

ABSTRACTS OF PAPERS

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**Abstracts of Papers for the Fifth Annual Meeting of the
Society of Toxicology, Williamsburg, Virginia,
March 14-16, 1966**

1. *Lysozymuria Induced in Rats by Nephrotoxic Agents*. T. BALAZS and R. R. ROEPKE, Department of Pharmacological Research, Experimental Therapeutics Research, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

The urinary excretion of lysozyme (muramidase) was investigated in rats treated with graded doses of mercuric chloride, uranyl nitrate, or sodium chromate. Renal concentration tests and urine glutamic-oxalacetic transaminase levels were used for the quantitation of nephrotoxicity [Balazs *et al.*, *Toxicol. Appl. Pharmacol.* 5, 661 (1963)].

Signs of mercuric- and uranyl nitrate-induced nephroses were accompanied by a moderate lysozymuria (0.04-0.6 mg/day). Sodium chromate caused a disproportionately high excretion of this enzyme (2-5 mg/day) for 3-4 days, exceeding normal renal lysozyme content (1.0-1.3 mg). Subnephrotoxic doses of sodium chromate did not induce lysozymuria.

Infusions of egg white lysozyme resulted in an increase in the amount of lysozyme in kidney and urine. Endogenous plasma lysozyme levels increased in nephrectomized rats, indicating an extrarenal source and a renal inactivation of this enzyme. Simultaneous measurements of renal clearances of exogenous lysozyme and creatinine demonstrated a decreased reabsorption of lysozyme in chromate- sodium—but a normal one in mercuric chloride-treated rats.

The data imply a decreased tubular reabsorption of lysozyme in the pathogenesis of lysozymuria, and also imply that the primary site of the sodium chromate lesion, the postglomerular portion of proximal convoluted, coincides with that of the lysozyme reabsorption.

2. *Myopathy and Fetal Death Due to Hemicholinium Injection into Chicken Eggs*. BERNARD A. BECKER and JEAN LINDER, Department of Pharmacology, University of Iowa College of Medicine, Iowa City, Iowa.

Hemicholinium (HC-3) antagonizes acetylcholine; therefore, HC-3 may affect embryonic development of systems in which acetylcholine plays some role. Because HC-3 is extremely toxic to mammals, the chicken embryo was selected as a test animal for this study. Particular attention was given to the heart and skeletal muscles.

Groups of 12 white Leghorn eggs were injected on days 1, 4, and 14 of incubation with 0.2 ml of an aqueous solution containing 1-1000 µg/egg of HC-3. Term chickens were examined and dissected; the skeletons were stained with Alizarin Red. Specimens of heart, skeletal muscle, and liver were examined histologically.

Striated muscle showed a dose-related effect which was particularly pronounced in the 14-day group. Muscles of high-dose animals were devoid of striations, with reduced numbers of nuclei. At lower doses, striations were seen but the muscle fibers were more homogeneous than control muscles. Karyorrhexis and increased numbers of nuclei were found. Grossly, the heart, particularly the auricles, of high-dose animals were smaller than controls. Striations were present at all doses, but an increase in the number of nuclei was seen at the highest dosage. Liver sections had cholesterol clefts and unrecognizable architecture. In a dose-related fashion, recognizable liver parenchymal cells were seen in increasing numbers with lower doses.

Embryo deaths were not dose related, but were more numerous in the 4-day group. Skeletal defects were few and not dose dependent. Defects seen were missing or excess ribs and uncinat processes.

3. *Ocular Drug Toxicities*. HOWARD N. BERNSTEIN, Eye Research Foundation of Bethesda, Bethesda, Maryland.

Two currently used drugs, the antimalarial chloroquine (a 4-aminoquinoline derivative) and the tranquilizer thioridazine (a phenothiazine derivative) produce a retinal toxicity. An analysis

of their similarities and differences provides an insight into the problems of detection and prevention of ocular drug toxicities, and clues to avenues of productive investigation.

Both drugs result in a pigmentary retinopathy in humans which can be reproduced in the experimental animal. Both drugs bind to melanin pigment in very high concentration and are stored there, in close approximation to the neuroreceptors of the eye—the rods and cones—for long periods of time. Yet chloroquine retinopathy is a manifestation of chronic toxicity occurring with a minimum of 9 months' daily administration, has a very low incidence of perhaps one in two thousand, is not dose related, and produces irreversible damage. Thioridazine retinopathy is a manifestation of subacute toxicity appearing within 30-60 days after administration of the drug and is directly dose-related with a 50% incidence when the dosage exceeds 1000 mg/day. In addition, much of the visual damage is reversible.

The role of melanin pigment in the physiology of vision appears to be a key to this type of toxicity, but investigation in this area is in its infancy. The effect of chloroquine on the production of free radicals by melanin pigment after irradiation will be discussed.

4. *Process Control Computer in the Automated Clinical Chemistry Laboratory.* MURRAY A. BLAIVAS and ANDRÉ MENCZ, Kings County Research Laboratories, Brooklyn, New York.

An IBM 1710 computer has been successfully programmed for: (1) simultaneous on-line data acquisition up to 20 Auto-Analyzers, (2) interpolation and validation of calibration curves, (3) calculation of specimen results, (4) accuracy control, and (5) continuous monitoring and control of instrument performance.

Rapid repetitive sequential scanning of output signals enables the computer to examine the peaks from each specimen as it is processed by the Auto-Analyzers. After the calculated final results have been validated according to pre-established criteria, they are recorded via an on-line printer or on punched cards. Invalid results or instrumental problems, even if as simple as an insufficient sample, are immediately communicated to the technician on a special output printer for prompt appropriate action. Conversely, the technician can communicate special requirements, such as priority for emergency samples, through the computer.

The system approaches the ideal of removing the technician's clerical burden of interpolation, calculation, and transcription, allowing him more time to exercise his technical skill. In addition, the possibility of human error has been restricted to a minimum, thereby increasing both the reliability of the data and the speed with which they are obtained.

5. *Toxicologic Studies on the Narcotic Antagonist Naloxone.* H. BLUMBERG, T. WERNICK, H. B. DAYTON, R. E. HANSEN, and D. N. RAPAPORT, Endo Laboratories, Garden City, New York.

Naloxone hydrochloride is a new and highly potent narcotic antagonist that is about 15-30 times as active as nalorphine hydrochloride and about 3-6 times as active as levallorphan tartrate in animals and man. Acute toxicity tests were carried out in mice, rats, guinea pigs, rabbits, cats, dogs, and monkeys by various routes of administration. The results indicated that naloxone has a comparatively low toxicity. The maximum nontoxic dose far exceeded the narcotic antagonist dose, e.g., the maximum nontoxic dose subcutaneously in rats was about 50 mg/kg, and the narcotic antagonist dose about 0.1 mg/kg. Despite its much greater potency, naloxone was only about twice as toxic as nalorphine and one-half as toxic as levallorphan. At toxic doses naloxone produced excitation, hyperactivity, salivation, tremors, and tonic-clonic convulsions. Respiration was slightly stimulated, as shown by minute volume measurements in rabbits. Subacute subcutaneous toxicity experiments in rats and monkeys, and a subacute intravenous toxicity experiment in dogs, demonstrated very little cumulative toxicity and no organic pathologic changes. When compared for acute subcutaneous toxicity in 24-hour-old rats, naloxone HCl was not any more toxic than nalorphine HCl, i.e., the LD₅₀ for naloxone HCl was 260 (228-296) mg/kg and for nalorphine HCl, 260 (234-289) mg/kg. Also, naloxone was only about twice as toxic in newborn as in 6-week-old rats.

6. *Uptake and Elimination of Carbon Monoxide from the Blood of Smokers and Nonsmokers.* ARTHUR W. BURKE, JR., and MARTHA J. BRIGGS, Department of Pharmacology, Medical College of Virginia, Richmond, Virginia. (Sponsor: P. S. Larson.)

The uptake and elimination of carbon monoxide (CO) by man has been estimated by analysis of venipuncture samples of blood using a modified microdiffusion method. Color development of palladium chloride-arsenomolybdic acid reagent by CO was determined spectrophotometrically. The percentage of saturation CO-Hb of subjects (smokers and nonsmokers) was determined before and after cigarette smoking, intermittent inhalation of measured quantities of pure CO, or a combination of both.

Upon abstinence from smoking or CO exposure (other than environmental background), all subjects at sedentary or ordinary laboratory activity eliminated CO from their circulation at the same rate, regardless of CO source, if adjusted to the same peak CO-Hb saturation. In the range of 6-12% CO-Hb, the elimination half-time is slightly less than 5 hours; 4-6% CO-Hb, about 5-1/2 hours; 2.5-4%, approximately 7 hours. By 16-18 hours, subjects with as much as 15% saturation CO-Hb returned to background (1.5-2% CO-Hb), while subjects with as much as 8% saturation CO-Hb returned to background within 12 hours.

Experiments indicate that the uptake of CO by man is influenced by cigarette smoking *only* to the extent that CO occurs in the tobacco smoke, and, further, that the degree of CO uptake is associated with the smoking habit of the subject.

7. *Evaluation of Methods for the Treatment of Nitrogen Dioxide-Induced Pulmonary Edema.* D. CAVALLI, J. DETLING, J. IRISH, and C. H. HINE, The Hine Laboratories, Inc., San Francisco, California.

An evaluation was made of candidate therapeutic agents for the treatment of acute pulmonary edema resulting from nitrogen dioxide (NO₂) exposure. Treatments consisting of hyperbaric air and oxygen; tracheal toilet; ethyl, isopropyl, and octyl alcohol vapors; hydralazine; bethanechol; physostigmine; and isoproterenol aerosols produced no change in the mortality, survival time, or lung:body weight ratios of rats suffering from NO₂-induced acute pulmonary edema. Rutin in large doses caused a decrease in mortality and an increase in survival time of exposed rats. Intravenous infusion of isoproterenol caused a decrease in mortality of rabbits exposed to NO₂. The effectiveness of hyperbaric oxygen, hydrocortisone, rutin, and bethanechol against moderate exposure to NO₂ was determined by solvent uptake measurements with rats. Oxygen administration 4 hours after exposure increased solvent uptake. There were no significant effects due to the other compounds.

8. *Interacting Responses following Inhalation of Ozone and Streptococci.* DAVID L. COFFIN and EARL J. BLOMMER, Laboratory of Medical and Biological Sciences, Division of Air Pollution, Public Health Service, U.S. Department of Health, Education, and Welfare, Cincinnati, Ohio.

Ozone has been incriminated circumstantially in outbreaks of spontaneous disease in animals under exposure, and in experimental trials in which bacteria have been artificially applied in association with ozone exposure.

The experiments described herein were performed to determine just how effective this interaction between ozone exposure and bacterial infection might be at atmospheric levels.

Groups of mice were exposed to ozone for 3 hours, then randomly interspersed with like groups receiving ambient room air only and subsequently exposed to aerosolized cultures of Streptococcus C for one-half hour in a concentration calculated to yield a dose of approximately 30,000 organisms per mouse.

This *infective system* produced mortality differences between the ozone-exposed and ambient air group at ozone concentrations ranging between 0.08 and 0.52 ppm with somewhat equivocal results below this range. The minimal effective dose probably lies between 0.05 and 0.08 ppm. Two inferences might be drawn from these results: (1) the infective system is an extremely sensitive indicator of a biologic effect of ozone not yet detectable by other means, and (2) such enhanced susceptibility to pulmonary infection might conceivably occur in a population exposed to a polluted atmosphere containing ozone.

9. *Chlorinated Insecticides in Human Serum*. WILLIAM E. DALE, AUGUST CURLEY, CIPRIANO CUETO, and WAYLAND J. HAYES, JR., Toxicology Section, Communicable Disease Center, Public Health Service, U.S. Department of Health, Education, and Welfare, Atlanta, Georgia.

The concentration and distribution of chlorinated insecticides and related materials in the blood of 10 women who had not had any occupational exposure to insecticides were determined by electron capture gas chromatography. The hexane-extractable chlorinated insecticides and related materials found in serum were *p,p'*-DDT (0.0077 ppm), *o,p'*-DDT (0.0016 ppm), *p,p'*-DDE (0.0152 ppm), β -BHC (0.0042 ppm), dieldrin (0.0013 ppm), and heptachlor epoxide (0.0008 ppm). The mean concentrations of these materials found in serum were greater than those found in the plasma, red blood cells, or whole blood. A comparison of the levels of insecticides in the serum of women with those of men (Dale *et al.*, *Life Sci.* in press) showed that men had statistically greater concentrations of *p,p'*-DDT (0.0105 ppm) but lower concentrations of *o,p'*-DDT (0.0009 ppm) than women. Even though the differences were statistically significant ($P < 0.025$ and < 0.01 , respectively), they may have been due to sampling.

Methods for the extraction of bound chlorinated insecticides and related materials in blood were also investigated.

10. *Metabolism of Ethyltin and Diethyltin and Its Oxide in Rats*. D. S. DAVIES and R. T. WILLIAMS, Department of Biochemistry, St. Mary's Hospital Medical School, London, England.

Few metabolic studies have been carried out on organotin compounds [Barnes and Stoner, *Pharmacol. Rev.* **11**, 211 (1959)]. Tetraethyltin is dealkylated in the rat to the toxic triethyltin which is believed to be stable *in vivo* [Cremer, *Biochem. J.* **68**, 685 (1958)].

We have found the intraperitoneal LD₅₀ of ethyltin trichloride (EtSnCl₃) dissolved in water to be 79 mg/kg in mice, with deaths occurring over 5 days. For diethyltin dichloride (Et₂SnCl₂) in water, the LD₅₀ was 19 mg/kg, with deaths occurring over 3 days. Et₂SnCl₂ is thus four times as toxic to mice as EtSnCl₃.

When given orally to rats, EtSnCl₃ (25 mg/kg) was largely excreted unchanged in the feces. When given intraperitoneally (12.5 mg/kg) it was largely excreted unchanged in the urine. Very little was excreted in the bile. EtSnCl₃ did not appear to be absorbed from the intestine, thus behaving like inorganic tin.

¹⁴C-Et₂SnCl₂ was prepared. On injecting an aqueous solution, about one-third of the dose (10 mg/kg) of Et₂SnCl₂ was excreted in the urine and about two-thirds in the feces. Reverse isotope dilution studies, paper chromatography, and fluorimetric estimation of EtSn⁺⁺⁺ showed that 80-85% of the urinary material was EtSn⁺⁺⁺ and 10-15% was Et₂Sn⁺⁺, while the fecal material was 75% EtSn⁺⁺⁺ and 25% Et₂Sn⁺⁺. Injected Et₂SnCl₂ was also excreted in the bile almost entirely as Et₂Sn⁺⁺. Thus Et₂Sn⁺⁺ was dealkylated to a considerable extent to EtSn⁺⁺⁺, and our evidence suggests that this may occur in the gut, and possibly to a lesser extent in the tissues.

The insoluble (Et₂SnO)_n is converted on keeping in aqueous solution, especially in acid, to Et₂Sn⁺⁺. Its metabolism in the rat is similar to that of Et₂SnCl₂, both EtSn⁺⁺⁺ and Et₂Sn⁺⁺ being found in the urine and feces.

Although ¹⁴C-Et₂Sn⁺⁺ is dealkylated in the rat, no ¹⁴C-CO₂ was found in the expired air. Oxidative attack at the Sn—C bond would be expected to be directed toward the Sn atom. Therefore, it is proposed that one ethyl group of Et₂Sn⁺⁺ is removed in the body as ethane. If the ethyl group had been attacked, ¹⁴C-CO₂ would be expected in the expired air. In our experiments nearly all the Sn was accounted for, but only just over 50% of the administered ¹⁴C. We conclude that the rest of the ¹⁴C was lost as ethane, which would not have been trapped in our experiments.

11. *Effect of Combinations of Pesticides on Reproduction of Mice*. W. B. DEICHMANN and M. L. KEPLINGER, Department of Pharmacology, and Research and Teaching Center of Toxicology, University of Miami School of Medicine, Coral Gables, Florida.

Pesticides were fed to mice during mating, gestation, and lactation and after weaning to determine effects on reproduction and on survival of the young. The first litters were maintained on

90% vs 1-2%
65-80%
0 m/feces
C¹⁴ # Sn
→ corn

the same pesticide-containing diet until 4 months of age. Then some were autopsied and some were sacrificed for chemical analysis. The parents were remated and the second litters treated as the first, with the exception that 18 mice were selected for replicate breeding when 120 days old. Breeding continued through five generations. Indices of fertility, gestation, viability, and lactation were calculated. Two other indices also were calculated, namely, number ~~alive~~ at 120 days expressed either as a per cent of number alive at weaning or as a per cent of the number born.

Pesticides used were aldrin, dieldrin, chlordane, and DDT. Twenty-nine different groups were studied. Mice died before becoming pregnant or delivering when fed aldrin 50 ppm or dieldrin 50 ppm. Aldrin 25 ppm, dieldrin 25 ppm, aldrin 25 ppm plus chlordane 25 ppm, aldrin 25 ppm plus DDT 25 ppm, aldrin 25 ppm plus chlordane 25 ppm plus DDT 25 ppm, and aldrin 10 ppm plus chlordane 100 ppm caused very marked effects. Some parents died before mating or delivery, and some did not become pregnant. There were fewer young per litter. None of the pups were alive at 4 days. Chlordane 100 ppm, DDT 250 ppm, dieldrin 10 ppm, aldrin 5 ppm plus chlordane 50 ppm, aldrin 10 ppm plus DDT 10 ppm, chlordane 100 ppm plus DDT 100 ppm, dieldrin 10 ppm plus chlordane 100 ppm, and dieldrin 10 ppm plus DDT 100 ppm caused some effects, particularly decreased viability and lactation indices.

12. *Effects of Certain Aryloxisobutyrate on the Rat Fetus.* R. M. DIENER and B. HSU, CIBA Pharmaceutical Company, Summit, New Jersey. (Sponsor: Alfred E. Earl.)

Rat reproductive studies were performed with six aryloxisobutyrate compounds, i.e., ethamoxy-triphetol, triparanol, clomiphene citrate, clofibrate, Su-13320 (a basic ether derived from an estrogenic phenol), and Sn-13437 (a substituted phenoxyisobutyric acid).

Comparable doses of each compound were administered daily to groups of rats during the 6th to 20th day of gestation. Dosages ranged from 0.6 mg/kg/day incorporated in the feed, to intubated amounts of 1-140 mg/kg/day.

Feed intake and body weight gains of the medicated dams were calculated and compared to controls. The number of uterine resorption sites, litter size, and average fetal size and weights were also determined. All fetuses were examined, and stained with Alizarin Red S to detect skeletal deformities.

Only the offspring from dams medicated with clofibrate and Su-13437 were comparable to controls. The progeny from rats medicated with the other four drugs exhibited a wide spectrum of defects including weak or edematous fetuses, skeletal deformities, and cleft palates. The incidence of *in utero* deaths was also increased.

13. *Data Analysis and Punched Cards.* E. V. DIETRICH, McNeil Laboratories, Inc., Fort Washington, Pennsylvania.

As a result of today's increased requirements to prove efficacy and safety of a new drug, it has become necessary to resort to means that will provide a clear, complete, accurate, and relatively brief compilation of the data. Punched cards and related hardware provide the means to facilitate the evaluation of these data. The mechanics for processing clinical data from a veterinary study will be defined.

Briefly, clinical data are submitted on report forms designed for the transfer of information to IBM cards by nonscientific personnel. All the charted data are generated using an IBM 083 sorter, an IBM 1401 computer, and a Monroe calculator. The data are organized in such a manner as to obtain detailed safety and efficacy information with a minimum of searching.

14. *Effect of Cyanide Administration on Pulmonary Clearance Processes.* J. V. DILLEY and W. J. BAIR, Biology Department, Pacific Northwest Laboratories, Battelle Memorial Institute, Richland, Washington. (Sponsor: H. A. Kornberg.)

Because tobacco smoke is said to inhibit ciliary activity in the tracheobronchial tree, it was of interest to determine whether one of the components of tobacco smoke, cyanide, altered the clearance of inhaled particles from rat lungs. Cyanide, one of the more prevalent components of tobacco smoke, is present at levels of about 80 mg/100 g of American tobacco burned.

aldrin 10 ppm
born

It was shown that the continuous intake of cyanide, ad libitum, in the drinking water by female rats for several months had no gross effect on the clearance of inhaled Fe^{59}O_3 particles immediately after an inhalation exposure. This suggests that the ciliary clearance processes were not markedly affected by cyanide ingestion. However, in animals sacrificed at 25 days after the inhalation of an Fe^{59} aerosol, there was a significantly higher retention of the inhaled particles in the lungs of the cyanide-treated rats, 72%, than in those of the controls, 43%. While the mechanism for this inhibition of long-term clearance is unknown, there is a possibility that a minor metabolite of cyanide, 2-iminothiazolidine-4-carboxylic acid, may be altering the connective tissue collagen in the lung.

15. *Urinary Excretion of Metabolites as Indicators of Exposure to Parathion and DDT.* W. F. DURHAM, H. F. WOLFE, and J. F. ARMSTRONG, Toxicology Section, Communicable Disease Center, U. S. Public Health Service, Wenatchee, Washington.

The urinary excretion of *p*-nitrophenol and of dichlorodiphenylacetic acid (DDA) was measured in spraymen applying formulations containing both parathion and DDT. In each instance, the excretory level of the metabolite correlated well with exposure to the pesticide. *p*-Nitrophenol excretion showed changes of greater magnitude in response to a specific exposure than did DDA excretion. However, in general, the excretory patterns for *p*-nitrophenol and DDA were similar. Both metabolites reached peak levels at about the same time interval (mean about 6 hours) after cessation of spraying. On the basis of relative concentrations present in the use-formulation, comparatively more parathion-derived than DDT-derived material was recovered in the urine.

p-Nitrophenol excretion increased with increase in ambient temperature. Bathing after exposure was associated with a rapid decrease in *p*-nitrophenol excretion. The *p*-nitrophenol excretion curves tended to show a diurnal variation during the period after exposure has ended. Thus, excretion of this metabolite was insignificant during the late evening and early morning hours, but reached higher levels during the middle of the day.

Tests allowing only one route of exposure at a time indicated that excretory levels of *p*-nitrophenol for orchard spraymen using a liquid formulation of parathion in an air-blast machine was greater when exposure by the dermal route only was involved than when exposure by the respiratory route alone was allowed.

16. *Effects of Various Potassium Salts on the Gastrointestinal Tract of Monkeys.* A. E. EARL, R. M. DIENER, and D. H. SHOFFSTALL, CIBA Pharmaceutical Company, Summit, New Jersey.

Various salts of potassium were compared to KCl for their ability to produce primary, non-specific ulceration of the gastrointestinal tract in rhesus monkeys.

The salts, which were administered in capsule or tablet form twice daily for nine consecutive treatments, contained the same quantity of potassium ion as is contained in 1000 mg of KCl, an amount which produced a 100% incidence of gastrointestinal ulceration in monkeys [Diener *et al.*, *Toxicol. Appl. Pharmacol.* **7**, 746 (1965)]. All lesions were observed grossly and confirmed by histologic examination.

Some of the salts, namely, the bicarbonate, phosphate, and chloride, produced a 100% incidence of ulceration of the gastrointestinal tract. The remaining ten potassium salts, namely, the citrate, tartrate, bitartrate, pyruvate, aspartate, fumarate, glutamate, gluconate, glycolate, and acrylate, produced no ulcerations.

The localized lesions produced by potassium salts could be increased, decreased, or transferred to different sections of the gastrointestinal tract by pharmaceutical manipulations which altered the rate or location of potassium release.

17. *Increased Susceptibility of Guinea Pigs to Histamine Poisoning following Exposure to Ozone.* RICHARD EASTON and SHELDON MURPHY, Department of Physiology, Harvard School of Public Health, Boston, Massachusetts.

Two hours after the end of a 2-hour exposure to 5 ppm of ozone (O_3), the subcutaneous LD_{50} of histamine dihydrochloride to male guinea pigs was 0.89 mg/kg compared to 1.25 mg/kg for un-

exposed controls. When 0.9 mg/kg of histamine dihydrochloride was injected 15 minutes after the end of 2-hour exposures to 5.6, 1.1, 0.5, or 0.0 ppm of O₃, mortality rates were 83, 50, 33, or 0%, respectively. No evidence of pulmonary edema was detected by wet and dry lung-weight measurements. Hypersusceptibility to histamine persisted for 12-24 hours after the end of a 2-hour exposure to 5 ppm of O₃. Increased susceptibility to histamine was also detected by respiratory function measurements. Guinea pigs exposed for 2 hours to 5 ppm of O₃ had increased respiratory flow resistance and breathing frequency, and decreased tidal volume and lung compliance, but these measurements returned to normal values within 1 hour after the end of O₃ exposure. Histamine dihydrochloride (0.25 mg/kg), injected subcutaneously 90 minutes after the end of O₃ exposure, increased flow resistance by 100% and decreased lung compliance by 40%. The same dose of histamine given to animals which had not been exposed to O₃ caused only 25% increase in flow resistance and 10% decrease in compliance.

The results of these experiments indicate that after O₃ exposure, guinea pigs are more susceptible to the functional and lethal effects of exogenous histamine at times when other residual effects of O₃ are not apparent.

18. *Evidence against Humoral Mediation of Tolerance Induction by Edemagenic Agents.* E. J. FAIRCHILD, G. A. BOBB, and L. G. ANDERSON, Division of Occupational Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Cincinnati, Ohio.

Development of tolerance for otherwise lethal effects of edemagenic agents is well documented, but the causative mechanisms remain to be elucidated. Gregory and Ripperton reported (*Am. Ind. Hyg. Conf.*, 1965) that mice thymectomized at birth were later unable to develop tolerance after a prechallenge exposure to ozone. This finding evokes a possible immune mechanism since others have shown that neonatal thymectomy renders mice immunologically incompetent. Dixon and Mountain [*Toxicol. Appl. Pharmacol.* **7**, 756 (1965)] have considered certain metabolic alterations as favorable for tolerance induction and suggest that the pentose phosphate pathway may provide the system.

We report here experiments with parabiotic and adrenalectomized rats, results of which offer evidence discordant with the above concepts. Littermate rats were joined by muscle-skin graft and used after establishment of cross-circulation via capillary anastomosis. One twin of a series was given prechallenge exposures to ozone for tolerance induction. Assessment of pulmonary edema after challenge showed that tolerance was not transmitted. Since it is well known that parabiotic rats can transfer immune responses, we can infer that tolerance for ozone is not mediated by circulating antibodies. Neither does it appear to be mediated by a metabolic shift toward the pentose phosphate pathway. Others have shown this shunt, normally operative during the reparative process after injury to muscle, to be nonfunctional in adrenalectomized rats. We found that adrenalectomy did not inhibit tolerance. Therefore, if we assume that the shunt was nonfunctional in lung as well, then tolerance should not have developed.

19. *Comparative Toxicity of Cytosine Arabinoside.* H. FEINMAN, E. R. HOMAN, and T. TUSING, Hazleton Laboratories, Inc., Falls Church, Virginia, and National Cancer Institute, Bethesda, Md.

The antimetabolite, 1-β-d-arabinofuranosylcytosine hydrochloride, has been found to induce complete remission in acute myelogenous leukemia and acute lymphocytic leukemia. Although the mechanism of action is not known, it has been postulated that this activity occurs by phosphorylation of the nucleoside to a nucleotide, with the conversion of cytidine diphosphate to deoxycytidine diphosphate.

Preclinical studies of this compound were conducted in mice, rats, dogs, and monkeys to evaluate the effects of various routes of administration and dose sequences and to characterize its toxicity. In mice or rats, this compound shows similar toxicity following acute intravenous, oral, or intraperitoneal administration. Single intravenous injections of 5-20 mg/kg caused depressor responses in anesthetized dogs, but no significant alterations of responses elicited by standard reference neurohumoral agents or bilateral carotid artery occlusion. Electrocardiographic records were unaltered.

Repeated intravenous dose studies in mice, dogs, and monkeys indicated that the compound

is more toxic to the dog. In dose-sequence studies, repeated doses, and particularly a continuous intravenous infusion, of this compound are more toxic than single doses. In the dog and monkey, leukopenia was well developed after single or repeated intravenous doses. Microscopic findings indicate depression of the hematopoietic system and a marked effect on the gastrointestinal tissues. Results of studies comparing the hydrochloride and the triacetate salt of cytosine arabinoside will be presented.

20. *Hepatotoxicity of Coumarin*. G. FEUER, L. GOLBERG, and K. I. GIBSON, British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey, England.

Oral administration of coumarin to rats (7 daily doses of 20-200 mg/kg) induced the following changes in the enzyme activities of liver microsomal fractions: glucose-6-phosphatase, inorganic pyrophosphatase, and adenosine diphosphatase were reduced; inosine-, guanosine-, cytosine-, and uridine diphosphatases and thiamine pyrophosphatase were increased. Investigation of the mechanism of these effects revealed that the major metabolites of coumarin, *o*-hydroxyphenylacetic acid and *o*-hydroxyphenyllactic acid, are powerful noncompetitive inhibitors of glucose-6-phosphatase and inorganic pyrophosphatase.

Attention was also directed to the microsomal phospholipids, whose level was increased by coumarin treatment of the rats, and the changes in microsomal enzyme activity obtained *in vivo* could be reproduced *in vitro* by treatment of isolated microsomes with phospholipase A or C. In the latter case, partial restoration of enzyme activity could be brought about by addition of phospholipid. A further and possibly related aspect was the effect of coumarin in increasing the activity of iron-stimulated NADPH-dependent lipid peroxidation in liver microsomes.

It was demonstrated that the increase in relative liver weight brought about by coumarin was not associated with enhanced activity of liver microsomal processing enzymes (hexobarbitone oxidase, aminopyrine demethylase, and nitroanisole demethylase).

21. *Cytogenetic Studies of Rats Injected with Lindane*. ROBERTA D. FRIBERG and VERNON N. DODSON, Department of Industrial Health, School of Public Health, The University of Michigan, Ann Arbor, Michigan. (Sponsor: Herbert H. Cornish.)

The insecticide lindane is currently under suspicion of being a leukemogenic agent. Chromosome changes have been demonstrated in the leukemias. Recently, cytogenetic variations have been detected in the preleukemia stage of human leukemia. This study was initiated to investigate the possibility of detecting early cytogenetic changes in animals injected with lindane.

A single dose (75 mg/kg) of lindane, prepared by toluene extraction from a commercially available pesticide, was injected intraperitoneally into 36 male, Sprague-Dawley rats. Groups of injected and normal rats were sacrificed at 3 days, and at 4-, 6-, 8-, and 12-week intervals. Leukocyte cultures were established, and direct bone marrow preparations were made for each rat. Leukocyte cultures were also made for a second group of rats bled serially at these same intervals.

Cell growth, as expressed by mitotic rates, and chromosome number and morphology were studied on those cultures and bone marrow preparations yielding material suitable for study.

The direct bone marrow preparations demonstrated depressed mitotic rates in the injected animals during the first month. A rebound phenomenon was evident by the sixth week, with injected animals exhibiting mitotic rates higher than their controls. The growth rates of the leukocyte cultures showed an initial lowering for injected rats. The increase of mitotic rate to more normal levels coincided with the increased bone marrow activity.

22. *Effect of Dietary Potassium on the Rate of Thallium Excretion by Rats and Dogs*. P. J. GEHRING and P. B. HAMMOND, Biochemical Research Department, The Dow Chemical Company, Midland, Michigan, and the Department of Veterinary Physiology and Pharmacology, University of Minnesota, St. Paul, Minnesota. (Sponsor: H. C. Spencer.)

The relationship between the level of dietary potassium and the excretion of thallium was studied in rats and dogs given a single intravenous injection. It was found that the disappearance

of thallium from animals was a first-order process. The rate of disappearance of thallium increased as the dietary level of potassium increased. The increased rate of disappearance resulted primarily from an increased rate of excretion of thallium in the urine, with no significant increase in the rate of fecal excretion. The rate of disappearance of thallium from rats exhibiting signs of thallosis decreased markedly just prior to and concurrent with the development of signs of thallosis.

23. *Toxicity and Biologic Effects of Malathion, Phosdrin, and Sevin in the Chick Embryo.* M. GHADIRI and D. A. GREENWOOD, Utah State University, Logan, Utah.

O,O-Dimethyl *S*-(1,2-dicarbethoxyethyl) phosphorodithioate (malathion), *O,O*-dimethyl *O*-(2-carbomethoxy-1-methylvinyl) phosphate (Phosdrin), and 1-naphthyl *N*-methylcarbamate (Sevin), organic pesticides, were injected singly or in various combinations into the yolk sacs of 720 fertile chicken eggs and incubated for 21 days. These experiments were repeated three times. The chemicals were injected at the level of 0, 1, 2, and 4 mg/egg, and the hatchability of eggs ranged from 0 to 70%. When combinations of malathion and Phosdrin, or malathion and Sevin, or Phosdrin and Sevin were injected into the eggs prior to incubation at levels of 1-4 mg/egg, some of the embryos developed striking abnormalities such as dwarfism of legs and wings, irregular beaks, and edema of the brain and other parts of the body. A few of the embryos injected with Sevin alone and incubated for 21 days developed edema in different parts of their bodies. Studies are being conducted to determine whether the administration of these substances to pregnant mice or other species during different stages of their gestation period will produce abnormal offspring.

The studies suggest that caution should be exercised in exposure of chickens to combinations of these substances.

24. *Enigma of Antibiotic-Induced Diarrhea in the Laboratory Rabbit.* J. E. GRAY and C. LEWIS, Research Laboratories, The Upjohn Company, Kalamazoo, Michigan.

A new antibiotic, lincomycin hydrochloride, which is particularly effective against gram-positive organisms, caused diarrhea and death of rabbits when given in an amount as little as one one-hundredth of that tolerated by the rat and dog. Penicillin and erythromycin similarly produced this effect in the rabbit. Lincomycin caused diarrhea by both intravenous (0.5 mg/kg) and oral (5-150 mg total) routes of administration in 100 Dutch rabbits which were maintained essentially free of spontaneous diarrhea. In most cases, 1-2 days after treatment the appetite became depressed. The fecal pellets decreased noticeably in size and quantity. Normal activity of the animal lessened. On the 4th to 7th days after treatment, the rabbit developed profuse diarrhea and usually died in less than a day.

At necropsy, a dilated stomach filled with static contents and hemorrhagic suffusions of the cecum were the characteristic findings. No significant evidence of a direct toxic effect of the antibiotic on the gastrointestinal tract was obtained by microscopic examination.

Bacteriological studies have demonstrated that the flora of the cecum changed from predominantly gram-positive to gram-negative organisms at about the same posttreatment interval (1-2 days) that the gastrointestinal stasis was recognized clinically. Efforts to alter the clinical course by floral supplement (*Lactobacillus* culture) and by oral transfusion of cecal contents of normal rabbits were not successful.

By the various methods employed in these studies, we have been unable to differentiate between spontaneous and antibiotic-induced diarrhea. The peculiar susceptibility of this species to diarrhea is believed to be related to its vital dependence upon a very labile gram-positive bacterial flora in the cecum.

25. *Blue VRS-Induced Rhabdomyosarcomas.* H. C. GRICE, I. DUPUIS, M. DENNERY, and W. A. MANNELL, Pathology and Toxicology Section, Research Laboratories, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Ontario, Canada.

Multiple injections of the triphenylmethane dye, Blue VRS (C. I. 42045), into the posterior thigh muscles induced malignant tumors at the site of injection in male and female rats. The

tumors first metastasized to regional lymph nodes and then to distant nodes and organs in the abdominal and thoracic cavities.

Tumor spread appeared to be principally by way of the lymphatics, although tumor cells were identified in smears of peripheral blood. The tumors were diagnosed histologically as pleomorphic rhabdomyosarcomas. They were first observed in male animals. However, the average latent period and incidence were higher in female rats.

Tumors were transplanted into male weanling rats of the same strain, and primary transplants developed in 1-2 months. Secondary transplants developed rapidly, and several rats died as a consequence of massive tumor-spread in less than 2 weeks.

26. *Inhibition of Experimental Silicosis by Beryllium in Ores.* DAVID H. GROTH, GUNTHER E. KRAUS, L. McMILLAN, and L. D. SCHEEL, Division of Occupational Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Cincinnati, Ohio.

Each of seven groups of rats was injected intratracheally with 50 mg of one of seven different beryllium ores. The animals were sacrificed, and the tissues were examined at 3, 12, and 18 months postinjection. Although the ores contained 20-40% of free crystalline silica, no silicotic lesions and little or no fibrosis was seen at any time in the lungs and tracheobronchial lymph nodes.

Pathologic lesions produced by intratracheal injections of silica and silica plus beryllium hydroxide will be compared with the lesions produced by the beryllium ores.

27. *Improved Tests for Chemical Carcinogens. Report on Aromatic Amines, Nitrosamines, Phenols, Nitroalkanes, Amides, Epoxides, Aziridines, and Purine Antimetabolites.* Z. HADJIAN, T. N. FREDRICKSON, E. K. WEISBURGER, and J. H. WEISBURGER, Mason Research Institute, Worcester, Massachusetts, and Carcinogenesis Studies Branch, National Cancer Institute, Bethesda, Maryland.

Weanling cesarean-derived Fischer rats of both sexes were used in all experiments. The test compounds, suspended in steroid-suspending vehicle, were administered by gavage 5 days/week for 52 weeks to animals maintained on a commercial laboratory chow and water. Rats were observed for 6 months longer (total, 18 months) and then sacrificed, unless their condition required earlier necropsy. The maximum tolerated dose (MTD) was established in a preliminary study, using 3 males per dosage. The definitive study involved 6 levels, beginning with the MTD and decreasing by two-thirds at each step. The groups at one-third of the MTD contained 30 rats; the others, 6 each. Appropriate vehicle and untreated controls were used. At necropsy, selected organs were weighed and tissues were preserved for histologic examination.

Vehicle-control and untreated control rats exhibited a similar pattern of occurrence of tumors. Male rats over 400 days old had a high incidence of testicular interstitial cell tumors. Females had a low proportion of mammary fibroadenoma and adenocarcinoma and pituitary adenoma.

Thirty-six compounds and 12 binary mixtures were tested in about 5000 rats. Ten compounds were definitely carcinogenic: (1) 3-methyl-2-naphthylamine; (2) 3-nitro-2-naphthylamine; (3) 1,2-dichloro-3-nitronaphthalene; (4) 4-(*p*-dimethylaminostyryl)quinoline; (5) 2-(4-dimethylaminostyryl)benzothiazole; (6) *N,N'*-diethyl,*N,N'*-dinitrosoethylenediamine; (7) *N,N'*-dimethyl,*N,N'*-dinitroso-1,3-propanediamine; (8) 1,4-dinitrosopiperazine; (9) 3,3'-dimethoxybenzidine; and (10) *N*-4-dinitroso-*N*-methylaniline. With 11 other chemicals, a type of tumor not seen in controls was noted.

28. *Malignant Neoplasia in the Rat following Administration of 5-Nitro-8-hydroxyquinoline.* R. A. HALL and P. MARTORANA, Pathology Department, Charles E. Frosst and Company, Montreal, Canada.

The compound, 5-nitro-8-hydroxyquinoline, was administered orally, 5 days/week, to young albino rats for one year. Three dosages were used, namely, 50, 100, and 200 mg/kg, with one control group receiving the drug vehicle, 6% gum acacia. During the test period, 2 rats were sacrificed, one was found dead at the 50 mg/kg level, and another was found dead at 100 mg/kg.

4
 All these animals presented at autopsy intraperitoneal tumor formation consisting of diffuse nodular growths throughout the mesentery, with larger masses in proximity to the intestine, but with no apparent involvement of the intestinal wall. Macroscopically, on section after fixation, these nodules presented uniformly a whitish surface with some necrotic and frequently hemorrhagic areas, and a well-differentiated capsule. Histologically, the tumors were classified as lymphosarcomas of the lymphoblastic type, with a high degree of malignancy. Although the observation of tumor formation in a small number of animals during the course of a toxicity test is by no means conclusive, the relatively low doses of 5-nitro-8-hydroxyquinoline required to induce malignancy in this test, coupled with previously published reports on the possible carcinogenicity of 5-hydroxyquinoline and its derivatives, suggest that the use of the compound as a urinary antiseptic be viewed with the utmost caution.

few mm →
 1.5 x 1.4 cm

29. *Acute Toxicity of Alkyl-Substituted 2-Aminoethanols*. ROLF HARTUNG and HERBERT H. CORNISH, Department of Industrial Health, School of Public Health, The University of Michigan, Ann Arbor, Michigan.

The alkyl-substituted 2-aminoethanols have found widespread use in industry as emulsifying, curing, and flotation agents. These substances have a close chemical relationship to the normal precursors of choline in the animal.

During this study, the acute oral and intraperitoneal toxicities of five alkyl-substituted 2-aminoethanols and their respective alkyl-substituted choline derivatives were investigated and compared.

The oral LD₅₀'s ranged from 1.2 g/kg for 2-ethylaminoethanol to 7.1 g/kg for 2-*n*-butylaminoethanol. Intraperitoneal LD₅₀'s were as low as 120 mg/kg for 2-di-*n*-butylaminoethanol. A number of these compounds increased gastric motility and secretory activity, while respiratory rates and heart rates were frequently depressed. Chromodacryorrhea and excessive salivation were frequent findings, suggesting an effect on cholinergic activities. Some of the observed changes could be correlated with the inhibitory effect of these compounds on choline oxidase and cholinesterase activity *in vitro*.

Even though the symptoms produced by many of the compounds in this series appeared to be, in part, due to interferences with normal cholinergic functions, these effects alone were not sufficient to account for the observed toxicity. In this series of compounds, toxicity could not be correlated with any systematic alterations in chemical structure.

30. *Tissue Storage in Mice Fed Combinations of Pesticides*. M. L. KEPLINGER, I. A. DRESSLER, and W. B. DEICHMANN, Department of Pharmacology, and Research and Teaching Center of Toxicology, University of Miami School of Medicine, Coral Gables, Florida.

Reproduction studies with mice fed combinations of pesticides are being conducted in our laboratory. The fat, liver, and brain from these mice (parents and progeny after several generations) were analyzed for pesticide content after feeding aldrin and DDT individually and in combination.

→ 20 # 11

The method of analysis included homogenization of tissue and extraction of pesticides with petroleum ether. Extracts were diluted to concentrations of 5-10 ng/ml and analyzed by gas-liquid chromatography without further clean-up. A 6-foot stainless steel column packed with 60-70 mesh Chromport XXX coated with 3% QF-1, was used. Purified nitrogen was the carrier gas. Column, inlet, and detector temperatures were 190-195°C, 200-210°C, and 185-190°C, respectively. An electron capture detector, containing tritium foil (polarization voltage 15 volts) was used. Sensitivity setting was 32×10^2 .

After ingestion of different doses of aldrin, there was little difference between the levels of dieldrin in the brain and liver. Levels of dieldrin in fat, however, were dose related. At the same level of feeding, males stored more dieldrin in the fat than females. From feeding DDT, the DDT levels in fat were increased after 2-4 weeks of feeding and then decreased during the fifth to eighth week before starting to rise again. After ingestion of aldrin plus DDT, the levels of both dieldrin and DDT in fat and liver were lower than in tissues from animals fed twice the dose of either compound individually.

31. *Toxic and Teratogenic Effects of Insecticides in Duck and Chick Embryos*. K. S. KHERA, Research Laboratories, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Ontario, Canada.

Insecticides representative of different chemical groupings were injected at 10-1000 µg/egg in 0-, 4-, 7-, 10-, and 13-day-old duck and chick embryos.

When injected at an early stage of embryonic development, parathion, diazinon, Trithion, and Ruelene induced defects of the cartilaginous and osseous skeleton, eye cataracts, ascites, and hepatic degenerative changes. The skeletal defects consisted of dwarfism, micromelia, ectrosyndactyly, stunted growth of cervical vertebrae with or without fusion, and irregular beak growth. Histologically, there was slow cartilaginous growth, retarded osteoid tissue formation, delayed enchondral and membranous ossification and mineralization.

In both species injected near the middle of embryogenesis with Phosdrin, DDVP, diazinon, Sevin, Trithion, Bayer 37344, or Bayer 39007 there were congenital foot deformities, some of which were permanent. These consisted of medial or plantar flexion of phalanges at the interphalangeal or metacarpophalangeal joints, with medial rotation of the tarsus. A few cases of retarded bone growth at the interfrontal and frontoparietal sutures with encephalocele or hydrocephalus were noted.

The toxicity of the insecticides in relation to the embryonal development of the two hosts was considered.

32. *Metabolism of Stearoyl Propylene Glycol Hydrogen Succinate*. W. R. KING and R. H. COORS, Miami Valley Laboratories, The Procter and Gamble Company, Cincinnati, Ohio.

Metabolism of stearoyl propylene glycol hydrogen succinate (SPGHS), an example of a compound composed of edible components, has been studied *in vivo* in rats, and *in vitro* with digestive enzymes. Both 1-C¹⁴-stearic acid-labeled SPGHS and 1-C¹⁴-succinic acid-labeled SPGHS were used so that the metabolic fates of both the stearic acid and succinic acid moieties could be determined. The SPGHS data from the studies *in vivo* were compared with those obtained when either 1-C¹⁴-stearic acid-labeled soybean oil, or free 1-C¹⁴-succinic acid was fed.

The data clearly indicate that hydrolysis of SPGHS takes place to a very large extent prior to absorption. The stearic acid moiety of the compound was absorbed primarily via the thoracic duct (85%), while greater than 90% of the succinic acid moiety was absorbed by a nonthoracic duct pathway. The distribution of radioactivity among respiratory CO₂, urine, feces, and carcass was determined. Respiratory CO₂ was shown to be the major end product of catabolism. No intact SPGHS was found to be deposited in the animal body.

The extent of hydrolysis *in vitro* of SPGHS after 6 hours' digestion in the presence of fresh rat pancreatic juice plus bile was 54-56%. Both stearic acid and succinic acid were cleaved from the SPGHS molecule, indicating that the enzyme(s) were capable of attacking either end of the molecule.

The data clearly show that SPGHS is efficiently metabolized by the animal body.

33. *Effects of Subacute Administration of Some Pesticides on Microsomal Enzyme Systems*. F. K. KINOSHITA, J. P. FRAWLEY, and K. P. DUBOIS, Toxicity Laboratory, University of Chicago, Chicago, Illinois, and Hercules Powder Company, Wilmington, Delaware.

Measurements of induced synthesis of hepatic microsomal enzymes by various chemical agents would be facilitated by quantitative procedures that are readily applicable under the experimental conditions generally used in subacute or chronic toxicity studies. The present investigation was undertaken to develop and test the applicability of practical procedures for measuring enzyme induction. Assay systems utilizing whole liver homogenates were the most suitable. The methods selected for this study were the EPN-detoxification system of Neal and DuBois [*J. Pharmacol. Exptl. Therap.* **148**, 185 (1965)], a modification of the O-demethylase system of Netter and Seidel [*J. Pharmacol. Exptl. Therap.* **146**, 61 (1965)], and the N-demethylation system of LaDu *et al.* [*J. Biol. Chem.* **214**, 741 (1955)]. The ability of some pesticides to induce synthesis of microsomal enzymes was first determined by oral intubation of daily sublethal doses

for 5 days, and measuring the enzyme levels in the livers at 24 hours after the last dose. Agents capable of causing induction were then fed at various levels in the diet for periods up to 13 weeks to determine the rate and extent of enzyme induction. Two substituted urea herbicides (Herban and Diuron) were shown to induce enzyme synthesis by both procedures. Dose-dependent increases occurred over a range of doses. Maximal induction was noted with repeated oral doses of 300 mg/kg and dietary levels of 3000 ppm. Similar dose-dependent increases were produced by toxaphene and a known inducing agent, DDT [Hart *et al.*, *Toxicol. Appl. Pharmacol.* 5, 371 (1963)], DDT being the most effective.

best demonstration yet - (Hays) Damn

34. *Studies in Chemical Carcinogenesis. III. α -Aminoanthraquinone.* S. LAHAM, H. C. GRICE, and J. W. SINCLAIR, Environmental Toxicology Programme, Occupational Health Division, Environmental Health Centre, Department of National Health and Welfare, Ottawa, Ontario, Canada.

In continuation of earlier studies from this laboratory [*Toxicol. Appl. Pharmacol.* 7, 489 (1965)] the carcinogenic activity of α -aminoanthraquinone has been investigated in Sprague-Dawley rats. The present report describes the preliminary results obtained with this compound over a period of 14 months.

Corn oil suspensions of this compound (10 mg/0.5 ml/rat) were administered orally once a week to 20 male and 20 female rats. Six adenomas of the mammary gland and other benign tumors were observed in the females, whereas only one small cell sarcoma of the intestine and a neurofibrosarcoma developed in the males.

Preliminary studies on the metabolism of this compound in the Sprague-Dawley rat will be described, and a correlation between structure and carcinogenic activity of related polycyclic hydrocarbons will be outlined in this report.

35. (Abstract withdrawn.)

36. *Successive Generation Studies with Pesticides Comparing Mice and Rats.* G. J. LEVINSKAS, I. J. MORICI, and W. E. RIBELIN, Environmental Health Laboratory, Central Medical Department, American Cyanamid Company, Princeton, New Jersey.

Successive generation studies were undertaken with mice and rats to compare the results obtained by the use of these two species. At the same time, consideration was given to the design of successive generation studies so that various experimental approaches could be evaluated as to their practicability and utility in assessing possible effects on reproductive performance. *O,O*-Dimethyl *S*-(*N*-methylcarbamoylmethyl)phosphorodithioate (dimethoate) and *O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate (phorate) were the test compounds employed. Dietary levels of dimethoate were 5, 15, and 50 ppm for mice, and 10, 30, and 100 ppm for rats. Phorate was fed at 0.6, 1.5, or 3.0 ppm to both mice and rats. Weanlings of each species were started on test, and they and their offspring were mated in turn until a third generation had been produced.

3 gen FDA design

Phorate

Indices were calculated for fertility, gestation, lactation, and viability. These will be presented, together with a description of the results obtained from gross, microscopic, and skeletal examination of animals. The advantages, drawbacks, and limitations of the experimental design selected will be discussed.

F₁ only, antigen F₂, worms only 4/2/68
histology

37. *Automated Techniques in Clinical Pharmacology and Toxicology.* DONALD G. LEVITT, Division of Medical Information, Bureau of Medicine, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C.

The Division of Medical Information in the U. S. Food and Drug Administration has in the past two years developed a project for the collection, storage, analysis, and dissemination of clinical reports involving adverse experiences with drugs. The problems which were faced in the implementation of such a project are the subject of this paper.

The specific difficulties which will be discussed are: (1) Data collection in hospitals and university centers and from the pharmaceutical industry. (2) The relevant evaluation of these

	mice	rats
FI	100	71-100
VI	71-95	74-98
LI	96-98	90-100
SI	- mixed data	

data in order that they may be subject to analysis. (3) The problems which arise in the analysis of this information. (4) The training and organization of medical and paramedical personnel for utilization of a complex medical system. (5) The adaptation of this material to computer processing with the attendant problems of vocabulary control and the production of meaningful reports.

The current status of this problem both internationally and from an international viewpoint will be discussed.

38. *Rectal Absorption of Salicylates*. WERNER LOWENTHAL and JOSEPH F. BORZELLECA, Department of Pharmacology, Medical College of Virginia, Richmond, Virginia.

Studies were conducted to determine the process(es) by which drugs may be absorbed from the rectum, and the kinetics of this absorption. Dogs were used since their rectal anatomy resembles that of humans. The rectal contents were evacuated prior to inserting the suppositories. Plasma salicylate levels from the absorption of sodium salicylate or salicylic acid incorporated into 4 different suppository bases were determined. At least 3 dogs per compound per suppository base were employed. It was found that both salicylates were absorbed to about the same degree from cocoa butter. However, from bases composed of a mixture of synthetic glycerides, polyoxyethylene glycol, or polyoxyethylene-4-sorbitan monostearate (PSMS), higher plasma salicylate levels were noted from the salicylic acid suppositories. The rates of absorption from the rectum and elimination from the blood, and the specific apparent body volumes were determined using a modification of Teorell's equation No. 25. To facilitate the calculations involved, a program in Fortran with Format was used in the IBM 1410 digital computer system. The rate of absorption of sodium salicylate was found to be greater than that for salicylic acid from all bases tested except the PSMS base. The rates of elimination from the blood for the two drugs do not vary significantly. The specific apparent volumes for salicylic acid were less than those for the sodium salt, suggesting a lesser degree of storage for the acid form. Since Teorell's equation assumes apparent first order rates of absorption and elimination from the blood, and since this equation describes the data presented, this suggests that salicylic acid and its sodium salt are absorbed into the blood from the four bases tested, and eliminated from the blood by apparent first-order kinetics.

39. *Effects of Respired Polyvinylpyrrolidone Aerosols in Rats*. H. B. LOWSMA, R. R. JONES, J. PRENDERGAST, and L. J. BODENLOS, U. S. Navy Toxicology Unit, National Naval Medical Center, Bethesda, Maryland. (Sponsor: J. Siegel.)

Rats were exposed to aerosols of polyvinylpyrrolidone (PVP) 5 days/week, 8 hours/day, for 30 exposures in an effort to produce a recently described pulmonary granulomatosis referred to as "thesaurosis" [Bergmann *et al.*, *New Engl. J. Med.* **258**, 471 (1958)]. Animals were sacrificed at predetermined intervals, and histologic, histochemical, and chemical analyses were performed on the lungs and reticuloendothelial tissues.

Histologic sections revealed no inflammatory response. Large numbers of macrophages laden with granular material were present throughout the alveoli. Fixation and staining in iodide-iodate solution revealed brown particles within and without the macrophages, compatible with those described for PVP. Strains of periodic acid-Schiff, Congo Red, and Chlorazol-Fast Pink were negative, and not in agreement with previously published results. Chemical determination revealed a large amount of PVP within the pulmonary tissue. No PVP was found in the reticuloendothelial tissues.

40. *Factors Modifying the Hepatotoxic Action of Neptunium-237 in the Rat*. D. D. MAHLUM, Biology Department, Pacific Northwest Laboratories, Battelle Memorial Institute, Richland, Washington. (Sponsor: H. A. Kornberg.)

Neptunium-237 was shown to exert in the rat a potent hepatotoxic action that is characterized by an increase in liver lipids, especially in the triglyceride fraction. The elevation of liver lipids was more marked in the female than in the male. The fatty liver response in the female could be decreased by various dietary and hormonal manipulations, including hypophysectomy, adrenal-

ectomy, the feeding of glucose or butylated hydroxytoluene, or the injection of nicotinic acid. Hypophysectomy and adrenalectomy also minimized cellular changes in the liver, other than fat deposition, as evidenced by microscopic examination and blood levels of isocitric dehydrogenase and serum glutamic-pyruvic transaminase.

Ovariectomy or testosterone administration did not decrease the susceptibility of the female rat to Np^{237} , and castration or estradiol treatment failed to increase the sensitivity of the male. Choline, methionine, and adenine supplements in the diet were without effect on fatty liver development in the female.

The mechanism of fatty liver development will be discussed with regard to the effect of the modifying influences examined.

41. *Metabolism of Linear Alkylate Sulfonate in Rats.* W. R. MICHAEL, Miami Valley Laboratories, The Procter and Gamble Company, Cincinnati, Ohio.

These studies were conducted to determine the metabolic fate of linear alkylate sulfonate (LAS), and to compare its metabolism in rats with that of tetrapropylene alkylbenzene sulfonate (ABS). Normal male rats were fed, by oral intubation, several dosages of either LAS^{35} or ABS^{35} . The urine and feces were collected separately 24, 48, and 72 hours after administration of the surfactant, and each was assayed for its S^{35} content. The LAS^{35} -fed animals excreted 36-57% of the administered S^{35} in the urine and 39-56% in the feces. The ABS^{35} -fed animals excreted 7-10% of the administered S^{35} in the urine and 53-79% in the feces. The relative distribution of the S^{35} activity between urine and feces was partially related to the quantity of the surfactant fed. The animals were all sacrificed 72 hours after dosing. Little or no S^{35} was detected in any of the organs assayed or in the remaining carcass.

In one phase of this study, male rats were fitted with cannulas in their thoracic or bile ducts, and in another phase the bile ducts of male rats were ligated. These studies showed that both surfactants were readily absorbed from the gastrointestinal tract (80-90% of a 1.2-mg dose) and that both were probably transported, after absorption, via the portal venous blood. In addition, these studies suggest that the LAS metabolites are probably highly polar and probably actively secreted by the kidney tubule into the urine, whereas the ABS metabolites are less polar and are reabsorbed by the kidney and excreted primarily in the bile.

The metabolites of LAS found in the urine and feces have been isolated by a combination of ion exchange and column chromatographic techniques. Characterization of these materials has involved the use of infrared, NMR, and mass spectrometry. It appears that the metabolites are a mixture of sulfophenyl alkylcarboxylic acids of various chain lengths. These findings suggest that the mammalian system is capable of oxidizing the linear alkyl chain much in the fashion reported for microbiological systems.

42. *Comparison of Malathion-Esterase and Acetylcholinesterase Activities in Animals Treated with Malathion.* SHELDON D. MURPHY, Department of Physiology, Harvard School of Public Health, Boston, Massachusetts.

Manometric techniques were used to measure the enzymatic hydrolysis of malathion (malathion-esterase) and acetylcholine (cholinesterase). Groups containing 4 or more male guinea pigs were injected intraperitoneally with 400, 200, 100, 50, 25, or 12.5 mg/kg of malathion in corn oil. The animals were sacrificed 60 minutes later. Average malathion-esterase activities of the livers were, respectively: 28, 27, 36, 41, 62, and 75% of the activity of livers from corn-oil controls. Only the 400 mg/kg dosage produced appreciable inhibition of brain and red blood cell cholinesterase activities (49 and 60% inhibited). The time of onset of depression of malathion-esterase preceded the inhibition of cholinesterase by several minutes. The reduction of liver malathion-esterase activity persisted for several days after a single injection. Two groups of 8 guinea pigs were pretreated with 200 mg/kg of malathion or corn oil. Sixteen hours later, one-half of the animals in each group were sacrificed. The remaining animals were given a second dose of 200 mg/kg of malathion and were sacrificed 1.5 hours later. The results of enzyme measurements are summarized in the table.

Pretreatment	Challenge	Per cent of corn-oil controls		
		Liver malathion-esterase	Brain ChE	RBC ChE
Malathion	—	20.5	96.2	95.1
Corn oil	Malathion	24.4	102.0	87.6
Malathion	Malathion	8.8	39.2	31.4

Malathion injections also reduced liver malathion-esterase activities in rats and mice. The results suggest that malathion has a self-limiting effect on its own hydrolytic detoxication, which may be of importance in subacute poisoning.

43. *Some Toxicologic Effects of High Doses of the Nonaddicting Analgesic Agent Methotrimeprazine.* J. F. NOBLE and B. M. SPARANO, Departments of Pharmacological Research and Experimental Pathology, Experimental Therapeutics Research, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York. (Sponsor: K.-F. Benitz.)

Previous studies in dogs with subcutaneous doses of 3 and 15 mg/kg/day of methotrimeprazine for 30 days failed to produce definite signs of drug-induced toxicity. In order to establish toxic dosages, the present high-dose studies were carried out. All doses used in these studies were in considerable excess of the usual human therapeutic doses of 20 mg, one to four times a day. Methotrimeprazine was administered to 24 dogs by the oral route at doses of 20, 40, or 80 mg/kg/day for 90 days, and to 6 dogs at doses ranging from 30 to 800 mg/kg/day for up to 75 days. Two additional dogs received single intravenous doses of 50, 100, or 480 mg/dog. The major toxic effects from the 90-day oral-dosing study occurred mainly at the 80 mg/kg/day dosage. The drug-related findings included: (1) death of one of the six dogs; (2) central nervous system depression; (3) extrapyramidal, autonomic, and sensory dysfunction; (4) electrocardiographic changes which were suggestive of myocardial anoxia; and (5) arrest of spermatogenesis, atrophy of the prostate, vacuolation of lymphoid tissue, involution of the thymus, and increased liver weights. Toxic effects at doses of 20 and 40 mg/kg were essentially limited to foci of atrophic tubules and to some giant cells in the testes. Repeated doses of 200-800 mg/kg were found to cause marked lowering of blood pressure, emesis, hematemeses, bloody urine, and death. Single intravenous doses of 50, 100, or 480 mg/dog produced a marked transient drop in blood pressure. In addition, 480 mg caused tachycardia, emesis, salivation, muscle tremors, unsteadiness, tonic convulsions, choreoathetoid behavior, inability to stand, sedation, and bloody urine.

44. *Data-Processing Techniques in Toxicology.* GARETH OWEN and H. P. K. AGERSBERG, JR., Wyeth Laboratories, Inc., Radnor, Pennsylvania. (Sponsor: Richard F. Tislow.)

The increasing demand for detailed data, including individual animal protocols, in toxicology reports has led to a formidable quantity of clerical work. The methods which will be described are designed to produce data directly in a form which can be accepted by a computer. The calculation and preparation of mean values, statistical analyses, and individual animal protocols then becomes a completely automatic process carried out by a machine.

Body weight and food consumption data are recorded as follows: Each animal is identified with animal number, sex, type of test, drug number, and dose by means of a 10-digit code punched into a permanent plastic badge which hangs on the outside of its cage. When the animal and its food jar are to be weighed, the badge is inserted into an electronic reader which causes the information to be punched into paper tape. The weights are then entered into the tape by utilizing an electronic balance, the output of which feeds into the tape punch.

Hematology, blood chemistry, and organ weight data are transferred to paper tape by means of Friden "Flexowriters." These machines produce a printed copy of the information being punched, thus making simultaneous verification possible. The nonvariable data on each animal are entered by means of edge-punched cards.

The punched tapes can be accepted directly by some computers, or can be converted into punched cards if necessary.

45. *Plutonium Particle-Induced Neoplasia of the Canine Lung*. J. F. PARK, W. J. CLARKE, and W. J. BAIR, Biology Department, Pacific Northwest Laboratories, Battelle Memorial Institute, Richland, Washington. (Sponsor: H. A. Kornberg.)

To determine the long-term translocation and biological effects of inhaled plutonium, 40 beagle dogs were given a single 10- to 30-minute exposure to $\text{Pu}^{239}\text{O}_2$ aerosols. Thirteen dogs died or were sacrificed when clinical signs indicated death was imminent 29-66 months postexposure. The body burdens at death ranged from 0.5 to $3\ \mu\text{C}$ with 40-75% of the body burden in the lungs and 20-50% in the bronchial and mediastinal lymph nodes. The liver contained 2-21%, and the skeleton, 1-7%. Cardiopulmonary insufficiency and lymphopenia were the primary clinical signs. Pathology in the lungs consisted of severe fibrosis followed by alveolar cell hyperplasia and by bronchiolar and squamous types of metaplasia. Seven of the animals showed bronchioloalveolar carcinomas, an incidence of 18% as compared to a primary lung tumor incidence of 0.2% in canine necropsy material. The bronchial lymph nodes were composed of dense sclerotic connective tissue devoid of any lymphoid element. Metastases of the pulmonary tumor to the bronchial lymph nodes were seen in three animals.

46. *Toxicologic Evaluation of the n-Heptyl Ester of p-Hydroxybenzoic Acid*. O. E. PAYNTER, L. W. HAZLETON, and E. A. GARLOCK, Hazleton Laboratories, Inc., Falls Church, Virginia.

An interpretation and evaluation of acute, subacute, and metabolic studies of the heptyl ester of *p*-hydroxybenzoic acid correlate well with those of the better known lower esters of the series. It has also been reported that the antifungal activity of this series of esters increases to approximately the heptyl member. Thus, it may well be that with greater understanding of the antimicrobial action, metabolic fate, and chronic toxicity of this series of esters, the higher esters will, in fact, prove to be more effective and safer food additives than the lower esters.

Chronic feeding studies in albino rats and beagle dogs, and three-generation reproduction studies with rats were conducted as part of the toxicologic evaluation of *n*-heptyl *p*-hydroxybenzoate for use as a food additive. The compound was fed at levels of 1.0, 3.0, or 5.0% of the basal diet to albino rats. Similar levels were used in the three-generation rat reproduction studies. A level of 5.0% in the diet was toxic to rats, whereas only minimal evidence of effect on food consumption and body weight was observed at the 3.0% and 1.0% dietary levels. The 5.0% test group of the reproduction study showed an abnormal skin effect on the pups, and a marked growth suppression in the first (F_{1a}) litter nursing phase. Growth suppression during nursing was also present in the subsequent litters in the 3.0% and 1.0% test groups. In spite of this, the fertility index, gestation index, live birth index, and lactation index of the test groups were comparable to those of the controls.

The compound was administered by capsule to beagle dogs 7 days/week at dosages equivalent to 0.15, 1.0, or 2.5% of the diet. At the higher levels, diarrhea and emesis were occasionally observed. Other than this, all criteria were comparable for the test and control groups. These include body weight, food consumption, behavior, survival, and clinical studies.

47. *Diet and Caffeine Toxicity in Rats*. J. M. PETERS and E. M. BOYD, Department of Pharmacology, Queen's University, Kingston, Ontario, Canada.

Dietary components have been reported to alter toxic reactions toward penicillin in rats [Boyd *et al.*, *Can. J. Physiol. Pharmacol.* **43**, 47 (1965)]. The study reported herein dealt with the effect of diet on susceptibility to caffeine administered daily, in oral doses of 185 mg/kg, to young adult female albino rats, 130-160 g, isolated in metabolism cages. Purified diet 1 (18% casein, 68% sucrose, and 10% vegetable oil plus vitamins) and purified diet 2 (30% egg white, 48% cornstarch, 14% cottonseed oil, and 3% cod liver oil plus vitamins except biotin) were compared with laboratory chow. No deaths occurred in controls given no caffeine. The fortnight mortality of rats given caffeine was 33% in 18 fed chow, 85% (P from chow < 0.02) in 29 fed diet 1, 70% ($P < 0.02$) in 27 fed diet 2, 70% in 10 fed diet 2 + 200 μg of biotin. Death occurred mostly in the first week with clinical and pathological signs similar to those described by Boyd [*Toxicol. Appl. Pharmacol.* **1**, 250 (1959)]. In addition, automutilation and hemorrhagic shock were seen.

Stress reactions and hypertrophy of the salivary glands and liver occurred in survivors. When rats were aggregated their sensitivity to the toxic reaction decreased.

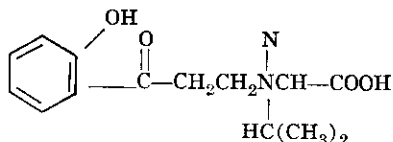
48. *Toxicity and Biologic Effects of Six Carcinogenic Polynuclear Aromatic Hydrocarbons in Chick Embryos and Mice.* F. RENO, H. MILLAR, and D. A. GREENWOOD, Utah State University, Logan, Utah.

3,4-Benzpyrene, 1,2,5,6-dibenzanthracene, 3-methylcholanthrene, 3,4,9,10-dibenzpyrene, 3,4-benzofluoranthene, and 1,2,7,8-dibenzacridine were dissolved in corn oil and injected singly or in various combinations into the yolk sacs of fertile chicken eggs and incubated for 21 days. The compounds were injected at levels of 0-10 µg. The hatchability of the eggs decreased as the levels of the carcinogens increased.

Six young adult mice received a single intraperitoneal injection of 5 mg of 1,2,5,6-dibenzanthracene dissolved in corn oil. They were fed a normal mouse diet. Four to six months after injection, mammary gland squamous cell adenocarcinomas developed in five of the six animals. The tumors ranged from one-tenth to one-third of the body weight.

49. *Urinary Bladder Irritation Subsequent to Short-Term Drug Treatment.* N. RIESER, T. M. LEES, G. W. ANTOGNOLI, and C. S. DELAHUNT, Pharmacology Department, Chas. Pfizer and Co., Groton, Connecticut.

L-N-[β-(*o*-Hydroxybenzoyl)ethyl]valine (GS-5740) has biologic activity as a urinary tract anti-infective. The chemical structure is shown below.



The compound has an oral LD₅₀ in mice and rats of greater than 2000 mg/kg. In a 2-week oral preclinical toxicity investigation, this agent was irritating to the urinary bladder of beagles, as evidenced by marked hematuria. The degree of hematuria could not be correlated with the drug dosages or with the number of doses received. Anorexia was the only other sign of toxicity observed. Clinical pathologic determinations performed were within normal limits. When five different analogs of this drug were screened for bladder irritation in dogs, all produced hematuria. The onset was extremely rapid, occurring a few hours after only one oral treatment. Chromatographic evaluation of the urine revealed no obvious common metabolites; consequently, the toxicity was believed related to the basic moiety. Antibiotic activity of the five different analogs, as measured by urinary bioassay, varied considerably. There was no correlation between the degree of activity and the amount of irritation produced by the individual drugs. The rapid onset of hematuria, coupled with metabolic work, indicated that the mechanism of action was due to direct irritation.

50. *Comparative Subacute Oral Toxicity of Some Organic Phosphates in Rats and Dogs.* M. S. ROOT and J. DOULL, Toxicity Laboratory, University of Chicago, Chicago, Illinois.

10+10/ 8 wks at least
 Weanling male and female Sprague-Dawley rats, and young male and female beagle dogs were fed diets containing 0, 2, 5, 10, or 20 ppm of *O,O*-dimethyl *S*-(ethylsulfinyl)ethyl phosphorothioate (Meta Systox), 0, 2, 3, 5, 25, or 50 ppm of *O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate (Tiguvon), 0, 1, 2, 5, 10, or 20 ppm of *O,O*-dimethyl *O-p*-(methylsulfinyl)phenyl phosphorothioate (B-25141) 0, 0.25, 0.5, 1, 2, 3, 5, or 10 ppm of *O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl phosphorodithioate (Ethyl Guthion), and 0, 5, 10, 20, 50, or 100 ppm of *S,S,S*-tributyl phosphorotriothioate (DEF) for 3 months during which frequent observations of the growth rate, food consumption, physical condition, and mortality were made. Measurements of the serum and erythrocyte cholinesterase activity were made weekly in the dogs and at 8 weeks in

16 in
test

7/6

the rats. Blood and tissue cholinesterase activity determinations were also made at the end of the feeding period when all the animals were sacrificed for gross and microscopic pathologic examination. There was no indication of toxic effects in the animals fed Meta Systox at dietary levels of 10 ppm or less, or in the animals fed B-25141 at 2 ppm or less. The corresponding no-effect level for Tiguvon in the diet was 5 ppm for rats and 2 ppm for dogs. The no-effect level for the phosphorotriothioate derivative, DEF, in the diet was 5 ppm, whereas that of the phosphorodithioate derivative, Ethyl Guthion, was 1 ppm for rats and less than 1 ppm for dogs. ^{12, 16 weeks}
? 0.25 ppm

51. *Preliminary Observations on the Toxicology of Metals in the Rabbit Eye.* MILTON G. ROSS, ROBERT M. HEHR, WILLIAM R. BRYON, and E. WILLIAM LIGON, JR., Division of Toxicological Evaluation, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C.

Experimental data are lacking on the behavior of many metals in the eye. A technique has been utilized which permits implantation of metallic fragments with minimal trauma into the transparent tissues of the eye, e.g., cornea, aqueous and vitreous humors. Observation of implanted metals was possible without the disturbing effects of trauma or infection. Silver, iron, steel, tin, copper, nickel, and aluminum have been studied thus far. Metals appear to fall into two general categories, namely, reactive or inert. Silver, tin, and aluminum appear to be biologically inert, while iron, steel, copper, and nickel are reactive. The reactive metals cause a moderate to severe reaction in the surrounding tissues almost immediately. Inert metals, on the other hand, may lie *in situ* without apparent reaction for indefinite periods. Color transparencies were taken during the course of this experiment with standard and fundus reflex cameras. It appears likely that the method used for implantation will prove useful in testing the toxicity of other solid substances in the eye.

52. *Application of Automatic Data Acquisition and Digital Computation to the Reduction of Data Generated during Animal Toxicity Studies.* T. J. RUSSELL, JR., W. C. WAGGONER, and E. B. GASSER, Bio/dynamics, Inc., East Millstone, and Colgate-Palmolive Research Center, Piscataway, New Jersey.

The large number of animals and experimental parameters examined in current preclinical toxicity studies poses the steadily increasing problem of data reduction. In order to accurately compile the data generated by such studies, a system for automatic acquisition of body weight, food consumption, and organ:body weight ratio data has been developed. These input data are automatically typed for inclusion in a laboratory notebook while simultaneously punched in paper tape for subsequent computer processing. In addition, programs have been designed to accommodate hematologic, biochemical, and behavioral data following their manual conversion to machine-readable language. As the data are submitted for digital processing at appropriate intervals, the parameters chosen for computation are stored on a magnetic history tape for recall upon termination of the experiment.

Among the programs devised for the computer handling of toxicologic data is one which attempts to place the accuracy of dietary administration of test materials on a plane with that of parenteral administration. This is accomplished by the computer assessment of each animal's growth performance during the week prior to the mixing of new food-compound batches. New growth coefficients (positive or negative) are continuously calculated to predict each animal's weight during the next week in order to assure accurate dosing, regardless of changes in body weight.

53. *Alcohol Metabolism in Methanol- and Ethanol-Pretreated Rats.* RICHARD C. RYAN and HERBERT H. CORNISH, Department of Industrial Health, School of Public Health, The University of Michigan, Ann Arbor, Michigan.

In the present study, the effect of fasting on the rate of liver slice metabolism of alcohols has been investigated. In addition, an attempt has been made to induce enzyme synthesis in rats by a prior injection of ethanol or methanol.

The rate of ethanol disappearance from rat liver was extremely rapid. The intraperitoneal dose of 2 g/kg was completely metabolized in about 4 hours. This is an overall metabolic rate of approxi-

mately 400 mg/kg/hr which is somewhat less than the value of 600-900 mg/kg/hr reported by Forney for mice. Following the injection of a similar dose of methanol, the concentration in the liver of fed or fasted rats remained relatively constant during the first 5 hours, then began to drop slowly. Small amounts were still detectable 24 hours after the injection. None was found 48 hours after the injection.

On the basis of liver protein, the rate of ethanol or methanol metabolism in rat liver slices was not different in fed and 24-hour-fasted animals. On a wet weight basis, considerable differences may be noted between fed and fasted rats, since liver weights may be reduced in the fasted rat.

The data on the effect of a previous injection of ethanol or methanol suggest that some stimulation of activity occurs with respect to the ability of liver slices to metabolize the corresponding alcohol. The present study also demonstrates an approach to this and similar problems through the study of induced enzyme synthesis. The effect of repeated doses of alcohol may provide more significant results with respect to enzyme induction and the interrelationships of ethanol and methanol metabolism.

54. *Comparative Acute Toxicities of Isoproterenol and Metaproterenol*. W. J. SCOTT, J. W. JOHNSTON, C. D. JOHNSTON, and R. P. BELLES, Woodard Research Corporation, Herndon, Virginia. (Sponsor: R. L. Hollingsworth.)

The LD₅₀'s of isoproterenol, expressed as mg/kg, resulting from intravenous administration to various species were as follows: mouse, 126; rabbit, 27.0; adult male rat, 95.7; adult female rat, 112; and dog, 50. In comparison, the LD₅₀'s of metaproterenol were: mouse, 87.8; rabbit, 81.3; adult male rat, 67.2; adult female rat, 91.0; and dog, 30.

The LD₅₀'s of isoproterenol administered orally were: mouse, 1260; rabbit, 3070; adult male rat, 2230; adult female rat, 2840; and dog, 600. Those for metaproterenol were: mouse, >8130; rabbit, 3110; adult male rat, 5280; adult female rat, 3370; and dog, 125.

Oral LD₅₀'s of isoproterenol for rats at various ages were determined as follows: 1-2 days, 610; 7 days, 1300; 21 days, 4550; and 42 days, 3880. Likewise, those of metaproterenol were: 1-2 days, 610; 7 days, 3000; 21 days, 9300; and 42 days, 5537.

55. *Biochemical and Electron Microscopic Changes Observed in Rats and Monkeys Medicated Orally with Carbaryl*. D. M. SERRONE, A. A. STEIN, and F. COULSTON, The Institute of Experimental Pathology and Toxicology, Albany Medical College of Union University, Albany, New York.

The comparative toxicology of carbaryl (1-naphthyl *N*-methylcarbamate) was studied in rats and monkeys. In acute studies, monkeys appeared to tolerate much larger oral doses of carbaryl than rats or even dogs. Monkeys orally tolerated doses up to 1.0 g/kg. Monkey plasma cholinesterase was inhibited at the high dosage (600 mg/kg) in a 6-month study. Little or no inhibition was found at lower dosages. Plasma and tissue cholinesterase levels were reduced in rats given carbaryl orally for 3 months. Both brain and plasma cholinesterase levels were decreased within 15 minutes after a single oral administration in rats. Alterations in hepatic microsomal demethylase activity were also observed in rats medicated with carbaryl for 14 days or 21 days - not at 72 hrs for single dose.

Electron microscopic studies of the kidneys of rats and monkeys medicated with carbaryl demonstrated a marked vacuolation of the epithelium of the proximal tubules. However, no disturbance in urinary function was observed other than a discoloration of the urine, due possibly to the presence of a metabolite.

56. *Comparative Toxicity of DDT, Dieldrin, and Heptachlor to Japanese and Bobwhite Quail*. T. S. SHELLENBERGER, JEAN LEE, BLAIR UDALE, and G. W. NEWELL, Stanford Research Institute, Menlo Park, California.

Japanese and bobwhite quail were fed diets containing various levels of DDT, dieldrin, and heptachlor. Growth, feed consumption, and mortality rates were recorded for 6 weeks for the bobwhite quail and 10 weeks for the Japanese quail. Egg production and hatchability of the Japanese

55.
300
600

75 hr obs.
974

single dose
autofluorescence?

quail were studied for weeks 7 through 10. Tissue specimens of fat, liver, brain, and muscle were obtained for residue analysis.

At comparable dietary levels, the Japanese quail appeared to be more sensitive to these pesticides than the bobwhite quail. Mortality rates for Japanese quail fed diets containing DDT or heptachlor were greater than those for bobwhite quail, but were not appreciably different in the dieldrin-fed birds.

Egg production of Japanese quail was not adversely affected by dieldrin at 10 ppm, DDT at 300 ppm, or heptachlor at 50 ppm (in surviving females). Egg fertility was not affected by dieldrin at 10 ppm or DDT at 300 ppm. Hatchability of eggs during weeks 9 and 10 was markedly reduced in birds fed dieldrin at 10 ppm and DDT at 300 ppm. Egg fertility and hatchability were not determined in heptachlor-fed birds, as all males in this group died during week 3.

?
Dieldrin limited by # birds

57. *Method for the Study of Neurotoxicologic Effects Produced by the Chronic Perfusion of Drugs through the Cerebrospinal Fluid Space of Unanesthetized Monkeys.* ROBERT A. SHEPARD, JR., Life Sciences Division, Arthur D. Little, Inc., Cambridge, Massachusetts. (Sponsor: C. J. Kensler.)

The tip of a stainless steel cannula is placed in the lateral cerebral ventricle of the rhesus monkey by inserting the cannula through a stereotaxically oriented guide hole. The guide hole is located in a stainless steel ball which is held in an expandable disk secured beneath the scalp in a trephine opening in the skull. This arrangement permits repeated cannulation of the lateral ventricle without fixing the animal's head in the stereotaxic instrument on each occasion, and has the added advantage that no part projects above the animal's scalp. With the monkey restrained in a primate chair, the perfusion is carried out by connecting inflow tubing to the ventricular cannula and outflow tubing to a cannula inserted in the subarachnoid space of the lumbar spinal cord.

Intraventricular pressure, EEG, ECG, pulse rate, respiration, and temperature are recorded on a polygraph during the perfusion and interperfusion period. Alterations in some aspects of the animal's neurological state may be directly observed during the perfusion. Appropriate conditioning techniques may be employed to detect more subtle changes in CNS function. Artificial cerebrospinal fluid (Elliott's "B" solution) is used as the vehicle for drug administration. Experimentation with this solution alone has indicated no apparent ill effects in tranquilized monkeys perfused for 2 hours with an inflow rate of about 0.06 ml/min. The results of perfusing various concentrations of methotrexate will be reported.

58. *Congenital Malformation of Calves Associated with Maternal Ingestion of Range Plants.* JAMES L. SHUPE, LYNN F. JAMES, WAYNE BINNS, LEW DELL BALLS, and RICHARD KEELER, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Logan, Utah.

Crooked calf disease is a complex syndrome. Varied opinions are held relative to cause(s) and lesions associated with this entity. Specific range areas, management practices, and types of range plants can be correlated with the crooked calf syndrome.

Animal species may differ widely in their response to a given toxic agent or teratogen. Even within a species, considerable interstrain differences occur in susceptibility. Many factors influence the individual animal and its reactive responses.

The disease appears to involve some arrest or interference with the normal sequential differentiation and specialization of cells, tissues, and organs in embryonic and/or fetal development. The spectrum of lesions associated with this entity indicates that differentiation is interrupted at various stages of embryogenesis. Percentage and degree of malformations cannot always be correlated with the intensity of maternal clinical symptoms.

Plants collected from endemic range areas were fed to pregnant heifers under controlled conditions during various stages of gestation. Some of the congenitally malformed calves from these heifers were typical of the crooked calves that have been observed by livestockmen for more than 50 years.

A number of congenital anomalies in calves have been confused with the crooked calf syndrome. Based on information accumulated to date, it appears that a number of causes may be associated with congenitally malformed calves. The normal formation of the central nervous system, muscles,

bony structures, and palate in an animal can be adversely affected by environmental as well as hereditary factors.

59. *Toxicologic and Pharmacodynamic Studies with Tenuazonic Acid.* E. R. SMITH, T. N. FREDRICKSON, and Z. HADJIAN. Mason Research Institute, Worcester, Massachusetts.

The toxic effects of sodium and *N,N'*-dibenzylethylenediamine (DBED) salts of tenuazonic acid (NSC-525,816 and NSC-82,260, respectively), an antiviral and antitumor agent, were studied in several species by the oral and intravenous routes. The actions of these compounds were similar, but the DBED salt had an additional convulsant effect. The same effects were encountered following oral or intravenous and single- or repeated-dose administration: salivation, emesis, anorexia, erythema, hemoconcentration, BSP retention, elevated serum glutamic-oxalacetic transaminase (SGOT) activity, cardiovascular collapse with hemorrhagic enteritis and death. The single-dose LD₅₀'s of the sodium salt in rats by the oral and intravenous routes were 174 and 152 mg/kg, respectively, and of the DBED salt, 225 and 85 mg/kg, respectively. The results with mice were similar. Repeated-dose experiments were conducted with the DBED salt. Emesis was encountered in dogs at 1.4 mg/kg/day, and death at 8 mg/kg/day, administered intravenously. This compound was much better tolerated by monkeys than by dogs. It was nearly as toxic orally as intravenously. Anesthetized dogs receiving a single lethal dose (≥ 60 mg/kg) showed over 8 hours a gradual decline in blood pressure and a rise in hematocrit and SGOT activity. Serial ileal biopsies showed progressive engorgement of villar capillaries and degeneration of reticular tissue in the villar core.

60. *Tissue Distribution of Inhaled Turpentine in Rats.* FREDERICK SPERLING and WILLIAM L. MARCUS, Department of Pharmacology, College of Medicine, Howard University, Washington, D. C.

Rats were exposed for one hour or to prior death to concentrations of turpentine vapor which ranged from 8 to 18 mg/l. After exposure, the animals were thoroughly washed to remove any turpentine which might remain on the body surface. Immediately after exposure, or after 15, 30, or 60 minutes, surviving rats were decapitated and blood collected. The brain, liver, kidneys, lungs, and spleen of these rats and of those which died during exposure, were removed and homogenized. All tissues, including blood, were diluted to constant volume within equal-volume flasks which were tightly covered, and the contents stirred and allowed to stabilize to equilibrium between tissue-released and tissue-retained turpentine. Relative tissue concentrations of turpentine were measured by withdrawing and analyzing a constant volume of air-vapor from above the sample surface. Analysis was by gas chromatography using a column of 5% squalene on Gas-Chrom A. The relative concentrations between tissues were then compared. The highest turpentine concentration, immediately after exposure, was found in the spleen, followed in descending order by kidney, lung, brain, liver, and blood. The time-decay curves, in all tissues except brain, were essentially parallel. The time-decay curve for brain was less steep; after 1 hour postexposure, its concentration was higher than in all other tissues except spleen. In addition, a correlation was found between brain concentration and convulsions and death.

61. *Cardiovascular Effects of 2-Pyridinealdoxime Methochloride.* W. B. STAVINOH, L. C. RYAN, J. A. RIEGER, L. B. HINSHAW, and P. W. SMITH. Civil Aeromedical Research Institute, Federal Aviation Agency, Oklahoma City, Oklahoma.

Earlier investigators have shown that 2-pyridinealdoxime methochloride (2-PAM) is capable of inducing cardiovascular responses which are unrelated to its predominant action of cholinesterase reactivation. Increased cardiac contractility, a diphasic rise in systemic arterial blood pressure (initial spike followed by a sustained rise), and release of catecholamines have been reported. In an initial series of experiments, dogs were anesthetized with sodium pentobarbital and prepared for total body perfusion. 2-PAM was injected into the perfusion preparation at a dosage of 50 mg/kg. Five of 6 dogs showed a marked rise of systemic arterial pressure within 5 minutes. In one animal, the rise was smaller and delayed. All animals showed a significant increase in total systemic blood flow. Cardiac stroke volume and calculated total peripheral resistance increased in most, but not all,

of the animals. Measurements of total catecholamines made at 5, 30, and 60 minutes after administration of 2-PAM showed no significant increase above control values. In a separate series of 9 intact animals anesthetized with sodium pentobarbital, there was no increase in catecholamines 5 minutes after administration of 2-PAM despite a pronounced rise in pulse pressure and mean arterial pressure in all cases.

62. *Cardiac and Ocular Toxicity Associated with a Thyromimetic Agent in Dogs.* R. L. STEELMAN and R. SEKELLA, Pathology and Toxicology Section, Smith, Kline and French Laboratories, Philadelphia, Pennsylvania.

On the basis of metabolic studies in the rat, SKF L-12276, in which an isopropyl radical has replaced iodine at 3' on the L-triiodothyronine molecule, was shown to have thyromimetic activity. Subacute toxicity studies were performed in rats and dogs and the results of the 18-week dog study are the subject of this report.

SKF L-12276 was administered orally by capsule, 6 days/week, to beagle dogs at starting levels of 20 or 100 $\mu\text{g}/\text{kg}/\text{day}$. The high dose was increased to 150 $\mu\text{g}/\text{kg}/\text{day}$ on the 66th day and to 200 $\mu\text{g}/\text{kg}/\text{day}$ on the 73rd day of the study.

Electrocardiographic abnormalities consisting of T-wave changes in the low-dose dogs, and ventricular extrasystoles as well as T-wave changes in the high-dose dogs were observed at the 2-month interval. The most severe extrasystoles were diagnosed as parasystole in one surviving dog and premature ectopic beats with coupling in one dog which died.

Ophthalmoscopic examination on day 89 revealed the appearance of bilateral posterior polar triangular cataracts in one high-dose dog. Another high-dose dog had a posterior polar capsular opacity of the left eye associated with a persistent hyaloid remnant. A final examination on day 127 showed a progression of the bilateral triangular cataracts. A third high-dose dog showed early cataracts which appeared to be forerunners of the triangular posterior polar opacities previously mentioned. Although these cataracts may occur spontaneously, they were of an unusual type and appeared to be associated with the administration of SKF L-12276.

63. *Comparisons of Biochemical and Electron Microscopic Alterations in Rats and Monkeys Medicated Orally with TDE.* A. A. STEIN, D. M. SERRONE, and F. COULSTON, The Institute of Experimental Pathology and Toxicology, Albany Medical College of Union University, Albany, New York.

The comparative toxicology of TDE [2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane] was studied in rats and rhesus monkeys. Similar quantitative changes in hepatic lipids and alterations in ultrastructure were observed in both species. Hepatic neutral lipids were depressed, particularly the triglycerides. Enlargement of mitochondria and accumulation of cytoplasmic fat vacuoles were observed in liver sections. Alterations in hepatic demethylase activity were also demonstrated in both species. These biochemical and ultrastructural alterations will be compared to those previously described with the chlorinated hydrocarbon pesticide, methoxychlor.

64. *Experimental Human Exposure to Trichloroethylene Vapor.* RICHARD D. STEWART, HAROLD H. GAY, HUGH C. DODD, DUNCAN S. ERLEY, and ARNOLD W. SCHAFFER, Biochemical Research Laboratory, Medical Department, and Chemical Physics Research Laboratory, The Dow Chemical Company, Midland, Michigan.

Volunteer, technical employees were exposed to carefully controlled concentrations of trichloroethylene (TCE) vapor for periods of time ranging from 1 hour to a work-week. Pre- and post-exposure clinical findings were tabulated, as well as the subjective and physiological responses occurring during exposures to 50 ppm, 100 ppm, or 200 ppm of TCE vapor. The concentration of TCE in the blood and expired air was determined using infrared and gas chromatographic techniques. The concentrations of trichloroacetic acid and trichloroethanol were measured in the urine during and after the exposures.

The measurement of TCE in the expired air during exposure provided a means for determining the amount of the compound absorbed by each subject. Serial measurements of TCE in the post-

also seen

in rats
not made
+ thion
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not made
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exposure alveolar air provided an accurate means for estimating the concentration of solvent vapor to which the subject had been most recently exposed, and for estimating his time-weighted vapor exposure.

Of the methods used for determining the magnitude of TCE vapor exposure, expired air analysis proved superior. It most accurately reflected the differences in the total amount of TCE absorbed by various subjects exposed to identical vapor concentrations. The measurement of the urinary metabolites of TCE proved to be a poor index of the magnitude of vapor exposure because of the great variation between individuals, and the variation in the same individual identically exposed at different times. The data acquired by periodic monitoring of TCE vapor concentration in the work environment, while useful in assessing the overall hazard of exposure, were of little use in assuring that overexposure had not occurred between monitoring periods.

65. *Effects of Alcohol, Drugs, and Lowered Environmental Temperatures on Conditioned Avoidance Response in Rats.* G. S. STONE, K. C. LEUNG, and C. H. HINE, The Hine Laboratories, Inc., San Francisco, California.

Rats were tested for Sidman avoidance behavior under four conditions: (1) with a drug alone at room temperature, (2) with a drug plus alcohol at room temperature, (3) with a drug alone in a 0-5°C environment, and (4) with drug plus alcohol in a 0-5°C environment. With the dose of alcohol used, rats given no other drug showed an improved efficiency of avoidance response. Chlorpromazine, morphine, and promethazine abolished this enhancement, replacing it with a moderate depression beyond that seen with the drug alone. Chlordiazepoxide acted synergistically with alcohol to produce a substantially greater depression than that seen with either drug alone. The stimulant effect of amphetamine was reduced by alcohol. Under all conditions of drug testing, unacclimatized rats performed more poorly in the cold environment than in the warm. The effects of the drugs appeared in all cases to be additive with the depressant effect of cold, i.e., depressant drugs led to even greater depression when testing was carried out in the cold, and the stimulant effect of amphetamine was eliminated. Cold acclimatization abolished the differences between tests carried out in the warm and in the cold.

66. *Histologic Lesions Occurring Spontaneously in Chr-CD Rats on Two-Year Feeding Studies.* EDWIN F. STULA, GARY M. ZWICKER, WILLIAM C. KRAUSS, and GROVER V. FOSTER, Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, Newark, Delaware.

In a safety evaluation of chemicals, using the rat in chronic studies, it is essential to know the incidence of the various spontaneous lesions which occur throughout the lifetime of a given strain of rat. It is well known that the high incidence of neoplastic and nonneoplastic disease during the second year of a rat's life complicates the safety evaluation of specific chemicals. Such data would supplement information derived from the control groups on a specific experiment.

During 1961 to 1964, the Charles River-Cesarean Derived rat was used at Haskell Laboratory on three different two-year feeding studies and on one 19-month feeding study. Histopathologic examination of 174 male and 174 female rats from control groups of the above tests forms the basis of this presentation. The environmental conditions remained relatively constant throughout this period. The diet was freshly ground Purina Laboratory Chow to which 1% corn oil had been added, with water ad libitum. Sections of up to 30 organs from each rat were fixed in Bouin's solution and stained by the Haskell Quadrachrome procedure.

Of 316 rats on the two-year tests, 69 (44%) of 158 females and 82 (52%) of 158 males died or were killed in extremis in the one- to two-year period on test. Fourteen (20%) of the 69 females and 28 (34%) of the 82 males died or were killed in extremis prior to completing 19 months on test. Of 32 male and female rats on a 19-month feeding study, four (12%) died or were killed in extremis prior to the terminal sacrifice. The sharp increase in mortality rate and histologic lesions from 19 through 24 months on test suggests the desirability of the inclusion of a 19-month sacrifice in the design of a two-year test.

An illustrated description of the more commonly occurring lesions will be presented together with the incidence. Prominent lesions observed were tumors of the pituitary, mammary, and adrenal

glands in females, and chronic nephritis in males. A comparative incidence of histologic lesions will be given among rats that died, that were killed in extremis, and that were killed by design in the succession of two-year tests.

67. *Subacute Oral Toxicity of Sodium Diethyldithiocarbamate*. F. WILLIAM SUNDERMAN and ORVILLE E. PAYNTER, Division of Metabolic Research, Jefferson Medical College, Philadelphia, Pennsylvania, and Hazleton Laboratories, Inc., Falls Church, Virginia.

In previous studies, sodium diethyldithiocarbamate (dithiocarb) has been shown to be an effective chelating agent in the treatment of acute nickel carbonyl poisoning, and in the mobilization of nickel and copper in hepatolenticular degeneration. The present studies were undertaken to evaluate the changes in the serum components of adult dogs after the administration of dithiocarb in dosages of 30, 100, or 300 mg/kg/day for 90 days. Studies of the subacute oral toxicity of dithiocarb were also undertaken in albino rats.

Our studies demonstrated that after administration of dithiocarb, the concentration of serum copper increased, the concentration of serum calcium decreased, and the concentration of serum iron was not significantly changed. In 2 male dogs that received high doses of dithiocarb, the concentration of serum glucose decreased, and the concentrations of urea nitrogen, alkaline phosphatase, and serum glutamic-oxalacetic transaminase increased. The other components of the serum that were measured remained essentially within the normal range of values.

The administration of the lowest dosage of dithiocarb to dogs during the 90-day period was without adverse effect as judged by general appearance and behavior, body weight, survival, periodic hematologic and biochemical determinations, urinalyses, and gross and microscopic pathology. The dogs receiving the intermediate and high dosages had intermittent episodes of emesis and tremors. No adverse effects on hematologic values were noted in these animals during the 90-day period. The dogs receiving the highest dosage were continued beyond the 90 days at increased dietary levels. The increased levels produced changes in cell volume, hemoglobin, and erythrocyte count. Microscopic examination of tissues revealed no distinct histologic alterations during the 90-day period.

68. *Nickel Carcinogenesis. Inhibition of the Induction of Pulmonary Benzpyrene Hydroxylase by Nickel Carbonyl*. F. WILLIAM SUNDERMAN, JR., Department of Pathology, University of Florida College of Medicine, Gainesville, Florida.

Parenteral administration of nickel carbonyl to rats in a dosage of 20 mg/kg has been found to inhibit the induction of pulmonary benzpyrene hydroxylase, an enzyme which detoxifies 3,4-benzpyrene. Induction of the enzyme is likewise inhibited by inhalation of nickel carbonyl at an atmospheric concentration of 80 ppm for 15 minutes. The inhibition of benzpyrene hydroxylase induction is maximal at 48-72 hours and persists for approximately 5 days. Inasmuch as nickel carbonyl and 3,4-benzpyrene are both constituents of tobacco smoke, a cocarcinogenic relationship of these compounds is suggested.

69. *Ornithine Carbamyl Transferase as a Liver Function Test: Comparative Toxicity Studies in the Beagle Dog, Miniature Swine, and Man*. A. S. TEGERIS, H. E. SMALLEY, JR., F. L. EARL, and J. M. CURTIS, Special Pharmacological Animal Laboratory, Division of Pharmacology, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C.

Ornithine carbamyl transferase (OCT) transcarbamylates ornithine to citrulline in the Krebs-Henseleit cycle of urea formation. Being thus involved in a highly specific hepatic activity, it has been proposed as a superior test for liver function. The purpose of this study was to evaluate this test in swine and dogs, and compare the findings with those in man.

A group of 6 dogs and 6 swine was given a toxic dose of carbon tetrachloride orally, while a similar group was given a toxic dose of the nephrotoxic agent, uranyl nitrate, parenterally. The control group also consisted of 6 dogs and 6 swine. The following parameters were monitored at frequent intervals during the experiment: (a) OCT, (b) both transaminases (SGOT and SGPT), (c) lactic and isocitric dehydrogenase (LDH and ICDH), and (d) blood urea nitrogen (BUN). The peak elevation of the various enzymes occurred 48 hours after CCl_4 administration, at which time

the various assays (expressed as multiples of the baseline value) were as in the accompanying tabulation:

<i>Swine</i>					
Test:	SGPT	LDH	ICDH	SGOT	OCT
Level:	9x	10x	93x	125x	488x
Range:	(3x—14x)	(6x—22x)	(74x—160x)	(72x—218x)	(250x—1420x)
<i>Dogs</i>					
Test:	ICDH	LDH	SGOT	SGPT	OCT
Level:	23x	33x	324x	762x	2375x
Range:	(16x—23x)	(25x—65x)	(100x—548x)	(127x—960x)	(1380x—3450x)

In the uranyl nitrate-treated animals, there was a marked elevation of BUN in all animals, a small elevation of ICDH in the swine and of SGPT in the dog, while the OCT remained normal.

An illustrative human case with an ECG-diagnosed diaphragmatic myocardial infarction had an eightfold elevation of SGOT and a thirtyfold elevation of LDH, while the OCT remained normal. These findings agree with similar published data for humans. This phase of the study is continuing.

70. *Evaluation of Spontaneous Activity of Dogs by Time-Lapse Photography.* ANTHONY A. THOMAS, Toxic Hazards Division, Biomedical Laboratory, 6570th Aerospace Medical Research Laboratory (MRMPT), Wright-Patterson Air Force Base, Ohio.

A semiautomatic recording process for spontaneous activity was developed utilizing a time-lapse movie camera and a photocell scanning device to translate the video information into audio pulses suitable for recording by simple tape-recording techniques. The magnetic audio pulses are then rerecorded on a chart recorder for permanent simultaneous display of the 24-hour cycle. This technique does not require any restriction of the animals, and can be adapted to computer use. Sample recordings of unexposed and exposed dogs will be used for discussion and demonstration of normal and depressed spontaneous activity.

71. *Behavioral Studies on Carphenazine during Chronic Toxicity Testing in Rats. The Value of Paratoxicologic Work.* RICHARD F. TISLOW, SUE P. WILSON, CORNELIUS M. KNEBEL, and ANNE L. GILLEN, Wyeth Laboratories, Inc., Philadelphia, Pennsylvania.

Behavioral studies carried out during toxicologic testing (paratoxicologic studies) can yield valuable data not obtainable with customarily smaller doses of drugs employed in pharmacology. During the course of testing the chronic toxicity in rats of the antipsychotic compound carphenazine dimaleate, a correlation was found between long-term administration and locosomatomotor activity. Twenty-five female and 25 male rats (Wistar strain) were used at each of six dosages and in one control series. The performance of the animals was evaluated on a numerical scale (0-4) according to their ability to cling to and walk up a screen inclined at a 45° angle. Each rat was evaluated once per week for one year. Analysis of the data at weekly intervals revealed a linear relationship between the logarithm of the dose and response. The slope of the dose-response curves decreased with the duration of treatment until there was no longer any significant regression. As the slope decreased, so did the intercepts. The linear relationship of average score to log dose indicates that observed scores are valid measurements of response to the drug. This finding has stimulated further mathematical work to define response in terms of duration of treatment as well as of dose.

72. *Effect of Thoracic X-Irradiation on the Composition of Lung Tissue.* E. G. TOMBROPOULOS and K. E. McDONALD, Biology Department, Pacific Northwest Laboratories, Battelle Memorial Institute, Richland, Washington. (Sponsor: H. A. Kornberg.)

The short-term effects of acute thoracic X-irradiation were examined. Forty-day-old female rats were exposed to 800 r thoracic X-irradiation. The animals were killed at intervals of 1, 2, 4, 8, 10, 14, 18, 24, 31, 38, 45, or 52 days postexposure. The lung tissues were homogenized and

analyzed for collagen, elastin, lipid, and lipid phosphorus content. The lung collagen content of animals exposed to thoracic X-irradiation increased 100% over the control animals by the second day postexposure. Collagen levels in the irradiated animals remained high for 2 weeks, and then dropped sharply to the levels of the control animals for the duration of the experiment. The lung elastin content of irradiated animals increased to 50% above those of the controls between the eighth and eighteenth day postexposure. After the eighteenth day, the lung elastin content returned to normal levels. The ratio of lung lipid phosphorus to total lung lipids remained constant during the whole experiment. The implication of these findings to the sensitivity and repair mechanisms of lung tissue will be discussed.

73. *Central Effects in Dogs of Intracisternally Administered Triamcinolone Acetonide 21-Phosphate.*

C. E. TRAITOR and J. F. NOBLE, Department of Pharmacological Research, Experimental Therapeutics Research, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York. (Sponsor: D. W. Hallesy.)

A previous study with triamcinolone acetonide 21-phosphate administered intravenously to dogs daily for 30 days has established the safety of this compound for systemic use. In the present study, triamcinolone acetonide 21-phosphate was administered intracisternally in phosphate buffer or normal saline to dogs anesthetized with pentobarbital, and in normal saline to conscious dogs. In the anesthetized state, the administration of 15 mg/kg of steroid in phosphate buffer resulted in marked stimulation of the central nervous system, and—upon recovery from anesthesia—depression and death. At a lower dose (1.5 mg/kg) similar central involvement was noted, followed by a prolonged recovery period. Phosphate buffer alone was found to produce similar effects, but of decreased severity and duration and followed by rapid and complete recovery. The intracisternal administration of steroid (1.5 mg/kg) in normal saline and saline alone produced no appreciable effects. In conscious dogs, the intracisternal administration of 1.5 mg/kg of steroid in normal saline resulted in convulsions, postconvulsive depression, and death. These effects were not seen with vehicle alone. Serum steroid levels were found to be comparable following intracisternal or intravenous administration. Central stimulation induced by triamcinolone acetonide 21-phosphate in dogs appeared to be restricted to the intracisternal route of administration. Intravenous doses of up to 50 mg/kg were found to be without effect on the central nervous system.

74. *Protection by Aldrin against the Toxicity of Some Organophosphate Anticholinesterases.* A. J. TRIOLO and J. M. COON, Department of Pharmacology, Jefferson Medical College, Philadelphia, Pennsylvania.

Aldrin orally at 16 mg/kg protects mice against parathion, paraoxon, and physostigmine given 16 hours later, but not against OMPA [Triolo and Coon, *Federation Proc.* **22**, 189 (1963)]. In the present studies aldrin-pretreatment reduced the mortalities of mice given TEPP, DFP, EPN, Guthion and tri-*o*-tolylphosphate, but not neostigmine. The protective effect of aldrin against parathion and paraoxon was reversed by ethionine. The 16-hour delay in the onset of protection, and its reversal by ethionine, suggested that aldrin may stimulate the synthesis of enzymes which detoxify the anticholinesterases. A-esterase, which detoxifies paraoxon, was increased 33% in the liver, but decreased 50% in the plasma of aldrin-treated mice. An increase in A-esterase by aldrin would make less paraoxon available for inhibition of cholinesterase (ChE). However, inhibition of plasma ChE by oral paraoxon was the same in aldrin-treated animals and controls, though brain ChE was less inhibited by paraoxon after aldrin. Aldrin did not protect against OMPA and neostigmine which are poor inhibitors of brain ChE. The anticholinesterases whose toxicities were decreased by aldrin are good inhibitors of brain ChE. Thus, a decreased inhibition of brain cholinesterase appears to be important in the protective action of aldrin against anticholinesterase.

75. *Vasopressor Responses in Hepatotoxic Beagles.* E. E. VOGIN, D. L. BOKELMAN, and P. A. MARRIS, Merck Institute for Therapeutic Research, West Point, Pennsylvania.

This investigation was undertaken to study the influence of chemically induced hepatotoxicity in beagles upon the pressor responses to angiotensin, norepinephrine, and epinephrine. Dogs were

administered either CCl_4 (2 ml/kg for 2 days) or ethionine (25 mg/kg for 18-20 days). Hepatotoxicity was confirmed clinically by elevation of SGOT, alkaline phosphatase, and plasma half-life of indocyanine green. The animals were then anesthetized with pentobarbital sodium, and the pressor responses to graded doses of angiotensin, norepinephrine, and epinephrine were determined and compared to responses obtained in normal, anesthetized animals. Ethionine-treatment resulted in a reduction of systolic pressor responses to angiotensin of about 35%. Diastolic pressor responses were decreased approximately 25%. The systolic pressor responses to norepinephrine were reduced by approximately 25%, while the diastolic pressor responses were not significantly altered. Ethionine-treatment did not alter the pressor responses to injected epinephrine. CCl_4 -treatment did not affect the pressor activities of angiotensin or norepinephrine. However, a twofold potentiation of the epinephrine responses was noted. In a second series of ethionine-treated dogs, pressor responses to angiotensin and norepinephrine in the denervated, vascularly intact, perfused femoral bed were determined. Pressor responses to intra-arterial injections of norepinephrine were reduced by about 30%. Histomorphologic data confirmed the clinical finding of hepatotoxicity.

76. *Comparative Chronic Inhalation Toxicity of Two Beryllium Ores, Beryl and Bertrandite.* WILLIAM D. WAGNER, DAVID H. GROTH, and HERBERT E. STOKINGER, Division of Occupational Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Cincinnati, Ohio.

The exploitation of beryllium-bearing ores of mineralogical assay different from the commonly mined beryl ore (BeAlSiO_3) necessitated an evaluation of the relative potential toxicity of the "new" ores as compared to that assumed for beryl.

Two groups of animals, each of three species (monkey, rat, and hamster), were exposed in separate chambers, 5 days/week, 6 hours/day, for periods of 17-23 months to a "bertrandite-type" (BeSiO_3 , 0.5% Be) or beryl ore (3.0% Be) at concentrations of 15 mg/m³. Ninety-six per cent of the particles of the utilized ore samples was less than 0.3 μ in diameter. Biweekly body weights of all species showed a 7% average decrease only in the rats exposed to beryl beginning at the 12th month. Biochemical evaluations for serum and tissue alkaline and Mg-activated alkaline phosphatase showed a decreased activity, $P < 0.01$, at 17 months of exposure for bertrandite-exposed rats. Spectrographic analysis indicated a shift of Be from the lungs to bone, liver, and kidney in the beryl-exposed animals.

Histologic evaluation of pulmonary tissues of bertrandite- and beryl-exposed rats, hamsters, and monkeys showed marked focal areas of dust deposition and phagocytic response. Bertrandite exposure produced dust lesions in only the rats and hamsters. In contrast, beryl ore produced granulomatous lesions in monkeys and adenomatous and granulomatous tumors in 21% of 17-month exposed rats. Pathologically, the beryl ore, under the conditions of exposure of this study, would appear to present an appreciably greater toxic potential than the bertrandite.

77. *Effects of Mirex and DDT on Reproduction in the BALB/c and CFW Strains of Mice.* GEORGE W. WARE and ERNEST E. GOOD, Department of Zoology and Entomology, Ohio State University, Columbus, Ohio. (Sponsor: Roger A. Yeary.)

Two tests involving 100 pairs of mice per diet are described. In the first test, in addition to a control diet, three organochlorine insecticides, Telodrin, Mirex, and DDT, were fed at 1, 5, and 7 ppm, respectively, to virgin BALB/c males and females for 1 month. The animals were then paired and continued on the treated diets for 90 days, during which reproduction data were collected.

In the second test the experiments with Mirex and DDT were duplicated with the BALB/c mice, and replicated with 8-week-old virgin mice of the CFW strain for a comparison in reproductive response.

Observations were made on litter size and frequency, postnatal mortality, sex ratio, and parental mortality. Statistical treatment included analysis of variance, chi square, and Duncan's multiple range tests.

In the first test Mirex produced: (1) significant mortality in the parents, (2) reduced fecundity, (3) fewer litters per producing pair, and (4) increased number of days to first litter. DDT produced: (1) reduced fertility, (2) fewer litters per surviving pair, and (3) the largest first and average litter sizes. Telodrin produced: (1) the highest level of fertility, (2) greatest number of

litters per producing pair and surviving pair, and (3) fewest days to first litter. A theory is offered which attributes reduced fertility and fecundity to gonadotropin imbalance.

The second test was incomplete at the time of writing.

78. *Induction of Emesis by Detergent Ingredients and Formulations.* J. E. WEAVER and J. F. GRIFFITH, Ivorydale Technical Center, The Procter and Gamble Company, Cincinnati, Ohio. (Sponsor: F. H. Snyder.)

Experimental results and human experience from accidental ingestions have shown that synthetic detergent formulations are effective emetics. This study was designed to determine which components of detergent formulations are responsible for the emetic activity, and to give indication of the site through which the emesis is mediated.

Fasted beagle dogs were used throughout the study. Materials were administered by gastric intubation.

Doses of less than 100 mg/kg of two granular, built detergent formulations (50% w/v aqueous) evoked prompt emesis. Less than 50 mg/kg of sodium silicate (21.5% w/v aqueous), sodium triphosphate (20% w/v aqueous), and tetrapotassium pyrophosphate (20% w/v aqueous)—ingredients in built detergent formulations—also evoked prompt emesis. Two other detergent ingredients, alkylbenzene sulfonate (45% w/v aqueous) and sodium sulfate (30% w/v aqueous), and a nonbuilt, liquid detergent formulation (undiluted) were slower acting and less potent than the above materials.

Subcutaneous chlorpromazine hydrochloride, which completely prevented emesis after normally effective intravenous administration of apomorphine hydrochloride, did not prevent emesis induced by the detergent materials. Chlorpromazine effectively inhibited delayed emesis produced by syrup of ipecac.

Of the materials tested, it is concluded that the phosphate builders are the ingredients which primarily are responsible for the prompt, potent, emetic action of the built detergent formulations. It tentatively is concluded that the emetic response to detergent formulations and their ingredients is mediated by a direct action within the upper gastrointestinal tract. Work to define more precisely the site of the detergent-induced emetic action is in progress.

79. *Age and Latent Period in Carcinogenesis.* G. S. WIBERG, M. DENNERY, and H. C. GRICE, Pathology and Toxicology Section, Research Laboratories, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Ontario, Canada. (Sponsor: W. A. Mannell.)

A single dose of dimethylnitrosamine was used to produce renal tumors in rats and thus provide an experimental model to study (1) the latent period of carcinogenesis in animals of different age groups, and (2) the dose-response relationship at different ages. Dimethylnitrosamine, at various dosages, was given subcutaneously to groups of at least 30 male rats of the following ages: *in utero*; 1, 7, 21, and 65 days; 6 months; or 1 year. The tumor-induction time was determined. The relative reliability of various methods for early detection of tumors was evaluated. These included urinary enzymes, palpation, and X-ray. Structural variants of the tumors found in the various age groups were assessed.

80. *An Acylamidase in Mammalian Liver Hydrolyzing the Herbicide, 3,4-Dichloropropionanilide.* CLARA H. WILLIAMS and KEITH H. JACOBSON, Division of Toxicological Evaluation, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C.

Enzymes which hydrolyze aryl-substituted acylamides, such as acetanilide, phenacetin and various naphthylamides, *in vitro* have been reported to be present in animal tissues. This study describes an enzyme in rat, rabbit, dog, and mouse liver which hydrolyzes the herbicide 3,4-dichloropropionanilide to 3,4-dichloroaniline. It has high activity in barbital buffer at pH 8.7, and can be stored for several days at 4°C and for several weeks at -20°C without loss of activity. It occurs chiefly in the microsomal fraction of the liver, and is not found in rat brain, erythrocytes, or plasma. It also hydrolyzes acetanilide, but at a slower rate.

With the cholinergic insecticides 6-chloro-3,4-xylyl methylcarbamate, 4-benzothienyl-*N*-methyl-

carbamate, and 1-naphthyl *N*-methylcarbamate, and *O,O*-diethyl *O-p*-nitrophenyl thiophosphate, the amidase was inhibited *in vitro* in very low concentrations. When these compounds were administered acutely or subacutely to rats, the *in vitro* acylamidase activity with 3,4-dichloropropionanilide as substrate was considerably reduced, and the extent of reduction was dose- and time-related. As the incubation of these liver homogenates with the substrate was prolonged, the activity of the carbamate-inhibited enzyme was restored. Livers from rats on a chronic feeding of 6-chloro-3,4-xylol methylcarbamate at levels of 0, 75, 300, or 600 ppm showed normal acylamidase activity at 6 months and at 1 year.

The livers from rats given chlordane as a microsomal stimulant did not have an increased acylamidase activity.

81. *Aspergillus flavus* Toxicosis in Ducks. H. G. WISEMAN, W. T. SHALKOP, and B. H. ARMBRECHT, Agricultural Research Center, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Beltsville, Maryland.

Chemical analyses for the aflatoxins were used to set the dosages, and duckling feedings to monitor the toxicity, of acetone-attenuated cultures of *Aspergillus flavus* Link (Hodges M-93) fed to swine. This strain was isolated from a toxic sample of wheat. The fungus was initially grown by surface culture on shredded wheat, and later on a rice, wheat and sucrose substrate. The results of the feeding tests with ducklings are reported in this paper.

In these tests the ducklings were fed ad libitum. The dosages listed in the table represent the sum of aflatoxins B₁ and G₁ for the test feeds.

Culture number	Dietary level (ppm)	Mortality ratio ^a	Time interval (days)	Number taken for pathology
M-93-1 ^b	1	15/20	5—9	5
	0.75	12/15	5—9	3
	0.50	5/15	13—19	9
	0.20	1/5	2—20 ^c	4
M-93-10	1	9/15	3—27	7
M-93-12	1	13/15	6—12	2
M-93-14	1	9/10	19—30	1
M-93-17-5	1	9/10	7—22	1

^a Number of deaths divided by the total number in the group.

^b This culture was attenuated with ethanol, all others were treated with acetone. The basal ration was broiler mash.

^c The early death of one bird was probably not caused by toxins since the remaining birds grew well and were sacrificed after 20 days.

The usual early liver damage caused by the aflatoxins was observed. This is referred to as aflatoxicosis in this report. In addition, other symptoms noted were damaged gizzard lining, enlarged kidneys and spleens, and petechiae of the pancreas in those birds that survived over 2 weeks. If the bird lived 4-6 weeks, the liver developed a nodular surface. These injuries will be illustrated by slides.

82. *Nature of Sensory Evoked Response in Relation to Consciousness*. C. XINTARAS, M. F. SOBECKI, and C. E. ULRICH, Pharmacology and Toxicology Section, Laboratory of Medical and Biological Sciences, Division of Air Pollution, Public Health Service, U. S. Department of Health, Education, and Welfare, Cincinnati, Ohio.

Brain responses to flashes of light in the unrestrained rat can be examined with on-line summation techniques [Xintaras *et al.*, *Toxicol. Appl. Pharmacol.* 7, 504 (1965)]. This development encouraged a study to furnish additional information about response morphology, particularly as it might vary as subjects move from alert wakefulness through drowsiness and into deeper stages of spontaneous or induced sleep.

Male rats were implanted with cortical recording electrodes and placed in an electrically shielded chamber. Photic stimuli were presented to the rat, and conventional EEG recordings obtained. Computer-summed responses (28-350 light flashes) were also graphically displayed by an X-Y plotter.

In the transition from wakefulness to sleep, specific components of the evoked response were attenuated whereas others were markedly augmented. Temporal changes in the visual evoked response recorded while the rat pressed the lever for food reinforcement were similar to the changes that occurred when the rat was going to sleep. Carbon monoxide and pentobarbital induced changes in the response that appeared similar to the changes recorded during the subject's normal transition from wakefulness to sleep. No consistent relationship was noted between the spontaneous EEG and the magnitude of the visual evoked response.

The findings suggest that the alterations in the evoked response during lever pressing and light spontaneous or induced sleep may be associated with a general lowering of the level of vigilance, and do not reflect habituation to the photic stimulus.

83. (No abstract.)

84. *Effect of Chronic Safrole Administration on Hepatic Enzymes and Functional Activity in Dogs.*

M. S. WEINBERG and S. S. STERNBERG, Food and Drug Research Laboratories, Inc., Maspeth, New York, and Sloan-Kettering Institute for Cancer Research, New York, New York.

At the conclusion of a 7-year study of the effects of feeding safrole (4-allyl-1,2-methylenedioxybenzene) to dogs, several parameters of hepatic function were studied. Among these were (a) the bromsulfalein excretion curve, (b) the soluble enzyme content in various portions of the central lobe of the liver, (c) the lipid and glycogen content of the same tissue segments, and (d) their nitro-reductase activity. Comparisons were made with the histologic appearance of the same fragments. Despite the number of morphological changes seen microscopically, the data suggest a lack of correlation with functional and enzyme tests made after the long-continued (7-year) period of insult. During the early phases of feeding, however, the functional tests were diagnostic of some degree of tissue injury. The terminal findings indicate that adaptation to the continuous intake of safrole may have occurred, and that the regenerated tissue had essentially the same functional and biochemical characteristics as that of untreated animals.

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