triangle Park, North Carolina 27709

S. typhimurium SV3 (*F*⁻, *trp-294*, *thr-115*, *pyr-B92*, *ara-531*) is a L-arabinose negative and L-arabinose sensitive mutant. Its growth is severely inhibited by L-arabinose in the presence of glycerol as the sole carbon and energy source; glucose prevents and cures the inhibition. It is proposed that SV3 is affected in the structural gene for L-ribulose 5-phosphate-4-epimerase (gene D) in the *araBAD* operon, with the accumulation of L-ribulose 5-phosphate responsible for the inhibition. This accumulation can be prevented by interfering with the A,B structural genes, the regulatory, permease or repressor genes, or by reversion of the original mutation. Thus, the assay based on the change from arabinose sensitivity to resistance is a simple, direct, forward mutation system with several loci involved. Its use with a number of selected mutagens will be described.

The system is being calibrated from the viewpoint of its sensitivity in the detection of mutagens and carcinogens. The spon-

aneous mutation frequency is not affected by the usual problems found with other systems: Fluctuation, size of the plating samples and spurious results; not requiring time, the phenotypic expression of the resistants. In the same way, the response to known mutagens and carcinogens is being tested and the sensitivity of the system increased through the construction of new strains with additional mutations.

Up to the date, *in vitro* tests without metabolic activation protocols have been performed with chemicals belonging to different groups (*N*-nitrosoguanidine, β -propiolactone, 2-nitrofluorene, captan, and others) and two physical agents; heating and sonication.

Intravenous Host-Mediated Assay for Detection of Mutagenesis with *Saccharomyces cerevisiae*. DOMENICO FREZZA AND ERROL ZEIGER, Laboratory of Environmental Mutagenesis, NIEHS, Research Triangle Park, North Carolina 27709

A considerable improvement in the short term mutagenicity test *in vivo* (host-mediated assay) is achieved by the injection of microorganisms into the blood stream and their recovery from different organs.

After an injection of 3×10^8 cells of *S. cerevisiae* into the tail vein of a mouse, the distribution in different organs was studied. Up to 20% of the injected cells can be recovered from the lungs after 30 min, decreasing to 4% after 18 hr. The maximum recovery of cells from the liver (40%) occurs at 4 hr, decreasing to 10% after 18 hr; the cells are trapped in the reticulo-endothelial system. Recovery of cells from the kidney is low but constant, with a maximum of 5% after 4 hr, decreasing to 1% after 18 hr. The distribution within the organs was studied by tagging the yeast with a fluorescent dye.

Dimethylnitrosamine, at levels of 50 and 5 mg/kg administered by gavage-induced gene conversion in *S. cerevisiae* strain D4, was recovered from the liver, lungs, and kidney.

Plant-Mediated Activation of Mutagens. BARRY R. SCOTT, Laboratory of Environmental Mutagenesis, NIEHS, Research Triangle Park, North Carolina 27709 A. H. SPARROW,* S. S. LAMM, AND L. A. SCHAIRER, Brookhaven National Laboratory, Upton, Long Island, New York

Various microbial test systems which exhibit a positive response to liquid 1,2-dibromoethene (EDB) give negative results when the mutagen is delivered in a gaseous form. These observations have led to the hypothesis that this compound induces genetic alterations by two routes. One route apparently involves "breakdown" product(s) *in vitro* which are active in liquid exposures and a second route which involves metabolic activation by plants. Other promutagens/procarcinogens have also been activated by plant metabolism.

These results indicate the need for further studies on plant metabolism of environmental chemicals (pesticides) for a clear understanding of potential genetic hazard to man, either direct by ingestion or indirectly by integration into the ecosystem.

*Deceased.

Gene Mutations in Cultured Cells. S. HUANG,

Laboratory of Environmental Mutagenesis, NIEHS, Research Triangle Park, North Carolina

Lesch-Nyhan syndrome is a heritable disorder of purine metabolism in man. The responsible gene is sex-linked and recessive. The primary product of the gene is the enzyme, hypoxanthine guanine phosphoribosyl transferase which is defective or absent in this syndrome. With knowledge of this inherited genetic defect a system using this locus has been established to monitor the induction of mutations by environmental agents. There are, however, variations in the response of the system. Our objectives have been to identify and elucidate the sources of variation in this genetic testing system and to carry out a model study.

Induction of 6-thioguanine (6TG) resistance was studied in human fibroblasts treated with the direct-acting chemical carcinogen NA-AAF. At low concentrations of NA-AAF (2.5–7.5 μM), induction of resistance to 6TG was linear and followed 1 hit kinetics. A study of about 50 resistant clones revealed that most had lower levels of hypoxanthine-guanine phosphoribosyl transferase (HGPRT) activity (12–85% of controls) and were able to use exogenous hypoxanthine for growth; a few had little HGPRT activity (1–7% of controls) and were unable to use exogenous hypoxanthine effectively. Use of $9\text{-}^{14}\text{C}$ NA-AAF made it possible to examine the frequency of induction of thioguanine resistance as a function of DNA damage (i.e., $\mu\text{mole AAF/mole DNA-P}$). Calculations from these data suggest that most "hits" on the HGPRT locus do not result in detectable mutations.

Genetic Activity of Oil-Spill Photoproducts. D. F. CALLEN, Laboratory of Environmental Mutagenesis, NIEHS, Research Triangle Park, AND D. A. LARSON, Stroud Water, Research Center, Avondale, Pennsylvania 19311

Simulated environmental ultraviolet irradiation of a #2 fuel oil resulted in the production of water soluble oxygenated compounds. The toxicity and genetic activity of irradiated fuel oil samples were tested in the diploid yeast (*Saccharomyces cerevisiae*) strain D4. This strain monitors gene conversion at the *ade2* and *try5* loci. Increasing toxicity to stationary phase cells of the strain D4 was associated with increases in the time of irradiation of the fuel oil. After irradiation of the fuel oil for 12 and 24 hr, the samples were also genetically active.

It has been established that a large proportion of the light induced toxicity can be attributed to the production of hydroperoxides. Cumene, *tert*-butyl, and tetralin hydroperoxide were tested and were all highly toxic to the cells. The hydroperoxide of *tert*-butyl was also genetically active.

Computer Assistance for the Screening of Mutagenic Compounds. L. D. CLAXTON, Laboratory of Environmental Mutagenesis, NIEHS, Research Triangle Park, North Carolina 27709

Bacterial test systems have provided a means for rapid, relatively inexpensive methods for screening for mutagens and potential carcinogens. These systems are among the tierone systems of proposed hierarchical testing schemes. Since these systems will be used to test thousands of chemicals per year, highly standardized protocols and strict quality will have to be assured. This pilot study demonstrates that computer-assisted screening procedures can be developed and utilized. The system provides for (1) a basic (but automatically modified) protocol for the technician's use, (2) the use of appropriate pilot tests for the

selection of more appropriate definitive test conditions, (3) the storing of preliminary information concerning the chemicals to be tested, (4) the performing of routine calculations during each experiment, (5) the objective evaluation of each test based on predetermined criteria, (6) the coding of information so that blind testing can be done, and (7) the collecting, storing, analysis, and retrieval of all generated data. The programming was written mainly in COBOL and the programming task was divided into three individual programs with each utilizing the time sharing option (TSO) for an online interactive system. The presentation presents a summary of the decision processes automatically executed by computer software, the files created for program execution and data storage, and an example of a printout used by the laboratory technician.

Characterization of Newly Arising Biochemical Mutations. C. H. LANGLEY AND R. A. VOELKER, Laboratory of Environmental Mutagenesis, NIEHS, Research Triangle Park, North Carolina 27709

This experiment is aimed at answering two questions about low dose-rate ionizing radiation: (1) What is the qualitative nature of the gene mutations induced at low dose rates? (2) How much variation is there among loci in sensitivity? The design takes advantage of genetic manipulations possible in *Drosophila* to expose genes continuously to low dose rates of γ -radiation for 14 generations (7 months).

A strain of *Drosophila* composed of balanced lethal heterozygotes, +/SMI was separated into 1000 lines and brother-sister mated each generation while being exposed to γ -radiation (ca 7 rad/hr). This strain is heteroallelic (electrophoretically) for seven soluble enzyme loci (*Got-2*, *α Gpdh*, *s-Mdh*, *Adh*, *Dip-A*, *Hex-C*, and *Amy*). When subjected to electrophoresis, the unmutated lines give codominant phenotypes. If one of the alleles in a particular line has sustained a null mutation (loss of enzyme activity) the phenotype will appear dominant (or homozygous).

These lines have now been screened for mutations. The next step taking place now is to characterize the mutation as to the effect on viability, associated cytogenetic and biochemical effects. At completion it is hoped that the spectrum of lesions induced by ionizing radiation at this dose rate can be contrasted with the preponderance of large deletions observed at high dose rates. Further information or variability among loci will also be examined. These two observations should help in the extrapolation of high dose rate animal experiments to human risk.

Estimation of Frequencies and Characterization of Heritable Enzyme Deficiencies in Natural Populations of *Drosophila*. R. A. VOELKER, C. H. LANGLEY, A. LEIGH-BROWN, AND S. OHNISHI, Laboratory of Environmental Mutagenesis, NIEHS, Research Triangle Park, North Carolina 27709

The objective of this experiment is to further describe the naturally occurring genetic variation so that some base line information is available from which to assess the effects of environmental mutagens. Because of its genetic manipulability and convenience of handling as an experimental organism, this study is being done with samples from natural populations of the fruit fly, *Drosophila melanogaster*.

One type of mutation, termed a "null" allele, results in an enzyme deficiency. At present almost nothing is known about the frequency of "nulls" in natural populations or their effects

on viability. By utilizing specially constructed stocks in a specified mating scheme, it is possible to recover genes from natural populations. Flies carrying these genes can then be mated to specially constructed tester stocks and their progeny examined electrophoretically for their ability to produce the enzyme in question. Genes which demonstrate an inability to produce a functional enzyme will be further analyzed to determine: whether no enzyme is produced or whether an enzyme is produced which is nonfunctional; whether "nulls" producing no enzyme at all are associated with the physical deletion of the structural gene; and whether the lack of enzyme activity is associated with lethality or other abnormality. The special stocks are nearly synthesized and samples will be collected in the near future.

The results of this study should begin to provide the background knowledge which is necessary to assess the amounts and types of mutations which are induced by environmental mutagens.

Use of Enzyme Heat Denaturation for Detection of Mutations. J. BURKHART, C.-Y. LEE, B. PEGORARO, AND H. V. MALLING, *Laboratory of Environmental Mutagenesis, NIEHS, Research Triangle Park, North Carolina 27709*

The objective of this project is to develop a system to detect induced mutations in mice using the high speed enzyme rate analyzer to monitor the thermal stability of enzymes. It is important to understand the relationships of structure and environment to apparent heat stability of the enzymes. Our approach has been to examine known electrophoretic variant enzymes in DBA/2J and C57BL/6J mice for conditions of tissue homogenization and heat denaturation that may help to define that relationship. Results indicate that enzyme thermal stability is closely correlated to pH, ionic strength, and endogenous concentrations of substrates and cofactors. The naturally occurring variants of isocitrate dehydrogenase, phosphoglycerate kinase, phosphoglucose isomerase, and phosphoglucosmutase as detectable with heat stability techniques. Pilot experiments are in progress to determine the biochemical condition necessary to monitor crude homogenates of mouse tissues for activity and thermal stability mutants of 20 enzymes. The development of this system is important because it could provide a rapid means to screen mammalian populations for mutations at many loci.

Biochemical Characterizations of Genetic Variants and Isozymes in the Mouse. B. PEGORARO, C.-Y. LEE, AND H. V. MALLING, *Laboratory of Environmental Mutagenesis, NIEHS, Research Triangle Park, North Carolina 27709*

The purpose of this project is to conduct a model study to differentiate the enzyme variants in mice by simple biochemical assays. Several isozymes and enzyme variants were purified from different inbred strains of mice. Two isozymes of phosphoglycerate kinase and isocitrate dehydrogenase have been characterized by kinetic studies, thermal and urea denaturation, and iodoacetate inactivation. Two genetic variants of cytoplasmic isocitrate dehydrogenase and phosphoglycerate kinase-B were also compared in detail. Most of the kinetic properties of the isozymes and variants were found to be similar. However, it was possible to observe differential thermal stability and urea inactivation for each pair of isozymes and for each pair of enzyme variants. The conditions to detect maximum difference by these means were established. Further studies are being con-

ducted to find whether other natural occurring enzyme variants in mice (electrophoretic variants) can also be detected by biochemical assay under some experimental conditions. We hope that we are able to generalize these experimental conditions so that it is possible to utilize them for rapid and simple biochemical assays to detect the mutant enzymes in mutagen-treated mice populations.

Mutagenicity of Trichloroethylene (TCE) in Yeast. G. BRONZETTI, E. ZEIGER, AND D. FREZZA, *Laboratory of Environmental Mutagenicity, NIEHS, Research Triangle Park, North Carolina 27709*

Trichloroethylene (TCE) (1-chloro-2,2 dichloroethylene; 1-1-2 trichloroethylene) is widely used in industry in the gaseous phase for degreasing, as a solvent, as an extraction medium in food processing, as an anesthetic, and as an ingredient in various products. TCE was tested for genetic effects using *Saccharomyces cerevisiae* strains D4 to evaluate gene conversion, and D7⁻ for gene conversion, mitotic recombination, and reverse mutation. Both strains were tested *in vitro* with and without metabolic activation and in the intrasanguinous host-mediated assay *in vivo*, using male Swiss albino mice.

The host-mediated assay was performed by injecting the yeast into the tail vein or supraorbital sinus of the mice; TCE was administered either subcutaneously or by gavage. We used an acute oral dose (400 ppm) and repeated oral administrations for a cumulative TCE dose of 3700 ppm. After 4 hr the test microorganisms recovered from the liver, lungs, and kidneys of the injected treated mice and evaluated for genetic effects.

Detection of Genetic Damage in Early Embryos. K. BÜRKI, *Swiss Institute for Experimental Cancer Research Epalinges, Switzerland*, AND WILLIAM SHERIDAN, *Laboratory of Environmental Mutagenesis, NIEHS, Research Triangle Park, North Carolina 27709*

After treatment of postmeiotic stages of spermatogenesis of the mouse with TEM, dose and stage of spermatogenesis dependent disturbances of early embryonic development can be observed both *in vivo*- and after *in vitro* culture of the embryos. The observations in both systems can be correlated. The rate of fertilized and cleaving eggs was unaffected, however, later stages of development were severely disturbed with a maximum effect observed on morulae. In addition, cytogenetic aberrations were observed in early embryos. Frequencies of first cleavage metaphases exhibiting structural aberrations (chromosome type), and the frequencies of 2 cell and 4-8 cell embryos containing nuclei accompanied by micronuclei or nuclei connected by bridges, show a close correlation to frequencies of preimplantation loss of embryos recorded in a dominant lethal test. The frequencies of morulae/blastulae exhibiting blastomeres with micronuclei show a close correlation to the frequencies of total loss of embryos.

Sequence Diversity of Total Poly(A)-Containing RNA from Mouse Simple Embryoid Bodies and Teratocarcinomas. S. E. HARRIS, S. GIPSON, D. TULLY, AND A. B. SILVERBERG, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

Understanding the molecular basis for differential gene expression is necessary if one hopes to gain further insights into the mechanism underlying damage to specific organs or cells. These processes are critical to understanding cancer development and to elucidate the basic phenomena of how the single fertilized egg differentiates into the adult organism. With that in mind we have undertaken a study of some of the molecular aspects of gene expression during cellular differentiation. The mouse embryo body/teratoma system OTT 6050 developed by Stevens appears to be an ideal system for our studies. Essentially, the embryo bodies (EB) are grown as an ascites in the peritoneal cavity of strain 129 mice. The EB's consist of an inner cell mass of embryocarcinoma cells and an outer ring of endoderm-like cells. Frequently, an EB will "implant" on the liver or spleen, or gut mesentery and this seems to trigger the embryocarcinoma cells to rapidly divide and differentiate into neuroepithelial-like teratomas which are 0.2-1.0 cm in diameter. Our initial studies have been concerned with the isolation and characterization of total poly(A)-containing RNA from the embryo bodies (EB) and the teratomas. This RNA fraction, which can be isolated by affinity chromatography using oligo-dT cellulose, is highly enriched in cytoplasmic messenger RNA as well as nuclear RNA's which contain poly(A) tracts. We have determined the content of poly(A) (2-3%) using ³H-poly dT as a probe. Likewise, the size of the poly(A)-containing RNA was determined on denaturing formamide sucrose gradients and found to be 1800-2100 NT (nucleotides). The size of the poly(A) tract was determined by RNase A + T1 treatment and then the size of the poly(A) RNase-resistant material was determined on polyacrylamide gels. Finally, ³H-complementary DNA probes were made to the poly(A)-RNA from embryo bodies and teratomas using reverse transcriptase. By nucleic acid hybridization analysis (RNA excess) of the respective ³H-cDNA with the RNA from which it is synthesized (back hybrid), an evaluation of the sequence complexity of the preparation could be made by computer analysis of the hybridization curves. Three classes of poly(A)-containing RNA could be resolved in both embryo bodies and teratomas based on their relative abundance in the respective RNA preparations. In general, all classes of sequences increase in the differentiation process from EB to neuroepithelial teratomas, and, in particular, the low abundance-high complexity class goes from about 3000 different sequences (of approximate complexity of 2000 NT) in the embryo bodies to some 7000 different sequences in the teratomas. Thus, the differentiation process has resulted in at least 5000 new sequences being expressed at the genetic level.

Heterologous *in vitro* Protein Synthesis for Detection of Specific mRNAs. A. B. SILVERBERG AND S. E. HARRIS, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

Heterologous *in vitro* systems for the translation of exogenous mRNA have been developed because: (1) complete removal of all mRNA fragments from ribosomes is difficult to demonstrate in homologous systems; (2) in a reconstituted system of ribosomal subunits and translation factors, the reinitiation of protein synthesis is suboptimal; (3) the protein factors, which are required for the initiation of protein synthesis in eucaryotic cells, are difficult to isolate and characterize. The heterologous systems that have been defined included: (1) S-30 preparation (30,000 g supernatant) of ascites tumor cells; (2) frog oocyte; (3) rabbit reticulocyte lysate; and (4) S-30 preparation from wheat germ. The latter two will be used in our laboratory. The advantages of the wheat germ S-30 system are the low level of en-

dogenous mRNA activity and the simple, inexpensive means of setting up the procedure. The low level of endogenous mRNA activity means increased sensitivity to exogenous mRNA. The disadvantages of this *in vitro* heterologous system are failure to reinitiate protein synthesis, inability to release completed proteins efficiently, difficulty in synthesizing large proteins (>40,000 daltons) and the presence of proteases. The rabbit reticulocyte lysate system reinitiates protein synthesis, completes proteins, releases proteins efficiently, and synthesizes large proteins (up to 200,000 daltons). The disadvantages of the reticulocyte lysate system are the high endogenous mRNA activity of reticulocytes (globin mRNA), the considerable effort and expense to prepare the system, and the variability in translation efficiency from preparation to preparation. A modification of the lysate system eliminates the major disadvantage of high endogenous mRNA activity. Lysate is incubated with a calcium dependent nuclease which will destroy the more sensitive mRNA species. The nuclease can be inactivated by EGTA at the end of the incubation. We plan to use the heterologous *in vitro* translation systems for the detection of specific mRNA's and as assay systems for characterizing total poly(A)-containing mRNA from embryo bodies, teratomas, and fetal and adult rat livers. The mRNA's for α -feto protein, glial fibrillary acidic protein, and S-100 are the specific messenger species in which we are interested.

Isolation and Characterization of Chromatin from Embryo Bodies and Teratomas: Role of Nonhistone Chromosomal Proteins in Differentiation. D. B. CARTER, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

The embryo body/teratocarcinoma system simulates many of the differentiative processes occurring during embryogenesis and might provide an alternative to intact embryos for the study of early mammalian development. Differentiation involves the activation and repression of different gene systems. Studies of the regulation of eukaryotic gene systems such as the globin, ovalbumin and histone gene systems show that the nonhistone chromosomal (NHC) proteins are the major determinants of the specificity of gene expression. Compounds having teratogenic potential usually alter the differentiative processes in embryonic tissues. Thus, the effects of compounds having the ability to interfere with normal differentiative processes may exert some detectable effect on the system of proteins controlling specific gene expression; the nonhistone proteins of mammalian chromatin. High resolution two-dimensional gel electrophoretic systems have been recently developed which have resolved up to 450 proteins on autoradiographic plates from the HeLa NHC protein system. Many NHC proteins were found to be present in 500 to 2000 copies haploid genome, a quantity consistent with expectations for specific gene regulatory proteins in eukaryotic systems. In order to prove the feasibility of the analysis of NHC proteins as an assay for putative teratogens the following paradigm is under development: (1) isolation of embryo body and teratoma chromatin from purified nuclei; (2) blocking chromosomal protease with 2mM phenylmethylsulfonyl fluoride; (3) extraction of 80% of NHC proteins by 5M urea-50mM NaPO₄, pH 7.5; (4) isoelectric focusing of NHC proteins on pH gradients (4 to 7.5); (5) equilibration of isoelectric gels in SDS buffer for second dimension SDS electrophoresis; and (6) autoradiography of slab gels. The comparison of NHC protein patterns of embryo bodies and teratomas grown with and without the presence of

known teratogens can be used to test the feasibility of the system. Qualitative and quantitative changes in the NHC proteins can be measured with a two dimensional scanner. If the utility of the system proves feasible, one may assay unknown compounds for their ability to change the NHC protein complement by using the procedure described above in a period of 2 to 3 weeks for the *in vivo* mouse ascites culture system.

The Role of DNA Repair in Germ Cell Toxicity. I. P. LEE AND R. L. DIXON, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

The mutagenic and carcinogenic potential of drugs and environmental chemicals has become a major human health concern. Short term test systems employing certain bacterial mutants such as the Ames test as well as mammalian cell lines including human diploid cells have been developed. In spite of encouraging recent advances, there is currently no rapid test utilizing germ cells. In an attempt to better understand the mechanisms of action and usefulness of a germ cell approach, alkaline elution analysis was applied to define the DNA damage induced in germ cells by three classes of known chemical mutagens. Monofunctional alkylating agents (methylmethane sulfonate, cyclophosphamide, and procarbazine), polyfunctional alkylating agents (busulfan, triethylene melamine, and mitomycin C), and DNA intercalating agents (adriamycin and proflavin) were studied. Prepubertal rat spermatogenic cells were pre-labeled with tritiated thymidine (0.5 μ Ci/g body weight; specific activity; 61 Ci/mole) by treating animals intraperitoneally four times daily. At 18 hr after the last thymidine injection, the animals received the test chemical and were subsequently sacrificed. Results demonstrated that there were at least two main types of DNA (1) DNA single strand breaks which were primarily induced by monofunctional alkylating agents and (2) DNA inter- and intrastrand crosslinking which were primarily induced by polyfunctional alkylating agents. The crosslinking between complementary strands of DNA and/or between DNA and nucleoprotein prevents proper DNA replication and consequent mutation and/or germ cell death. Polyfunctional alkylating agents interact with DNA resulting in DNA which is very resistant to both ionizing radiation and monofunctional alkylating agents. This phenomenon does not occur for monofunctional alkylating agents. Therefore, alkaline elution analysis of pre-labeled germ cell DNA exposed to chemical mutagens might be a useful technique to screen chemical mutagens at mammalian germ cell level.

Ontogeny of Epoxide Metabolizing Enzyme Activities in Steroidogenic Tissues of the Rat. I. P. LEE, K. SUZUKI, H. MUKHTAR, AND J. R. BEND, *Laboratory of Environmental Toxicology and Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709*

Although the potential interaction of chemical carcinogens with germinal cells could be of critical significance, little attention has been accorded the potential ability of testicular tissue to metabolize reactive chemicals, such as alkene or arene oxides. The present report is concerned with ontogeny of epoxide hydrolase (EH) and glutathione-S-transferase (GS-T) activities in rat steroidogenic tissues as well as with the differential distribution of arylhydrocarbon hydroxylase (AHH), EH, GS-T, and cytochrome P-450 content in interstitial and spermatogenic cells. Mi-

croosomal EH and soluble fraction GS-T activities were determined with styrene 7,8-oxide (SO) and/or benzo[a]pyrene 4,5-oxide (BPO) in female ovary, adrenal, and male testis. Adult animals had high GS-T specific activity (SA) (nmole/min-mg protein) in all three tissues (about 50% hepatic activities with SO). EH activity was 3-4 times higher in testis with SO (SA, 1.8) than in ovary (0.43) and adrenals (0.65). Microsomes and soluble fraction prepared from testicular germ cells had about twice the EH and GS-T, SA of those from Leydig cells. By comparison, benzo[a]pyrene hydroxylase activity and cytochrome P-450 content was at least 2-fold greater in Leydig cells than in germ cells. Perinatal development of GS-T activity was followed in ovary, adrenal, and testis and that of EH in testis. In adrenals, SA of GS-T was high in 1-day-old rats (SA, 90.3 and 4.8 for SO and BPO, respectively) and did not change appreciably with age. Ovaries of 12-day-old rats had SA of GS-T of about 25% that of adult values for SO and BPO. Activity developed gradually and reached a maximum by 56 days of age. Testis of 7-day-old rats had GS-T activities (SA, 66) about 50% of adult levels. By contrast, EH activities developed slowly in testis (less than 21% adult SA by 21 days of age). By contrast, EH activities developed slowly in testis (less than 21% adult SA by 21 days of age). High GS-T activities in testis and ovaries may be important in protecting against chemical toxicity to germ cells mediated by circulating electrophiles, such as alkene or arene oxides.

Environmental Teratology Information Center (ETIC). R. E. STAPLES AND F. E. JORDAN, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

Existing teratology data on chemicals and other agents to which man is exposed are difficult to locate. No central file currently exists that specifies what chemicals or agents have been tested for teratogenicity, in what species, to what extent, and with what effects. Recognizing these needs, we have initiated development of a computerized system, the Environmental Teratology Information Center (ETIC). The information collecting and data processing activities are located at the Oak Ridge National Laboratories (ORNL). The center is made possible through an interagency agreement between NIEHS and the Energy Resources and Development Administration (ERDA), which operates ORNL. At this time, ETIC has identified and entered over 7500 references to teratology studies published since 1912. It is ETIC's goal to provide easy access to these references for all individuals and organizations needing this information. ETIC hopes to meet the diverse needs of teratologists, physicians, regulatory agencies, and industry. An arrangement with the National Library of Medicine (NLM) to have the ETIC bibliographic file included in TOXLINE (Toxicology Information On-Line) provides one easy access method for the biomedical community. TOXLINE is an interactive toxicology information retrieval system which can be accessed through remote terminals via both national and international communications networks. Information contained in the ETIC file also can be obtained by writing or calling the Environmental Teratology Information Center, National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, North Carolina 27709 (telephone, 919/541-3214 or FTS 629-3214). ETIC is also developing a teratology data bank which will provide an in-depth extract of information in tabular form for each reference. This additional information will be made available through publication of extracts in hard copy or microfiche. Eventually these data also may be made available to the biomedical community through an on-line system. Another goal of ETIC is to provide information about the teratogenic status of

the most important chemicals as evaluated by experts. It may soon be possible to have Teratology Information Response Centers similar to the existing Poison Control Centers. In the meantime, it is felt that the development of the Environmental Teratology Information Center has greatly enhanced the transfer of this technology from the laboratory to the scientific and medical communities.

Postimplantation Embryo Culture: a Model for the Study of Organogenesis. M. K. SANYAL, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

A primary source of developmental anomalies is presumably exposure to adverse environmental factors. Major organ defects are known to appear during the organogenesis phase of development. With the perspective of detection and study of mechanisms by which environmental agents induce developmental disorders, an *in vitro* culture system of embryos during the organogenesis phase of development in the rat paralleling *in vivo* development has been established. This system of embryo culture is modified from the initial method developed by New et al. [J. Reprod. Fert. 48: 219 (1976)]. It involves culture of 5 conceptuses of pregnancy day 11 (sperm positive, day 1) with embryos within yolk and amniotic sacs and intact ectoplacental cones in 60 ml serum bottles containing 10 ml heparinized heat inactivated male rat plasma. The bottles are rotated horizontally in a roller disc as described by Kochhar [Teratology 11:273 (1975)]. In this culture system, the embryos which were curved ventrally during the afternoon of pregnancy day 11 grew and differentiated optimally with gas phase of 5% CO₂, 20% O₂, and 75% N₂, and with bottle rotation speed of 40 rpm. In 24 hr of culture, the size of conceptuses increased from 2.64 ± 0.03 mm in diameter to 4.99 ± 0.06 mm. The embryos grew remarkably within this period from Witschi stage 16 (somites 14.5 ± 0.2) to stage 17 (somites 21.7 ± 0.2) with DNA content increasing from 2.4 ± 0.4 μg/embryo to 11.5 ± 0.7, and protein from 40.2 ± 2.3 μg/embryo to 96.3 ± 7.5. Histological examination revealed that major organs, brain, neural tube, sensory organs, heart and circulatory system, gut, liver and musculature, differentiated considerably. The organogenesis of these embryos *in vitro* was comparable to that *in vivo*. Further differentiation of the placentas, however, did not occur under the present culture conditions.

Genital Tract Abnormalities in Mice Following Gestational Exposure to DES. J. A. MCLACHLAN, R. R. NEWBOLD, AND J. C. LAMB, IV, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

Prenatal treatment of CD-1 mice with the synthetic estrogen, diethylstilbestrol (DES), resulted in abnormalities in the genital tract of mature female offspring. These abnormalities were detected after treatment on days 9-16 of gestation with DES (1-100 μg/kg-day) and included cystic endometrial hyperplasia, inflammatory disease of the oviduct, persistent cornification of the vaginal epithelium, edema and stromal hyperplasia of the cervix, ovarian cysts, and female hypospadias. A low incidence of genital tract neoplasia was observed in the treated female offspring; a case of vaginal adenocarcinoma and one of uterine adenocarcinoma were among these observations. Genital cancers were not seen in the corresponding control offspring. To further evaluate these lesions, the surface ultrastructure of the reproductive tract in female mice exposed prenatally to DES was studied

by scanning electron microscopy (SEM). Abnormalities in DES-treated mice observed by SEM at low power included urethral openings in the cervicovaginal area and untransformed vaginal epithelium. At higher powers of magnification, epithelial alterations included abnormal squamous cell configurations in the vagina, squamous metaplasia of the uterus and a "cobblestone" appearance of the cervicovaginal epithelium. Since cell surface changes are thought to be important in early neoplasia, ultrastructural surface changes may provide a morphological "marker" of preneoplastic development.

Diethylstilbestrol Metabolites and Analogs: *in vivo* and *in vitro* Estrogenic Activities. K. S. KORACH, M. METZLER, AND J. A. MCLACHLAN, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

The *in utero* toxic and carcinogenic nature of DES prompted our study of the biological activity of certain DES metabolites and analogs. Several metabolites (β-dienestrol and ω-hydroxy dienestrol) have been identified in mouse, rat and monkey urine, and two proposed metabolic intermediates (DES-α,α'-epoxide and α,α'-dihydroxy DES) were synthesized and their estrogenic activities determined. Analysis was also performed on two DES analogs, DES-dihydrodiketophenanthrene (DES-phenanthrene) and 1 (α-ethyl)-α-(4-hydroxy phenyl)indanyl-5-ol (Indanyl-DES). The compound's estrogenic activity was determined *in vivo* by using the immature mouse uterine weight bioassay. Analysis *in vitro* consisted of estradiol receptor binding activity using competitive equilibrium binding, sucrose gradient analysis and association rate inhibition assays. Bioassay results gave the following order of estrogenicity: DES > α-dienestrol > DES-epoxide > indanyl DES > dihydroxy DES > β-dienestrol > DES-phenanthrene. Results of the competitive equilibrium binding analysis of these compounds with 17β-estradiol for the mouse uterine cytosol receptor followed the same order seen for the bioassay, except for indanyl-DES which ranked second in the *in vitro* analysis and fifth in the bioassay. DES, indanyl-DES and α-dienestrol had the greatest affinities (K_a values, approximately 9.1 × 10⁹M⁻¹ ± 1.8), while DES-phenanthrene had the lowest K_a = 3.5 × 10⁷M⁻¹ ± 1.2. The interaction of these DES compounds with the receptor was shown to be competitive in nature by Lineweaver-Burk analysis. Displacement of ³H-estradiol from the receptor peak was demonstrated by sucrose gradient analysis and found to be receptor specific and concentration dependent. In addition, the most hormonally active substances demonstrated the greatest rate inhibition in the receptor association rate reaction (V₀). This study demonstrates that certain DES metabolites and analogs interact quite effectively with the uterine cytosol receptor. Our ability to rank order the estrogenicity of these compounds should be useful in evaluating alternative metabolic pathways of DES as well as distinguishing the hormonally active metabolites from the relatively inactive ones.

Perinatal Pharmacology of PCBs and PBBs. G. W. LUCIER, O. S. MCDANIEL, C. M. SCHILLER, AND H. B. MATTHEWS, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

Although much is known about the fate of specific PCB isomers in adult animals, little information is available concerning the pharmacology of these isomers in perinatal systems. Therefore, we have studied specific fetal and newborn tissue distributions of

several ¹⁴C-labeled chlorinated biphenyls: 4-chloro-(1-CB); 4,4'-dichloro-(2-CB); 3,4,3',4'-tetrachloro (4-CB); 2,3,6,2',3',6'-hexachloro-(6-CB'); and 2,4,5,2',4',5'-hexachloro-(6-CB). We have also studied 2,4,5,2',4',5' hexabromobiphenyl (6-BB), the major component of Firemaster BP-6. Pregnant rats were administered the labeled PCBs on day 18 of gestation and levels of radioactivity measured in maternal blood, liver, intestine, fat, skin, and muscle; fetal blood, liver, lung, intestine, kidney, brain, skin, and reproductive tract; and placenta. PCBs that do not accumulate significantly in adipose tissue do accumulate dramatically in fetal intestine. Fetal intestine to blood radioactivity ratios on day 21 of gestation (3 days after treatment) were as follows; 1-CB(41), 2-CB(22), 6-CB'(18), 4-CB(3), 6-CB(1). By 5 days after birth, intestine to blood ratios had decreased to approximately one for all PCBs. Approximately 8% of the fetal intestinal radioactivity following 1-CB treatment was parent compound, 10% 4'-hydroxy-4-chlorobiphenyl and most of the remainder represented conjugates. 6-CB, which does not accumulate in the fetal intestine, is secreted in the milk although significant newborn tissue levels of this isomer also results from placental transfer. Newborn skin and fat contained the highest concentrations of 6-CB after gestational and/or milk exposure. Significant levels of 6-CB were detected in brown fat immediately after birth. The brown fat is rapidly depleted causing a redistribution of 6-CB to other tissues. 6-BB crosses the placenta slowly but is secreted in high concentrations in milk. Further experiments are investigating the relative importance of placental transfer and milk secretion of 6-CB and 6-BB following treatment of female rats two weeks prior to mating.

The Small Intestine as a Target Organ for Developmental Toxicity. W. H. CURLEY, T. E. KEE, C. M. SCHILLER, R. WALDEN, AND G. W. LUCIER, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

The small intestine is an important target organ for the study of environmental toxicology. The gastrointestinal tract is often responsible for the initial metabolism of all ingested substances. The normal functioning of this organ is especially important during the perinatal period, when the organism must first rely on its own absorptive and active transport processes for nourishment. In humans, there is an increasing level of incidence of colon carcinomas and routine methods for early detection are virtually nonexistent. According to the theory of fetalism first proposed by Greenstein, there could be a link between the rapid growth conditions during early development and those associated with cancerous states. Some of our experiments were designed to explore the possibility that fetal or neonatal enzymic profiles are similar to those of tumor tissue. We have been interested in hydrazine and its metabolic derivatives dimethylhydrazine (DMH) and methylazoxymethanol (MAM), because they are known to be organ specific carcinogens for the colon and to be possible teratogens in mammals. In addition, these compounds are of environmental significance because they can be found in mushrooms, tobacco, jet fuel exhaust, and *Cycas circinalis* nuts which contain the toxic substance cycasin. The mammalian enzyme β -glucuronidase (BG) is involved in the enterohepatic detoxication-intoxication processes of a wide variety of chemicals, including the deglucuronidation of a cycasin derivative to its active form, MAM. This enzyme also exhibits a developmental peak of activity in the intestine during the neonatal period. We are investigating BG isozyme profiles in newborn and adult tissues as well as tumor tissue to determine the possible fetalism of BG in intestinal carcinomas. Lactase, sucrase, (Na, K)-ATPase, and alkaline phosphatase activities, which are biochemical indicators of intestinal function and membrane in-

tegrity, are changed after prenatal exposure to hydrazine and/or its derivatives.

Perinatal Programming and Hepatic Enzyme Development. C. A. LAMARTINIERE, E. BRIDGES, P. WATKINS, C. S. DIERINGER, AND G. W. LUCIER, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

Differentiation may be regarded as progressive development of specific enzymes with each differentiating tissue manifesting its own characteristic enzyme developmental pattern. The appearance of each enzyme and its subsequent developmental course is presumably dependent on differential and timed initiation of specific gene expression and subsequent regulation of translational, transcriptional and post-transcriptional processes. Hepatic histidase of the rat undergoes a complex postnatal developmental course and is therefore an excellent model system to study. Histidase activity is first detectable at parturition, increases in activity during puberty in both males and females, and attains levels that are approximately twofold higher in adult females than adult males. Estrogens are responsible for the higher adult enzyme levels characteristic of the female rat. We demonstrate that the synthetic estrogen, diethylstilbestrol (DES), affects rat liver histidase in a manner similar to 17 β -estradiol (E₂). Daily subcutaneous injections of 100 μ g/kg body weight E₂ or DES for one week to 28-day-old male rats elevated hepatic histidase activities to 177 and 175%, respectively, of control values. Adult ovariectomized rats receiving E₂ or DES on the same schedule exhibited histidase activities that were 150 and 140%, respectively, of controls. Oral administration of DES (100 μ g/kg body weight) to pregnant rats on day 15 of gestation had no effect on histidase activities in immature male and female offspring and adult male offspring. However, histidase activities of these intact adult female rats were decreased by 30% and approached activities of adult males. Ovariectomy of these prenatally DES treated adult females, and subsequent administration of E₂ resulted in elevated histidase activities that are similar to normal intact females. Thus, DES elicits a prenatal programmed response of hepatic histidase that appears to be reversible by exogenously administered estrogen.

This abstract was presented in part in Fed. Proc. 36: 927 (1977).

Effects of DES on Perinatal Development of Hepatic Steroid-Metabolizing Enzymes. C. S. DIERINGER, C. A. LAMARTINIERE, AND G. W. LUCIER, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

The "programming" of sexual differences in adult rat hepatic metabolism of steroid hormones and drugs appears to occur as a result of neonatal exposure to endogenous steroid hormones. Diethylstilbestrol (DES), a highly potent synthetic estrogenic compound, has been used both clinically and as a cattle feed additive. We have examined the effects of DES exposure on the developmental patterns of 5 α -reductase (5 α -RED) and 16 α -hydroxylase (16 α -OH) activities. Pregnant rats were given oral doses of DES (500 μ g/kg) on days 14 and 18 of gestation. Hepatic enzyme activities were determined in the offspring at various developmental stages. Prepubertal developmental patterns of 5 α -RED and 16 α -OH are similar in male and female rats. At 31 days of age, 5 α -RED activity for females increases

markedly, whereas 5 α -RED remains at relatively low prepubertal levels for males. Prenatal exposure to DES delays the onset of the dramatic rise in levels of 5 α -RED for females until 45 days of age; there is little effect on 5 α -RED development in males. In contrast, 16 α -OH activity is higher in the adult male rat than in the adult female rat, with an increase from low prepubertal levels occurring at 45 days in the male; female 16 α -OH activity remains low throughout development. Prenatal exposure to DES has little effect on either male or female developmental patterns of 16 α -OH. Postnatal treatment of 7-day old rats with 10 μ g DES (PO) resulted in a 51% decrease (masculinization) of 5 α -RED activity in 49-day old female rats, but had little effect on 5 α -RED activity in 49-day old male rats. Castration of 7-day old male rats resulted in a 51% increase (feminization) in 5 α -RED activity by 49 days of age. Thus, postnatal exposure to DES seems to exert a greater effect on the programming of adult rat hepatic 5 α -RED and 16 α -OH activities than does prenatal DES exposure, suggesting that the critical period of development for rat hepatic enzyme programming occurs during the early postnatal period of life. These data demonstrate that exposure to a hormonally active compound during the critical period of development can alter normal enzyme development.

Altered Regulation of Hepatic Heme Biosynthesis and Mixed Function Oxidase Activity in Ethionine-Fed Rats. JAMES S. WOODS, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

These studies investigated the relationship between hepatic heme biosynthesis and microsomal mixed function oxidase (MFO) activity during hepatocarcinogenesis in ethionine-fed rats. As compared with controls, marked decreases in the levels of microsomal heme (56%), cytochrome P-450 (59%), aminopyrine demethylase (55%), and in the activity of mitochondrial heme synthetase (64%), the final enzyme in heme biosynthesis, were seen in livers of rats fed ethionine (0.25% of diet) for 4-8 weeks. The activity of δ -aminolevulinic acid synthetase, the rate-limiting enzyme in heme biosynthesis in normal liver, was not altered in ethionine-fed rats. However, heme, which represses synthesis of δ -aminolevulinic acid synthetase, in normal liver, neither repressed nor prevented chemical induction of δ -aminolevulinic acid in ethionine-fed rats. Allylpropylacetamide, which induces δ -aminolevulinic acid synthetase but not heme synthetase, did not increase P-450 or aminopyrine demethylase levels in ethionine-fed rat liver. In contrast, drugs such as phenobarbital or CoCl₂, which alter levels of both enzymes, or of heme synthetase alone, produced concomitant changes in microsomal or P-450 levels and in MFO activity. These results suggest that the relationship between hepatic heme synthetase and MFO levels in ethionine-fed rats is characterized by impaired regulation of δ -aminolevulinic acid synthetase and a primary block in heme production at the level of heme synthetase leading to a relatively deficiency in microsomal heme levels and decreased MFO activity.

This work was originally published in Proc. Amer. Assoc. Cancer Res. 18: 8 (1977).

Renal Porphyrin Following Chronic Methylmercury Exposure in the Rat. JAMES S. WOODS AND BRUCE A. FOWLER, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

Previous reports have demonstrated the occurrence of elevated heme precursors in humans chronically exposed to methylmercury (MM) in the diet or to organomercurial diuretic drugs. In the present study the etiology of mercury-related porphyria was investigated in adult male rats exposed to 0, 3, 5, or 10 ppm MM in drinking water for 6 weeks. Activities of heme biosynthetic pathway enzymes in livers of MM-exposed rats were not substantially different from those of controls. In kidney, however, both uroporphyrinogen I synthetase and ferrochelatase were depressed at all dose levels, with dose-related decreases to a maximum of 58 and 69% of control levels, respectively. In contrast renal δ -aminolevulinic acid synthetase, the rate-limiting enzyme in heme biosynthesis, was elevated at all dose levels with a maximal increase of 2.5 times control values. This alteration of renal heme biosynthetic pathway enzymes in 6-week MM-exposed rats was accompanied by dose-related increases in urinary uroporphyrin and coproporphyrin levels, which reached 12 and 21 times control levels, respectively, at 10 ppm. Acute exposure studies (0.7 and 1.3 mg/kg given by IP injection for 2 days) revealed that depression of ferrochelatase and uroporphyrinogen I synthetase precedes elevation of δ -aminolevulinic acid synthetase activity in rat kidney. These studies suggest that MM acts primarily to depress ferrochelatase and uroporphyrinogen I synthetase activities, followed by a secondary induction of renal δ -aminolevulinic acid synthetase and an increase in urinary porphyrin levels. These results may have utility in the design of clinical tests for diagnosing pretoxic biologic responses to MM in human populations.

Originally published in Toxicol. Appl. Pharmacol. 39:154 (1977).

Ultrastructural Morphometric and Biochemical Studies of Chronic Arsenic Exposure on Hepatocyte Mitochondria of Rats and Mice. B. A. FOWLER, J. S. WOODS, AND C. M. SCHILLER, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

This investigation was undertaken to delineate the subcellular manifestations of arsenic toxicity following chronic exposure using combined ultrastructural morphometric and biochemical techniques. Male rats and mice were given access to deionized drinking water solutions containing 0, 20, 40, or 85 mg/l arsenic as arsenate (As⁺⁵) for 6 weeks. Increased mitochondrial volume density was observed in hepatocytes located near the periphery of liver lobules. Respiration studies indicated decreased state 3 respiration and respiratory control ratios (RCR) for pyruvate mediated respiration at all dose levels. Rat mitochondria were more markedly affected than those of mice. Specific activity of monoamine oxidase, which is localized on the outer mitochondrial membrane, showed dose-related increases of 120-150% of control for both species. Cytochrome oxidase and Mg⁺² ATPase, which are present on the inner mitochondrial membrane, showed increases in specific activity of 150-220% for rat liver mitochondria at the dose levels used whereas no changes were evident in mice. Malate dehydrogenase activity, which is localized in the mitochondrial matrix, was not altered in either species. These studies suggest that hepatic mitochondrial damage following chronic exposure to sodium arsenate is an important aspect of arsenic toxicity in rats and mice. Arsenic-mediated changes in important mitochondrial enzyme systems which participate in the regulation of respiration and other metabolic functions are hitherto unstudied aspects of toxicity from this agent following *in vivo* exposure.

Originally published in Toxicol. Appl. Pharmacol. 39:145 (1977).

Isolation and Partial Characterization of an Inducible Cadmium-Binding Protein from American Oyster. JAMES W. RIDLINGTON AND BRUCE A. FOWLER, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

American oysters, *Crassostrea virginica*, were exposed to 0.1 ppm Cd for 0 to 14 days in a flowing water exposure system and then placed into Cd-free water for 24 hr before sacrifice. Whole oysters were homogenized and centrifuged in 0.1M phosphate buffer. The heat-treated supernatant material contained 0.048, 0.047, 0.046, 0.51 and 0.86 μg Cd/mg protein, respectively for the days 0, 1, 3, 7, and 14. A highly anionic Cd-binding protein which did not bind Zn or Cu was purified to near homogeneity by further centrifugation, heat precipitation, G-75 Sephadex and DEAE column chromatography. The homogeneity of the protein and metal-binding specificity were determined using discontinuous disc gel electrophoresis. Amino acid analysis of the protein disclosed a composition of 15% aspartic acid, 18% glutamic acid, and 8% cysteine. A minimum molecular weight of 7400 was calculated on the basis of the amino acid composition. In contrast, amino acid analysis of rat kidney metallothionein revealed a composition of 7% aspartic acid, 4% glutamic acid, and 28% cysteine. These findings indicate that oysters possess an inducible cadmium-binding protein which appears to function in a manner similar to mammalian metallothionein but whose chemical properties and amino acid composition are quite different.

Originally published in *Pharmacologist* 19: 180 (1977).

TCDD(Dioxin)-Induced Alterations in Microsomal Hemoprotein-Mediated Enzyme Activity in Mammalian Liver. K. T. KITCHIN AND J. S. WOODS, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

These studies investigated the effect of TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), a potent hepatotoxin, on hepatic microsomal hemoprotein-mediated enzyme activities. Despite a 62% elevation of total cytochrome P-450 three days after administration of TCDD (2 $\mu\text{g}/\text{kg}$ PO) to female rats, no alterations were observed in heme biosynthesis or levels of cytochrome P-450-mediated benzphetamine-*n*-demethylase. In contrast the activities of hepatic aryl hydrocarbon hydroxylase and ethoxyresorufin-*O*-deethylase, both cytochrome P-448 mediated enzymes, were greatly elevated after administration of TCDD. TCDD increased aryl hydrocarbon hydroxylase activity as determined by 4 and 2 hydroxylation of biphenyl (4- and 10-fold), the hydroxylation of the novel substrate 4,4'-dimethylbiphenyl (24-fold), and the formation of polar metabolites of benzo[*a*]pyrene (37-fold). Hydroxylation of 4,4'-dimethylbiphenyl appears to be cytochrome P-448-dependent. This new aryl hydrocarbon hydroxylase substrate is noncarcinogenic and light stable in contrast to the more commonly used substrate benzo[*a*]pyrene, a photochemically unstable carcinogen. TCDD also induced ethoxyresorufin-*O*-deethylase activity 50-fold. Further evidence that TCDD specifically induces the cytochrome P-448 form of the heterogeneous cytochrome P-450 pool was obtained from studies of the ethyl isocyanide difference spectra of hepatic microsomes. The ethyl isocyanide 455 nm/433 nm absorption ratio was 0.50 and 1.13 in microsomes from control and TCDD-treated rats, respectively. This increase is similar to that observed by other investigators with 3-methylcholanthrene, a specific cytochrome P-448 inducer. These studies indicate that

TCDD induces the synthesis of a specific microsomal hemoprotein, cytochrome P-448, which mediates aryl hydrocarbon hydroxylase and ethoxyresorufin-*O*-deethylase in mammalian liver.

This study was supported by a CIIT postdoctoral fellowship.

The Effect of Differential Inhibitors of Both Types of Monoamine Oxidase on Various Organs and on Exploratory Behavior of the Albino Rat. E. A. ZELLER, W. E. WILSON, AND C. L. MITCHELL, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

Aminergic systems appear to be involved in the development of behavioral alterations which are produced by certain pollutants, either as primary targets or as essential links in the chain of reactions initiated by the toxic agent. Neuronal function may be affected in several ways as a consequence of changes in the metabolism of monoamines. Neuronal transmission is markedly influenced by the synaptic levels of norepinephrine and related amines. In turn, these levels are modulated by the rate-limiting steps of synthesis and degradation. Initial studies analyzed the two types of monoamine oxidase (MAO) which exhibit different substrate specificity patterns and biofunctions. As a basis for future studies, we attempted to decrease *in vivo* activity of one type of cerebral MAO with minimal effect on the other. This goal was reached with the help of clorgyline, an inhibitor of MAO type A, and deprenyl, an inhibitor of MAO type B. A sensitive isotope method was used to measure accurately the A- and B-activities. We tested the animals in the open field after treating them for various lengths of time with the inhibitors. When MAO type A in brain was inhibited by more than 80%, exploratory and psychomotor activities increased, while inhibition of MAO type B was without effect. However, simultaneous 80% inhibition of MAO types A and B did not affect these behaviors. On the basis of these and preceding experiments, we suggest that the two enzymes act in concert and that B-type substrates (possibly phenylethylamine) may modulate the consequences of A-type substrate accumulation. Finally, we developed a unified model for the mechanism of substrate oxidation and for inhibitor action to provide a rational basis for the *in vivo* experiments.

The Blood-Testis Barrier. R. L. DIXON, K. OKUMURA, AND I. P. LEE, *Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

Setchell and others have described the existence of a blood-testis barrier (BTB) surrounding the seminiferous tubules of the mammalian testis. Our studies were initiated to better define the role of the BTB with regard to the penetration of exogenous chemicals to male germ cells. Fluid was collected from the cannulated rete testis. Test chemicals or drugs were usually administered by continuous intravenous infusion. Permeability of nonelectrolytes of various molecular sizes were studied as well as ionized molecules. The penetration of drugs, such as salicylic acid, barbiturates, and sulfonamides with varying partition coefficients and values pK_a from blood to germ cells was also investigated. Permeability of nonelectrolytes was demonstrated to be dependent upon this molecular size, suggesting bulk flow through water-filled pores. On the other hand, permeability of acid drugs with varying pK_a values depended upon their partition coefficients. Transport of these chemicals from

blood to seminiferous tubules closely resembled their transport from blood to cerebrospinal fluid. The BTB is apparently a complex multicellular system composed of membranes surrounding the seminiferous tubules and the several layers of spermatogenic cells organized within the tubules, which restrict the permeability to the male germ cells of many foreign compounds. Thus, the BTB must be considered along with detoxication mechanisms and unscheduled DNA synthesis when extrapolating *in vitro* test results to whole animals.

Effects of Various Toxic Compounds on the Consummatory Behaviors of the Rat. D. MITCHELL, *Behavioral Toxicology, Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

Changes in consummatory behaviors often accompany toxicosis in a variety of species. For example, many organisms characteristically show a pronounced hypophagia (decrease in food consumption) when poisoned. In a recent series of experiments my colleagues and I have been using changes in consummatory behaviors as behavioral assays of toxicosis. The three consummatory behaviors we have employed include eating (food consumption, drinking (fluid consumption), and geophagia (soil consumption). We have found that various toxic substances (methyl mercury, cyclophosphamide, alcohol, lithium chloride, and cardiac glycosides) have characteristic effects on these various consummatory behaviors. For example, when rats maintained with food (NIH diet No. 23), fluid (room temperature tap water), and kaolin (hydrated aluminum silicate) freely available are administered daily intraperitoneal injections of 0.15M lithium chloride (127.2 mg/kg-day) food consumption decreases, but fluid and clay consumption increase. However, when cyclophosphamide (Cytosan) is administered via daily intraperitoneal injections (10 mg/kg-day) to rats maintained on a similar regimen, there is a similar progressive increase in geophagia but a progressive decline in both food and fluid consumption. Moreover, preliminary studies show that whereas the hypophagia and geophagia caused by lithium poisoning occur in close temporal proximity to treatment, the polydipsia (increased drinking) occurs throughout the day. These findings suggest that both consummatory behaviors and their temporal characteristics might be useful as relatively inexpensive and sensitive behavioral assays of toxicosis in the rat.

Some Neurotoxic Effects of Polybrominated Biphenyl (PBB) Compounds in Rodents. H. A. TILSON, P. A. CABE, D. MITCHELL, T. MOFFITT, AND R. RHODERICK, *Behavioral Toxicology, Laboratory of Environmental Toxicology, NIEHS, Research Triangle Park, North Carolina 27709*

Male and female rats (CDF) and mice ($B_6C_3F_1$) were given various doses of Firemaster (FF-1), a mixture of brominated biphenyls, and 2,2',4,4',5,5'-hexabromobiphenyl (HBB) (major component of FF-1) orally for 30 days. On days 28 and 29 of dosing, the animals were given a battery of tests designed to detect a wide spectrum of neurotoxic effects. Gross physical examination of the animals revealed no signs of autonomic nervous system dysfunction, abnormal gait or incoordination, irritability, or loss of the visual placement response. Some depression of reflexes and responses to pain may have been present in animals treated with the high dose of FF-1 (30 mg/kg). Rectal temperature and body weight were taken as general indicators of

physiological dysfunction. Rats and male mice receiving 30 mg/kg of FF-1 weighed less than controls, while HBB had no influence on the weight of any of the animals. Except for a significant hypothermia observed in male mice at 30 mg/kg of FF-1, no effects on body temperature were observed. The primary behavioral effect of FF-1 and HBB in rodents appears to be on neuromuscular function. Graded measures of forelimb strength showed a significant decrease in rats after 3 and 30 mg/kg of FF-1 and in male mice given 30 mg/kg of FF-1. Except for an unexpected increase in the strength of female mice exposed to 16.8 mg/kg of HBB, no other effects were observed. Attempts to measure fine motor fasciculations of animals in restraint indicated that FF-1 and HBB had no consistent effect in rats. Mice, however, had elevated tremor scores after 30 mg/kg of FF-1 and 16.8 mg/kg of HBB. Open field activity was depressed in female rats by 3 and 30 mg/kg of FF-1 and 2.7 mg/kg of HBB, but male rats and mice were not significantly affected. Gross motor impairment or loss of balance was not detected in animals forced to walk on a rotating rod. These studies indicate that 3-30 mg/kg of FF-1, and in some cases, 16.8 mg/kg of HBB given orally for 30 days produce signs of neuromuscular dysfunction. In general, FF-1 was more toxic than HBB, and rats were affected more than mice.

Pharmacokinetic Modeling of Xenobiotic Disposition Data. D. TUEY, S. KATO, N. MORALES, M. ANDERSON, AND H. MATTHEWS, *Pharmacokinetics Section, Biometry and Pharmacology Branches, NIEHS, Research Triangle Park, North Carolina 27709*

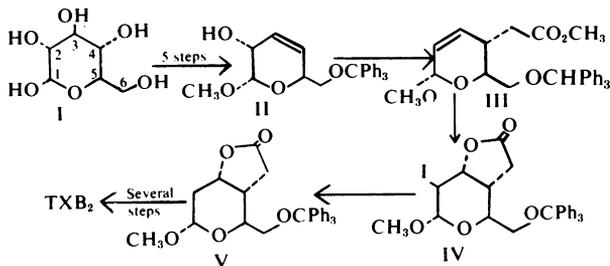
Pharmacokinetic modeling has become an established method for assessing and predicting drug disposition. Similar techniques have been used to study the disposition kinetics of several polychlorinated biphenyls (PCBs) and of a polybrominated biphenyl (PBB) in rats and mice. Quantitative estimates of the biological persistence of these environmental contaminants were obtained by first applying some classical methods of pharmacokinetic analysis to experimental body burden data. Each of these halogenated xenobiotics had a characteristic biological half-life. Some PCBs have relatively short half-lives, while others are eliminated so slowly that repeated exposure would lead to significant accumulation in the body. The degree and positions of chlorination determined each PCB's individual disposition kinetics in both rats and mice. The PBB half-life was so long in rats that their body burden of this compound would not decrease significantly during their lifespan. Physiological pharmacokinetic models have also been developed to more precisely describe the detailed disposition kinetics of these xenobiotics. These models were constructed from physiological and physicochemical information such as tissue and organ volumes, blood flow rates, distribution ratios, transport times, and metabolism and clearance rates. The time courses of tissue distribution, metabolism, and excretion generated by these mathematical models were compared to experimental data in the rat. The PCB models were also scaled and used to predict the disposition kinetics of PCBs in the mouse. Preliminary results suggest that physiological pharmacokinetic models can describe the proper dynamic behavior of these compounds in mammals, and may provide a operational strategy for extrapolating and predicting the fate of these and other xenobiotics from one animal species to another, and ultimately to man.

Chiral Synthesis of Thromboxane B_2 . O. HERNANDEZ, J. D. MCKINNEY, J. R. BEND, T. E.

ELING, AND M. W. ANDERSON, *Chemistry Section, Environmental Biology and Chemistry Branch and Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina 27709*

The potent platelet aggregator Thromboxane A₂ (TXA₂) is hydrolyzed, presumably by a nonenzymatic process, to the more stable Thromboxane B₂ (TXB₂). By using D-glucose (I) as starting material an efficient synthesis of TXB₂ was achieved. The synthetic strategy involves selective functionalization of the hydroxyl groups on I and culminates with the stereospecific introduction of the acetic acid side chain at C₁ (D-glucose numbering) by a Claisen rearrangement of the allylic alcohol II to produce III. The side chain controls the stereochemistry of the following transformation which results in introduction of the third asymmetric center *via* an iodolactonization reaction to form IV. Removal of the iodo substituent on IV yields V which contains all the required asymmetric centers, and with the absolute enantiomeric purity needed for full biological potency. Compound V is converted to TXB₂ by established literature procedures.

This synthetic method constitutes an efficient preparation of optically pure TXB₂ and it also provides a potential route for the synthesis of TXA₂ and TXA₂ analogs.



Effects of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) on Host Resistance to Infectious Agents. J. E. THIGPEN, E. E. MCCONNELL, J. A. MOORE, AND R. E. FAITH, *Environmental Biology and Chemistry Branch, NIEHS, Research Triangle Park, North Carolina 27709*

TCDD, a possible contaminant in the production of chlorinated phenols has been shown to cause thymic atrophy and to suppress cell-mediated immunity in laboratory animals. To investigate possible effects on other aspects of host defense, 4-week-old mice were dosed via gavage with subclinical levels of TCDD (0.5, 1, 5, 10, or 20 µg/kg of body weight) once weekly for 4 weeks and then infected with either *Salmonella bern* or *Herpesvirus suis* (pseudorabies virus). In a second study, mice were exposed to TCDD, (5 µg/kg body weight; same schedule) and then induced with thioglycollate intraperitoneally. Five days later, heart blood, and peritoneal exudate cells were collected aseptically. Total cell concentration, cell viability, differential cell counts and macrophage phagocytic function were determined.

Dose schedules of 1 µg or more, followed by *Salmonella bern* infection, resulted in significant increases in mortality and decreases in the time from infection to death. However, TCDD had no significant effect on mortality in the pseudorabies-infected mice.

TCDD exposed mice contained significantly fewer peritoneal cells (macrophages and lymphocytes). *In vitro* phagocytic studies showed that macrophages from TCDD exposed mice phagocytized *S. bern* at a reduced rate at 1 hr, but not at other times. No differences were observed in the bactericidal ability of these macrophages. Hematologic data revealed no difference in differential cell counts. These findings indicate that subclinical levels of TCDD have a subtle, yet definite effect on the phagocytic defense system of the mouse and that the reduction in cell numbers is the primary cause of the increased susceptibility to infection with *S. bern*. This suggests that even subclinical levels of TCDD are potentially dangerous and may present definite public health problems by rendering exposed individuals more susceptible to infectious agents.

Toxicity of Chlorinated Dibenzo-*p*-dioxins in Mice, Guinea Pigs, and Rhesus Monkeys. E. E. MCCONNELL, J. A. MOORE, M. W. HARRIS, AND J. K. HASEMAN, *Environmental Biology and Chemistry Branch, NIEHS, Research Triangle Park, North Carolina 27709*

A single oral dose of various chlorinated dibenzo-*p*-dioxin (CDD) isomers were given to mice and guinea pigs to establish and compare the LD₅₀ and clinical and pathologic manifestations of toxicity. Rhesus monkeys received only 2,3,7,8-TCDD. It was apparent that the 2,3,7, and 8 positions must be chlorinated to achieve the greatest degree of toxicity. Additional chlorine atoms at an *ortho* position reduced toxicity but not nearly to the degree caused by deletion of a chlorine atom at one of the lateral positions. A decrease in body weight gain was the most sensitive clinical parameter and animals severely intoxicated showed a marked weight loss, especially guinea pigs. The median time to death at a LD₅₀ was 17–20 days in guinea pigs, 22–25 days in mice, and 42–47 days in monkeys. At the toxic dose the spectrum and severity of lesions and organ weight effects were similar for all isomers within the same animal species; however, there were interspecific differences. The thymus was greatly reduced in size in all animal species due to a reduction in the number of cortical lymphocytes. Significant macroscopic and histopathologic hepatic effects including porphyria were observed only in the mouse and were found at dose levels well below the LD₅₀. Another lesion suspected as being a primary effect of dioxin toxicity was hyperplasia of the transitional epithelium in the urinary tract of guinea pigs and monkeys. Epithelial lesions with involvement of the sebaceous glands of the eyelids, external ear canal and surrounding hair follicles were the hallmarks of toxicity in monkeys. There was a reduction of total serum protein in the mouse due to lower levels of α-globulin and in the monkey due to reduction of the albumin fraction.

Modulation of Immune Function by Chemicals of Environmental Concern. R. E. FAITH AND M. I. LUSTER, *Environmental Biology and Chemistry Branch, NIEHS, Research Triangle Park, North Carolina 27709*

These studies were undertaken to investigate the effects of selected environmental chemicals on the immune competence of experimental animals. The chemicals investigated were 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), lead, polybrominated biphenyls (PBB), and diethylstilbesterol (DES). In all cases, animals were exposed either prenatally, postnatally or pre- and postnatally.

Results of studies with TCDD have shown that rats exposed prenatally and postnatally have depressed body weights and thymic weights as well as depressed cellular immune function. Cell mediated immunity was suppressed as determined by the *in vitro* response to T-cell mitogens and delayed hypersensitivity reactions. Humoral immune responses in TCDD exposed rats to bovine gamma globulin (BGG) were not suppressed, indicating that TCDD does not interfere with the normal functioning of all T-cell subsets as BGG is a T-dependent antigen which requires functioning T-helper cells for a normal response.

Pre- and postnatal lead exposure in rats was shown to cause suppression of cell mediated immune competence. *In vitro* mitogen responsiveness of splenic and thymic cells to the mitogens Con A and PHA were suppressed as were *in vivo* delayed hypersensitivity responses.

Cattle exposed to PBB *in utero* through contaminated feed were found to have enhanced cell mediated immune function as exhibited by increased *in vitro* mitogen responsiveness of peripheral blood lymphocytes.

In utero DES exposure has been found to cause modulation of immune function in a manner dependent upon sex. Male offspring from treated mothers have enhanced humoral responsiveness to a T-independent antigen while female offspring have suppressed humoral responsiveness to the same antigen.

These studies have shown that chemicals of environmental concern can modulate various immune functions.

Use of Negative-Ion Chemical Ionization Mass Spectrometry in the Determination of Polychlorodibenzo-*p*-dioxins at the Part-per-Trillion Level in Tissue Samples. J. R. HASS, M. D. FRIESEN, C. E. PARKER, AND D. J. HARVAN, *Environmental Biology and Chemistry Branch, NIEHS, Research Triangle Park, North Carolina 27709*

Polychlorodibenzo-*p*-dioxins (PCDD) comprise one of the most toxic classes of compounds found in the environment. Analytical methods employed for the determination of these chemicals must be extremely sensitive and specific. Furthermore, it is desirable to minimize the time and expense per analysis. The methods presently employed for PCDD analysis at the part-per-trillion (ppt) level involve an extensive sample extraction and clean-up method followed by measurement of the PCDD by use of a relatively high performance mass spectrometer. We describe in this paper the application of more rapid extraction and clean-up method in combination with PCDD measurement by gas chromatography-negative ion chemical ionization mass spectrometry (NICI). The NICI method is sufficiently sensitive for the PCDD that analyses requiring the detection of 10-100 pg (10^{-11} - 10^{-10} g) of material injected on the gas chromatographic column can be accomplished through the use of a relatively inexpensive quadrupole mass spectrometer which was modified for negative ion detection.

The analysis of dairy cow livers and fat from animals housed in a bar constructed of pentachlorophenol treated lumber resulted in finding levels between 10 ppt for a hexaCDD to approximately 50 ppb for octaCDD with intermediate values for the heptaCDD present. When PCDD levels were sufficiently high for confirmation by electron impact mass spectrometry, the agreement was quite good between the two methods. Furthermore, the NICI method was shown to give a linear response between 10 pg and 10 ng PCDD injected on the gas chromatographic column.

Effects of Pure Polychlorinated Biphenyl(PCB) Isomers on Drug-Metabolizing Enzymes. J. A. GOLDSTEIN, P. HICKMAN, J. D. MCKINNEY, M. P. WALKER, J. R. HASS, AND D. J. HARVAN, *Environmental Biology and Chemistry Branch, NIEHS, Research Triangle Park, North Carolina 27709*

Commercial PCB mixtures induce enzyme characteristics and spectral changes characteristic of both cytochromes P-450 and P-448. When pure PCB isomers were administered to rats (30, 140 or 700 nmole/kg \times 3), they could be divided into two separate classes of inducers. PCB isomers chlorinated in the *ortho* and *para* positions (2,4,5,2',4',5'-; 2,3,4,2',3',4'-; and 2,4,6,2',4',6'-hexa- and 2,4,2',4'-tetra-) induced cytochrome P-450 and associated enzymes (aminopyrine *N*-demethylase), but had no effect on the ethyl isocyanide (ETNC) difference spectra, and little effect on aryl hydrocarbon hydroxylase (AHH). PCB isomers chlorinated in the *meta* and *para* positions (3,4,3',4'-tetra- and 3,4,5,3',4',5'-hexa-) shifted the peak of the CO-difference spectrum to 448 nm, increased the ratio of the 455/430 peaks of the ETNC difference spectra, and increased enzymes associated with cytochrome P-448 (AHH), but decreased aminopyrine *N*-demethylase. These PCBs were also the most toxic as measured by weight loss at 700 nmole/kg. A commercially available lot of one isomer (2,4,5,2',4',5'-HCB) was found to be contaminated with 50 ppm of chlorinated dibenzofurans (CDFs) and dibenzodioxins (CDDs). The contaminated isomer was a mixed-type inducer; however, the pure isomer induced only cytochrome P-450. The induction of cytochrome P-448 by the contaminated isomer was apparently due to the presence of CDFs and CDDs. No pure isomer gave a mixed response. PCB isomers which were relatively inactive as inducers include: 2,3,4,5,3',4',5'-hepta-; 2,3,6,2',3',6'-hexa-; 2,3,2',3'-, 3,5,3',5'-, 2,6,2',6'-, 2,5,2',5'- and 2,5,3',4'-tetra-; and 2,2'-, 3,3'-and 4,4'-dicBs.

Newer Methods in the Analysis of Polychlorinated Biphenyls (PCB's). P. W. ALBRO, K. CHAE, J. B. HASS, K. KOHLI, D. HARVAN, J. HASEMAN, T. CLEMMER, AND B. CORBETT, *Environmental Biology and Chemistry Branch, NIEHS, Research Triangle Park, North Carolina 27709*

Analysis of fractions from the HPLC chromatography of commercial mixtures of PCB's revealed that the use of electron capture detectors can introduce an error of up to 300% in quantitation when there is a qualitative difference between the sample mixture and the mixture used as reference standard. We have developed a radioisotope dilution assay for the determination of levels of "total PCB's" in tissues that avoids this problem. In addition, the GLC retention indices for all 210 PCB's have been calculated relative to 13 different liquid phases, permitting reliable identification of the components of PCB mixtures. A computer search program has been written to utilize this body of data. Optimized procedures for the clean-up of tissue extracts for analysis of PCB's and other mixtures of halogenated aromatics have been selected on the basis of efficiency and high recovery.

Studies of the Effects of Chronic Inhalation Exposure of Rabbits to Chlorodifluoromethane. E. W. VAN

STEE, AND E. E. MCCONNELL, *Environmental Biology and Chemistry Branch, NIEHS, Research Triangle Park, North Carolina 27709*

Male and female rabbits were exposed to 6% CHClF_2 for 5 hr/da, 5 da/wk for 8–12 weeks in an effort to establish an animal model of supraventricular arrhythmias seen in man under similar circumstances. Many of the rabbits were placed on 0.5 g/l. of sodium phenobarbital in the drinking water to stimulate drug metabolizing enzyme systems, since the original epidemiology suggested the possibility that a product of the biotransformation of the compound may have been the proximate arrhythmogen.

One female rabbit on both phenobarbital and CHClF_2 developed well-defined cardiac arrhythmias of probable supraventricular origin.

We observed pale livers in some rabbits during the first experiment which prompted us to monitor serum enzymes reflecting liver damage, in subsequent experiments. After four weeks exposure to CHClF_2 these enzyme levels began to rise, reaching modestly elevated plateaus after 10–12 weeks. Comparing the serum enzyme elevations and the relatively benign histopathological changes, we concluded that most or all changes observed would be expected to be reversible, a hypothesis not yet tested.

Femurs of exposed animals contained slightly more fluoride than controls. The differences were statistically significant at the 5% level, but of questionable biological significance. The differences could have been attributable to a minute amount of defluorination of CHClF_2 or trace-level fluorinated contaminants. We would expect that if a significant amount of biotransformation takes place it probably does not involve defluorination. No differences were detected between animals treated with and without phenobarbital and exposed to CHClF_2 . Indocyanine green elimination was unaffected by the CHClF_2 .

The matter of quantitatively limited biotransformation and toxicologic response to the presence of trace quantities of metabolites that may be formed, remains worthy of careful consideration. The fact that we have not yet demonstrated biotransformation (using crude estimates) may mean only that we need more sensitive methods. Furthermore, the biotransformation may not include defluorination, but rather deprotonation or dechlorination. The usually observed biological stability of $-\text{CF}_2-$ in an alkane does not favor metabolic defluorination anyway.

One incontrovertible fact remains: supraventricular arrhythmias were observed in one rabbit of 14 exposed so far to CHClF_2 , for which no explanation other than an effect of CHClF_2 is available. The phenomenon may have represented a rare occurrence in the rabbit, after the pattern of postanesthetic hepatic failure in man attributed to halothane. Judging from the Speizer Report (1975) on arrhythmias in man attributed to CHClF_2 , theirs was by no means a rare occurrence in the same sense. We may have identified an interspecies differential response to CHClF_2 , the rule rather than the exception for most compounds, or we may not have established the right set of conditions to reproduce the phenomenon seen in man, in the rabbit.

Meantime, prudence dictates that measures be undertaken to protect workers from unnecessary inhalation exposure to CHClF_2 . Furthermore, in view of the liver effects observed in rabbits exposed to CHClF_2 , and keeping in mind the still unresolved matter of a possibly halothane-induced post-anesthetic hepatic failure, we conclude that it would be wise to check serum GOT and GPT in people exposed to CHClF_2 in the work environment.

Radioimmunoassay for 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin. P. W. ALBRO, K. CHAE, M. LUSTER,

J. D. MCKINNEY, S. CHAUDHARY, G. CLARK, J. FAWKES, AND J. CORBETT, *Environmental Biology and Chemistry Branch, NIEHS, Research Triangle Park, North Carolina 27709*

Antibodies were raised in rabbits against 1-amino-3,7,8-dibenzo-*p*-dioxin coupled through a C-6 spacer arm to either bovine thyroglobulin, bovine serum albumin, or human serum albumin. Although differences in affinity occurred, each of eleven rabbits immunized responded with the production of usable antisera. Titers of $>1:32$ were seen on Ouchterlony plates, while 1:3200 dilutions of antisera could be used in the radioimmunoassays. Tetrachlorobiphenyls, dibenzofuran, chlorobiphenyl ethers and chloroanilines did not interfere even when their amine derivatives were similarly coupled to protein, and they were present at a 10^3 - 10^4 -fold excess over the dioxin. A unique method for accommodating the extremely insoluble dibenzodioxin in the system was developed, utilizing a nonionic detergent. The assay is still being refined, but presently will respond to 4 picomoles of free 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and has much greater sensitivity to protein bound haptens.

Development of an Automatic Small Animal Inhalation Facility. M. P. MOORMAN, AND E. W. VAN STEE, *Environmental Biology and Chemistry Branch, NIEHS, Research Triangle Park, North Carolina 27709*

This project involves the design and implementation of an automatic data processing system to monitor and control gas concentrations in a small animal inhalation exposure facility. Included in this effort are the development of hardware, software and operational procedures necessary for the complete utilization of the system.

Data acquisition and feedback control theory have been used to develop an electronic calculator based sampled data control system capable of regulating 9 chambers on a time multiplex basis. Since this system must regulate gases generated from compounds with different physical properties, it has been necessary to design a control system capable of measuring certain characteristics of each generating system and adapting the control equations to optimize responses for each particular compound. The realization of full performance from this system requires further improvement in the adaptive control functions and the addition of operation and status monitoring equipment to provide the man-machine interface.

Machine control of inhalation chambers can produce an accurately controlled and documented exposure resulting in reduced technical error. Documentation of the exposure conditions and profiles can be summarized in a compact and meaningful format eliminating the need for hand analysis of chamber output data. More complicated exposures such as time varying concentrations of multiple compounds are possible for machine control than with human operators.

Association Between Chloroform Levels in Finished Drinking Water Supplies and Various Site-Specific Cancer Mortality Rates. MICHAEL D. HOGAN, PI-YEONG CHI, TOBY J. MITCHELL, AND DAVID G. HOEL, *Biometry Branch, NIEHS, Research Triangle Park, North Carolina 27709*

This research was concerned with some of the statistical and biological problems that are likely to be encountered when an indirect or ecological approach is used to assess the possible public health impact of general population exposures to environmental agents. For purposes of illustration the potential for association between various site-specific cancer mortality rates and chloroform levels in public drinking water supplies was considered.

The analyses that were performed demonstrated that, for the data sets under consideration, there were some definite associations between chloroform levels and cancer mortality for specific sites such as the rectum-intestine and bladder. However, the marked extent to which these results were dependent on (1) the weighting scheme adopted in the analysis, (2) the presumed appropriateness of the data, and (3) the characteristics of the statistical model was also clearly illustrated. Because of these dependencies the quantitative, causal interpretation of results generated from an indirect study would appear to be a very tenuous and questionable practice in most instances.

Environmental Contaminants of Foods for Infants. **BETH GLADEN AND WALTER ROGAN, *Biometry Branch, NIEHS, Research Triangle Park, North Carolina 27709***

Ubiquitous in the environment are a number of very stable man-made compounds, such as DDT and the industrial insulating compounds, the polychlorinated biphenyls (PCBs). These compounds tend to be stored in fat in humans and are very slowly excreted. Thus, even very low levels of contamination in water, or food, can eventually result in a measurable level in human fat tissue. Human levels of these compounds have been measured in many studies and all indicate that a small but measurable amount of these compounds are present in a high proportion of adult humans. To date, no human illnesses have been linked directly to these residues.

A particularly bothersome aspect of this problem is the presence of residues in breast milk. Breast milk is about 3% fat, a far higher proportion than any other substance we excrete. A number of studies have shown that PCB and DDT find their way into a very high proportion of breast milk samples. The health consequences of these residues are not known. Alternative methods of feeding infants, like cows' milk and formula, are regulated and do not show this contamination. However, most health professionals feel that breast feeding is the optimal way to feed infants.

Last summer, the National Institute of Environmental Health Sciences began the design of a study in this area. The study has three purposes; first, to determine the extent to which this phenomenon occurs in North Carolina; second, to see if any of the health effects that occur in animals or have occurred in human exposures to these compounds can be seen in infants; and third, to establish a cohort of children on whom exposure is well known so that a long term study would eventually be possible.

The study is a "follow-up" study. Mothers giving birth in three or four hospitals will be asked to participate. A questionnaire will be administered to her by a trained nurse. This concerns any problems that might have gone on during the pregnancy, and questions about diet, medications, etc. She will be asked to give a sample of blood, and also breast milk or colostrum if she can. Her baby will be examined according to a protocol, and samples of cord blood and placenta will be collected. Follow up examinations will be scheduled for 6 weeks, 3 months, and 6 months. These will involve a brief questionnaire, an examination of the child, collection of breast milk or formula at all visits, and a blood sample at one visit.

Computing and Data Processing. T. A. CLEMMER, *Biometry Branch, NIEHS, Research Triangle Park, North Carolina 27709*

The computing and data processing activities of NIEHS are located within the Biometry Branch. Computing facilities available to the Institute staff range from programmable calculators and special-purpose laboratory minicomputers, through Biometry's PDP11/40 computer, to the large IBM/370 and DECsystem-10 computer systems at the main NIH campus in Bethesda, Maryland.

Data processing efforts within the Biometry Branch fall into two distinct areas—those directed principally to tasks within the branch, and those dealing primarily with providing data processing and computer engineering services to the Institute at large.

Numerous projects in the latter category receive support. Those of an administrative nature include a computerized warehouse inventory management system, a periodically updated listing of all radioisotopes held at NIEHS, a budget reporting system listing financial transactions and billings with summaries for each branch, and an animal ordering and inventorying system.

In support of and in collaboration with intramural researchers, major efforts are conducted in the fields of protein receptor analysis, assessment of toxicological synergism, gene frequency deviation detection with Monte Carlo simulation of related phenomena, and interspecies extrapolation of low-level, long-term pharmacokinetic profiles.

A capability of providing computer engineering support to the laboratories of the Institute is presently being developed within the Biometry Branch. Solutions will be sought to engineering problems related to all aspects of computer hardware and instrumentation programming.

Within the Biometry Branch, substantial efforts are directed toward improving the general data processing capability, particularly as it affects the computing needs of those providing statistical data analysis in support of the intramural research program. The Institute's PDP11/40 computer continues to be significantly expanded with the addition of various peripherals and the implementation of new operating systems. This computer is heavily used both to provide high-speed telecommunications to the NIH IBM/370 system and to accomplish a great variety of local computing activities.

Statistical Analysis of Binary Data Arising from Certain Toxicological Experiments. J. K. HASEMAN, B. GLADEN, F. O. LIN, AND L. KUPPER, *Biometry Branch, NIEHS, Research Triangle Park, North Carolina 27709*

In certain toxicological experiments with laboratory animals, the outcome of interest is the occurrence of dead or malformed fetuses. In the past this type of binary data frequently has been analyzed by procedures that assume an underlying binomial or Poisson model. However, both of these models were found to be inappropriate when large sets of mouse control data and other data sets from the literature were examined. Thus, statistical tests based on these models may be suspect.

Several nonparametric procedures were derived for use when no assumptions are made concerning the specific functional form of the underlying distribution. These tests are simple to use and have been shown in a variety of situations to operate at the correct level of significance and to have good power. An alternative approach, which has the advantage of weighting according to litter size, is to use jackknife-based tests. These procedures

were shown to be very competitive with other methods of analysis.

Finally, several two-parameter models were investigated that provide a markedly better fit than the simple one-parameter binomial and Poisson. A correlated binomial model was derived that has certain advantages over the alternative Beta-binomial model proposed by Williams [Biometrics, 31: 947 (1975)]. Likelihood ratio tests based on either of these models can then be used to test for treatment vs. control differences in dead or malformed fetuses.

Low Dose Extrapolation in Carcinogenesis. D. HOEL AND H. GUESS, *Biometry Branch, NIEHS, Research Triangle Park, North Carolina 27709*

Statistical methodology based on the Armitage-Doll multistage model has been developed and used to analyze dose-response data from animal experiments with a number of chemical compounds and from computer simulations of such experiments. This work has produced the following conclusions applicable to carcinogens that act directly on cellular DNA. (1) If the dose-response trend is linear in the range where test data are available, then the total statistical uncertainty on the risk at low dose levels will be small, provided that the sample sizes are fairly large (e.g., 200 animals per dose). (2) If the dose-response trend in the range where test data are available is nonlinear, then the upper confidence limit on the difference between the risk at a given dose and the background risk will often be as high as one in ten thousand at dose levels where the best estimate of this increased risk is around one in one million. (3) At low dose rates the upper confidence limits on increased risk are approximately linear functions of dose, even when the best estimate curve of increased risk versus dose is nonlinear.

Computer Engineering at NIEHS. JAMES F. DIX, *Biometry Branch, NIEHS, Research Triangle Park, North Carolina 27709*

Several projects have been undertaken at NIEHS to support research programs with minicomputer hardware and software development. The main tasks being performed in these projects include the analysis and design of laboratory computer systems, the design of special purpose computer interfaces for laboratory instruments or experiments, and the development of computer programs for real-time data acquisition, control, and biomedical applications.

A small network of minicomputers to control and acquire data from a number of independent, simultaneously-running behavioral experiments is currently being developed. Plans include systems for controlling Skinner box experiments and acquiring activity data with expansion capability for an analog-interfaced system for electrophysiology experiments.

Another project is the development of an automated scanning microscope for research in mutagenesis. This instrument will be used for measuring the frequency of somatic mutations in sperm cell and red blood cell samples and for detecting the occurrence of translocations in sperm cells. Presently, photometric measurements of cells are being made to verify the feasibility of this system with plans for semi-automatic and eventually fully-automatic scanning. This instrument will find uses in monitoring human populations and screening compounds for mutagenic effects.

A multipurpose minicomputer system being developed for the population genetics laboratory will be used for automatic acquisition and storage of spectrophotometer data. Later, an x-y

coordinate digitizer will be interfaced to the computer for measuring the length of DNA molecules and the area under scintillation and densitometer scan curves.

A data communication facility is being created for the NIEHS central computer to allow direct and telephone access from laboratory terminals and minicomputers.

Future plans for the computer engineering effort at NIEHS include both larger projects and smaller microprocessor applications.

Ion Transport in the Cochlea of the Guinea Pig. TERUZO KONISHI, *Laboratory of Environmental Biophysics* AND PHILIP E. HAMRICK, *Research Resources Program, NIEHS, Research Triangle Park, North Carolina 27709*

The scala vestibuli and/or tympani in anesthetized guinea pig was perfused with artificial perilymph containing ^{42}K and ^{22}Na or ^{36}Cl for periods ranging from 5 to 90 min. Sound-evoked responses were recorded during the perfusion. The perfusion of scala vestibuli and/or tympani did not result in appreciable changes in electrical responses. The introduction of ^{42}K and ^{22}Na or ^{36}Cl into the scala tympani resulted in the rapid appearance of these isotopes in the perilymph of the scala vestibuli. With perfusion of the scala vestibuli, the concentration of these isotopes in the perilymph of the scala tympani remained low. This is in agreement with our findings that the clearance of these isotopes was faster in scala tympani than in scala vestibuli. Our results further indicate that the endolymph took up ^{42}K with a rate constant of 0.013 min^{-1} and extruded ^{22}Na against the concentration gradients. There was no marked difference in concentration of ^{-}Cl between the endolymph and perilymph. The endolymph took up ^{36}Cl with a rate constant of 0.01 min^{-1} . Uptake of ^{42}K in the endolymph from perilymph of the scala vestibuli was comparable to uptake from the perilymph of scala tympani but uptake of ^{36}Cl in the endolymph from perilymph of the scala vestibuli was greater than from perilymph of the scala tympani. The transport of ^{42}K , ^{22}Na , or ^{36}Cl across the endolymph-perilymph barrier was inhibited by anoxia or local application of ouabain. Our results suggest that ^{+}K is actively transported from perilymph to endolymph and ^{+}Na is extruded from endolymph to perilymph and that the transport of ^{+}K and ^{+}Na take place in the stria vascularis. In addition the results suggest that Reissner's membrane is more permeable to ^{-}Cl than the rest of the endolymph-perilymph barrier and ^{-}Cl is actively transported from endolymph to perilymph.

A part of this report was published in *J. Acoust. Soc. Amer.* 60: S79 (1976).

Effects of Microwave Radiation Exposure on Japanese Quail During Embryonic Development. DONALD I. MCREE, *Laboratory of Environmental Biophysics*, AND PHILIP E. HAMRICK, *Research Resources Program, NIEHS, Research Triangle Park, North Carolina 27709*

Japanese quail embryos were exposed to 2.45 GHz microwave radiation during the first 12 days of development at an incident power density of 5 mW/cm^2 and specific absorption rate of 4.03 mW/g . No change in hatchability was found in the exposed embryos. No gross deformities were observed in the exposed quail when sacrificed and examined at 24-36 hr after hatching. No significant changes in the total body weights or weights of the

heart, liver, gizzard, adrenals, and pancreas were found in the treated birds. Hematological parameters were also measured in the 24–36 hr. old quail. The results showed a small but statistically significant increase in hemoglobin and statistically significant decrease in monocytes in birds treated with microwave radiation. No statistically significant changes in hematocrits, red blood cells, total white blood cells, lymphocytes, heterophils, basophils, or eosinophils were detected.

The decision was made to investigate the effects of microwave radiation on humoral immunity since a decrease in monocytes had been observed in the newly hatched quail. Another group of developing quail embryos were exposed to the same conditions as those stated previously. After hatching the quail were reared to five weeks of age. Weekly body weight measurements were made to compare the growth patterns of exposed and control quail. The weights of the exposed males at the ages of 4 and 5 weeks were 12 and 7%, respectively, less than the control males. At 5 weeks of age the quail were challenged with sheep red blood cells (SRBC), and the levels of the anti-SRBC antibodies were determined. The levels of specific anti-SRBC antibodies, determined four days after antigen challenges, were of the same magnitude for both the exposed and control quail. Following this assessment of humoral immunity, the quail were sacrificed and the bursa of Fabricius and spleen were removed and a comparison was made of exposed and control birds. The weights of bursa of Fabricius and spleen were not altered significantly by the microwave exposure.

A Simple Fiber Optic Lever for Sub-Angstrom Displacement Measures. REGINALD O. COOK, *Laboratory of Environmental Biophysics, NIEHS, Research Triangle Park, North Carolina 27709*

Very simple fiber optic levers whose performance rival those of laser interferometers and optical heterodyne spectroscopy in measuring audio frequency motion of the auditory system have been constructed. The primary feature of these levers are their simplicity, small size, and mobility.

While it has long been recognized that the internal reflection characteristics of optic fibers could be exploited for both "static" and dynamic motion measurements based on the geometry of the emerging light, commercially available instruments based on this principle had marginal resolution and separation distance capability. Since the technique potentially possessed a combination of very desirable features, an analysis of the relevant electro-optic parameters was undertaken. As a result of this analysis, the relevant parameters were separated such that future design of levers can be approached on a sound theoretical basis allowing optimization for a given application. Subsequent ossicular chain optimized levers having a resolution of approximately 0.1 Å and five times commercially available working distance were constructed. Instantaneous oscillographic waveform visualization (10 kHz BW) is possible from these instruments at 10–20 Å. These gains were made possible by the use of very intense light sources, optimized optic fibers and very low noise miniaturized electronic circuitry.

During the process of investigating these levers it became apparent that the measurement technique possesses great versatility beyond the intended application in making Ångstrom-level auditory system measurements. Possibly due to the lack of a ready analysis of the electro optic parameters, the potential of these devices in biomedical and mechanical applications has been only minimally exploited.

While the first generation levers were optimized for ossicular chain applications, it seems possible to design levers of sufficient

miniaturization for basilar membrane measurements. Sound-initiated basilar membrane motions range from 0.01 Å to more than 1000 Å. The basilar membrane is the site of analog to digital signal conversion, and questions still remain concerning anatomical/physiological structures associated with detection and encoding of complex auditory signals. Degradation of complex signal detection is the first manifestation of noise induced hearing loss.

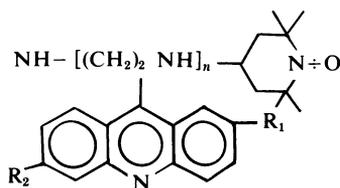
A Review of Impact Noise. ADNAN AKAY, *Laboratory of Environmental Biophysics, NIEHS, Research Triangle Park, North Carolina 27709*

Sounds with very high intensity and rapid rise time are generated by mechanical impacts or impulsive pressure changes. The human auditory system cannot accurately perceive the peak levels of this class of sounds by loudness comparisons because the integration time of the central auditory system is much longer than the impact peak duration. In addition the protective transmission mechanism of the middle ear cannot react fast enough to protect the inner ear from these short duration impulses.

In this paper available literature concerned with impact noise generation and effects is reviewed to give a better understanding of the mechanism of impact sounds. The academic studies are considered under four different classes according to the mechanism of sound generation due to impact processes. These are air ejection, rigid body radiation, radiation from impulsive surface deformations, and pseudo-steady state radiation. The information generated from this literature survey will be used in the design of an impact noise generator whose output can be manipulated to replicate the most common impact noise signatures for hearing loss studies.

Spin-Labeled Analogs of 9-Aminoacridine as Probes for Nucleic Acids. B. K. SINHA AND C. F. CHIGNELL, *Section on Molecular Pharmacology, Pulmonary Branch, NHLBI, and Laboratory of Environmental Biophysics, NIEHS, Research Triangle Park, North Carolina 27709*

Sedimentation and velocity measurements indicated that levels I–III caused unwinding of calf thymus DNA. The T_m value of DNA was increased from 66.5 °C to 70, 76 and 73°C by I, II, and III, respectively. At a concentration of 1 µg/ml, I, II, and III produced 24%, 56%, and 16% inhibition of the growth of leukemia L1210 cells *in vitro*. At 50 µg/ml, labels II and III inhibited *E. coli* DNA-dependent RNA polymerase by 23% and 58% respectively, while I stimulated enzyme activity by 20%. The electron spin resonance (ESR) spectra of I bound to DNA, poly dA–poly dT and poly dC–poly dC were characteristic of highly immobilized spin labels with $2A_{||}$ values of 59 G, 62.5 G, and 59 G, respectively, while the $2A_{||}$ values for label II in the same system were 55.5 G, 55.5 G, and 62.5 G, respectively. These findings suggest that labels I and II are intercalated into DNA. In the presence of DNA, the ESR spectrum of III was characteristic of a nitroxide undergoing rapid motion about its x-axis, indicating that there was little interaction between the piperidine ring and the base pairs. Similar results were obtained when labels I and II bound to single-stranded RNA. These observations indicate that labels I–III are useful probes for nucleic acids and their biologically important complexes such as histones.



- I: $n = 0$, $R_1 = \text{OMe}$, $R_2 = \text{Cl}$
 II: $n = 0$, $R_1 = R_2 = \text{H}$
 III: $n = 1$, $R_1 = R_2 = \text{H}$

Spin-Label Studies of Rat Peritoneal Mast Cells, Mouse Mastocytoma Cells, and Compound 48/80. MARY J. ORTNER AND COLIN F. CHIGNELL, *Section of Molecular Pharmacology, Pulmonary Branch, NHLBI, and Laboratory of Environmental Biophysics, NIEHS, Research Triangle Park, North Carolina 27709*

5-Doxylstearic acid, which probes the lipid-protein interface of the plasma membrane, was readily incorporated into mast cells and P-815 ascites mastocytoma cells grown in CDF-1 mice. The $2T_{||}$ values obtained from mixed rat peritoneal cells (58 G), mast cell depleted peritoneal cells (57 G), purified mast cells (57 G) and mastocytoma cells (56 G) at 23°C indicated that this membrane region in mast cells and mastocytoma cells was similar in viscosity to normal leukocytes. When measured as a function of temperature, similar $2T_{||}$ values for mast cells and mastocytoma cells occurred between 3°C (64 G) and 35°C (51 G). In contrast, fluorescence polarization studies with diphenylhexatriene indicated that the hydrophobic region of the mastocytoma cell membrane was significantly more fluid than that of the normal mast cells. This difference was consistent throughout the temperature range of 3-40°C. No abrupt lipid phase transitions were indicated by either probe. Compound 48/80 had no effect on the fluidity of any of the cell membranes studied, although evidence of a direct interaction with membrane intercalated stearic acid was indicated. The signal from spin-labeled 48/80 bound to normal peritoneal cells and mastocytoma cells was not influenced by ferricyanide ions in the extracellular medium. These data indicate that 48/80 may penetrate these membranes as far as the protein-lipid interface.