ABSTRACTS OF PAPERS

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Abstracts of Papers for the Fourth Annual Meeting of the Society of Toxicology, Williamsburg, Virginia, March 8–10, 1965

1. *Toxicity and Biological Effects of 1,2-Benzpyrene, 1,2,5,6-Dibenzanthracene and 3-Methylcholanthrene in the Chick Embryo.* R. ABAZA, A. DENNEY, H. C. MILLAR, and D. A. GREENWOOD, Utah State University, Logan, Utah.

1,2-Benzpyrene, 1,2,5,6-dibenzanthracene and 3-methylcholanthrene were studied singly and in various combinations for toxic and other biological effects by the technique of McLaughlin *et al.* (*Toxicol. Appl. Pharmacol.* **5**, 760-71, 1963). The polycyclic aromatic hydrocarbons were determined by ultraviolet spectrophotometric and gas chromatographic procedures. Results indicate that there was a general correlation between maximum quantity of the polycyclic aromatic hydrocarbons that permits chicks to hatch and the average acute oral toxicity for mice. Various combinations of the compounds were more toxic than single administrations. Evaluations of the histopathological data and statistical analyses will be presented.

2. *Toxicological Studies on Tylosin: Its Safety as a Food Additive.* R. C. ANDERSON, H. M. WORTH, R. M. SMALL, and P. N. HARRIS, Lilly Toxicology Laboratories, Greenfield, Indiana.

Tylosin, a macrolide antibiotic, inhibits many Gram-positive bacteria, spirochetes, protozoa, pleuropneumonia-like organisms, and several large viruses. It is used in a swine growth ration, and to control turkey sinusitis and chicken chronic respiratory disease. Tylosin is useful for controlling spoilage in heat-processed foods.

In mice and rats, the LD_{50} estimates by intravenous and intraperitoneal routes were approximately 600 mg/kg, and by oral administration $>$ 5000 mg/kg. Dogs tolerated single 800 mg/kg oral doses. Rats fed 2 years on diet containing 10,000 ppm of tylosin had no significant alterations in growth, viscera, or hematology; and in reproduction studies, those fed the same level showed no diminution in the fertility, viability, gestation, or lactation indices. Oral doses to dogs of 200 and 400 mg/kg/day for 2 years produced no visceral damage. The post-rotatory nystagmus response of cats was not altered by 3 months' daily subcutaneous injections of 200 mg/kg.

Tylosin and its metabolite, Desmycosin, have been shown to be safe for use as a food additive.

3. *Experimental Aflatoxicosis in Peking White Ducklings and Coturnix Quail.* B. H. ARMBRECHT, G. W. BIERBOWER, and P. C. UNDERWOOD, Food and Drug Administration, Washington, D. C.

Several laboratories have described lesions found in the liver, kidney, spleen, and pancreas of ducks fed rations containing peanut meal contaminated with *Aspergillus flavus*. In pilot studies in which chloroform-soluble extracts of *Aspergillus flavus* cultures were incorporated in the diet of ducklings and mature quail, we found the typical liver lesions reported caused by aflatoxin. However, lesions were not observed in the kidney, pancreas, spleen or other organs examined.

Liver lesions were not apparent in *Coturnix* quail on levels up to 50 ppm of aflatoxin extract for a period of three months. At levels of 100 ppm for periods of 28–93 days, proliferation of bile duct epithelial cells, slight to moderate fibrosis, vacuolation of hepatic cells and variation in hepatic cell size were observed. Aflatoxin was cumulative with respect to mortality in quail.

In the duck, lesions were not detectable until the third month at toxic feeding levels of 1.0 ppm. Progressively severe liver lesions were found in surviving ducks held for additional periods of 3–6 months after discontinuance of the toxic feed. Liver lesions observed in ducks were proliferation of bile duct epithelial cells, focal parenchymal cell vacuolation and slight to moderate fibrosis.

4. *The Marmoset as an Experimental Animal in Toxicology.* R. E. BAGDON and J. A. F. DE SILVA, Department of Pharmacology, Research Division, Hoffmann La Roche, Inc. Nutley, N. J.

One approach towards the development of new techniques in experimental toxicology is the use of species other than those conventionally employed in conducting toxicity measurements. The

cotton-eared marmoset (*Hapale aurita*) originating in Brazil exhibits highly emotional behavior, suggesting that this low order primate may serve as an interesting animal for conducting chronic toxicity studies of psychotropic drugs. Groups of 3 marmosets were administered 20–40 mg/kg or 5 mg/kg of diazepam (Valium®) orally 5 days per week for 132 weeks admixed in a cereal-powdered milk vitamin diet. The animals were easily maintained under laboratory conditions and body weights were not significantly altered. One animal expired during the 5th month due to an intense parasitic infestation. The vicious, aggressive behavior of the animals was attenuated by the tranquilizing effect of diazepam; the taming activity of the drug usually had an onset 1 hour after administration and persisted for 16 hours after each dose. Sedation and ataxia, effects seen with these large doses of diazepam in canines, were not elicited in these primates. Diazepam-treated animals did not show significant hematologic or liver functional changes from normal. Blood levels of diazepam and the *N*-demethylated metabolite were determined after 33 months chronic administration by gas-liquid chromatography. The levels of diazepam were 0.02–0.05 γ/ml whereas the *N*-demethylated metabolite was present in 6- to 10-fold higher concentrations of 0.20–0.30 γ/ml, suggesting that stimulation of enzyme systems involved in *N*-demethylation may have occurred.

5. *Adrenocortical Function Test in Toxicity Studies.* T. BALAZS and D. KUPFER, Departments of Pharmacological Research and Chemical Pharmacology, Experimental Therapeutics Research, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

The estimation of cortisol production rate was used for the assessment of adrenocortical function. The guinea pig was the animal of choice because its adrenals secrete cortisol, which is excreted in the urine in measurable quantities (Burstein and Kimball, *Analytical Biochem.* **4**, 132, 1962). The metabolism of injected labeled cortisol was found to follow the pattern of the secreted cortisol (Kupfer *et al., Life Sci.,* in press), indicating a common pool and permitting the use of the isotope dilution method. The specific activity of urinary free cortisol was selected for the determination of secretory rates. The 24-hour secretory rate of cortisol varied widely among different guinea pigs (0.3–2.4 mg); however, repeated examinations of the same animal revealed relatively constant values. Chemically induced elevation and depression of cortisol production rates were demonstrated by this method.

6. *Unsymmetrical Dimethylhydrazine-Induced Diuresis.* M. L. BARTH, C. L. GEAKE, and H. H. CORNISH, Department of Industrial Health, School of Public Health, University of Michigan, Ann Arbor, Michigan.

During studies of the toxicity of unsymmetrical dimethylhydrazine (UDMH), it was noted that a marked diuresis commonly occurred. An investigation of this diuresis has been carried out in an attempt to identify the biochemical mechanism involved.

Diuresis begins 1–1½ hours after the intraperitoneal injection of 80 mg/kg of UDMH into 250-g male albino rats and persists for 3–3½ hours. During the period of diuresis, an average of 7 ml of urine with a specific gravity of 1.017 is produced, compared with average normal urine output of 1.8 ml with a specific gravity of 1.041 during a similar time period.

Intraperitoneal injection of 100 mg/kg (0.49 mM/kg) of pyridoxine hydrochloride reverses the diuresis caused by 100 mg/kg (1.67 mM/kg) of UDMH. Ten mg/kg (0.05 mM/kg) of pyridoxal hydrochloride given intraperitoneally prevents the diuretic effect of 80 mg/kg (1.33 mM/kg) of UDMH. Since 0.05 mM/kg of pyridoxal hydrochloride reverses the diuretic effect of 1.33 mM/kg of UDMH, it appears that B_6 is acting at an enzymatic level and is not combining chemically with UDMH to reduce its effective concentration.

Intracerebral injection of 25–35 mg/kg of UDMH results in a definite but variable diuresis. This effect is minimal when 35 mg/kg of UDMH is given by other parenteral routes. Since low doses of UDMH given intracerebrally produce diuresis in rats, it was of interest to determine the effect of anti-diuretic hormone (ADH) on UDMH-induced diuresis. A dose of 5 mU of ADH per rat has a marked anti-diuretic effect upon water-loaded rats. Doses of ADH up to 1 U given intraperitoneally do not affect UDMH diuresis. This seems to indicate that UDMH does not interfere with either the synthesis or the release of ADH. Studies are underway to determine if B_6 plays a role in ADH activity.

7. *Serum Enzyme Response to Specific Organ Damage.* L. D. BEATTY, V. N. DODSON, and H. H. CORNISH, Department of Industrial Health, School of Public Health, University of Michigan, Ann Arbor, Michigan.

The relationships of serum enzyme and protein responses to tissue damage are receiving increased attention. The present study aims at developing simple, sensitive techniques for the early detection of specific organ damage. Serum protein and enzyme patterns produced by polyacrylamide gel electrophoresis were stained for protein esterase, lactic dehydrogenase (LDH) and malic dehydrogenase (MDH). These patterns were studied in rats after specific organ injury caused by (a) crushing with blunt forceps, (b) injection of 10% hydrochloric acid, (c) injection of carbon tetrachloride, and (d) anoxia produced by interrupting the blood supply to the organ.

After liver and kidney injury by these techniques, serum protein patterns varied only slightly from normal controls, the most distinctive difference being a decreased density of protein bands following injection of HCl into the kidney.

Serum esterase patterns showed four new bands after injection of CCl_4 into the liver. Other methods of producing liver injury were ineffective.

Changes in serum LDH patterns seem to be the most sensitive indicators of tissue damage for both liver and kidney. Crushing, CCl_4 injection, and anoxia increased the serum levels of LDH isozymes. Injection of 10% HCl noticeably lowered the serum concentration of LDH isozymes.

To date, serum MDH shows insufficient electrophoretic pattern variance to be useful in detecting tissue damage. However, an extra band appears following injection of CCl_4 into the kidney.

Human enzyme electrophoretic patterns were studied in serum obtained from an individual who had ingested approximately 90 ml of CCl_4. Serum protein, esterase, LDH and MDH all showed distinctive electrophoretic pattern changes during the first few days following ingestion. Enzyme levels appeared to be below normal at nine days but were approaching normal two weeks after the CCl_4 ingestion.

8. *The Metabolic Fate of 1-Methyl-3-pyrrolidyl α-Cyclohexylmandelate Methobromide, A New Antiperspirant.* R. B. BRUCE, J. H. NEWMAN, L. B. TURNBULL, Research Laboratories, A. H. Robins Company, Richmond, Virginia.

The fate of hexopyrronium bromide, 1-methyl-3-pyrrolidyl α-cyclohexylmandelate methobromide (Franko and Lunsford, *J. Med. Chem.* **2**, 523, 1960), an anticholinergic and antiperspirant substance (Stoughton *et al., J. Invest. Dermatol.* **42**, 151, 1964); Stoughton, *ibid.* **42**, 287, 1964) is reported. A sensitive nonisotopic method of detection of drug-related substances in urine is presented.

Urine and feces of dogs that received 1-methyl-C^{14} drug (10 mg/kg, 1 mg/kg, and 0.1 mg/kg) intravenously contained approximately 55% and 40% of the radioactivity, respectively. The urinary radioactivity was divided between two major components as demonstrated by paper chromatograms and counter-current distribution. These substances were separated from urine by methylene chloride extraction of bromcresol purple and dipicrylamine dye complexes, respectively. Removal of the dye anions by anion exchange resin afforded clean metabolite fractions for chromatography and isotope dilution experiments which established the identity of the two components as the administered drug and a hydrolysis product, 3-hydroxy-1,1-dimethylpyrrolidinium ion.

A sensitive spectrophotometric method for quantitative assay of urine was developed. After extraction of α-cyclohexylmandelic acid from base-hydrolyzed urine, lead tetraacetate oxidation produced cyclohexyl phenyl ketone which was determined photometrically. These lead tetraacetate values agreed substantially with the isotope results using the urine from dogs. Urine of humans receiving oral doses as low as 1 mg/day of the drug was assayed by this method.

9. *Analytical Control in the Clinical Toxicology Laboratory.* S. CARSON, M. S. WEINBERG, and B. L. OSER, Food and Drug Research Laboratories, Inc., Maspeth, New York.

Quality control procedures have been used fairly extensively for evaluating reliability and establishing normal ranges in human clinical laboratory work. These procedures have been applied to assessing blood, urine and tissue analyses in several species of laboratory animals used in toxicological studies. Control samples for each species, containing the various constituents at critical

levels are employed as standards, and recovery tests are run routinely. The analytical data have been computed to establish confidence limits. The value of this system of control for assessing the reliability of within laboratory data and for comparing data between laboratories will be discussed.

10. *The Salivary Excretion of Drugs. Antibiotics.* H. M. CHERRICK and J. F. BORZELLECA, Department of Pharmacology, Medical College of Virginia, Richmond, Virginia.

Hairy tongue, a condition characterized by hypertrophy of the filiform papillae of the tongue, is not infrequently seen by dentists and physicians. Although the precise etiology is unknown, suppression of the normal flora of the oral cavity appears to be involved. This suppression could result from exposure to high concentrations of antibiotics as a result of either the prolonged use of lozenges or the salivary excretion of antibiotics following administration for systemic purposes.

The following agents were administered intravenously to anesthetized dogs: sulfanilamide, 20 mg/kg; sodium crystalline penicillin G, 100,000 units/kg; tetracycline, 10 mg/kg. Blood, urine and salivary samples (from the cannulated parotid ducts) were then collected periodically. The analytical procedure used was a modification of the method of Osgood (*J. Lab. Clin. Med.* **32**, 446, 1947). The results obtained indicate that the drugs under investigation are excreted in significant quantities in the saliva, that there is correlation between blood and salivary levels, and that the quantity excreted in saliva is dependent upon the dosage and the route of administration. Further evidence of the importance of this route of drug excretion was obtained in dogs with bilateral nephrectomies where it was found that the quantity of drug excreted in saliva was considerably greater than in controls.

11. *Effect of Phenobarbital on the Metabolism of Dieldrin.* C. CUETO, JR., and W. J. HAYES, JR., Toxicology Section, Communicable Disease Center, U. S. Public Health Service, Atlanta, Georgia.

Evidence has been presented that the administration of chlordane or DDT to rats produces a stimulatory effect on hepatic microsomal drug-metabolizing enzymes (Hart *et al., Toxicol. Appl. Pharmacol.* **5**, 371, 1963; Hart and Fouts, *Proc. Soc. Exp. Biol. Med.* **114**, 388, 1963). However, the effects of stimulation of the microsomal enzymes on the metabolism, storage, and elimination of pesticides has not been investigated. These possible effects were studied in rats by repeated administration of phenobarbital (50 mg/kg/day) as the enzyme stimulator and dieldrin (0.5 mg/kg/day) as the chlorinated pesticide. Fat, liver, feces and urine samples were collected at intervals of 2, 8 and 16 weeks after the initial dose of dieldrin. The samples were analyzed by gas chromatography using a microcoulometric detector.

Dieldrin and unknown dieldrin-derived metabolites were detected in all samples except urine. The fat of rats on chronic phenobarbital-dieldrin administration always contained significantly less dieldrin-derived material than that of rats on dieldrin alone. Treatment with phenobarbital also reduced storage in the liver but not to a significant degree. However, no significant difference in the rate of fecal excretion of dieldrin metabolites was detectable by the method used.

12. *Some Comparative Pharmacological Studies in Man and the Monkey with Thalidomide.* C. S. DELAHUNT, N. KISS, E. FELDMAN, and M. OAKES, Pharmacology Department, Chas. Pfizer and Co., Inc., Groton, Connecticut.

Thalidomide administered to pregnant monkeys, after implantation but before limb bud formation, produced congenital malformations in this species strikingly similar to those reported in man (Delahunt *et al.*, First Annual Meeting of the American College of Clinical Pharmacology and Chemotherapy, New York, October, 1964). When this compound was administered to man and monkeys, the drug blood levels were similar. Thalidomide was detected in "cord" blood of a monkey fetus. Following an oral dose of 10 mg/kg to monkeys, in Foringer chairs inside a sound proof isolation "chamber," brain electrophysiologic tracings revealed a typical sleep pattern. Many investigators have observed that sleep was induced in patients given thalidomide. The conclusion drawn from this limited comparative study is that the preferred animal for experimental investigations of thalidomide is the monkey. It closely correlates with man in biochemical, pharmacologic, and teratologic responses to this drug. In comparative investigations of other drugs, the monkey may not parallel man in its response. The thalidomide disaster has pointed out the necessity for

comparative pharmacological studies in man and a variety of experimental animals. The selection of an animal species with a pharmacological profile similar to that of man will govern the accuracy of predicting from animal data a drug's toxic or teratogenic effect in man.

13. *The Chronic Toxicity of Ruelene and a Ruelene Derivative in Beagle Dogs.* W. H. DIETERICH, O. E. PAYNTER, and R. J. WEIR, Hazleton Laboratories, Inc., Falls Church, Virginia.

Young beagle dogs were maintained for two years on Ruelene and a phenol derivative of Ruelene at daily dietary levels varying from 10–2000 ppm. The animals receiving 2000 ppm of Ruelene evidenced gross signs of compound effect and elevated alkaline phosphatase and serum transaminase values. These animals as well as those fed 200 ppm of Ruelene showed depressed plasma and erythrocyte cholinesterase values. Other hematological and biochemical findings were comparable to those of the controls.

At the two-year sacrifice, the male dogs which had received the phenol derivative at the 200 and 2000 ppm levels showed atrophy and degenerative changes in the seminiferous tubules of the testes. There were no other gross or microscopic autopsy findings which would indicate an effect due to the feeding of either Ruelene or its phenol derivative.

14. *The Role of Autoimmunity in Carbon Tetrachloride Intoxication: I. Influence of Route of Administration.* V. N. DODSON, I. MITCHELL, R. FRIBERG, and D. KETCHUM, Department of Industrial Health, School of Public Health, University of Michigan, Ann Arbor, Michigan. (Sponsor: H. H. Cornish.)

Anti-liver antibodies have been detected in patients having various liver diseases, especially those leading to cirrhosis. This study was designed to see whether liver autoimmunity is significantly involved in carbon tetrachloride intoxication and, if so, whether it mediates tissue damage, modifies biochemical responses, or varies with the portal of entry, dose, frequency and temporal relationships.

Male Sprague-Dawley rats were given CCl_4 by quantitative inhalation, gastric intubation or subcutaneous injection in doses from 0.25–4.75 ml for periods up to 148 days. They were sacrificed at intervals following exposure from 6 hours to 120 days.

The sera were tested for anti-liver antibodies by tanned red blood cell hemagglutination and complement fixation tests. Routine tissues were taken for tissue pathology and stained by standard methods using hematoxylin-eosin, periodic acid-Schiff and Oil Red O. Liver lipids were determined gravimetrically. Liver connective tissue was measured by hydroxyproline determination (Leach's method).

Anti-liver antibodies were detected by hemagglutination but not by complement fixation regardless of the portal of entry, but most commonly when CCl_4 was given by gastric intubation. Post-exposure pathologic and chemical lipidosis was dose-dependent, declined with time, and was most severe following the gastric CCl_4 poisoning. Necrosis correlated with dose, proximity to last exposure and its severity was greatest from the gastric route, least by inhalation. Within these dose ranges, portal of entry and dose produced no significant difference in hydroxyproline values. Anti-liver antibodies best correlated with involvement of interlobular and trinity areas.

15. *DDT and DDE Content of Complete Prepared Meals.* W. F. DURHAM, J. F. ARMSTRONG, and G. E. QUINBY, Toxicology Section, Communicable Disease Center, U. S. Public Health Service, Wenatchee, Washington.

The DDT and DDE contents of 29 complete prepared meals were determined. Seven meals contained no detectable DDT or DDE. One additional meal did not contain measurable DDT. No DDE was found in two other meals. In general, the products of animal origin (meats, gravies, meat combinations and eggs) contained the larger quantities of DDT and DDE. Dairy products were an exception to this generality.

The household meals in this study contained somewhat higher concentrations of DDT and DDE than did the restaurant meals. Since they represented a more average type of food selection and did not contain specialty or home-grown items, the restaurant meals were considered to be more typical of the average American diet than were the household meals. Based on the restaurant meals, the present mean daily intake for the general population of the U. S. was estimated to be

0.038 mg of DDT and 0.044 mg of DDE. This amount of DDT represents a mean concentration of about 0.016 ppm in the total wet diet. This DDT intake is equivalent to a daily dosage for a 70-kg man of about 0.0006 mg/kg.

Comparison of the results of the present study with earlier surveys indicate that dietary exposure to DDT has not increased during the past 10 years and may even have decreased.

16. *The Effect of Spinal Cord Lesions on the Pathophysiology of a Lung Irritant, Ozone.* E. J. FAIRCHILD and G. A. BOBB, Division of Occupational Health, U. S. Public Health Service, Cincinnati, Ohio.

Spinal cord lesions in rats have been shown to inhibit the development of pulmonary edema resulting from acute inhalation of ozone, a lung irritant gas. Quantitation of the inhibitory response was standardized by gravimetric analysis of lung fluid content; comparison with sham-operated controls gave relative assessment of response following a standard gas exposure. Transverse cord section, as well as partial cord destruction, at the 6th cervical and 2nd thoracic vertebrae, markedly inhibited edemagenesis, whereas similar lesions at mid- or low-thoracic levels did not inhibit lung edema. Transverse cervical section always produced deep hypothermia, whereas partial lesion of the cord produced little, if any, hypothermia. Transverse section at mid- or low-thoracic levels produced mild hypothermia.

Hypometabolism, which accompanies hypothermia, has been shown to be the controlling factor in reduction of CCl_4-induced hepatotoxicity in cord-sectioned rats (Larson and Plaa, *Experientia* **19**, 604, 1963), but does not appear to be the determinant in our work. Otherwise, inhibition of lung edema would be expected only if hypothermia ensued, and this was not the case. Rather, our results indicate that the effect of nerve cord lesions upon edemagenesis is a result of disrupted nervous pathways which alter hemodynamics and possibly respiratory mechanics.

17. *A Radiotracer Balance Study of C^{14} Polyvinyl Methyl Ethermaleic Anhydride Copolymer (PVM/MA) in the Rat.* I. M. FORD, Stauffer Chemical Company, Mountain View, California.

Two male and two female rats were treated with single oral doses of C^{14} PVM/MA. The use of individual glass metabolism cages and tail cups permitted the separation and collection of urine, feces and exhaled CO_2 with essentially no cross contamination. This enabled an accurate determination of the absorption-distribution-excretion pattern of the material or its metabolites.

A rapid loss of administered radioactivity occurred through excretion in the feces of each animal during the first 12 hours after dosage. Very small amounts were found in the feces for an additional 48 hours. Trace quantities were also observed in the urine and expired air. No radioactivity could be detected in any of the selected body tissues.

18. *Ring Aromaticity Requirement for Enhanced Toxicities (Mouse) of Tropanol Phenylacetates.* S. L. FRIESS and L. J. REBER, Naval Medical Research Institute, Bethesda, Maryland.

Previous toxicity studies in mice (acute, intravenous route) with phenylacetate esters of tropine (T) and pseudotropine (pT) have shown a high degree of stereospecificity in lethal response, with potency in the isomeric sequence T > pT. Now, the role of aromaticity in the phenyl ring in contributing to receptor binding has been assessed by synthesis and toxicity testing of the two ring-saturated esters T-cyclohexylacetate and pT-cyclohexylacetate, for comparison with the corresponding potencies of the phenylacetate esters in which aromaticity still exists.

The cyclohexylacetate esters produce death in mice *via* a convulsion-paralysis syndrome, are equipotent in terms of LD_{50} indexes (T-ester·HCl, 36.0 ± 1.6 mg/kg; pT-ester·HCl, 34.0 ± 1.2 mg/kg), and lie at the same potency level as pT-phenylacetate (as HCl salt, 42.3 ± 1.6 mg/kg). Saturation of the aryl ring appears to wipe out stereospecificity in receptor response to the triggering ester, and reduces the intensity of response to that characteristic of the unfavored pT configuration. Interestingly, the same sensitivity to aryl structure is also evident in the ester-mediated responses of neuromuscular chemoreceptors in the rat phrenic nerve-diaphragm preparation; with the phenyl esters the strength sequence for twitch amplification is T > pT, whereas with the cyclohexyl esters stereospecificity disappears.

These results are interpreted structurally in terms of binding increments at receptor surfaces controlling the biological responses.

19. *Acute Ethanol Toxicity in Dogs.* J. C. GARRIOTT, R. B. FORNEY, F. W. HUGHES, and A. B. RICHARDS, Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana.

The comparative effect of continuous ethanol or pentobarbital infusion on the concentration of plasma free fatty acid (FFA), blood glucose, and serum glutamic oxaloacetic transaminase (SGOT) was studied in dogs. Periodic blood samples were collected for above analyses as well as for arterial and venous blood alcohol determination. Blood pressure was recorded continuously during the experiment.

The first group of seven dogs was administered ethanol intravenously at a rate of 1.25 g/kg/hr. The ethanol was diluted with normal saline so that each dog received 50 ml of solution per hour. Infusion was continued until death.

The second group of four dogs was given pentobarbital intravenously at the rate of 25 mg/kg/hr until death.

The third group of four control dogs was infused with normal saline intravenously for a comparable period.

SGOT, as well as FFA, and blood glucose levels showed highest increases in the alcohol-treated animals, whereas SGOT and FFA levels decreased in the pentobarbital group.

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

0,0-dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate (malathion), 2-carbomethoxy-1-methylvinyl dimethyl phosphate (Phosdrin), and 1-naphthyl *N*-methylcarbamate (Sevin) were examined singly and in various combinations for toxic and/or teratogenic effects by the procedure of McLaughlin *et al.* (*Toxicol. Appl. Pharmacol.* **5**, 760-71, 1963). Data obtained indicate that there was a general correlation between the maximum quantity of pesticide which permitted some chicks to hatch, and the average acute oral toxicity for mice. Combinations of the organophosphorus compounds produced teratogenic effects. Histopathology studies will be discussed.

21. *Convenient Procedures for Checking the Accuracy of Commercial Gas Detector Tubes.* R. N. HARGER and R. B. FORNEY, Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana.

Sealed detector tubes for testing workroom air for various toxic gases, which are now available from commercial sources, should be checked with known concentrations of the particular gas before using the results for medicolegal purposes. Well-ground, dry, glass syringes have proved very useful for making specific gas-air mixtures.

On repeatedly passing a gas between two such syringes, it was found that the leakage between plunger and barrel was almost nil, making the syringes suitable for measuring, transferring and mixing gases.

For vaporizing a liquid into air, a measured droplet of the former is placed on the end of a vertical syringe plunger by means of a micropipette with a metal needle delivery tube, the syringe outlet is closed, and the plunger withdrawn to give a partial vacuum. An all-glass apparatus for equilibrating air with a volatile liquid is also reported. Determinations were made of the relative permeability of certain toxic gases in air through the walls of storage bags made of various plastics or of aluminum foil, the edges of which are sealed with a thermo-setting plastic.

A dry, 100-ml syringe, equipped with small, mercury-sealed valves, or with a reversing glass stopcock, provides a very convenient gas meter, particularly for introducing a given volume of air into the bag which will contain a particular gas-air mixture.

A mercury-operated device for introducing a constant micro-flow of a gas into an air stream, is described.

22. *Some Toxic Effects of Ingested Polluting Oils on Waterfowl.* R. HARTUNG, Department of Industrial Health, University of Michigan, Ann Arbor, Michigan and G. S. Hunt, Department of Wildlife Management, University of Michigan, Ann Arbor, Michigan. (Sponsor: H. H. Cornish.)

Examination of the literature indicates that oil pollution presents a serious problem for waterfowl. Effects were studied by autopsies of ducks which had been killed by oils in the wild, and

by experimental feeding of oils to ducks. Oil ingestion was found to produce a high incidence of lipid pneumonia. Many oils were highly irritating to the gastro-intestinal tract. The blood loss into the intestine was often sufficient to produce slight anemia. The number of zymogen granules in the pancreas was greatly reduced, especially after the ingestion of some cutting oils and diesel oils. The livers showed a high incidence of fatty infiltration and degeneration, but the plasma transaminase levels and BSP liver function tests showed changes only after the ingestion of high doses of diesel oil. The kidneys of many ducks showed a toxic nephrosis. Non-protein nitrogen levels in the blood were frequently elevated. Stresses imposed by oil ingestion resulted in adrenal cortical hyperplasia and the general adaptation syndrome. Some oils contained cholinesterase inhibitors. Approximate mean lethal doses were determined for several oils under varying environmental conditions.

23. *Tests on Mice for Evaluating Carcinogenicity.* H. C. HODGE, E. A. MAYNARD, W. L. DOWNS, J. K. ASHTON, and L. L. SALERNO, The University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

The Food Protection Committee (1959) suggested that tests for evaluating carcinogenicity include (a) repeated skin applications, (b) single subcutaneous injections in mice, (c) life-time feeding studies in rats and mice, and (d) dog feeding studies of at least 4 years' duration. Six compounds were selected on which rat-feeding and dog-feeding (at least one year) data were available: 3 carcinogenic and 3 non-carcinogenic in rat-feeding studies. These six compounds were tested in mice.

Single subcutaneous injections of 5 percent solutions (in tri-*n*-caprylin) of aminotriazole, aramite, butylated hydroxyanisole, di-octyl adipate, flectol, and methoxychlor were administered to C_3H mice (10 mg/mouse). The mice were maintained on a basal diet for their lifetime without further treatment. One hundred mice (50 of each sex) were used for each test compound and a similar number were given tri-*n*-caprylin only and served as controls (total of 700 mice).

In a second study, C_3H mice were treated with a weekly application of the compounds, in acetone solution, some with the low dose-0.10 mg/mouse (0.20 ml of 0.05 percent soln) and others with the high dose-10.0 mg/mouse (0.20 ml of 5.0 percent soln) applied until the mouse died. One hundred mice (50 of each sex) were used for each dose group and for the control group which received a weekly application of acetone (0.20 ml/mouse) (total 1300 mice).

All mice were maintained in plastic cages (12–13 to a cage) and were examined and weighed weekly. At death, each mouse was examined for gross change at the site of treatment; sections of the skin or subcutaneous tissue were taken for histopathological study. Incidence of tumors detectable on gross examination was remarkably low.

24. *Effects of Diet on Toxicity of Safrole in Rats.* F. HOMBURGER, T. F. KELLEY, and P. D. BOGDONOFF, Bio-Research Institute and Bio-Research Consultants, Inc., Cambridge, Mass.

Previous studies showed (Homburger *et al., Medicina Experimentalis* **4**, 1-11, 1961, and *Arch. Path.* **73**, 40-47, 1962) that CFN male rats fed 12% casein diets, deficient in riboflavin, are exceptionally susceptible to hepatotoxic and carcinogenic effects of safrole, and that increased protein content of the diet results in lessened fibrous and ceroid deposition with larger tumors. Addition of riboflavin had no effect.

It has now been observed that increase of the fat content of the diet from 5% to 15% will reduce the toxicity of safrole but not that of butter yellow.

The control rats on high-fat diets alone usually showed fatty livers, whereas the livers of animals on high-fat diets containing butter yellow or safrole never showed such changes.

Toxic manifestations of hepatotoxic agents clearly are modified by nutritional factors, and such nutrition-dependent variations of response may be different for various agents.

25. *Experience with the Guinea Pig in Screening Primary Irritants and Sensitizers.* D. B. HOOD, R. J. NEHER, R. E. REINKE, and J. A. ZAPP, JR., Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, Newark, Delaware.

Early experience in producing sensitization in the guinea pig with simple chemicals has been used to develop a test to detect the weak as well as the moderate and strong sensitizers, thus pro-

viding information as to the nature of the risk of using a given chemical in a new product. Primary irritation is first evaluated by applying the test material to the shaved skin of 10 guinea pigs. Several concentrations are used including one which provides a minimum response. A series of scratches on the dorsal skin of five guinea pigs and intradermal injections on another five are carried out for a one- to three-week period. Following a two-week rest period, the challenge test is carried out on intact and abraded skin. Variability in response is reduced by carefully controlling environmental conditions, diet and age as well as the nature of the solvent used, concentration, techniques and other factors. The use of the guinea pig screening test to determine whether a patch test on human subjects should be carried out and the correlation between the two are discussed for different classes of compounds.

26. *Acute Toxicity in the Young Adult and the Newborn Mouse.* J. O. Hoppe, L. P. Duprey, and E. E. Dennis, Biology Division, Sterling-Winthrop Research Institute, Rensselaer, New York.

The hazard of accidental poisoning in infants and young children emphasizes the importance of the acute toxicity determination in the infant animal. An estimate of the magnitude of the difference in acute subcutaneous toxicity in the young adult mouse (30 days) compared with the newborn mouse (1 day) was made among a group of 16 compounds including morphine sulfate, meperidine HCl, pentobarbital sodium, atropine sulfate, chloramphenicol sodium succinate, nalidixic acid, chlorpromazine HCl, tetracaine HCl, procaine HCl, sodium iodide, sodium chloride, sodium salicylate, methanol, ethanol, *n*-propanol and *n*-butanol. LD_{50} values were calculated at 24 hours and again at 7 days after single injection. Thirteen of the 16 compounds were found to be significantly more toxic in the newborn than in the young adult mouse. The acute toxicities of meperidine HCl, tetracaine HCl and sodium iodide in the newborn mouse were not found to be significantly different from that in the young adult mouse. The ratio of adult LD_{50}: newborn LD_{50} varied from 0.7 with sodium iodide to 6.2 with morphine sulfate at 24 hours, while at 7 days this ratio ranged from 0.5 with sodium iodide to 25.5 with chlorpromazine HCl. The acute subcutaneous LD_{50} for morphine sulfate in the newborn was found to be 56 $\pm$ 16 mg kg with no significant change at 4 and 8 days, while at 16 days the LD_{50} increased to 205 $\pm$ 47 mg/kg and to 350 $\pm$ 36 mg/kg at 30 days of age.

27. *Histochemical Differentiation between Concentration of Free Cholesterol and Cholesterol Ester for the Assessment of Pesticide-Induced Demyelination.* H. Johnson and L. R. Weiss, Division of Toxicological Evaluation, Food and Drug Administration, Washington, D. C. (Sponsor: O. G. Fitzhugh.)

The Schultz histochemical reaction for the detection of cholesterol and cholesterol esters was used to study the possibility that changes in their concentration in nerve tissue might be induced by certain organophosphorus compounds along with myelin degeneration of the nerves. The sciatic nerve of the adult chicken was used for this purpose. Chickens were given an acute neurotoxic dose of tri-ortho-cresylphosphate (TOCP). They were sacrificed, 16–21 days later, when they became paralyzed. Frozen sections of the sciatic nerve were prepared and relative amounts of total and free cholesterol estimated by visual comparison of depth of color caused by reaction of cholesterol with the iron-acetic acid anhydride reagents. Esterified cholesterol was removed, when necessary, by acetone treatment of sections which were pre-treated with digitonin.

There was relatively more ester cholesterol in nerve tissues of paralyzed birds than in those of controls.

It is suggested that in myelin degeneration, induced by neurotoxic cholinesterase inhibitors, free cholesterol reacts with fatty acids to form an increased amount of cholesterol esters in the nerve. The reaction appears to be useful for investigating the neurotoxic potential of pesticides, as well as to confirm myelin degeneration in nerve tissue.

28. *Analyses of Organ Weights and Organ to Body Weight Ratios for 500 Untreated Purebred Beagle Dogs.* J. W. Johnson, M. W. Woodard, and K. O. Cockrell, Woodard Research Corporation, Herndon, Virginia. (Sponsor: G. Woodard.)

Mean organ weights and organ-to-body-weight ratios of hearts, lungs, livers, kidneys, thyroids, adrenals, and brains, together with range and distribution data, are presented for 500 untreated purebred dogs maintained under the same dietary and environmental conditions.

The data were obtained over a period of six years from control dogs in standard repeated dose toxicity studies, and coded onto standard 80-column punch cards. The cards may be sorted by animal number, sex, body weight, organ weights, and age at time of sacrifice, and the information listed on a print-out as needed.

Also presented are statistical analyses of these data, indicating those organs which should be compared separately, with respect to sex and those that may be combined, when evaluating results of repeated dose toxicity studies in beagles.

29. *Subacute Oral Toxicity of Biodegradable Santomerse 85-b.* J. H. KAY, F. E. KOHN, and J. C. CALANDRA, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois.

A linear, biodegradable, commercial dodecylbenzene sodium sulfonate (Biodegradable Santomerse 85-b) was fed to rats at dietary levels of 0.02%, 0.1% and 0.5% for a period of 90 days. No adverse effects were found upon the following parameters: growth, food consumption, food utilization, survival, hematologic values, urinary values, organ weights and organ/body weight ratios. There were no significant gross or microscopic tissue changes attributable to ingestion of the test material.

30. *Toxicological Problems in the Field of Agriculture.* K. KAY, Department of National Health and Welfare, Ottawa, Canada.

Less than 20 years ago, the main toxic agents recognized in agriculture were lead arsenate and nicotine. Now there are many potentially toxic chemicals used, and it has come to be realized that there are as well, toxic hazards from plants, animals and physical agents. Lately cases of ill-effects to agricultural workers from these causes have increased as chemicals and mechanical equipment have become more and more widely applied. For instance, in California in recent years compensable occupational disease from pesticides has run at an incidence around 10 times as high as in the general work population. Furthermore, changes in social attitudes toward the health care of agricultural workers have had the effect of bringing to light other toxic conditions suffered by workers in this class. The result is that a substantial body of knowledge on toxicological problems in the field of agriculture has developed in the past few years, particularly in relation to environmental factors.

It is the purpose of this paper to assess the current state of knowledge of ill-effects from the new pesticides and their interactions with one another as well as environmental associates, drugs and alcohol. Recent findings will be presented on other aspects of the environmental toxicology of agriculture such as farmer's lung, silo filler's disease, contact dermatitis from plants, zoonotic infections and climate.

31. *Toxicity of Combinations of Pesticides.* M. L. KEPLINGER and W. B. DEICHMANN, Department of Pharmacology, University of Miami, School of Medicine, Coral Gables, Florida.

These studies were conducted to determine the effects and lethal doses from combinations of two or more pesticides, or from the combination of a pesticide with a drug or other chemical. Sherman or Osborne-Mendel strain rats and Swiss mice were used. The LD_{50} of each compound as well as the LD_{50} of two or more compounds administered at the same time were determined. The expected LD_{50} was calculated for each combination from the LD_{50}'s of the individual compounds, assuming strictly additive toxicity. The observed LD_{50} of the combination was recorded and the ratio of expected to be observed LD_{50} was calculated.

The pesticides used were Aldrin, Aramite, Chlordane, DDT, Delnav, Diazinon, Dieldrin, Endrin, Malathion, Methoxychlor, Parathion, Sevin, Toxaphene, Trithion and VC-13. Pharmacodynamic classes of drugs which might obviously alter the effects of those pesticides were selected and compounds representative of that class were used. The drugs were Caffeine, Chlorothiazide, Dimenhydrinate, Diphenhydramine, Diphenylhydantoin, Ephedrine, Ethanol, Paraldehyde, Pentachlorophenol, Pentobarbital, Pentothal, Phenobarbital, Promazine, Sodium Bromide, Trimethadione and Tripelennamine.

Most of the combinations of pesticides showed additive or synergistic effects, although not strictly in a mathematical sense. The effects in rats and mice were not significantly different with

most of the combinations. The combination of Aldrin and Chlordane, however, showed a potentiating action in mice but not in rats.

Many of the combinations of a drug and a pesticide showed essentially an additive or synergistic effect. The drugs tested which depress the CNS, with the exception of Paraldehyde, were antagonistic when administered with the chlorinated hydrocarbon pesticides tested. Ephedrine also showed an antagonistic effect. Promazine and Diphenylhydantoin showed some potentiating action.

32. *Evaluation of the Safety of 3,5-Dinitrobenzamide.* K. B. KERR, P. R. ABRAHAMSON, and J. R. IPSON, Dr. Salsbury's Laboratories, Charles City, Iowa.

The compound, 3,5-dinitrobenzamide, is primarily used as a coccidiostat for chickens, but it also has considerable value against salmonella infections. To evaluate the toxicity, acute and subacute and chronic toxicity tests were conducted.

The oral LD_{50} for chickens is 900 mg/kg, for turkeys 1150 mg/kg and for rats 1250 mg/kg. The intraperitoneal LD_{50} for chickens and rats is 280 and 320 mg/kg, respectively.

Subacute toxicity tests in chickens resulted in a no-effect dosage between 500 and 1000 ppm with the maximum tolerated dosage about 4000 ppm. For rats, the no-effect dosage lies between 600 and 1000 ppm. The same no-effect dosage was found for dogs.

The chronic toxicity test in rats essentially confirmed the no-effect dosage found in the subacute toxicity test. Similarly, the dog chronic toxicity test results revealed histopathologic changes at 1100 ppm but not at 520 ppm. Other parameters at these dosages were negative, thus confirming the previously found results in the subacute toxicity test.

In all tests, the growth parameter was the most sensitive index.

33. *Toxic Effects Induced by Inoculation of EPN and Systox into Duck Eggs.* K. S. KHERA, Q. M. LAHAM, and H. C. GRICE, Research Laboratories, Food and Drug Directorate, Department of National Health and Welfare, Ottawa.

Ducklings hatched from eggs inoculated at 13 days of incubation with μ/egg of EPN or Systox had partial to complete loss of voluntary control of one or both hind legs. The symptoms gradually disappeared about 5 days after hatching, but the growth of treated ducklings remained retarded. Inhibition of "acetylcholinesterase" activity of motor end-plates was detected from 20 days of age until the day of hatching. Histologic examination of the skeletal muscles from affected ducklings revealed areas of degenerative change alternating with areas of marked regenerative activity.

34. *Chronic Toxicity Study of Anapregnone in Rats.* T. O. KING and J. LUBANSKY, Ortho Research Foundation, Raritan, N. J.

A 2-year oral chronic toxicity study was conducted on 390 rats to determine the effects of anapregnone (6 α-methyl-4-pregnene-17α-ol-20-one acetate) incorporated in the diet. The animals were divided into 6 groups: Control diet (male and female), drug groups (female) at 0.2, 1.0, 10 mg/kg/day and males at 10 mg/kg/day. Rats from each group were sacrificed at 6 weeks, 6 months, 1 year and 2 years following onset of treatment. Histopathological evaluation of tissues from all animals was made at death or sacrifice. Urinalysis, hematology and clinical chemical tests were made on selected animals. Organ weight/body weight ratios were calculated and evaluated statistically.

Increasing doses of anapregnone resulted in increased weight and food consumption in drug-treated females but not in males. No differences in hematological chemical tests and urinalysis results between treated and control animals were detected. Polyuria and polydipsia were observed at the 20 mg/kg/day dose level. Neoplasms observed grossly and histopathologically were distributed with similar frequency in both treated and control animals.

35. *Subacute Oral Toxicity of Zinophos.* F. E. KOHN, J. H. KAY, and J. C. CALANDRA, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois.

The subacute oral toxicity of the pesticide Zinophos (0,0-diethyl 0-2-pyrazinyl phosphorothioate) was studied in albino rats and beagle dogs for a period of 90 days.

Initial dietary levels for rats were: 0, 0.5, 2, 8 and 25 ppm. After four weeks of testing, the highest level was raised to and maintained at 50 ppm. Parameters affected at the high dose included:

39. *A Species Difference in the Oxidation of Methanol.* G. J. MANNERING, A. MAKAR, D. R. VANHARKEN, and T. R. TEPHLY, Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota.

The first step in the oxidation of methanol can proceed in two ways:

1) CH_3OH + catalase·H_2O_2 ⟶ HCHO + $2H_2O$ + catalase

2) CH_3OH + DPN $\underset{\longleftarrow}{\xrightarrow{\text{alcohol dehydrogenase}}}$ HCHO + DPNH

A controversy exists as to which enzyme system is responsible for methanol oxidation *in vivo*. In the current studies rats and monkeys were treated in various ways, injected with C^{14}-methanol and placed in metabolism chambers which permitted the collection of both labeled methanol and $C^{14}O_2$. The rate of $C^{14}O_2$ collection was used as a measure of C^{14}-methanol oxidation. Three approaches were employed to determine the role of the two systems *in vivo*: (1) advantage was taken of the known differential rates of oxidation of methanol, ethanol and 1-butanol by the two enzyme systems, (2) 3-amino-1,2,4-triazole was injected to depress hepatic catalase activity, and (3) the rates of oxidation of methanol at different dose levels were used to calculate an "apparent *in vivo* Michaelis constant" that could be compared with the known Michaelis constants of the two enzyme systems *in vitro*. From the results it was concluded that the monkey and the rat oxidize methanol primarily by different mechanisms. The peroxidative system plays a major role in the oxidation of methanol in the rat while in the monkey this mechanism does not appear to be important. Instead, the evidence indicates that alcohol dehydrogenase is responsible for the initial step in the oxidation of methanol in the monkey.

40. *A Comparison of Toxicity Data Obtained for Twenty-One Pesticides by the Chicken Embryo Technique with Acute, Oral LD_{50}'s in Rats.* J. P. MARLIAC, M. J. VERRETT, J. MCLAUGHLIN, JR., and O. G. FITZHUGH, Division of Toxicological Evaluation, Food and Drug Administration, Washington, D. C.

The toxicity data for twenty-one pesticides, obtained by the chicken embryo technique have been compared to the acute oral LD_{50} values obtained with rats (*Pesticide Index,* D. E. H. Frear, 1961, College Science Publishers, State College, Pennsylvania). A rank correlation was established between these two types of data. The toxicities to the chicken embryo of the pesticides, methyl parathion, Guthion, lindane, demeton, heptachlor epoxide, endrin, dieldrin, DDT, DiSyston, parathion, malathion, and EPN correlate well with the rat data. The ratios of chick to rat toxicity ranged from about 0.5 to 2. However, a few exceptions exist in which the chicken embryo was found to be much more sensitive than the rat to the effects of certain pesticides. For example, carbaryl and Diazinon were found to be about 30 and 300 times more toxic, respectively, to the chicken embryo than to the rat. The data for several other pesticides, as well as the implications of these findings, will be discussed.

41. *Toxicology of 4-Tert-butyl-2-chlorophenyl Methyl Methylphosphoramidate (Ruelene).* D. D. MCCOLLISTER, F. OYEN, K. J. OLSON and V. K. ROWE, Biochemical Research Laboratory, The Dow Chemical Company, Midland, Michigan.

Ruelene is used for control of external and internal parasites in cattle, sheep and goats. The LD_{50}'s by single oral administration to laboratory animals range from 490 to greater than 1000 mg/kg. Ruelene is moderately irritating to the eyes. It is essentially non-irritating to intact or abraded skin, but the LD_{50} by single 24-hour skin exposure is about 4000 mg/kg. In rabbits treated topically, three consecutive daily applications of 10 mg/kg had no effect on erythrocyte cholinesterase. Treatment with 100 mg/kg/day caused decreases to 50-60% of control.

Single oral doses of Ruelene were administered jointly with each of 14 commercial cholinesterase-inhibiting insecticides. There was no effect other than additive (ratios 0.7-2.2) except for Ruelene-

malathion (five-fold increase). Feeding studies in dogs for 12 weeks with diets containing 100 ppm or 10 ppm malathion plus 20 ppm Ruelene showed no evidence of significant potentiating effects.

In 90-day rat dietary feeding, no effect occurred between 30 and 1000 ppm Ruelene except for a peculiarly uniform (40-60%) decrease in blood and/or brain cholinesterase activity. A 75-day dietary feeding study with dogs showed no effect whatsoever from diets containing 40 ppm Ruelene. At 250 ppm, blood cholinesterase activity was decreased slightly and slight changes were observed upon microscopic examination of liver sections.

In two-year rat diets, 1000 ppm Ruelene caused slight growth retardation, degeneration of testes, atrophy of hind quarters and some sciatic nerve degeneration. The only effect at 100 ppm was blood cholinesterase depression. Three-generation studies in rats showed no effect on reproduction indices at dietary levels up to 500 ppm. Based upon these data, and other available information, a dietary concentration of 40 ppm (2 mg/kg/day) is suggested as a reasonable "no-ill-effect" level for safety evaluation calculations.

42. *The Adrenal Responses to Stress of Rats Treated with Certain Progestagens.* G. R. McKinney and J. H. Weikel, Jr., Mead Johnson Research Center, Evansville, Indiana.

Since it is known that certain progestagens can induce adrenal hypoplasia in rats and can alter their response to stress, it was of interest to examine this matter by measuring the corticosterone levels in rats treated with various progestational steroids and then subjected to appropriate challenge or stress. Female, Sprague-Dawley rats were stressed with ACTH injection, etherization, or exposure to cold either after they had received the progestagen in their diet for two weeks or two hours after a single, large dose of the steroid. Blood samples were obtained from stressed and nonstressed rats either by retro-oribital bleeding (2-week test) or by decapitation (acute). Plasma levels of corticosterone were determined spectrophotofluorimetrically. In 2-week feeding studies steroids showed adrenal suppression (including lower adrenal weights) in the following increasing order of potency: megestrol acetate $<$ dimethisterone $<$ medroxyprogesterone acetate $<$ melengestrol acetate. Norethindrone was not suppressive. Plasma corticosterone levels 2 hours after single subcutaneous dose were markedly reduced by all compounds, but increased relative to basal level (no stress) after intravenous ACTH and etherization. Since the dosages used in this study were 20-300 times the human dosage, depending on the particular steroid, it does not appear that the progestagens which are useful in clinical medicine offer a threat to pituitary-adrenal integrity.

43. *Toxicity of Some Food Additive Chemicals as Measured by the Chick Embryo Technique.* J. McLaughlin, Jr., J. P. Marliac, M. J. Verrett, and O. G. Fitzhugh, Division of Toxicological Evaluation, Food and Drug Administration, Washington, D. C.

Several food additive chemicals have been tested by the chick embryo technique (*Toxicol. Appl. Pharmacol.* **5**, 760, 1963): propylene glycol, Paraplex G-60, Paraplex G-62, di(2-ethylhexyl) phthalate, 2-ethylhexyl diphenyl phosphate, 3,5-dinitro-*o*-toluamide, and acrylamide. The "no-effect" levels obtained were (in ppm): propylene glycol, 2000; Paraplex G-60, 1000; Paraplex G-62, 2000; di(2-ethylhexyl) phthalate, 2000; 2-ethylhexyl diphenyl phosphate, 1000; 3,5-dinitro-*o*-toluamide, 10; and acrylamide, 10.

A comparison of values from the chick embryo technique with those of published 2-year feeding studies in rats will be presented. A discussion of results from the chick embryo technique and their significance will be given.

44. *Glyceryl Trinitrate. II. Chronic Toxicity.* J. C. Munch and B. Friedland, Medical Department, Key Pharmaceuticals, Inc., Miami, Florida.

Chronic exposure is considered in connection with (1) clinical use, (2) pharmaceutical manufacture, and (3) commercial production for explosives.

Long-continued sublingual or oral ingestion of glyceryl trinitrate in the treatment of angina pectoris or other diseases may produce headache, cyanosis and methemoglobinemia occasionally, but no evidence of death has been found in humans. Subcutaneous injections of large doses to

animals were reported to produce fatty degeneration in the liver and heart, albuminuria and cerebral hemorrhages.

Glyceryl trinitrate reactions among pharmaceutical workers following inhalation or dermal absorption include intense throbbing frontal headaches extending toward the occiput. Longer exposure may produce severe cramps, nausea, vomiting and various psychic disturbances.

Workers engaged in the manufacture of glyceryl trinitrate and dynamite (especially if the concentration in the air is above 5 mg/m^3) develop obstinate headaches, giddiness, nausea, vomiting and a marked fall in blood pressure. All of these are exaggerated by ingestion of alcohol. With continued exposure tolerance develops to the headache, and to a lesser degree to the depressor response. Workmen rub small quantities of glyceryl trinitrate on their hatbands to maintain contacts over the weekend, to prevent "Monday morning headaches." Various investigations have confirmed the reports that chronic effects develop in the "Haut, Hirn, und Herz" (skin, brain and heart), but there are no evidences of characteristic pathological changes.

45. *Adaptive Enzymes and Glycogen in Livers of Rats Subjected to Toxic Stress.* S. D. MURPHY, Department of Physiology, Harvard School of Public Health, Boston, Mass.

Recent studies have suggested that the capacity of glucocorticoids to stimulate the synthesis of glucose and glycogen in rat livers is related to the capacity of these hormones to induce the synthesis of certain adaptive liver enzymes (*Adv. Enz. Regul.* **2**, 1964, G. Weber, Ed.). Recent studies in this laboratory have shown that adrenal-mediated induction of liver tyrosine transaminase (TT) and alkaline phosphatase (AP) occurs in rats treated with chemical irritants and organophosphate insecticides (Murphy, *Toxicol. Appl. Pharmacol.* **6**, 355, 1964, and *The Pharmacologist* **6**, 189, 1964). These findings stimulated an investigation of the relationships between liver glycogen and induced enzyme activities in rats treated with toxic chemicals.

Male rats were fasted from 6 p.m. until sacrifice at 2 p.m. the following day. Experimental groups and controls were injected intraperitoneally at selected times during the period of fasting. A group of 28 rats was treated with varied doses (20-50 mg/kg) of the organophosphate, Delnav®, 15 hours before sacrifice. Liver TT and AP activities ranged from control levels to 8 times the control values. Liver glycogen values for control rats ranged from 0.02-0.09%. Glycogen levels in 60% of the Delnav-treated rats exceeded the maximum control value. In further experiments, liver glucose-6-phosphatase (G-6-Pase) was also assayed. With another organophosphate, Guthion®, it was found that liver glycogen levels averaged 1.02% at 5 hours after 3 mg/kg compared to an average of 0.06% for controls. Adrenalectomy prevented the Guthion-stimulated glycogen deposition. Livers of rats injected with moderate doses of acrolein or allyl alcohol had glycogen levels exceeding control values. TT and AP but not G-6-Pase activities were also elevated.

The findings indicate that time, dosage and type of chemical are important determinants of the metabolic changes that occur in response to toxic stress, and that toxic chemicals may serve as useful tools in studying metabolic regulatory mechanisms.

46. *The Effects of Adrenergic Drugs and Certain Adrenergic Blocking Agents on the Uptake of Substrate by the Canine Myocardium.* D. NAMM and I. ROSENBLUM, Department of Pharmacology, Albany Medical College, Albany, New York.

It has been demonstrated that certain adrenergic amines can cause myocardial necrosis and that these amines differ in their capability to produce such lesions. (Rosenblum *et al., Toxicol. Appl. Pharmacol.,* 1965, in press). Many workers have attempted to implicate abnormal myocardial metabolism as a causal factor of amine necrosis. In this study the changes in myocardial substrate uptake caused by amines of both low and high lesion-producing capabilities were measured in order to determine whether they affected substrate metabolism adversely. A-V differences of total and individual nonesterified fatty acids and glucose were measured as well as triglyceride content of the left ventricle. Analysis of the uptake data showed that the uptake of glucose followed the direction of change of the total tension developed by the heart. However, this correlation did not exist for the uptake of the nonesterified fatty acids, which appeared to be nearly constant for a wide range of tensions developed by the myocardium. All the drugs used produced a depletion of myocardial triglycerides. The data indicate that for glucose uptake, myocardial sub-

strate utilization under the influence of the amines adjust to the changing demands made on the myocardium. A further study of this substrate uptake-work correlation was made using blocking drugs to alter the cardiovascular effects of the amines, and these results are presented.

47. *A Comparison of the Toxic Effects of the 21-Phosphate Esters of Triamcinolone Acetonide, Dexamethasone and Hydrocortisone Following Daily Intravenous Administration to Dogs for One Month.* J. F. NOBLE and W. M. LAYTON, JR., Departments of Pharmacological Research and Experimental Pathology, Experimental Therapeutics Research, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York. (Sponsor: D. W. Hallesy.)

The toxic effects of the compounds tested were qualitatively similar. Quantitatively the amount of dexamethasone-21-phosphate producing equivalent effects was about 1/5 to 1/25 that of triamcinolone acetonide-21-phosphate and less than 1/250 that of hydrocortisone-21-phosphate. The major findings related to drug effects were polydipsia and polyuria, increased plasma transaminase activity, altered serum proteins, increased erythrocyte sedimentation rate, changes in the number of circulating erythrocytes and leukocytes, slightly increased bromosulphalein retention (dexamethasone-21-phosphate dogs only) and increased urinary nitrogen and electrolyte excretion. Drug induced morphological changes were seen in liver, skeletal muscle, cardiac muscle, lymph nodes and the adrenal cortex.

48. *The Effect of Carbaryl on Blood Glucose and Liver and Muscle Glycogen in Fed and Fasted Rats.* R. A. ORZEL and L. R. WEISS, Division of Toxicological Evaluation, Food and Drug Administration, Washington, D. C. (Sponsor: K. H. Jacobson.)

The changes in blood glucose levels in fed and fasted rats following intraperitoneal administration of 5 and 25 mg/kg doses of carbaryl (1-naphthyl N-methyl carbamate), and the behavior of liver and muscle glycogen at these dosage levels were determined.

These two dosages were selected because administration of 5 mg/kg to rats produced minimal, if any, cholinergic signs, while pronounced pharmacological effects appeared at the 25 mg/kg dose. A significant rise in blood glucose of fed and fasted rats occurred following intraperitoneal injection of 5 and 25 mg/kg doses of carbaryl. No significant changes in liver glycogen of fed and fasted rats occurred following administration of 5 or 25 mg/kg of carbaryl. However, there was a definite decrease in muscle glycogen of fed rats at both the 5 and 25 mg/kg doses, but there was no change in the muscle glycogen of fasted rats.

The hyperglycemic response following injection of carbaryl seems to be independent of the level of liver glycogen in fed and fasted rats.

49. *Hemorrhagic Involvement in the Toxicity of Sodium 4-Hydroxybutyrate.* G. OWEN, A. DERVINIS, and H. P. K. AGERSBORG, JR., Wyeth Laboratories, Inc., Radnor, Pennsylvania. (Sponsor: R. F. Tislow.)

Sodium 4-hydroxybutyrate, a central nervous system depressant, is capable of inducing sleep when administered in large doses. During toxicological studies, the daily administration of 1600 mg/kg intramuscularly to rats resulted in external bleeding from the urethral orifice in 7 out of 20 animals on the first and second days, but not thereafter despite continued treatment. Twenty rats autopsied 24 hours after a single intramuscular injection of 1600 mg/kg revealed blood in the urinary bladder and petechial hemorrhages in the bladder wall in 11 of the animals. Serial autopsies performed during daily drug administration showed hemorrhages in the bladder and sometimes in the gastrointestinal tract on the first four days only. The lesions occurred more in males than in females but did not occur at intramuscular dose levels below 400 mg/kg or after oral administration of 1600 mg/kg.

Eight unanesthetized rats cannulated for blood pressure measurement did not show blood pressure changes when injected with 1600 mg/kg of the drug, but 3 did show the hemorrhagic phenomena. Hematological values obtained in 10 rats on the 1st, 2nd, 3rd, and 6th days during daily drug treatment showed a temporary decrease of hemoglobin, total red cells, total white cells and thrombocytes in about one-third of the animals. The data suggest that the hemorrhagic effects are due to a temporary depression of hematopoietic activity.

50. *Studies on the Delayed Toxicity of Phthalanilides and Other Compounds.* P. E. PALM, W. I. ROGERS, D. W. YESAIR, and C. J. KENSLER, Life Sciences Division, Arthur D. Little, Inc., Cambridge, Massachusetts.

The phthalanilides are a new group of cancer chemotherapeutic agents which show strong activity against a broad spectrum of experimental tumor systems. With several of these compounds it has been found that low doses produced renal damage which was not apparent until two or more weeks after drug administration had ceased. In dogs and monkeys administered daily intravenous dosages for 14–28 days, or a single massive dosage by the intramuscular route, we have observed delayed renal toxicity as evidenced by increases in blood urea nitrogen with four of these agents. Renal damage appeared to be reversible at lower dosage levels in several animals observed for up to six months after the last dose. These findings were later confirmed by histopathology. It appears that monkeys can tolerate a higher drug concentration in the kidney without functional impairment than can dogs.

Physiological disposition studies indicated the greatest quantities of drug were found in kidneys with large amounts also in liver and spleen immediately after the last dose. Oculomotor muscles contained several times as much drug as diaphragm and gastrocnemius muscles. Considerable drug remained in kidneys of animals sacrificed after 5–6 months of observation. Delayed toxicity produced by other compounds will also be discussed.

51. *Human Caffeine Fatality.* R. F. PARISH, C. FRICK, A. B. RICHARDS, and R. B. FORNEY, Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana.

It has been estimated that 5–10 g of caffeine taken orally may be fatal in man (Krantz and Carr, *Pharmacologic Principles of Medicine,* 5th Edition). However, distribution data in body tissues of fatal doses are not known. The opportunity for study of tissue distribution was presented to our laboratory in a suicidal case possibly involving caffeine. Various methods of identification and quantitation of caffeine were performed with the submitted tissues using ultraviolet and infra-red spectrum, spot tests, and thin-layer chromatography. The quantitative results obtained from the various tissues in mg of caffeine alkaloid per 100 g of tissue were: kidney, 12.96; liver, 32.90; brain, 7.47; stomach, 150.66. Comparative studies of tissue distribution levels of fatal dosages of caffeine were extended using rats. Ten male rats averaging 200 g in weight were given an oral dosage of 1 g/kg of caffeine alkaloid suspended in a 2% solution of acacia, which was kept warm to insure uniform suspension. The time of administration was recorded as well as the time of death. Immediately following death, kidney, liver, brain, and blood samples were obtained for analysis. Brain and kidney tissues were pooled for analysis whereas blood and liver analyses were performed separately. Identical means of identification and quantitation were used in determining rat tissue levels as in the suicidal case. The following results were obtained for rat tissue distribution expressed as mg of caffeine alkaloid per 100 g of tissue: kidney, .879; brain, .64; blood (average of 10 samples), 1.03; liver (average of 10 samples), 1.41. If distribution data from the rat experiment are compared to those of the suicidal case, it is apparent that the distributions of caffeine in both are in the same sequential order

52. *Organ Weights and Water Levels in Albino Rats Following Fortnight Starvation.* J. M. PETERS and E. M. BOYD, Department of Pharmacology, Queen's University, Kingston, Ontario, Canada.

Reduced food intake and weight loss are common findings in toxicity studies. To establish the effects of starvation on organ weights and water levels, food intake was varyingly reduced in adult, female, albino rats. Water intake was not limited. Autopsy followed a fortnight starvation with the loss of initial body weight ranging from 5–40%. The gram weight of cardiac and pyloric parts of the stomach and of brain were most resistant to starvation and increased as percent of body weight. Small bowel, cecum, colon, heart, kidney, lung and skin did not consistently change in percent of body weight. Liver, spleen and thymus decreased in percentage with increasing loss of body weight. Muscle (left abdominal wall) and submaxillary glands showed a decrease after an initial increase in percent of body weight while ovaries and adrenal glands showed the reverse. There was a general tendency towards lower water levels at 10 and 20% loss of body weight and

higher levels at greater loss of body weight. Exceptions were spleen (continuous decrease in water level), and muscle, carcass and pyloric part of stomach (early rise).

53. *Effect of Hypothermia on α-Naphthylisothiocyanate-Induced Cholestasis.* G. L. PLAA and R. J. ROBERTS, Department of Pharmacology, College of Medicine, University of Iowa, Iowa City, Iowa.

Previous work in this laboratory has shown that hypothermia can alter markedly the response of the liver parenchyma to CCl_4. Presently, we have obtained evidence which indicates that hypothermia can also modify the effects of another hepatotoxin, α-naphthylisothiocyanate (ANIT). Male Sprague-Dawley rats (200–300 g) were treated with ANIT (300 mg/kg, p.o.); 24 hours later the bile duct was cannulated and bile flow measured. Bilirubinuria was present in all rats 24 hours after ANIT, suggesting the presence of hyperbilirubinemia. Rats given ANIT become hypothermic if they are placed in a Bollman-type rat restrainer for the 24-hour postadministration period, particularly if a fan is employed to conduct away body heat. In 18 rats the mean rectal temperature just prior to bile duct cannulation was 28°C; only 2/18 of these rats exhibited cholestasis (no bile flow). On the other hand, segregated, but unrestrained, ANIT-treated rats (9 animals) had a mean rectal temperature of 34.5°C; 7/9 of these rats exhibited cholestasis. When restrained, ANIT-treated rats were placed in an incubator to prevent hypothermia, 5/5 rats (mean rectal temperature, 38°C) exhibited cholestasis. Therefore, the cholestatic effect of ANIT seems to be dependent upon body temperature.

54. *A Possible Cholinergic Influence on the Action of Certain Central Nervous System Depressants.* C. D. PROCTOR, Departments of Pharmacology and Psychiatry, Meharry Medical College, Nashville, Tennessee.

In previous reports the author and his co-workers have reported that extension of both barbiturate and tranquilizer action could be effected by prior administration of low doses of certain organophosphorus anticholinesterase compounds (Proctor *et al., Toxicol. Appl. Pharmacol.* **6**, 1, 1964; Proctor, *Arch. Intern. Pharmacodyn.* **150**, 41, 1964).

Using methods analogous to those employed in the references cited, the possibility of other CNS-active cholinergic agents effecting extension of the response to CNS depressants has been investigated. Each experimental group consisted of ten 20–30 g albino mice, made up of equal numbers of each sex. Hydration volume control, using physiological saline "sham" injections where necessary, was maintained in all animals.

With careful attention to dosages and latent periods, results analogous to those yielded with the organophosphorous compounds were obtained. For example, 0.5 mg/kg physostigmine, given s.c. 15 minutes after 20 mg/kg atropine methyl bromide i. p. and 20 minutes prior to 50 mg/kg hexobarbital i.p., converted this subthreshold hexobarbital dosage (control mean sleep time: 0 minutes) to threshold response (mean sleep time ± S.E.: 15.0 ± 0.63 minutes). Substituting 5 mg/kg atropine sulfate i.p. for the atropine methyl bromide in this experiment yielded results like those obtained with the controls (mean sleep time: 0 minutes). Analogous results were obtained in experiments employing pentobarbital in place of hexobarbital.

In experiments involving subthreshold chloroform anesthetic dosage (10-minute exposure of each mouse to about 9710 ppm of chloroform), 5 mg/kg pilocarpine s.c., given 10 minutes prior to the chloroform and 10 minutes after 20 mg/kg atropine methyl bromide, converted subthreshold chloroform effect (control mean sleep time: 0 minutes) to threshold response (mean sleep time ± S.E.: 24.2 ± 2.01 minutes). Methacholine, 10 mg/kg s.c., given in place of the pilocarpine in this experiment yielded no enhancement of the response to chloroform (mean sleep time: 0.8 minutes). Substitution of 10 mg/kg atropine sulfate i.p. for the atropine methyl bromide significantly reversed the pilocarpine enhancement of chloroform action (mean sleep time ± S.E.: 5.5 ± 1.2 minutes). Analogous results were obtained in experiments employing ether in place of chloroform.

55. *The Effect of Depressed Body Weight Gain on Relative Organ Weights in Rats.* E. B. ROBBINS, R. M. SMALL, and R. C. ANDERSON, Lilly Toxicology Laboratories, Greenfield, Indiana.

In chronic toxicity studies, organ weights are usually expressed as organ weight/body weight ratios which disregards the possibility that organ weights are also a function of age. In these

studies when there is a marked depression of weight gains in treated groups, there might incorrectly appear to be a drug effect unless the ratios are adjusted for this effect of reduced weight.

A simple, straight-line relationship evolves when the independent variable, x, is normal weight divided by reduced (or, under certain conditions, increased) body weight, and the dependent variable, y, is the organ weight/body weight ratio. When 1.0 is subtracted from each x, the parameter, a, becomes zero and $y = bx$, wherein b is the appropriate constant for adjusting ratios.

To estimate these constants for various organs in rats, a three-month study was designed in which *ad libitum* feeding in one group and restricted feeding in two others produced 100, 70 and 40% rates of gain, respectively.

Constants obtained in this study were:

	Liver	*Kidney*	*Heart*	*Spleen*	*Thyroid*	*Adrenals*
Male	—.37	.093	.028	—.022	1.80	6.29
Female	—.97	.175	.074	.090	2.64	8.12

56. *Treatment of Acute Cyanide Intoxication.* C. L. Rose and K. K. Chen, Department of Pharmacology, Indiana University School of Medicine, Indianapolis, Indiana.

Thirty years ago we demonstrated that the combination of sodium nitrite and sodium thiosulfate had a potentiation of antidotal action against acute cyanide poisoning in dogs. (*Am. J. Med. Sci.* **188**, 767, 1934). The nitrite alone detoxified 5 LD_{50}'s of sodium cyanide; the thiosulfate alone, 3 LD_{50}'s; and the combination, 18 LD_{50}'s. The function of the nitrite is to form methemoglobin, which conjugates with CN ions to become cyanmethemoglobin. Thiosulfate acts, in collaboration with the enzyme rhodanese, to oxidize CN ions to thiocyanate, which is excreted in the urine. Because it causes death rapidly, cyanide poisoning in man must be treated promptly; first with repeated inhalations of amyl nitrite followed by intravenous injection of an aqueous solution of sodium nitrite, and this in turn by a solution of sodium thiosulfate, without removing the needle from the vein. Using this method, sixty-three clinical cases, with only one death, have been reported in the literature. Hydroxycobalamine has some antidotal action in cyanide poisoning, but it was not possible to administer such amounts as to save dogs' lives from multiple LD_{50}'s. Several cobalt salts were similarly tried, and were found ineffective.

57. *Toxicity Studies on Ammonium Sulfamate: Review of the Literature.* D. E. Rosen and C. J. Krister, Industrial and Biochemicals Department, E. I. du Pont de Nemours and Company, Wilmington, Delaware.

A review is presented of the toxicity investigations which have been conducted with ammonium sulfamate, the active ingredient of a commercial, contact-type weed and brush killer. Acute and subacute data on a number of animal species are included, as well as findings on skin and eye tests. An evaluation of results is presented.

58. *Long-Term Safety Evaluation Studies on Benzyl N-Benzyl Carbethoxyhydroxamate.* R. A. Scala, T. W. Tusing, S. Margolin, and F. M. Berger, Hazleton Laboratories, Inc., Falls Church, Virginia, and Wallace Laboratories, Division of Carter Products, Inc., Cranbury, New Jersey.

Oral administration of benzyl *N*-benzyl carbethoxyhydroxamate (W-398) to rabbits and rats fed hypercholesterolemic diets resulted in a reduction of blood cholesterol levels and, in rabbits, led to reversal of atherosclerotic lesions (Berger *et al., Proc. Soc. Exptl. Biol. Med.* **114**, 337, 1963). No untoward effects were noted in the test animals at the effective doses, and long-term studies were conducted in rats and dogs to evaluate the effects associated with chronic administration of the compound.

Groups of 25 male and 25 female albino rats received W-398 at dietary levels of zero (control), 0.05, 0.1, 0.25, and 0.5% for 78 weeks. The criteria evaluated were general appearance, body weight, food consumption, food efficiency, hematology, clinical chemistry, urine analysis, and gross and microscopic pathology.

Grossly observed signs of effect in the rat study at the 0.5% level included decreased growth, food consumption and food efficiency in the females during the first 26 weeks, an early decrease

in blood sugar followed in the females by an increase, and an increase in liver weight and/or ratio to body weight in both sexes.

The blood sugar level was also depressed in the female rats at 0.25% at 26 weeks and in the male rats at 0.05% at 78 weeks. There was an increase in blood sugar in the females at 0.25% at 78 weeks. The liver weight was increased in male rats at the 0.1% level at 78 weeks, and at the 0.25% level at 13 and 78 weeks and in the female rats at the 0.25% level at 78 weeks.

Grossly observed signs of effect in the dog study were confined to the highest level, and consisted of an increase in liver and kidney weight ratios. The histologic findings in both species will be presented.

59. *Biochemical and Electron Microscopic Changes Observed in Rats and Monkeys Medicated Orally with Methoxychlor.* 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.

Changes in hepatic metabolism and hepatic subcellular structure were found in rats and monkeys medicated orally with large doses of methoxychlor [*2,2-bis*(*p*-methoxyphenyl)-1,1,1-trichloroethane]. Early biochemical alterations included enhancement of hepatic microsomal demethylase activity. Electron microscopy revealed an increase in hepatic endoplasmic reticulum. These changes appeared to be transient as enhanced enzyme activity and proliferation of the endoplasmic reticulum were not found in animals treated with methoxychlor over a prolonged period of time.

Enlargement of hepatic mitochondria was observed early in the methoxychlor treatment. This change may have been related to later alterations found in liver lipid patterns. These included a depression in the quantity of triglycerides and the suggestion of an increase in the amount of cholesterol and diglycerides. An increase in the incorporation of labeled linoleic acid into phospholipids and diglycerides was also suggested by radioactive tracer studies.

Possible relationships between biochemical alterations and observed electron microcopic changes will be discussed.

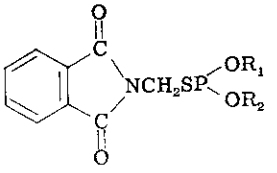
60. *Toxicity Studies of Ammonium Sulfamate.* H. SHERMAN and E. F. STULA, Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, Newark, Delaware.

Ammonium sulfamate has been fed to male and female rats, starting with twenty-nine day old animals, for 19 months at dietary concentrations of 0, 350 and 500 ppm of an adequate nutritional diet without any clinical or nutritional evidence of toxicity.

A three-generation reproduction study has been conducted with rats receiving 0, 350 and 500 ppm ammonium sulfamate in the diet without any evidence of toxicity as measured by histopathologic appraisal and reproduction and lactation indices.

61. *Toxicity of Imidan® and Five Analogs to the Chick, Rat, and House Fly.* M. SHERMAN, R. HERRICK, M. T. Y. CHANG, and J. J. MENN, College of Tropical Agriculture, University of Hawaii, Honolulu, Hawaii.

The compounds investigated have the structure



they were: Imidan® (CH_3, CH_3); R5723 (CH_3, C_2H_5); R5092 (CH_3, $CH_2CH_2CH_3$); R5722 (C_2H_5, $CH_2CH_2CH_3$); R5725 [CH_3, $CH(CH_3)_2$]; and R5724 [C_2H_5, $CH(CH_3)_2$]. Acute toxicity to the chick, rat, and house fly were compared. Imidan was the most toxic compound to the house fly and the least toxic to the chick and rat. Subacute toxicity to the chick with the effect on blood plasma cholinesterase was also investigated. No clear correlation between cholinesterase inhibition and toxicity of this limited series of materials could be determined.

62. *Crooked Calf Syndrome.* J. L. SHUPE, W. BINNS, and L. F. JAMES, Agricultural Research Service, Animal Disease and Parasite Research Division, U. S. Department of Agriculture, Logan, Utah.

Many congenital malformations have been associated with cattle. Based on scientific findings of some conditions and general field observations of others, most of these congenital anomalies were assumed to be hereditary. Because of this, some livestockmen have in the past 40–50 years disposed of valuable breeding stock and altered their management operations. Although breeding stock has been changed, and, in some instances, different breeds of cattle obtained and used on various range areas, the crooked calf syndrome still persists.

To date, it appears that a number of causes of congenital malformations in cattle have been or can be confused with the crooked calf syndrome. The cause of some of these conditions has been determined while others are of unknown etiology.

Varied opinions and thinking exists relative to the cause, signs, and lesions associated with the crooked calf syndrome. Based on information accumulated to date, the condition commonly seen in the western states and Alaska can be differentiated from other diseases with which it has been confused. The disease appears to be non-hereditary in nature, but is associated with the ingestion of some toxic substance(s) during some stage of embryonic or fetal development. Calves characteristic of the crooked calf syndrome, as commonly observed on the range, have been produced experimentally.

63. *Toxicological Studies on Beryllium Oxides.* H. C. SPENCER, J. C. JONES, S. E. SADEK, K. B. DODSON, and A. H. MORGAN, Biochemical Research Laboratory, The Dow Chemical Company, Midland, Michigan.

Several series of beryllium oxides were prepared by calcining crystalline α-$Be(OH)_2$, amorphous β-$Be(OH)_2$, and $BeSO_4 \cdot 4H_2O$ at temperatures varying from 500–1600°C for periods of time varying from a few minutes to several hours. In general, as the calcining temperature and time were increased there was a trend toward a decrease in surface area, an increase in crystallite dimensions, an increase in crystallinity, and an increase in density. Special studies were made of surface characteristics, particle size relationships, and solubility under various conditions.

Striking differences were observed in the biological response of animals following intratracheal administration depending upon the sample of beryllium oxide used. The lungs of rats treated with beryllium oxide, produced by calcining α-$Be(OH)_2$ for ten hours at 500°C, showed a widely dispersed focal pneumonitis of granulomatous nature. The lesion has a dense central core of proliferated histiocytes containing beryllium oxide particles surrounded by endothelioid cells with scattered lymphocytes and plasma cells enmeshed in a fine reticulum. Multinucleated giant cells, fibrosis and collagen formation were irregular features of the lesion and usually appeared two to three months following the injection. In many cases, the lung parenchyma showed thickening alveolar walls with compensatory emphysema. Tumors developed in the lungs after seven to eight months. Analyses showed a relatively high level of beryllium in the liver, kidney and bone from these rats. In contrast, the lungs of rats treated with beryllium oxide, produced by calcining α-$Be(OH)_2$ for 10 hours at 1600°C, showed minimal pathological changes. The beryllium oxide particles were observed lying free in the alveoli or within the septal walls with minimal infiltration of lymphocytes or plasma cells. Very little, if any, beryllium was found in the liver, kidney and bone from these animals.

The significance of these findings will be discussed.

64. *Pulmonary Responses of Rats to Turpentine Vapor.* F. SPERLING and W. MARCUS, Howard University Medical School, Washington, D. C.

Rats were exposed individually to turpentine vapor in the inhalation system previously discussed (*Toxicol. Appl. Pharmacol.* **6**, 360, 1964). The animals were exposed for 2, 4, or 6 hours. Various concentrations were used. Respiratory rates and patterns were periodically monitored before and during exposure, as well as immediately after for the survivors. Similar determinations were made for a group of control rats which received only air for six hours at the same flow rates and pressure as the experimental rats which received a mixture of air and air-vapor. The controls

showed small increases in minute and tidal volumes. After 2 and 6 hours the surviving experimental rats showed decreases in both these responses, some of which were very large. Gross autopsies did not reveal any lung lesions with which such changes could be associated. LC_{50}'s after 1, 2, 4 and 6 hours of exposure were 19.9, 16.5, 13.7, and 11.5 mg/l, respectively. The log intervals between the LC_{50}'s were proportionately equal. The regression lines were all parallel with a slope of 1.2. These two factors serve to indicate that the mechanism of lethal action in the period of 1–6 hours of exposure was similar.

65. *Comparison of Biological and Chemical Assay Methods for Estimating Acetylcholine Concentration in Rat Brain.* W. B. STAVINOHA and L. C. RYAN, Civil Aeromedical Research Institute, Oklahoma City, Oklahoma.

Recently a chemical assay for acetylcholine was developed which utilizes gas chromatography (Stavinoha *et al., Life Sci.* **3**, 689-693, 1964). In the present study this chromatographic method and a bioassay utilizing the guinea pig ileum were compared. Rat brains were frozen in a dry ice-ether mixture and the acetylcholine was extracted using the method of Crossland (*Methods Med. Res.* **9**, 125-129, 1961). The extract of each rat brain was analyzed by both methods. The results were as follows:

Treatment	Number of Animals	Gas Chromatograph[a]	Guinea Pig Ileum[a]
Unanesthetized controls	4	3.81 ± 0.10	2.65 ± 0.11
Pentobarbital 50 mg/kg, i.p.	8	4.22 ± 0.36	3.27 ± 0.45
Phosdrin 1.5 mg/kg, i.v.	4	5.44 ± 0.20	4.54 ± 0.10

[a] Results are expressed as acetylcholine, μg/g ± S.D.

These results indicate that the chemical method is applicable to the estimation of acetylcholine in biological samples, although it consistently gives results which are slightly higher than those obtained by biological assay.

66. *Safety Evaluation of Methoxychlor in Human Volunteers.* 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.

Following a number of basic studies in rodents and monkeys, a human volunteer study was planned. Capsules and placebos were prepared and four groups of patients were given daily doses of 0.5, 1 and 2 mg/kg of methoxychlor [2,2-*bis*(*p*-methoxyphenyl)-1,1,1-trichloroethane] and a placebo for eight weeks. These patients were carefully observed clinically, and repetitive samples for hematology, biochemistry and urinalysis were obtained. At the end of the experiment, biopsies of fat, testis, bone marrow, liver and small intestine were obtained for multiple basic studies including histochemistry, electron microscopy and gas-liquid chromatographic lipid analyses.

These studies indicate the safety of methoxychlor in dosages 200 times the maximum permissible limit.

67. *Chronic Toxicity Study on Interaction in a Combination of Food Additives and Pesticides.* J. M. TAYLOR, E. C. HAGAN, and R. T. HABERMANN, Division of Toxicological Evaluation, Food and Drug Administration, Washington, D. C.

The average human diet contains many chemical additives. The safety of the additives has been demonstrated on an individual basis, but little work has been done on the effects of combinations of food additives. A chronic (2-year) rat feeding study was designed to determine whether combining a number of flavoring compounds (allyl heptylate, anethole, amyl butyrate, cinnamic aldehyde, citral, ethyl methyl phenyl glycidate, eugenol, methyl salicylate) and pesticides (DDT,

aldrin, pyrethrin, piperonyl butoxide, malathion, 2,4-D) would result in interaction. Five test levels of the combination were fed: the lowest level contained each component in an amount equivalent to its occurrence in the human daily diet and the highest level contained each component in an amount slightly less than that producing effects in individual toxicity studies. Two control groups were utilized: one received the basal diet, and the other received the basal diet with the pesticide components of the combination in an amount equal to that at the highest test level. With the exception of growth depression at the highest test level, the test groups and pesticide control group were similar in all respects to the untreated control group.

68. *Methoxychlor Toxicity: Comparative Studies in the Beagle Dog and Miniature Swine.* A. S. TEGERIS, F. L. EARL, H. E. SMALLEY, and J. M. CURTIS, Division of Pharmacology, Bureau of Scientific Research, Food and Drug Administration, U.S. Department of Health, Education and Welfare, Washington, D. C.

Thirty pure-bred beagle dogs and thirty Hormel miniature swine were distributed in four groups each. One group was given 1 g/kg of methoxychlor, another group 2 g/kg, and a third group 4 g/kg, with twelve animals from each species serving as controls. The pesticide was administered mixed in the feed and the experiment was conducted for six months.

The dogs showed a dose-related increase in the following parameters during the experiment: alkaline phosphatase, SGOT, SGPT and ISDH. Clinically, they exhibited a dose-related decrease in weight as well as CNS disturbances, with only one animal from the high-dose group surviving for the duration of the experiment. At autopsy the only pertinent gross pathology seen was in the small intestine, the mucosa of which exhibited a marked hemorrhagic involvement in the dosed animals.

The miniature swine showed a mild dose-related elevation in blood urea nitrogen. Clinically, the animals receiving the pesticide developed breast enlargement. At autopsy, there was enlargement of the uteri of the dosed females, as well as striking changes of chronic renal damage in both sexes.

69. *Protective Effects of Low Doses of Cadmium Chloride against Subsequent High Oral Doses in the Rat.* C. J. TERHAAR, E. VIS, R. L. ROUDABUSH, and D. W. FASSETT, Laboratory of Industrial Medicine, Eastman Kodak Company, Rochester, New York.

Testicular atrophy and death from orally administered $CdCl_2$ can be prevented by pretreatment with small oral doses of cadmium chloride.

Oral doses of 200 and 400 mg/kg of $CdCl_2$ killed 9/12 and 9/10 rats respectively, while 1/11 and 0/9 were killed when 20 mg/kg was given 24 hours prior to the high doses. At 800 mg/kg, 10/10 rats were killed regardless of pretreatment. However, the pretreated rats lived one to three days after the large dose, while those not pretreated died within 24 hours. Doses as low as 0.01 mg/kg given 24 hours prior to 100 mg/kg protected against massive testicular atrophy.

When 20 mg/kg was given not less than seven hours and not more than two weeks prior to 100 mg/kg, testicular atrophy was prevented.

Total anuria was noted for 24 hours in 6/6 rats given a single oral dose of 100 mg/kg, while in rats pretreated with 20 mg/kg only 1/6 was anuric. On the two successive days, 5/6 of the singly dosed animals remained anuric while all of the pretreated animals micturated.

70. *New Horizons in Space Cabin Toxicology.* A. A. THOMAS, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio.

The effects of truly uninterrupted, prolonged and continuous exposure to contaminants in the sealed cabin are known to be extremely deleterious, even at normal atmospheric pressure and composition. Since there are reasons to believe that reduced pressure atmospheres which, by necessity, are rich in oxygen, do cause pulmonary irritation and morphologic changes, it became necessary to obtain basic information on the true impact of such environment upon the resistance of the living organism to specific and non-specific toxic stress. This information must be developed to support the engineering design of long-range space systems. The facility described is radically different from the usual inhalation chambers. In addition to its low pressure capability,

it has the flexibility required for space cabin simulation studies. Life support systems and subsystems can be studied *in toto,* psychopharmacological studies can be performed without interrupting exposure, and maximum visibility has been incorporated to aid clinical and behavioral observations. If necessary, sensory deprivation can be simulated, or the day-night cycle can be reversed. The specific medical and safety considerations which are required to operate these chambers are also discussed.

71. *Proteolytic Activity of Snake Venoms.* A. T. Tu, G. P. James, and A. Chua, Department of Chemistry, Utah State University, Logan, Utah.

Proteolytic enzyme activities of 22 snake venoms were investigated by using the synthetic substrates, p-toluene sulfonyl-L-arginine methyl ester (TAME), N-benzoyl-L-arginine ethyl ester (BAEE), and N-benzoyl-L-tyrosine ethyl ester (BTEE). Eleven venoms of *Crotalidae* used are: *Agkistrodon actus, Trimeresurus mucrosquamatus, Crotalus viridis oreganus, Crotalus atrox, Bothrops jararaca, Crotalus adamanteus, Ancistrodon contrix mokasen, Trimeresurus okinavenisis, Ancistrodon contortrix contortrix, Ancistrodon contortrix laticinctus,* and *Ancistrodon piscivorus piscivorus.* Three venoms of *Viperidae* used are: *Vipera russellii formosensis, Vipera ammodytes,* and *Bitis arietans.* Eight venoms of *Elapidae* investigated are: *Naja naja atra, Naja naja samerensis, Naja haje, Naja melanoleuca, Bungarus multicinctus, Dendraspis angusticeps, Oxyuranus scutellatus scutellatus* and *Oxyuranus scutellatus canni.*

Venoms of *Crotalidae* and *Viperidae* hydrolyzed TAME and BAEE but none of the *Elapidae* venoms investigated showed enzyme activity. When pancreatic trypsin was used in the presence of ovomucoid and soybean trypsin inhibitors, the hydrolysis of TAME and BAEE were inhibited completely. However, none of the venom proteolytic activity was inhibited in the presence of these substances. This suggests that the proteolytic enzyme present in *Crotalidae* and *Viperidae* is similar to trypsin but not identical. Additional evidence is that they do not hydrolyze lysylglycine. Only 2 venoms, *Trimeresurus mucrosquamatus* and *Vipera russellii formosensis,* showed weak activity toward BTEE. Therefore, most of the venoms do not have chymotrypsin-like activity.

It was found also that various venoms contain a number of di- and tripeptidases. None of the venoms hydrolyzed glycylproline, glycylglycine, serylglycine, leucylglycylphenylalanine, and tetraglycine. However, phenylalanylglycine, leucylglycine, tryosylglycine, alanylphenylalanine, glycylleucine, glycyltyrosine, glycylleucyltyrosine, alanylglycylglycine, and leucylglycylglycine were hydrolyzed by certain venoms. Each venom demonstrated different peptidase activities. Glycylleucyltyrosine was a substrate of particular interest because some venoms hydrolyzed only glycylglycine linkage, others hydrolyzed leucyltyrosine linkage, and still others hydrolyzed both linkages.

All venoms investigated contain neither carboxypeptidase nor aminopeptidase.

72. *Enzymatic Dechlorination of Certain Chlorinated Compounds.* R. A. Van Dyke, Biochemical Research Laboratory, The Dow Chemical Company, Midland, Michigan. (Sponsor: M. B. Chenoweth.)

Two compounds, 2,2-dichloro-1,1-difluoroethylmethyl ether (methoxyflurane) and 1,1,1-trifluoro-2-bromochloroethane (halothane) labeled either with chlorine-36 or carbon-14, have been studied *in vivo* in rats. These were found to undergo biotransformation with the exhalation of $^{14}CO_2$ from ^{14}C-methoxyflurane and the excretion in the urine of ^{14}C-metabolites and chloride-36 from ^{14}C- or ^{36}Cl-halothane and methoxyflurane. The *in vivo* metabolism of these compounds was found to be enhanced by pretreatment of the animal with phenobarbital or 3-methylcholanthrene. *In vitro* studies were carried out using rat liver slices. Under these conditions, the isotopically labeled anesthetics were also found to undergo biotransformation with the formation of $^{14}CO_2$ and chloride-36 from the appropriately labeled methoxyflurane. Halothane was found to be dechlorinated but was not oxidized to CO_2. The dechlorination of these materials has been found to require viable liver slices since heated slices are not active. Thus, the dechlorination is enzymatic. The dechlorination also requires oxygen and is inhibited by cyanide. The dechlorination was also found to proceed in a microsomal preparation and to require reduced NADP. The dechlorination reaction also exhibits a substrate sensitivity which will be discussed. This de-

halogenation will be compared with reports of other dehalogenations which have appeared in the literature.

73. *Toxicology of Progestagens with Special Reference to Dimethisterone and Megestrol Acetate.* J. H. WEIKEL, JR., and A. G. WHEELER, Mead Johnson Research Center, Evansville, Indiana.

The effects of 6 potent progestagens upon target organs of rats were compared. The progesterone derivatives megestrol acetate (I), melengestrol acetate (II), and medroxyprogesterone acetate (III) were similar in causing depression of adrenals, thymus, uterus, ovaries, and leukocytes. The most potent in all parameters was II while I was least active. Norethynodrel (IV) and norethindrone (V), the 19-nortestosterone derivatives, had less effect on the thymus and little on the adrenals and leukocytes. Both IV and V depressed growth and stimulated the uterus. Dimethisterone (VI), a testosterone derivative, caused responses similar to I and III. Chronic toxicity studies showed I to cause obesity and adrenal depression in dogs while VI caused little change. In rats, however, I was very well tolerated while VI depressed growth and caused fat deposits.

74. *Some Aberrant Effects in Control Groups during Repeated-Dose Toxicological Studies and a Note on a Valid "N" in Fertility Studies.* C. S. WEIL, Mellon Institute, Pittsburgh, Pennsylvania.

The results of the quantitative criteria of effect in repeated-dose toxicological studies are usually compared by the application of appropriate statistical procedures. The aim is to denote which criteria result in alterations not attributable to chance. Hence, the data of the treated groups are compared to the concurrently obtained data of a specific control group of animals.

If the probability levels of 0.05 or 0.01 are used, differences that are merely random occurrences may be expected to occur 5 or 1 times in 100. These are readily apparent if changes are found only at the lower dosage levels of a chemical being tested but not at higher levels. However, even if only the high dosage level differs statistically-significantly from the control group, common sense must be applied. Instances where the aberrant results were definitely attributed to changes in the controls will be illustrated. These occurred in the criteria of body weight gain, mortality, tumorigenicity, hematological and biochemical measurements.

An indication of alternate methods of comparisons of some data in fertility studies will be presented. The exaggeration in interpretation of results using an incorrect "N" for number of observations will be discussed.

75. *Carcinogenesis by Simultaneous Action of Several Agents.* J. H. WEISBURGER, Z. HADIDIAN, T. N. FREDRICKSON, and E. K. WEISBURGER, Carcinogenesis Studies Branch, National Cancer Institute, Bethesda, Maryland, and Mason Research Institute, Worcester, Massachusetts.

Experiments were performed on the effect of binary mixtures of either a carcinogen and another agent of special interest, or of two carcinogens affecting normally different target organs.

Weanling male and female Fischer rats received by gavage appropriately determined doses of the agents singly or in mixture five times per week for a maximum of 260 doses. The animals were held for 9–18 months. The compounds and the daily doses were: *N*-2-fluorenylacetamide (1 mg) and/or one of the following: DDT (10 mg), CCl_4 (10 mg), dieldrin (0.1 mg), epoxy soya oil (100 mg), diethylnitrosamine (0.3 mg), dibutylnitrosamine (3 mg), diamylnitrosamine (3 mg), also 4-phenylacetanilide (2 mg) and/or CCl_4 (10 mg). All sacrificed animals and those dying during the experiment were necropsied. Selected organs were weighed and examined microscopically.

N-2-Fluorenylacetamide alone gave hepatoma in about 65% of the males and 10–35% of females in approximately 11 months. Diethylnitrosamine produced hepatoma in virtually all males and females in 9 months. Dibutylnitrosamine yielded hepatoma in practically all males and females and also tumors in lung, esophagus, and bladder after 11 months.

The percentage of hepatomas was increased or the latent period was decreased by joint administration of *N*-2-fluorenylacetamide and the following agents: DDT, CCl_4, diethylnitrosamine, dibutylnitrosamine, and diamylnitrosamine. Epoxy soya oil may have a slight enhancing effect on the carcinogenicity of *N*-2-fluorenylacetamide, but dieldrin had none. Mammary carcinoma seemed slightly higher in females receiving CCl_4 and 4-phenylacetanilide.

76. *Pharmacological Interaction of Drug Effects in Rats Chronically Intoxicated with Parathion.* L. R. WEISS, Division of Toxicological Evaluation, Food and Drug Administration, Washington, D. C. (Sponsor: E. C. Hagan.)

Six months' feeding of parathion to female rats at 25 ppm reduces brain cholinesterase activity to 50–60% of normal. This dosage does not influence mortality over a six-month period. These animals and corresponding controls were used to study the pharmacological activity of a number of drugs in order to assess their interaction with pesticides that inhibit cholinesterase.

For this purpose, hexobarbital sleeping time, morphine analgesia, phenobarbital anticonvulsant activity against electroshock, chlorpromazine hypothermia, and the heart rate in controls and in animals which were fed parathion chronically were evaluated. The effect of chlorpromazine on hexobarbital-induced sleeping time in animals intoxicated by parathion was also used as a method to demonstrate potentiation or other interaction. In none of these studies did statistical analysis show significant differences between parathion-treated and control animals, as regards response to the various drugs. In this experiment, potentiation or other drug interactions which have been reported following single, high doses of cholinesterase inhibitors could not be demonstrated at feeding levels of parathion causing minimal signs of toxicity in chronically treated animals.

77. *Further Studies of the Toxicity of the Venom of Pogonomyrmex Barbatus, the Large Brown Desert Ant.* M. W. WILLIAMS and C. S. WILLIAMS, Veterans Administration Hospital, Tucson, Arizona.

The venom of the large desert ant, *Pogonomyrmex barbatus,* has been collected by a new method and subjected to several toxicological tests. The collection method consisted of subjecting large numbers (*i.e.,* 150 or more) of the intact animals in distilled water to high-voltage repetitive shocks from a Harvard inductorium, over a period of fifteen minutes. Venom was collected in the water and after filtering through standard qualitative filter paper, the slightly opalescent solution was centrifuged.

Thirty-one dry weight determinations of 4-ml aliquots from venom extract of 13 separate groups of 150 or 200 ants each (total 2350 animals) resulted in mean solids content of 0.55 $\pm$ 0.02 mg per 4-ml sample. Aliquots of these same three samples were given intraperitoneally to young, adult, male mice following the dosage progression of Deichmann and LeBlanc (*J. Ind. Hyg. Toxicol.* **25**, 415, 1943). Mortality observed indicated an LD_{50} of 1.37 mg/kg. When the dried material in the weighing flasks was reconstituted with distilled water and given intraperitoneally to mice, it was found to be much less toxic, the average 48-hour LD_{50} being 9.44 mg/kg.

78. *Phenacetin: Long-Term Studies in Rats and Dogs.* G. WOODARD, K. F. POST, K. O. COCKRELL, and M. T. I. CRONIN, Woodard Research Corporation, Herndon, Virginia.

In an effort to determine whether or not phenacetin is responsible for the kidney damage alleged to be caused in man by the long-term excessive use of drug mixtures containing phenacetin, two-year feeding tests were conducted in rats and dogs.

Beginning with a total of 560 Charles River CD rats and 96 purebred beagles divided equally into 4 groups, daily doses of 200, 63, 20, and 0 mg/kg, respectively, were administered by dietary feeding to the rats and by gelatin capsules to the dogs. At six-month intervals, a portion of the animals from each group were sacrificed for organ weight data and histopathology. Hematological and clinical chemistry observations were also made at intervals throughout the study.

Significant findings were primarily limited to the high level animals and consisted of slightly reduced hemoglobins and hematocrits in some, increased frequency of degenerative or inflammatory liver and kidney changes, and some abnormal pigmentation of various tissues. Kidney lesions of the type observed in man and ascribed to the abuse of phenacetin-containing drugs were not seen in these animals.

79. *The Potentiation of Acute Ethanol-Induced Fatty Liver by Doxapram.* W. R. WOOLES, C. A. PRIORE, and G. W. BRANHAM, Department of Pharmacology, Medical College of Virginia, Richmond, Virginia.

Doxapram HCl has been previously reported to stimulate alcohol dehydrogenase activity *in vitro* and to enhance the intravascular clearance of alcohol. To evaluate whether stimulation of these

parameters by doxapram could ameliorate or prevent the fatty liver due to acute ethanol intoxication, doses of 5 mg/kg to 100 mg/kg were administered intraperitoneally to rats immediately following oral intubation with ethanol in the amount of 6 g/kg or to rats which received saline by stomach tube. In doses of 5 mg/kg and 25 mg/kg to normal rats, doxapram was not hepatotoxic but increased the liver triglyceride accumulation following ethanol administration 50% and 25% respectively. At doses of 50 mg/kg and 100 mg/kg any potentiation of ethanol-induced fatty liver was masked by the fact that at these doses doxapram was hepatotoxic and, when compared to normal animals, produced a mean increase in liver triglyceride concentration of 400% and 360% respectively. Liver triglyceride accumulation following the administration of 50 mg/kg of doxapram intraperitoneally began at 4 hours with peak accumulation occurring at 8 hours as indicated by a mean increase of 475% in doxapram-treated rats compared to the saline control group. By 24 hours liver triglyceride concentration of doxapram-treated rats had returned to control levels, demonstrating the reversibility of the hepatic lipid derangement. At the peak of liver triglyceride accumulation following the administration of 100 mg/kg of doxapram, hepatic parenchymal cell function, as indicated by BSP retention, was unaltered. Further studies are in progress to elucidate the mechanism(s) whereby doxapram produces an acute, reversible fatty liver.

80. *Application of the Evoked Response Technique in Air Pollution Toxicology.* C. XINTARAS, B. L. JOHNSON, and C. E. ULRICH, Pharmacology and Toxicology Section, Laboratory of Medical and Biological Sciences, Division of Air Pollution, U. S. Public Health Service, Cincinnati, Ohio. (Sponsor: C. L. Punte.)

The evoked response to flash in the specific visual cortex and in the superior colliculus of unrestrained and unanesthetized albino rats, bearing indwelling bipolar electrodes, was investigated with the use of an on-line digital computer. It was anticipated that this method may provide a useful technique that may not only be more sensitive and reliable than the conventional methods, but may, additionally, provide some insight into the site of action of toxic agents on the central nervous system. For additional information the behavioral response of pressing a lever for food reinforcement was observed simultaneously.

The effect of exposure to carbon monoxide as recorded by the technique of the evoked response was similar to that of exposure to pentobarbital. The response to respiratory exposure to ozone differed from that induced by carbon monoxide and pentobarbital.

It was concluded that the technique of the evoked response could be a helpful tool in air pollution toxicology. The results are reproducible and the method shows great sensitivity. Data can be accumulated in a reasonably short time, especially if animals with indwelling electrodes are maintained continuously in the laboratory.

81. *A Comparison of the Acute Toxicities of Drugs in Newborn and Adult Rats.* R. A. YEARY and R. A. BENISH, Department of Pharmacology, Lakeside Laboratories, Division of Colgate-Palmolive Company, Milwaukee, Wisconsin.

The acute toxicities of drugs of current clinical interest were investigated in newborn (1–3 day old) and adult (> 100 g) rats of the Charles River C-D or Sprague-Dawley strain. Either the oral, intraperitoneal or subcutaneous routes of administration were employed to duplicate as closely as possible the method of administration in humans.

The adult:newborn LD_{50} ratios were as follows: phenobarbital 2.7, mephenoxalone 5.9, meprobamate 4.4, ferrous sulfate 1.3, iron-dextran 1.2, mepenzolate bromide 4.0, pipenzolate bromide 5.8, acetaminophen 5.7, acetylsalicylic acid 2.7, meralluride 0.6, chlormerodrin 1.0, neomycin sulfate 1.1, dicoumarol 10.0, fencamfamin hydrochloride 1.6, protokylol hydrochloride 0.8, menadione 1.1, menadiol sodium diphosphate 0.7, desipramine hydrochloride 2.3.

Many of the drugs investigated were more toxic in the newborn than in the adult rat. However, the acute toxicities of the iron preparations, mercurial diuretics, CNS stimulants and vitamin K compounds were similar in both age groups. Although each compound must be evaluated individually, there appears to be some consistency in the newborn-adult toxicity relationships within a given pharmacologic class of compounds. Additional studies to substantiate this observation and correlate these findings to help explain the basis for the similarities and/or differences in the acute toxicities of these compounds in newborn and adult rats will be discussed.

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