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**Abstracts of Papers for the Sixth Annual Meeting of
the Society of Toxicology, Atlanta, Georgia
March 23-25, 1967**

1. *Factors Influencing the Choice of the New Zealand White Rabbit in Teratology Studies.*
A. K. PALMER and A. N. WORDEN, Huntingdon Research Centre, Huntingdon, England.

Because of their long association with the laboratory, most strains of rats and mice tend to be more inbred than modern rabbit breeds. The latter have been derived with a minimal degree of inbreeding and a consequent wide dispersion of genetic background, so that often the difference between breeds rests mainly on one or two mendelian genes. This is especially the case with commercial varieties such as the New Zealand White, where the prime requisites of high fertility and rapid growth arise from the expression of numerous polygenes. Due to this wide genetic pool the rabbit produces a relatively high incidence (1% or more) of *varied* skeletal and visceral malformations. This suggests that development is susceptible to modification in a number of ways and by teratogens of differing action. Whilst many malformations may be common to the majority of rabbit stocks, it is particularly important to determine fully (i.e., both qualitatively and quantitatively) the characteristics of the strain under investigation to avoid misinterpretation of results.

Accumulated data illustrating these aspects of variability for our own line of New Zealand Whites will be reported. The relative advantages (known reproductive physiology, high fertility, large fetal size, susceptibility to teratogens) and possible disadvantages (difficulties of disease control, seasonal variation, susceptibility to stress, atypical response to certain agents) attendant on the use of the rabbit will be discussed.

2. *Experimental Teratology in the Monkey.* K. D. COURTNEY, D. A. VALERIO, and A. J. PALLOTTA, Bionetics Research Laboratories, Inc., Falls Church, Virginia.

The effects of acetylsalicylic acid, meclizine hydrochloride, and vincristine sulfate on fetal development of the *Macaca mulatta*, were studied.

The two monkeys administered 40 mg/kg of acetylsalicylic acid from day 25 of gestation to term, produced normal babies. Seven pregnant monkeys received 10 mg/kg of meclizine hydrochloride during various 5-day intervals of organogenesis. All the progeny were normal.

Two of the four monkeys which received vincristine sulfate produced offspring with congenital malformations. One of these monkeys received a single injection of 0.175 mg/kg on day 28 of gestation and the offspring had an encephalocoele. One administration of 0.15 mg/kg on day 25 of gestation produced syndactyly in the offspring of the second monkey. The other monkeys which received 0.15 and 0.20 mg/kg on days 32 and 36 of gestation, respectively, produced normal offspring.

Retrospective studies of the therapeutic agents used during various stages of pregnancy on the monkeys in our colony have shown that hydroxyprogesterone caproate, furazolidone, kanamycin sulfate, nalidixic acid, procaine penicillin G in dihydrostreptomycin sulfate with chlorpheniramine maleate and diphemanil methylsulfate, chloramphenicol, lincomycin hydrochloride, benzathine penicillin G and procaine penicillin G, and clomiphene citrate have no apparent effects on the developing monkey fetus.

3. *Application of Indocyanin Green as a Test for Hepatic Function in Mice and Rats.*
Y. NOGUCHI, Department of Pharmacology and Experimental Therapeutics (Toxicology Activity), School of Medicine, University of California at San Francisco, San Francisco, California. (Sponsor: C. H. Hine.)

Although many clinical hepatic tests have been published, they are not easily applied to mice and rats because of their blood volume. The withdrawable blood volume and the

administratable fluid volumes are limited. As the severity of liver damage caused by hepatotoxins is variable, mice and rats are convenient experimental animals in that large numbers can be used.

Indocyanin Green (ICG), a tricarbocyanine dye, has been reported by others to be an excellent and relatively simple test for assessing liver function in humans and dogs. We have confirmed this using monkeys. The present investigations were carried out to determine whether the ICG test can be applied to mice and rats and whether it is suitable for detecting liver damage. The normal values for ICG clearance from the blood of mice were determined following a single intravenous injection via the tail vein. The dye was administered in normal saline in a dose of 10 ml/kg. The concentration of the dye varied from 0.1 mg/ml to 5 mg/ml. Blood samples were withdrawn by heart puncture at various times from 5 to 60 minutes after injection. Plasma concentration of the dye was determined spectrophotometrically at 805 m μ .

The disappearance of ICG from blood is very rapid. The time to reach half maximum value was less than 6 minutes for all doses. At a dose of 10 mg/kg the plasma concentration after 15 minutes was less than 10 mg/100 ml and was essentially unaltered in the next 30 minutes. This dose is suggested as a convenient liver function test for mice.

In small animals, hemolysis is frequently associated with the withdrawal technique used to obtain the blood sample. The hemoglobin released interferes with the determination of bromsulfalein because of overlapping absorption bands. Since the absorption bands of ICG and hemoglobin do not overlap, the ICG test is more reliable. Another advantage of the ICG test is its simplicity. (Supported in part by U.S. Public Health Service Toxicology Training Grant No. 5 T01 GM01304-02.)

4. *Toxicologic and Teratologic Studies on Antibiotics in Rabbits.* H. MADISSOO, C. D. JOHNSTON, W. J. SCOTT, I. HOLME, and M. H. PINDELI, Research Division, Bristol Laboratories, Division of Bristol-Myers Company, Syracuse, New York, and Woodard Research Corporation, Herndon, Virginia.

DeSomer [*Antibiot. Chemotherapy* 5, 463 (1955)] studied the toxicity of penicillin G, chlortetracycline, and bacitracin in guinea pigs and concluded that the exquisite sensitivity of this species to these agents resulted from death of gram-positive organisms followed by overgrowth of gram-negative bacteria which elaborated toxins responsible for the lethargy, anorexia, obstipation, and death. Gray and Lewis [*Toxicol. Appl. Pharmacol.* 8, 342 (1966)] observed similar results with lincomycin, erythromycin, and penicillin in rabbits. Because of the implications of such toxicity to rabbits in relation to teratologic studies with newer penicillins, toxicologic studies were carried out on dicloxacillin, oxacillin, nafcillin, methicillin, penicillin G, penicillin V, and hetacillin, and teratologic studies on dicloxacillin, hetacillin, penicillin G, and penicillin V. All the penicillins tested produced typical toxicity when administered daily at only 100 mg/kg. Deaths occurred in all groups except methicillin. Similar toxicity observed in does at doses of 50, 200, and 500 mg/kg/day administered for only 10 days during gestation precluded meaningful interpretation of teratologic data, although surviving pups generally showed no abnormalities. Kanamycin, a nonabsorbable antibiotic highly active against gram-negative organisms, was studied toxicologically and teratologically to determine the effect of sterilization of the bowel of gram-negative bacteria. Kanamycin was then combined with dicloxacillin, hetacillin, and penicillin G to further delineate the role of gram-negative bacterial overgrowth on the symptoms produced generally by gram-positive antibiotics.

5. *Intensification of Fetal Bone in the Rodent.* M. HITE, M. JAEP, and C. M. CLARK, Merck Institute for Therapeutic Research, West Point, Pennsylvania. (Sponsor: P. A. Mattis.)

A relatively simple method for examining the skeleton of immature rodent fetuses will be described. The technique is based upon partial replacement of calcium by silver in order to enhance the radiographic detail of bones with a low mineral salt content. The laboratory and

X-ray procedures will be presented. A comparison of silver impregnation by immersion *in toto* in silver nitrate and by intraperitoneal injection into the viable fetus will be discussed. The use of this technique in combination with alizarin red staining of bone will be discussed.

6. *Teratogenicity of Cyclophosphamide in the Mouse.* J. E. GIBSON and B. A. BECKER, Department of Pharmacology, College of Medicine, University of Iowa, Iowa City, Iowa.

Cyclophosphamide in nontoxic dosages of 10 or 20 mg/kg, i.p., was administered to primigravid Swiss-Webster type mice on gestational day 9, 10, 11, 12, 13, or 14. Litters were delivered by cesarean section on day 18 of gestation. Fetuses were weighed and examined for gross defects. One-half of the examined fetuses were fixed in Bouin's solution and hand-sectioned for examination of internal structures. The other fetuses were fixed in 95% alcohol and the skeletons were stained with alizarin red. Treatment with 10 mg/kg on day 11 significantly lowered fetal body weights ($P < 0.001$). Treatment of 7 females on day 13 effected resorption of 27% (14/52) of the total number of implantations. Cyclophosphamide at a dosage of 20 mg/kg was teratogenic; the degree of teratogenicity was day dependent. Administration on day 10 to 8 pregnant animals yielded 74 implantations, 66% (49/74) of which were resorbed; each of the remaining 25 fetuses was malformed (100%). Six pregnant animals receiving the drug on day 11 yielded 55 implantations, 62% (34/55) of which were resorbed; all remaining fetuses were malformed (100%). Treatment of 6 animals on day 12 yielded 79 implantations, 12% (9/79) of which were resorbed; 9% (6/70) revealed external anomalies; and 38% (13/34) had cleft palate. The principal malformations are exencephaly, microcephaly, hydrocephaly, adactyly, polydactyly, ectrodactyly, ectromelia, and cleft palate. Injection of 6 animals on day 13 and 9 animals on day 14 yielded 61 and 94 implantations, respectively, of which 41% (25/61) and 17% (16/94) were resorbed; gross anomalies were not important. Significant lowering of fetal body weights in all high-dosage groups is associated with skeletal defects.

7. *Local Histologic Changes following Long-Term Well Tolerated Intramuscular Injections.* J. E. GRAY, Research Laboratories, The Upjohn Company, Kalamazoo, Michigan.

This report is based upon the results of 5 intramuscular toxicity studies in which antibiotics of low irritancy were injected into the posterior thigh of the dog and the cat. In most cases, injections of 1 to 1.5 ml were made bilaterally once or twice daily for 30-90 days.

Upon gross and microscopic examination, the muscle was found to be relatively unaffected. In response to "residue" and trauma from the long series of injections, a facultative lymphatic drainage is established. Certain distinctive histologic changes accompany this response. These are: (a) collections of lymphocytes are organized along the drainage pathway into a prenatal lymphofollicular response; (b) loose connective tissue is replaced by a more resistant granulation tissue. The removal of the content of the fat cells is facilitated by multinucleated giant cells; and (c) muscle fibers which immediately border the tract are walled away from the low-grade recurrent insult by a fibroblastic response which "caps" the exposed fibers.

A most important conclusion from these morphologic studies has been the recognition that the administration of an aqueous vehicle, such as physiologic saline, alone, when given under the above-stated conditions, may be accompanied by these adaptive changes as readily as in those cases in which a well tolerated antibiotic is injected serially.

8. *Cardiovascular Responses in Nephrotoxic Beagles.* E. E. VOGIN, A. H. PHELPS, and P. A. MARTIS, Merck Institute for Therapeutic Research, West Point, Pennsylvania.

Although Rosenthale and Baum [*Proc. Soc. Exptl. Biol. Med.* 120, 689 (1965)] reported no significant differences in the renal vascular bed response to pressor substances in glomerulonephrotic rats, Perry and Yunice [*Proc. Soc. Exptl. Biol. Med.* 120, 805 (1965)] showed that mercuric ion administered intra-arterially to rats resulted in a hypertensive episode. In the present study, nine dogs were administered 3 mg/kg of mercuric chloride intravenously. Two

days later, these animals had a blood pressure of 133 ± 7.3 mm while their controls had a blood pressure of 105 ± 5.5 mm. Pressor responses to intravenous doses of angiotensin of 0.5, 1.0, and 2.0 $\mu\text{g}/\text{kg}$ were reduced by 51, 66, and 46%, respectively. The pressor responses to similar doses of epinephrine were decreased in 4 dogs, increased in 4 dogs, and unchanged in one dog. In 3 additional mercuric chloride-treated dogs, renal blood flow was decreased about 52%, renal vascular resistance was markedly increased, and renal pressor responses to intra-arterial angiotensin (0.1 μg) were reduced about 50%. Electrocardiograms of the mercuric chloride-treated dogs revealed depressed S-T segments, prolonged QRS intervals and heightened T-waves. Histomorphologic examination of the kidneys showed marked tubular degeneration and necrosis. No significant changes were found in the myocardium.

9. *Ultrastructural and Biochemical Studies of Sodium Cyclamate*. A. A. STEIN, D. M. SERRONE, and F. COULSTON, Institute of Experimental Pathology and Toxicology, Albany Medical College of Union University, Albany, New York.

Diffuse, mild vesiculation of the endoplasmic reticulum associated with vacuolization was observed in the liver and kidney of monkeys given 4 or 8 g/kg of sodium cyclamate orally and sacrificed 1 or 48 hours later. No changes in various biochemical values were associated with these early morphologic alterations.

Similar ultrastructural alterations were observed in rats given oral doses of 2, 5, or 8 g/kg of sodium cyclamate for five consecutive days. A slight decrease in hepatic triglycerides was found in rats given 8 g/kg daily for 5 days. No changes in the activities of hepatic demethylase or desulfurase were observed following either single or multiple oral administration of sodium cyclamate. An increase in hepatic mitochondrial oxygen utilization was found in rats given 4 g/kg/day of sodium cyclamate by mouth for 4 days.

Studies in progress will determine whether the changes observed in these short-term studies persist or whether these early observations are adaptive responses which may disappear with continued administration of sodium cyclamate. (This research was supported by grants from the Sugar Research Foundation and from Abbott Laboratories.)

10. *Chronic Overexposure to Benzene Vapor*. R. D. STEWART, H. C. DODD, E. D. BARETTA, A. W. SCHAEFFER, and J. E. MUTCHLER, Department of Environmental Medicine, Marquette University School of Medicine, Milwaukee, Wisconsin, and Environmental Health Section, Biochemical Research Laboratory, The Dow Chemical Company, Midland, Michigan.

Ten workmen who for many years had been chronically exposed to benzene vapor (< 25 ppm) were accidentally overexposed to 85–115 ppm for 3 months. Six complained of fatigue and increased irritability while four remained asymptomatic. The peripheral blood of all featured increased mean corpuscular volume, hypersegmentation of the neutrophils, and a mild anemia. Nine of the ten men recovered in 4–8 months. Treatment with vitamin B₁₂ and folic acid did not appear to influence the clinical course. After their recovery, the workmen were allowed to return to the benzene area and were placed under a strict medical surveillance program which included continuous breathing-zone monitoring and frequent breath benzene analyses. The statistical correlation between the expired air benzene concentrations and the magnitude of the daily vapor exposures proved so reliable that postexposure breath analysis can now be considered a rapid diagnostic index of benzene exposure.

11. *Quantitative Analysis of Spontaneous Activity: Effects of Long Continuous Exposure to Space Cabin Atmospheres*. A. A. THOMAS, and J. L. NAYLOR, Toxic Hazards Division, 6570th Aerospace Medical Research Laboratories (MRBTT), Wright-Patterson Air Force Base, Ohio.

In a previous study, the author reported on the development of a new technique for the evaluation of spontaneous activity of unrestricted dogs by time-lapse photography and subsequent scan of the photographic information by a photoelectric sensor and associated circuitry [Thomas, *Toxicol. Appl. Pharmacol.* 8, 359 (1966)].

The evaluation of the level of activity at that time required visual comparison of the recorded trace and subjective interpretation. Since the recording process reflects changes in the degree of activity (the absence of pen movement indicates a steady state or no activity and conversely, pen movement indicates a frame-to-frame change in information content or the presence of activity), an accurate quantitation can be accomplished by measuring the total length of the trace on the recorder chart. Manual length of trace analysis proved to be too tedious and was simplified by the use of a highly water-soluble recording ink which is later eluted from the paper strip, and the quantity of deposited ink is measured colorimetrically. The length of the trace is directly proportional to the optical density (OD) of the eluted ink solution.

Variations in image density due to slight changes in lighting intensity and development are compensated by simultaneous scan of a neutral area of the image not occupied by animals. The OD of the eluted activity tracing (T_a) divided by the OD of the tracing obtained from the neutral area (T_n) furnishes an accurate index of spontaneous activity of the animals (I_a), i.e., $I_a = T_a/T_n$.

Reliability and reproducibility of the method will be illustrated by the quantitative study of time-lapse photographic material recorded during an 8-month continuous exposure of beagle dogs to a 5 psia 100% oxygen atmosphere.

12. *Inhalation by Small Animals of Fresh Cigarette Smoke Generated by New Smoking Machine.* F. HOMBURGER, P. BERNFELD, P. BOGDONOFF, T. KELLEY, and R. WALTON, Bio-Research Consultants, Inc., Cambridge, Massachusetts.

Exposure of experimental animals to cigarette smoke in conventional smoke chambers is inadequate [Stewart and Herrold, *Bull. Intern. Statist. Inst.* **39**, Report No. 135 (1962); Wynder and Hoffman, *Proc. Am. Assoc. Cancer Res.* **7**, 32 (1966)], resulting in inhalation of small amounts of stale smoke because of the nature of the apparatus and because of the retention of particles in the nasal passages of exclusively nose-breathing rodents.

A new smoking machine has been designed which burns cigarettes under positive pressure and delivers fresh mainstream smoke in any desired concentration to exposed animals. The gas phase and particulate phase of this smoke are identical with those of smoke produced by a conventional smoking apparatus.

The acute toxicity of cigarette smoke in normal mice as well as the increased toxicity in mice with occluded noses were reduced by pretreatment with an analog of nicotine, the hydrobromide of 2,3-dimethyl-2-azabicyclo[3.3.0]octanol-8.

Acute toxicity studies of fresh smoke of the type described here are suggested as a significant measure of the relative efficiency of cigarette filters and other devices intended to reduce the acute toxicity of tobacco smoke.

Chronic exposure studies are being done in rodents on possible carcinogenicity of native fresh mainstream smoke. (This work was done under contract with the Council for Tobacco Research, U.S.A.)

13. *Pulmonary-Cardiovascular Responses of Intact, Spinal-Lesioned, and Sham-Operated Rats Exposed to Ozone.* E. FAIRCHILD, II, S. PLUME, III, and C. PANTLE, Toxic Hazards Research Unit, Aerojet-General Corp., Dayton, Ohio, and Division of Occupational Health, U.S. Public Health Service, U.S. Department of Health, Education, and Welfare, Cincinnati, Ohio.

Respiration, heart rate, and blood pressure were recorded for each of a series of intact, spinal-lesioned, and sham-operated rats, half of which received ozone (O_3) exposures. Blood pressure was monitored by transducer connected to indwelling caudal artery catheters, while measurement of the other parameters was accomplished by means of pin electrodes connected to an impedance pneumograph and electrocardiograph pickup.

Graded O_3 exposures (40 down to 10 ppm) of intact animals produced a markedly significant reduction in heart rate and blood pressure prior to or by 30 minutes, whereas respiration showed a gradual increase subsequent to a brief, initial decrease. Lesioned animals (by electro-

cautery at the 6th cervical) exhibited precipitously low heart rates and blood pressures; these were unchanged by O₃ when the gas was administered 24 hours postoperative.

We previously reported [Fairchild and Bobb, *Toxicol. Appl. Pharmacol.* 7, 483 (1965)] that spinal-lesioned rats did not develop pulmonary edema following O₃ exposure, and considered that the inhibitory effect resulted from altered hemodynamics rather than from decreased respiratory rate. Results reported here show that "spinal" rats did ventilate similar to intact or sham-operated controls. In fact, the respiratory rate of spinal animals exposed to O₃ was increased over that of the controls. Thus, the reduced edemagenic response to pulmonary irritation observed in spinal animals appears to be associated with cardiovascular, but not respiratory effects. The implication of the findings will be discussed in detail.

14. *Further Studies of Metal Shifts from Inhaled Toxic Irritants: Trace-Metal Changes in Lungs in Tumor-Susceptible and Tumor-Resistant Mice Exposed to Ozone.* J. R. DIXON, R. E. KINSER, W. C. LANE, and H. E. STOKINGER, Occupational Health Research and Training Facility, Public Health Service, U.S. Department of Health, Education, and Welfare, Cincinnati, Ohio. (No abstract.)

15. *In Vivo Effects of Cigarette Smoke on Mucus Transport Activity and Mucus Secretion in Chronically Exposed Chickens.* S. P. BATTISTA, R. W. GRAHAM, and C. J. KENSLER, Life Sciences Division, Arthur D. Little, Inc., Cambridge, Massachusetts.

The chronic effects of tobacco smoke on ciliary transport activity and mucus secretion have been studied in the intact chicken. Eight 40-ml puffs of smoke (equivalent to the amount of smoke from 1 cigarette) were administered for 4 seconds directly into the larynx of each animal 5 days a week on either a once-a-day or twice-a-day schedule. Results show that despite the severe depression which occurred during and after each of 19 or 37 exposures, measurement of particle transit times indicated that ciliary function was usually completely restored the following day prior to the next scheduled exposure. Mucus collected at the larynx, was also measured and found to increase markedly ($P < 0.05$) from a control rate of approximately 0.4 g/hr to over 2 g/hr during the period of exposure to cigarette smoke.

16. *Shift in Circulating Neutrophil-Lymphocyte Ratios Associated with Pulmonary Injury in Rats Exposed to Ozone.* G. A. BOBB and E. J. FAIRCHILD, II, Division of Occupational Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Cincinnati, Ohio, and Toxic Hazards Research Unit, Aerojet-General Corp., Dayton, Ohio.

Ozone (O₃) behaves as a chemical irritant upon the respiratory bronchioles producing "stress." Many forms of stress, e.g. immunologic reactions, skin burns, wounds, and bacterial invasion, cause a shift in the neutrophil-lymphocyte ratio. Our evidence shows that exposure to O₃ induces a significant alteration of the circulating neutrophil-lymphocyte ratio in rats.

Adult male albino rats exposed to 12.5 ppm of O₃ for 2 hours showed a maximal decrease in lymphocytes (mean of 40%) and increase in neutrophils (mean of 300%) 3 hours post-exposure. This shift in blood-cell ratio persisted until the sixth hour; thereafter, it gradually returned to normal by the twelfth hour postexposure.

The relationship of these blood changes to the overall mechanism of O₃ toxicity is discussed.

17. *Performance in Human Subjects at Same Blood-Alcohol Concentrations on Rising vs. Falling Curve.* D. J. BROWN, R. B. FORNEY, F. W. HUGHES, and A. B. RICHARDS. Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana.

Several attempts have been made to substantiate claims that a difference in impairment exists with the same blood-alcohol concentration when determined in the rising blood level and in the falling blood level. In order to evaluate behavioral responses, and thereby measure impairment, delayed audio feedback techniques were used to compare human performance

during rising and falling blood alcohol levels of 75 mg/100 ml. Sufficient alcohol to achieve a 100 mg/100 ml blood level was administered to nine subjects and monitored with a Breathalyzer. Latin-square sampling of the three conditions of rising blood level, falling blood level, and control, provided a means of eliminating learning. The subjects exhibited significantly impaired performance when either alcohol condition was compared to the control condition. However, no significant difference in performance was seen when the rising phase and the falling phase were compared.

18. *Sleep: Changes in Rapid-Eye-Movement Phase in Chronic Lead Absorption.* C. XINTARAS, M. F. SOBECKI, and C. E. ULRICH, Pharmacology and Toxicology Section, Laboratory of Medical and Biological Sciences, Division of Air Pollution, Public Health Service, U.S. Department of Health, Education, and Welfare, Cincinnati, Ohio. (Sponsor: C. L. Punte.)

Rapid-eye-movement (REM) sleep, in contrast to no-REM or slow-wave sleep, is a period of intense activity of the central nervous system. Since the most serious manifestations of lead intoxication are those resulting from cerebral involvement, a study was designed to investigate the effect of chronic lead absorption on the electroencephalogram sleep record.

Male rats were implanted with cortical recording electrodes and placed in electrically shielded chambers. Water containing lead acetate (1.5 mg/kg) was available ad libitum. Observations were made regarding the rat's behavior (alert wakefulness, quiet, sleep, etc.) via a closed-circuit TV system in order to relate changes in the electroencephalographic record with changes in the rat's behavior.

In the transition from wakefulness to sleep, the normal adult rat exhibited a typical succession of electroencephalographic changes similar to those that are found in man. The REM phase was entered by way of 15-cycle-per-second "sleep spindles" and was characterized by regular waves of 6-10 cycles per second. REM epochs were more abundant during the later part of the sleep record, i.e., late afternoon. Lead acetate induced changes in the duration and stability of the REM periods. Excessive spindle-slow-wave complexes appeared during periods of REM sleep. Treated animals showed more REM sleep during the early sleep period as compared to control baseline data.

The findings suggest that the alterations in the REM phase in lead-treated animals may be directly or indirectly associated with an impaired neural control system.

19. *Approaches to Toxicity Evaluation of Plastic Materials for Medical Purposes.* J. AULTAN, Materials Science Toxicology Laboratory, College of Pharmacy and College of Dentistry, The University of Tennessee, Memphis, Tennessee. (Sponsor: T. O. King.)

Introduction of man-made devices and items into the medical and paramedical fields has increased at an astonishing rate in the last decade. All present indications point to an even greater rate of increase of these and similar items in the near future. Since man-made materials falling under the very broad classification of plastics may be synthesized for specific applications or can be formulated to give a variety of properties, an unlimited number of substances can be introduced into the medical world which can have either direct contact (prosthetic devices) or indirect contact (containers for drugs, nutritional products, diagnostic agents, etc.) with tissue. Often, rapid introduction of these items without proper evaluation has brought from very minor to possible serious consequences to the patient or recipient. This paper reviews some of the problems encountered in the use of polymeric materials for medical and paramedical applications and presents an approach to the toxicity evaluation of materials which should be helpful to eliminate those materials which may present potential toxic problems to the user or patient.

20. *Tissue Reaction to Plastic Implants in the Animal and Man.* J. C. DAVILA, Temple University School of Medicine, Philadelphia, Pennsylvania. (Sponsor: T. O. King.)

This presentation considers patterns of tissue healing in response to prostheses implanted in the circulation. The importance of total encapsulation of foreign body implants is stressed.

Stability of the scar is dependent on adequate blood supply to all its parts. The importance of structure of the implant in this regard is discussed. Histologic studies are used to describe the mechanisms of healing and the type of tissue reaction seen in relation to different materials and different structures.

21. *Research Approaches to Blood-Plastics Compatibility Problems.* R. D. FALB, G. A. GRODE, and R. I. LEININGER, Columbus Laboratories, Battelle Memorial Institute, Columbus, Ohio. (Sponsor: T. O. King.)

The use of polymeric materials in the body has resulted in a number of medical advances in recent years. However, associated with these advances, a number of problems concerning the compatibility of polymers with the body environment have arisen. Problems involving traumatic effects of polymers on the body are blood clotting, damage to blood formed elements, protein denaturation, tissue irritation, toxicity, and possibly carcinogenicity. In addition, many polymers are attacked by body enzymes and subsequently degraded. It is of primary importance that polymers for use in the body be free of toxic additives such as stabilizers, antioxidants, fillers, or plasticizers.

Research approaches to the above problems have involved studies of the factors thought to influence blood-plastics compatibility such as surface wettability, smoothness, electrical charge, and chemical structure. In addition, some success in attaining a compatible surface has been attained through attachment of a viable tissue layer to a velour material. Along other lines, a number of polymers which do not cause blood to clot have been developed at Battelle Memorial Institute. These polymers have been made nonthrombogenic by the attachment of heparin to their surfaces. A number of different techniques have been employed to effect heparin attachment including surface reactions, radiation grafting, and bulk incorporation of quaternary ammonium salts. Thickness of the attached heparin layers as measured by radioisotope techniques ranged from 100 to 1500 Å. Short-term *in vivo* studies on a selected number of these polymers showed that they did not cause clotting. The hemolytic activity of the heparin-grafted surfaces was found to be only slightly higher than that of the unmodified surfaces. (Supported by the National Heart Institute Contract Number PH43-64-496, Dr. Frank Hastings, Project Monitor.)

22. *Plastics and Carcinogenesis.* R. R. BATES, Biology Branch, National Cancer Institute, Bethesda, Maryland. (Sponsor: T. O. King.)

The carcinogenicity of solid films or sheets of a wide variety of materials, including plastics, metals, glass, silk, and ivory, has been well demonstrated by Oppenheimer *et al.* [*Cancer Res.* 15, 333 (1955); *ibid.*, 16, 439 (1956)], Nothdurft [*Naturwissenschaften* 42, 75 (1955)], and Nothdurft and Mohr [*Naturwissenschaften*, 45, 549 (1958)]. When these are implanted in rodents, a dense fibrous capsule forms around the implant. Subsequently, sarcomas develop within the fibrous capsule. The same materials when introduced as powders, textiles, or perforated films induce far fewer tumors. The extreme diversity of materials which are carcinogenic, the insolubility of these materials, and the importance of the physical form for tumorigenesis suggest that it is the physical state of the implants, rather than their chemical nature, which is responsible for their carcinogenicity. The greater porosity of forms which induce few tumors has suggested to some that the films may act as a barrier to intercellular exchange of macromolecules required for maintenance of normal differentiation. An alternative possibility is that the reaction of adjacent tissues to the smooth surface of films may differ from their reaction to the rougher surface of more porous materials. The importance of a smooth surface for carcinogenesis was tested by implanting two groups of polyethylene disks, one smooth and the other rough, into Balb/c mice. When the first tumor arose 7½ months after implantation, there were 71 survivors with smooth disks and 60 with rough disks. By 13 months after implantation, 35 tumors had developed adjacent to smooth disks but only 7 beside rough disks. Throughout the entire observation period, the rate of accumu-

lation of sarcomas was consistently greater for mice bearing smooth disks. This experiment reemphasizes the importance of the physical form of the implant in plastic carcinogenesis and demonstrates that the surface of the implant is an important aspect of its form.

In contrast to this physical mechanism of carcinogenesis by all adequately tested plastics, a few plastics may also act as chemical carcinogens. Hueper [*Am. J. Clin. Pathol.* 34, 328 (1960)]; *J. Natl. Cancer Inst.* 33, 1005 (1964)] observed adenocarcinomas of the colon in rats with intraperitoneal implants of polyurethane foam. Degradation of the foam was apparent, suggesting that breakdown products may have penetrated the intestinal wall to induce carcinomas.

23. *Evaluation of Mental and Motor Performance with the Anti-Anxiety Drug Ro 5-4556, Ethanol, and Drug-Ethanol Combination.* M. E. BERNSTEIN, F. W. HUGHES, A. B. RICHARDS, and R. B. FORNEY, Department of Pharmacology and Toxicology, Indiana University Medical Center, Indianapolis, Indiana,

Ro 5-4556, a chlordiazepoxide analog, was tested on mental and motor performance, with and without ethanol, as part of a continuing investigation into the interactions of drugs and ethanol on man. Sixteen male medical and graduate student volunteers were tested under four treatment conditions, namely, 10 mg of drug plus ethanol, 10 mg of drug plus placebo ethanol, placebo drug plus ethanol, and placebo drug plus placebo ethanol, which were double-blind and randomized. One hour before testing, each subject was given a capsule to swallow immediately as well as a beverage, 3.33 g/kg, to consume within 30 minutes. Blood-ethanol concentrations were determined by breath analysis just prior to testing and immediately after completion of the tests. Testing consisted of a delayed audio feedback situation in which the subject was tested on verbal output, reverse reading, reverse count, progressive count, addition, subtraction, and a color discrimination test. Motor performance was measured by means of a Pursuit Meter, whereby the subject had to superimpose a spot, controlled by himself, upon a moving pattern. Results indicated that, in general, there was no significant alteration in performance with administration of Ro 5-4556. Ethanol treatment produced significant impairment of performance in a number of test conditions when it was combined with either placebo drug or Ro 5-4556. (Supported in part by U.S. Public Health Service Training Grant PHS GM 1089 03.)

24. *Effects of Solvent Inhalation on Conditioned Avoidance Response in Rats.* C. H. HINE, G. S. STONE, K. C. LEUNG, and A. F. LAUDEL, The Hine Laboratories, Inc., San Francisco, and the Department of Pharmacology and Experimental Therapeutics (Toxicology Activity), School of Medicine, University of California at San Francisco, San Francisco, California.

Preoperant avoidance behavior was utilized to determine the effects of a series of industrial solvents on rats exposed to three concentrations, some of which bracketed the threshold limit value (TLV). The test apparatus included devices for generating and monitoring ambient air concentrations of the solvent, a test and exposure chamber, programming equipment, and a printing counter. Studies were carried out with a response-shock interval of 20 seconds, a shock-shock interval of 5 seconds, and no escape contingency. Exposures were for 2.5 hours. Treatment orders were varied to control adaptation. All data were grouped at 30 minutes, transferred to IBM cards and analyzed, using the FORTRAN IV program. Assessment was made of possible interaction effects between exposures, subject and exposures, and testing periods and exposures. Concentrations above the TLV decreased performance, with an increase in shocks received and a decrease in responses. Exposures at the TLV levels affected the mean response rate with some solvents. At concentrations below the TLV, mean responses were not affected, though responses of individual animals occasionally were.

We suggest that the procedure may give useful information regarding possible behavioral effects produced by solvent vapors. (Supported in part by a grant from the Shell Development Company, Emeryville, California.)

25. *Ethanol Effects on Chloral Hydrate Metabolism in Mice.* H. L. KAPLAN, R. B. FORNEY, F. W. HUGHES, A. B. RICHARDS, and N. JAIN, Department of Pharmacology and Toxicology, Indiana University Medical Center, Indianapolis, Indiana.

Concurrent administration of ethanol enhances the depressant action of chloral hydrate. Sleeping time of mice was measured after chloral hydrate alone or in combination with a subhypnotic dose of alcohol. The magnitude of the effect resulting from 4 g/kg of ethanol and 0.5 g/kg of chloral hydrate appeared too large to be accounted for simply by the additive action of the two depressants. This observation led to an investigation of the metabolism of the two compounds. Male Wistar mice were divided into three groups; ethanol was administered to one group, chloral hydrate to another, and both ethanol and chloral hydrate to the third. The dosage of ethanol was 4 g/kg and that of chloral hydrate, 0.5 g/kg. The route of administration was intragastric. At various times after administration, the animals were sacrificed and the total body content of chloral hydrate, trichloroethanol, and ethanol was determined using gas chromatographic techniques. Comparison of the above drugs and metabolites in the three groups demonstrated that a slower rate of degradation of ethanol as well as of trichloroethanol was evident when mice received both ethanol and chloral hydrate. Therefore, the synergism of chloral hydrate and ethanol appears to be one in which there is a mutual interaction. Not only is the metabolism of ethanol inhibited by chloral hydrate, but there is also a prolongation of the trichloroethanol levels.

26. *Effect of Hepatocarcinogens on the Metabolism of Some Radionuclides by Rats.* D. D. MAHLUM, Biology Department, Battelle Memorial Institute, Pacific Northwest Laboratory, Richland, Washington. (Sponsor: H. A. Kornberg.)

The effect of dietary administration of the hepatocarcinogens 2-(*N,N*-diacetamido) fluorene, *N,N*-dimethyl-*p*-phenylazoaniline, and ethionine on the distribution of cerium-144 and plutonium-239 was studied in the rat. In one series of experiments, carcinogen-feeding was initiated immediately after injection of the radionuclide. The animals were killed at several intervals and the nuclide distribution was determined. These animals exhibited an increased retention of the nuclide in the liver but not in other organs. Ethionine exerted the greatest effect on nuclide retention and *N,N*-dimethyl-*p*-phenylazoaniline the least. Increased retention appeared to be associated with a decreased secretion of nuclide in the bile.

In a second series of experiments, the rats were fed the carcinogenic diet 7-21 days prior to injection of the nuclide. Distribution of the nuclide was determined 24 hours after administration. Although the distribution varied with the nuclide and carcinogen studied, in general, carcinogen-feeding decreased the uptake of the nuclide by the major site of deposition, and increased uptake in other tissues. [This paper is based on work performed under U.S. Atomic Energy Commission Contract AT(45-1)-1830.]

27. *Joint Action of Uranium and Niobium on the Urinary Excretion of Electrolytes.* W. L. DOWNS and C. L. YUILE, Department of Radiation Biology and Biophysics, University of Rochester, Rochester, New York.

Preliminary investigations in this laboratory suggested that prior treatment with potassium niobate (11.3 mg/kg as niobium) reduced mortality in female rats following the intraperitoneal injection of a lethal dose (0.94 mg/kg as uranium) of uranyl nitrate.

Renal function studies were performed on rats treated with these nephrotoxic agents in an attempt to examine their interaction on the kidney. Groups of 3 rats were placed in metabolism cages and urine was collected for 24 hours. Measurements were made to determine urinary osmolality and electrolyte (Na, K, Cl) concentrations.

A significant increase in urine and electrolyte output, accompanied by a decrease in urinary osmolality was observed when niobium or uranium was administered to rats. These increases

peaked rapidly (24-48 hours) after niobium (11.3 mg/kg as niobium) but were observed to be more prolonged (3-7 days) after uranium (0.94 mg/kg as uranium). The injection of uranium in rats treated with niobium 22 days earlier produced changes in urinary function similar to those reported for uranium. However, uranium-poisoned rats (3 days after administration of the uranium) which were injected with niobium did not show the increase in electrolyte excretion during the next 24-hour period as was expected. In these rats, although urine output was elevated, excretion of Na and Cl was somewhat less than normal.

Studies on kidney weights show that increased kidney weight/body weight ratios occurred 2-4 days after niobium treatment and somewhat later (6-8 days) after uranium injection. The ratios found in rats given both salts were higher than predicted.

28. *Responses of Rats to Carbachol, Oxotremorine, Decamethonium, and d-Tubocurarine after Subacute Administration of an Organophosphorus Cholinesterase Inhibitor.* J. J. MCPHILLIPS, Department of Pharmacology, Medical College of Virginia, Richmond, Virginia. (Sponsor: P. S. Larson.)

Brodeur and DuBois [*Arch. Intern. Pharmacodyn.* 149, 560 (1964)] have observed that rats which have acquired tolerance to the cholinomimetic effects of Di-Syston [*O,O*-diethyl S-2-(ethylthio)ethyl phosphorodithioate] are more resistant than control rats to lethal doses of carbachol. In the present investigation, it has been shown that rats which have been treated with 0.9 mg/kg of Di-Syston for periods up to 30 days are more resistant than control rats to the hypotensive and negative chronotropic effects of carbachol. In addition, segments of ileum taken from Di-Syston-treated rats exhibited increased resistance to carbachol.

Rats treated with Di-Syston, 0.9 mg/kg, for 30 days were found to be more resistant than control rats to lethal doses of oxotremorine and carbachol. In control rats, the LD₅₀'s of oxotremorine and carbachol were found to be 4.4 mg/kg and 2.4 mg/kg, respectively. In Di-Syston-treated rats, however, the LD₅₀ of oxotremorine was 5.6 mg/kg and the LD₅₀ of carbachol was 3.2 mg/kg. Di-Syston-treated rats, however, were found to be more sensitive than control rats to lethal doses of decamethonium and *d*-tubocurarine. In control rats, the LD₅₀'s of decamethonium and *d*-tubocurarine were found to be 2.9 mg/kg and 0.20 mg/kg, respectively, whereas in the Di-Syston-treated rats the LD₅₀ of decamethonium was 1.5 mg/kg and the LD₅₀ of *d*-tubocurarine was 0.15 mg/kg. Differences between treated and control rats with respect to the LD₅₀'s of the drugs were significant to at least the 1% level of probability.

29. *Effect of Alkyl-Substituted 2-Aminoethanols on Cholinesterase and Choline Oxidase Activity.* R. HARTUNG and H. H. CORNISH, Department of Industrial Health, School of Public Health, The University of Michigan, Ann Arbor, Michigan.

The alkyl-substituted 2-aminoethanols bear an obvious structural relationship to choline. A number of these compounds are natural precursors in choline metabolism and several are readily incorporated into phospholipids and may take on some, but not all, of the functions of choline. The possibility of *in vivo* synthesis of choline analogs from these alkyl-substituted 2-aminoethanols still appears to be in doubt, but some of these analogs (e.g., triethylcholine) are known to interfere with normal cholinergic functions.

Therefore, effects of the alkyl-substituted 2-aminoethanols and their corresponding choline analogs on the choline oxidase enzyme system (from rat liver homogenates) and on acetylcholinesterase (purified from bovine erythrocytes) were investigated. Choline oxidase was not greatly inhibited by any of the materials examined; some of them were able to function to some extent as substrates. In contrast, acetylcholinesterase activity was readily inhibited by the test substances. The *n*-butyl-substituted choline analogs had the greatest inhibitory capacity followed by ethyl-substituted choline analogs and choline itself. The mono-substituted aminoethanols were less inhibitory, and unsubstituted aminoethanol was least inhibitory

in character. The relationships of the *in vitro* enzyme responses to acute toxicities of these substances will be discussed.

30. *Toxicity of Combinations of Glycerol Trinitrate and Sympathomimetics*. D. L. OPDYKE, C. M. BURNETT, and J. C. MUNCIE, Revlon Research Center, Inc., Bronx, New York.

LD₅₀'s of *d*-, *l*-, and racemic epinephrine and isoproterenol have been compared with corresponding values in rats preconditioned by daily doses of nitroglycerin.

Mixtures of each of the sympathomimetics with nitroglycerin have also been tested for acute toxicity.

Studies of a parallel nature in the dog have also been carried out monitoring pressor effects and respiratory rate.

31. *Effects of Prolonged Methylchloroform Inhalation on Drug-Induced CNS Activity*. H. LAL and H. SHAH, Department of Pharmacology and Toxicology, University of Kansas, Lawrence, Kansas.

After an exposure to methylchloroform-air mixture in a continuous flow (10 liters/minute) chamber, experimental rats and mice were tested for loss of righting reflex produced by short-acting barbiturates. Sleeping times measured concurrently in the control and the treated animals were compared.

Continuous inhalation of methylchloroform for 24 hours or more reduced the barbiturate hypnosis significantly (38-64% reduction in 24-hour groups, $P < 0.005$). Discontinuous exposure for the same total number of hours was ineffective. Both male and female animals were susceptible. The effect was optimal at 24 hours but could be still detected even at 48 hours after the exposure was terminated. The effect was always reproducible when 2000 ppm of methylchloroform were used. Lower concentrations produced variable results. Exposure in the same chamber to air not containing methylchloroform was ineffective. Postexposure study for 2 weeks did not reveal any loss in body weight, gross pathologic lesion, or lethality.

The supernatant fraction containing microsomes and soluble constituents of the livers dissected from the exposed animals did not differ from their controls in its ability to oxidize hexobarbital, demethylate aminopyrine, or reduce *p*-nitrobenzoic acid. (Supported by Contract N00014-66-C0006 from the Office of Naval Research.)

32. *Ultrastructural Study of the Absorption of Methoxychlor in Rat Jejunal Mucosa*. H. IMAI and F. COULSTON, Institute of Experimental Pathology and Toxicology and Department of Pathology, Albany Medical College of Union University, Albany, New York.

Thirty-four male, adult Wistar rats were given by gastric tube either single doses of 400 mg/kg or 3 consecutive daily doses of 1600 mg/kg of methoxychlor dissolved in corn oil or suspended in aqueous polyvinylpyrrolidone. Controls were given equal volumes of vehicle. Specimens of the jejunal mucosa were taken at 1 hour after the single feeding or on the fourth day from the first of three administrations, and were examined with light and electron microscopes. The probable methoxychlor product was identified by densities different from those of the vehicles. The routes of absorption of all administered materials through the mucosa were similar: apical pinocytotic vesicles, larger vacuoles toward the Golgi and nuclear level, and widened intercellular spaces at the paranuclear to basal zone. Absorption of methoxychlor was characterized by marked distention of the intercellular spaces that seemed to be more than enough to merely accommodate the dense material. Cellular changes attributable to methoxychlor included degeneration or structural fragility of mitochondria as manifested by individual gross swelling, either partial or diffuse, with rarefaction of the matrix. Such alterations were not accompanied by *in vivo* formation of microsomes, rarefaction of the cytoplasmic ground substance, or other indications of cell death. (This research was supported by the Food and Drug Administration, U.S. Department of Health, Education, and Welfare, Contract FDA 64-19.)

33. *Effects of Oral Doses of Carbaryl on Man.* J. H. WILLS, E. JAMESON, A. STEIN, D. SERRONE, and F. COULSTON, Institute of Experimental Pathology and Toxicology, Albany Medical College of Union University, Albany, New York, Clinton Prison, Dannemora, New York, and New York State Pesticide Control Board, Albany, New York.

Groups of men were given daily oral doses (7 days/week) of 0, 0.06, or 0.12 mg/kg of carbaryl in gelatin capsules during a period of 6 weeks. Thirty-two biochemical, histologic, or physical parameters of normal bodily function were measured before, during, and at the end of the exposures. None of these parameters, including the cholinesterase activities of red blood cells and plasma, underwent any significant change that can be attributed certainly to the ingestion of carbaryl. We conclude tentatively that daily ingestion of carbaryl in amounts up to 0.12 mg/kg (total daily intake of the order of 6-15 mg, depending on body weight) will be noninjurious to man.

34. *Metabolism of Carbaryl in Man.* J. B. KNAAK, L. J. SULLIVAN, and J. H. WILLS, Chemical Hygiene Fellowship, Mellon Institute, Pittsburgh, Pennsylvania, and Institute of Experimental Pathology and Toxicology, Albany Medical College of Union University, Albany, New York. (Sponsor: H. F. Smyth, Jr.)

Two volunteers were administered carbaryl (1-naphthyl *N*-methylcarbamate) orally at 2 mg/kg. Urine was collected over a 4-day period and analyzed for carbaryl metabolites by diethylaminoethylcellulose chromatography and fluorometry [Knaak *et al.*, *J. Agr. Food Chem.* 13, 537 (1965)]. The overall recovery of carbaryl was 26 and 28% of the dose for the two men. The metabolites identified were 4-(methylcarbamoyloxy)-1-naphthyl glucuronide (about 4.0%), 1-naphthyl glucuronide (about 15.0%), and 1-naphthyl sulfate (about 8.0%). Qualitative evidence was obtained for the presence of 1-naphthyl methylimidocarbonate *O*-glucuronide. Based on carbon-14 metabolism studies in the domestic pig, this metabolite could be present in the urine of man in concentrations up to 40% of dose (Knaak *et al.*, unpublished results). An analytical method for this metabolite is presently being worked on. The results obtained with the fluorometric method (26-28%) were lower than those obtained colorimetrically for total 1-naphthol (37.8%) [Best and Murray, *J. Occupational Med.* 4, 507 (1962)].

35. *Physicochemical Changes in Pesticides after Formulation Causing Health Hazards.* G. E. QUINBY, State of Washington Department of Health, Olympia, Washington.

The decomposition of pesticides after formulation resulting in decrease of active ingredients and lessening of toxicity has long been recognized and widely recorded by industrial and regulatory agencies concerned with buyers' rights to receive the quality of product being purchased. However, little attention has been given to the opposite phenomenon of changes in pesticides that result in increased toxicity with production of increased pesticidal efficiency or poisoning of man, animals, or plants exposed.

A number of pesticides have been discovered to change toxicity or stability interfering with planned use or becoming incompatible with other ingredients in the original formulation or with other formulations when mixed just before application.

Examples of physicochemical changes of this sort include formulations of DDT, dieldrin, Perthane, Di-Syston, diazinon, dimethoate, parathion, trichlorfon, Baygon, captan, and Phaltan.

Some mechanisms of change are unknown. Physical and chemical alterations known or suspected to cause these changes have been established in dusts, wettable powders, emulsifiable concentrates, granular fertilizers, and technical materials including insuspensibility, separation (from weak dilutions back to technical strength), oxidation, isomerizations, and other molecular rearrangements.

No doubt other examples of these kinds of physicochemical changes are known to other workers but may not have been reported as a potential health problem.

36. *Urinary Organic Extractable Phosphate after Phosphate Ester Poisoning*. E. G. COMSTOCK and C. WATTS, P. O. Box 2565, Houston, Texas. (Sponsor: J. A. Zapp, Jr.)

Urinary excretion of acidic organic metabolites of the organic phosphate ester insecticides has been measured in six cases of accidental poisoning of humans. The cases reported include poisoning by ethion, diazinon, DDVP, and malathion. The excretion of organic-extractable phosphorus is correlated with the clinical course and other parameters of organic phosphate ester poisoning. Its use in the differential diagnosis of the cholinergic crisis is discussed.

37. *Effect of Dieldrin Insecticide on Rhesus Monkeys after Three Years of Ingestion*. M. R. ZAVON, R. TYE, and K. STEMMER, Kettering Laboratory, University of Cincinnati, Cincinnati, Ohio. (Sponsor: C. S. Weil.)

The insecticide dieldrin has been used for more than a decade and is presently resident in the body fat of much of the human population. The significance of the trace quantities present in the population is a matter for speculation. We have attempted to evaluate the significance of long-term exposure by feeding the compound to monkeys at doses that are more nearly comparable to the human experience than has been true in previous studies.

Thirty-one rhesus monkeys have been fed dieldrin in their daily diet in concentrations of 0.01, 0.1, 0.5, 1.0, and 5.0 ppm for a period of 3 years in a study that is continuing. Before exposure to dieldrin, and at intervals during the experiment, clinical and laboratory examinations were made to evaluate bodily functions. These included dieldrin analyses of blood and fat and liver biopsies.

Weight and body function have remained normal throughout the three years for most of the animals. Correlation between the concentration of dieldrin in the fat and blood and between fat and dietary intake is indicated. Data on all clinical laboratory determinations are included.

38. *Comparative Anticholinesterase Action of Organophosphate Insecticides in Mammals, Birds, and Fishes*. S. D. MURPHY, R. R. LAUWERYS, and K. L. CHEEVER, Department of Physiology, Harvard School of Public Health, Boston, Massachusetts.

Manometric assays of acetylcholinesterase (ChE) were performed on homogenized brains from several mammalian, avian, and piscine species. Results of tests of the *in vitro* anticholinesterase activity of the oxygen analogs of malathion, parathion, and Guthion are shown in the table. The figures are the quantities, in millimicromoles ($m\mu$ moles), of each compound that were required to produce 50% inhibition of the ChE activity of brains from each species.

Compound	Mouse	Rat	Monkey	Guinea pig	Chicken	Sparrow	Sunfish	Bullhead
Malaoxon	0.410	0.420	0.400	1.100	0.092	0.130	0.200	0.760
Paraoxon	0.044	0.028	0.041	0.210	0.007	0.004	1.850	0.310
Gutoxon ^a	0.056	0.038	0.037	0.500	1.700	1.100	0.033	0.009

^a *O,O*-Dimethyl S-[4-oxo-1,2,3-benzotriazin-3(4H)-yl-methyl] phosphorothioate.

More than 50 $m\mu$ moles of malathion, parathion, or Guthion were required for 50% inhibition of rat, chicken, and sunfish brain ChE *in vitro*. Mice, sunfish, and chickens were given intraperitoneal injections of insecticides, sacrificed after 2 hours, and their brains assayed for ChE activity. The relative sensitivities to cholinesterase inhibition *in vivo* were: for malathion and malaoxon, sunfish > chickens > mice; for parathion and paraoxon, chickens > mice > sunfish; for Guthion, mice > sunfish > chickens; and for Gutoxon, sunfish > mice > chickens. The results suggest that differences in the sensitivities of cholinesterases from different species or classes of animals to inhibition by some organophosphates can be an important factor contributing to differences in species susceptibility to poisoning by these

insecticides. This factor may explain the lack of correlation between toxicity and liver metabolism for some insecticides that was observed for mice and sunfish [Murphy, *Proc. Soc. Exptl. Biol. Med.* **123**, 392 (1966)].

39. *Feeding of Malathion and Carbaryl to Laying Hens and Roosters.* M. GHADIRI, D. A. GREENWOOD, and W. BINNS, Animal Disease and Parasite Research Laboratory, Agricultural Research Service, Utah State University, Logan, Utah.

Three hundred twelve white Leghorn laying hens and 65 adult white Leghorn males were divided into 13 groups with 24 hens and 5 males in each treatment. The birds were fed a basal diet of a laying mash to which malathion and carbaryl were added singly and in combination at levels of 0, 75, 150, 300, and 600 ppm for 3 weeks. Birds were weighed at the beginning and at the end of the experiments.

Eggs were collected daily and incubated to determine hatchability and teratogenic effects. As the levels of pesticide in the diet increased, the hatchability decreased significantly. The percentage of deformities increased significantly as the concentration of pesticide in the diet increased. Marked deformities were observed in the chicks that developed.

At the end of a 3-week feeding period, the birds were sacrificed and tissues were taken for chemical and histopathologic studies.

Storage of malathion and carbaryl in the eggs increased significantly as the level of pesticides increased. Storage of the two compounds was greater in egg yolk than in egg white. The liver and kidney tissues of the hens stored more malathion and carbaryl than other tissues, such as the breast, leg muscles, and gizzard. Colored photographs illustrating the deformities are presented.

It should be noted that the levels of pesticide fed were excessive. Studies are in progress to determine the toxicologic effects of feeding lower levels of the pesticides. [This research was supported in part by a Training Grant (1179-02) in Toxicology supported by the General Medical Services Section, Research Grants Division, National Institutes of Health, Bethesda, Maryland.]

40. *Study of Liver Phospholipase Activity in Normal and Pesticide-Treated Rats.* C. H. WILLIAMS and K. H. JACOBSON, Division of Toxicological Evaluation, Bureau of Science, Food and Drug Administration, U.S. Department of Health, Education, and Welfare, Washington, D.C.

Many insecticides that are toxic by virtue of their inhibition of cholinesterase also inhibit other enzymes, but the toxicologic significance of this additional inhibition, while often suspected, has not been established. Some relationship exists between lecithin and nerve activity, and several anticholinesterases affect lipid metabolism in the nervous system. The present study was concerned with the possible action of several insecticides on a phospholipase, and whether such action was toxicologically significant.

Phospholipase D, an enzyme hydrolyzing lecithin to phosphatidic acid and choline, has been found only in plants. Using exogenous lecithin substrates, we were unable to demonstrate such an enzyme in rat liver but were able to measure endogenous phospholipid hydrolysis in liver homogenates and microsomes. Endogenous cephalin hydrolysis by rat-liver microsomes has been reported.

Chlordane, aldrin, and 6-chloro-3,4-xylyl methyl carbamate had no effect upon enzyme activity *in vitro*; parathion produced inhibition.

Effects on enzyme activity were absent after administration to rats of (1) acutely toxic doses of the carbamates 6-chloro-3,4-xylyl methyl carbamate or 4-benzothienylmethyl carbamate; (2) 6-chloro-3,4-xylyl methyl carbamate in a chronic feeding study; (3) aldrin or chlordane 8 days prior to testing; and (4) acutely toxic doses of 6-chloro-3,4-xylyl methyl carbamate or 4-benzothienylmethyl carbamate to aldrin- or chlordane-pretreated rats. Acutely toxic doses of parathion for short periods enhanced enzyme activity; the relationship of this action to toxicity is not now known.

41. *Cataractogenicity of Various Drugs in Rats*. S. GORDON and T. BALAZS, Toxicology Evaluation Department, Toxicology Research Section, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

Weanling rats, six per group, were fed triparanol in the diet at a level of 0.01 and 0.03% for 10 weeks, and at 0.1% for 6 weeks. Biomicroscopic (slit-lamp) examination of the lens during treatment revealed, as early as 4 weeks, a few striae in the cortex at the 0.01 and 0.03% dosages and marked diffuse striations reflecting the anatomic structure of the lens fibers at the 0.1% level. The changes in the intermediate-dose group became more pronounced 3 months after the discontinuance of dosing. Opacities visible to the naked eye developed in the high-dose group a few weeks after the end of dosing.

A hypocholesteremic alkylated steroid, 3 β -(β -diethylaminoethoxy)androst-5-en-17-one methoxime hydrochloride, given in the diet induced similar types of changes as those described above.

Hexesterol (0.0001%), diphenhydramine hydrochloride (0.15%), and chlorpromazine (0.1 and 0.2%) given for 12 weeks produced no evidence of lenticular changes.

42. *Use of the Rhesus Monkey for Predicting Human Response to Eye Irritants*. J. H. BECKLEY, T. J. RUSSELL, JR., and L. F. RUBIN, Colgate-Palmolive Co., New York, New York, Bio/dynamics, Inc., East Millstone, New Jersey, and University of Pennsylvania, Philadelphia, Pennsylvania. (Sponsor: T. O. King.)

Accurate prediction of human ocular response to eye irritants on the basis of the standard Draize rabbit-eye test is often difficult, if not impossible. A new method, using the monkey eye, has been developed to predict a substance's irritant potential in human eyes with a high degree of reliability. The new method has been documented by exposing monkey eyes to a given test substance, followed by instillation of the same material in the eyes of human volunteers.

Development of the new method employed, in sequential fashion, gross photography, slit-lamp examination, slit-lamp photography, fluorescein staining and photography, tonometry, and fundus examination.

The new scoring method incorporates the standard Draize readings, but slit-lamp examinations require detailed observations with localization of all abnormalities (i.e., cells in the anterior chamber, rubeosis iridis, status of the epithelium, and depth of vascularization).

A number of animal species were used to demonstrate the variation of reaction to a single test substance using the new scoring method. Included were the albino rabbit, the pigmented rabbit (since this animal simulates man more closely in coloration and reaction), the dog, the squirrel monkey, and the rhesus monkey.

43. *Interlaboratory Comparative Evaluation of Single Oral Test*. C. S. WEIL and C. J. WRIGHT, Chemical Hygiene Fellowship, Mellon Institute, Pittsburgh, Pennsylvania, and Biochemical Research Laboratory, The Dow Chemical Company, Midland, Michigan.

In recent years, regulatory procedures to predict relative hazard to man have been published which detail every part of the protocols to determine toxicity and irritation of chemicals on laboratory animals. An interlaboratory round-robin test was designed to indicate the degree of necessity to rigidly regulate such a procedure for the single oral test.

Ten chemicals were administered orally to male albino rats by each of a group of industrial, commercial, and university toxicology laboratories. In each laboratory, the LD₅₀ for each chemical was determined using three different procedures: (a) by reference protocol using a reference strain of rats, (b) by the same reference protocol using the rats commonly used by that laboratory, and (c) by the procedure and rat generally used by that laboratory.

The reference procedure selected was a compromise based upon protocols used in the majority of the laboratories. Comparisons which can be derived from these studies are the interlaboratory variation using the same procedure and the intralaboratory variation using different procedures, for example, fasted *vs.* nonfasted rats, rats of different ages or strains, and variations in the method of administration.

44. *Use of the Rhesus Monkey for Predicting the Toxic Effects of Detergents in Humans.* J. H. BECKLEY, T. J. RUSSELL, JR., and J. R. MCCOY, Colgate-Palmolive Co., New York, and Bio/dynamics, Inc., East Millstone, New Jersey. (Sponsor: T. O. King.)

The albino rat has commonly been used to predict the consequences of accidental human ingestion of detergents and related substances. Acute oral toxicity studies in rats, however, do not reflect the emetic characteristics of many soap and detergent materials.

The rhesus monkey has been employed for acute oral toxicity studies to demonstrate the protective action of emesis following administration of various detergents. This species has further been used to develop dissection techniques to investigate possible local tissue damage following oral administration of detergents.

Groups of male and female rhesus monkeys were force-fed a number of detergent products, while groups of equal numbers were intubated with aqueous solutions of the same materials. Animals in each group were sacrificed shortly following treatment to evaluate local tissue effects. The remaining animals in each group were sacrificed at varying time intervals thereafter to assess the possibility of delayed effects. Animals in all groups had blood samples drawn prior to test and at varying time intervals after treatment to evaluate possible systemic changes in electrolyte balance.

The results of these studies suggest that the rhesus monkey mimics human response to accidental acute ingestion of detergents and is, therefore, the animal of choice for predicting the intoxication potential of these materials in a human population.

45. *Toxicologic Observations on the Venom of the Desert Ant Pogonomyrmex barbatus.* M. W. WILLIAMS and C. S. WILLIAMS, Veterans Administration Hospital, Tucson, Arizona.

Venom yields by a new technique to be described averaged 12 μg per ant extracted from one pooled sample of 11,200 ants trapped between mid-September and mid-November, 1965, in the Tucson area.

The 48-hour intraperitoneal LD_{50} for 20-gram Swiss-Webster mice was 0.81 mg/kg \pm 30% as determined by the method of Deichmann and LeBlanc [*J. Ind. Hyg. Toxicol.* **25**, 415 (1943)]. Similarly, the 72-hour intraperitoneal LD_{50} for mongrel 2-kg cats was 2.95 mg/kg \pm 30%. Subcutaneously, the venom was much less toxic in the mouse, the 48-hour LD_{50} being 45 mg/kg \pm 30%. Thus, the intraperitoneal route is 55 times as toxic as is the subcutaneous.

Intense salivation, retching, vomiting, and diarrhea accompany toxic intraperitoneal doses of venom in the cat and dog, while similar subcutaneous doses produce only localized irritation with no general systemic toxicity.

Micro-Kjeldahl analysis of venom revealed a total nitrogen content of 51.4% protein (of total solids), and 24-hour acid hydrolysis gave the following amino acid percentage concentrations: aspartic, 8.9; threonine, 2.8; serine, 2.5; glutamic, 7.7; proline, 5.3; glycine, 2.9; alanine, 3.5; cystine, trace; methionine, 0.02; isoleucine, 7.9; leucine, 8.5; tyrosine, 1.4; phenylalanine, 2.3; lysine, 8.9; histidine, 1.5; and arginine, 2.6. (This project supported in part by National Institutes of Health grants GM 11991-02 and CA 07775-02 CY.)

46. *Comparison of the Toxicity of Thiopental Given Rectally versus Orally to Albino Rats.* J. SINGH and E. M. BOYD, Department of Pharmacology, Queen's University, Kingston, Ontario, Canada.

Previous studies from this department have shown that tannic acid and aspirin are more toxic when given rectally than when given orally to albino rats. In overnight-starved, young, male albino rats, the $\text{LD}_{50} \pm \text{SE}$ of thiopental sodium was found to be 102 \pm 16 mg/kg when administered rectally and 158 \pm 19 mg/kg when given intragastrically. The onset of anesthesia was faster and the degree of impairment of the corneal reflex was greater following rectal administration. Death occurred at 1-3 hours by both routes. In survivors at 8 hours, slowing of the respiratory rate and the degree of hypothermia were greater following rectal administration. The degree of hepatic edema at death in rats given the smaller doses

of thiopental rectally was similar to that following the larger doses given orally. The results indicate that the acute toxicity of thiopental sodium given rectally approximates that when given by slow intravenous infusion. (Supported by Canadian Public Health Research Grant 605-7-273.)

47. *Studies on Cobalt Toxicity.* G. S. WIBERG, I. C. MUNRO, and H. C. GRICE, Pathology and Toxicology Section, Research Laboratories, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Ontario, Canada.

The effects of cobalt on myocardial metabolism in rats and guinea pigs will be discussed. This will involve *in vivo* and *in vitro* studies on mitochondrial respiration with pyruvate, octanoate, and palmitate serving as substrates. A more general pathologic assessment will appraise the cardiotoxic potential of cobalt ion in rats and guinea pigs maintained on normal and thiamine-deficient diets. Toxicologic criteria will include growth patterns, hematologic, gross pathologic and histologic examination, including electron microscopy.

48. *Computer Management of Toxicologic Data.* T. J. RUSSELL, JR., Bio/dynamics, Inc., East Millstone, New Jersey. (Sponsor: T. O. King.)

Efforts to develop a toxicologically oriented FORTRAN library have resulted in a series of computer programs directed toward the management of subacute and chronic toxicity studies. The purpose of these programs is to rapidly assimilate the mass of data generated in the toxicology laboratory and present appropriate compilations and analyses in a manner allowing senior professional management to accurately monitor a number of experiments being simultaneously executed. This end is accomplished, in part, by instructing the computer to examine the data using a variety of analytical techniques, then verbalizing the results on separate output sheets for convenient and immediate reference.

Included in the FORTRAN series currently operating are programs for the management of hematology and clinical chemistry data. Normal clinical ranges established by sex and species in this laboratory are filed in the computer memory. The individual test values for each parameter examined are then compared to the preestablished normal ranges and a symbol placed by outlying values. Values out of normal range are further examined statistically in an attempt to determine whether the outliers are drug related.

The comparison of dosages with a control group, and of dosages among themselves, is based on the statistical analysis of paired differences. A paired difference is computed by subtracting the value of a given clinical parameter in any animal at a fixed point in the experiment from the same parameter obtained from that animal at baseline.

49. *Toxic Effects of Oral Administration of 3,4-Benzfluoranthene and Other Polynuclear Hydrocarbons on Mice.* F. E. RENO, H. C. MILLAR, and D. A. GREENWOOD, Utah State University, Logan, Utah.

One hundred thirty-two adult virgin female mice, 5-7 months of age, were distributed into six groups and treated with diets containing 3,4-benzfluoranthene, 3,4-benzpyrene, 1,2,7,8-dibenzacridine, 3,4,9,10-dibenzpyrene, and 1,2,5,6-dibenzanthracene. A control group served as the sixth group. The treated diets consisted of powdered laboratory feed blended with a corn oil solution of the hydrocarbon to give a concentration of 1 mg of hydrocarbon per gram of feed. The control received the corn oil and feed.

Animals receiving 3,4-benzfluoranthene showed toxic signs within 7 days and all receiving this compound died within 21 days. Those receiving the other hydrocarbons showed no toxic signs.

At the end of a 21-day period on treated feed, the animals receiving the other hydrocarbons were mated, and the effect of the hydrocarbons on their ability to conceive was noted. Those animals treated with 3,4-benzpyrene and 1,2,7,8-dibenzacridine produced fewer pregnancies than the control group.

Histopathology conducted on the animals receiving 3,4-benzfluoranthene showed a general systemic toxemia. Histopathologic studies on the animals will be presented. [This research was supported in part by a Training Grant (1179-02) in Toxicology supported by the General Medical Services Section, Research Grants Division, National Institutes of Health, Bethesda, Maryland.]

50. *Synergism among Oral Carcinogens. IV. Results of the Simultaneous Feeding of Four Tumorigens to Rats.* W. B. DEICHMANN, M. L. KEPLINGER, and E. M. GLASS, Department of Pharmacology and Research and Teaching Center of Toxicology, University of Miami School of Medicine, Coral Gables, Florida.

In this experiment, four compounds were fed to Osborne-Mendel strain rats, individually and in combination, each at a level of 50% of its liver-tumor-producing concentration (rat); that is, aramite and DDT were each added to the diet in a concentration of 200 ppm; methoxychlor at a concentration of 1000 ppm; and thiourea at 50 ppm. A fifth compound, aldrin, was added to these studies. It was fed at a level of 5 ppm, representing 50% of its mouse-liver tumorigenic dose.

There were 60 Osborne-Mendel strain rats in each of eight groups (30 males and 30 females), seven experimental groups and one control.

Our primary concern was directed toward the number and types of tumors. As in previous experiments, an animal, as a rule, showed only one tumor. Therefore, the number of tumors found coincides essentially with the number of rats so affected. Of the 60 rats fed aramite, 23% showed tumors. Of the 60 rats fed DDT, 17% showed tumors, while the ratio for methoxychlor was 18%, for thiourea 28%, and for aldrin 25%. Of the control rats, 23% showed tumors.

Of the rats in group 7 which were fed aramite, DDT, methoxychlor, and thiourea in a total tumorigenic dose of 200%, only 17% showed tumors. And, finally, of the 60 rats fed aramite, DDT, methoxychlor and aldrin in a total tumorigenic dose of 200%, only 10% showed tumors.

Most of the tumors in the experimental and control rats were nonmalignant. The organs primarily affected were the mammary glands, blood, lungs, liver, stomach, and skin.

Since the number of liver tumors was essentially the same in both experimental and control groups, it is concluded that these compounds, when fed in a total liver-tumorigenic dose of 200%, do not act in an additive manner.

51. *Metabolites of 1- and 2-Naphthylamine in the Urine of Dogs.* J. L. RADOMSKI and E. BRILL, Department of Pharmacology and Research and Teaching Center of Toxicology, University of Miami School of Medicine, Coral Gables, Florida.

A method for the simultaneous quantitative and qualitative analysis of the urinary metabolites of the naphthylamines utilizing ^{14}C -labeling has been developed. Using this procedure, a total of 9 labeled substances have been detected in the urine of dogs administered maximally tolerated doses of 2-naphthylamine. By far the greater part of the urinary activity (85-90%) is due to 2-amino-1-naphthyl sulfate. In addition, small concentrations of di-(2-amino-1-naphthyl) hydrogen phosphate were detected as well as 2-amino-1-naphthyl glucuronide and the free amine. Evidence was obtained of the presence of 2-amino-1-naphthyl *N*-glucuronide in addition. The other 4 metabolites remain unidentified.

For the first time, di-(2-amino-1-naphthyl) hydrogen phosphate has been prepared in pure form. It has been established that this substance does not decompose to 2-amino-1-naphthyl dihydrogen phosphate in *n*-butanol-acetic acid solvent systems as has been reported [Boyland, *Biochem. J.* 78, 175 (1961)]. Spontaneous decomposition to the monoester does occur with time, however. Pure di-(1-amino-2-naphthyl) hydrogen phosphate has also been prepared. The absence of this metabolite from the urine of dogs given 1-naphthylamine has been confirmed.

Previously reported results indicated that *N*-2-naphthylhydroxylamine was not a urinary metabolite of 2-naphthylamine in the dog. However, an unidentified purple band of lower electrophoretic mobility than the TPF complex of *N*-2-naphthylhydroxylamine was observed in the course of these studies. The results of our attempts to identify this band will be presented.

52. *Possible Complicity of Diphenylene in the Origin of Tumors in the Manufacture of Benzidine.* J. MARHOLD, M. HUB, F. RUFFER, and M. MATRKA, Industrial Toxicology Division, Research Institute for Organic Synthesis, Pardubice-Rybitivi, Czechoslovakia.

Benzidine is produced by the "benzidine rearrangement" of hydrazobenzene. In this operation, a considerable amount (10-15%) of benzidine isomers arise, principally diphenylene (2,4'-diaminobiphenyl). In the production of benzidine, an exposure to diphenylene, therefore, exists, and in its use to a much lesser extent, because technical benzidine contains only about 1% of diphenylene. Occupational tumors are more frequent in benzidine producers than in benzidine users.

In animal experiments, benzidine is only a comparatively weak carcinogen. The carcinogenicity of diphenylene has probably not yet been investigated thoroughly.

In our experiments, diphenylene administered orally to rats proved to be acutely more toxic and chronically much less toxic than benzidine. In rats, no tumors developed after its administration. In dogs (3 males and 3 females of the same litter), xenylamine (2 animals), benzidine alone (1 animal), diphenylene alone (2 animals), and benzidine with diphenylene (1 animal) were given in daily oral doses of 5 mg/kg. After 7 years of dosing, tumors of the spleen (2 cases) and of the skin and lungs (1 case) arose in diphenylene- and benzidine-diphenylene-treated dogs. A tumor of the urinary tract was found in a xenylamine-treated dog after 4 years of dosing. No tumor was found in the other dog after a short (6 months) administration of xenylamine (4-aminobiphenyl), or after 6 years of dosing with pure benzidine.

It would not be right, perhaps, to draw definite conclusions from these experiments, but it seems to be possible that diphenylene could be a stronger carcinogen than benzidine, and could play a role in the origin of tumors attributed to benzidine only.

53. *Fluoroacetate Toxicity by a New Antitumor Agent, Cyclohexyl Fluoroethyl Nitrosoourea.* E. R. HOMAN, V. T. OLIVERIO, and H. FEINMAN, National Cancer Institute, Bethesda, Maryland, and Hazleton Laboratories, Inc., Falls Church, Virginia. (Sponsor: D. P. Rall.)

3-Cyclohexyl-1,1-(2-fluoroethyl)-1-nitrosoourea (CFNU) is one of a series of nitrosoourea compounds with demonstrated antitumor activity in experimental animal systems. During the toxicologic investigation of this series of compounds in several animal species, a particular susceptibility of the rat to CFNU was noted. The 21-day single-dose LD₅₀ of CFNU in male albino mice was 111 mg/kg for oral administration and 51 mg/kg for intravenous administration. The comparable LD₅₀'s in male rats were 18.5 mg/kg orally and 12 mg/kg intravenously. Further study revealed that this nitrosoourea is distinguished by a more rapid onset of toxicity in all species tested. A similarity between the toxic signs of CFNU and fluoroacetate focused attention on the possibility of metabolic formation of fluoroacetate ion from CFNU.

This hypothesis was tested by evaluating the relative protective effects of monoacetin (glyceryl monoacetate) in rats treated with CFNU or fluoroacetate. Lethality from intraperitoneal fluoroacetate was markedly reduced when repeated intramuscular injections of monoacetin were given. In similar experiments, the lethality resulting from oral administration of CFNU was reduced when monoacetin was administered, and there was a marked prolongation of survival time.

Further confirmation of the fluoroacetate hypothesis of CFNU toxicity was provided by biochemical studies. A large body of research supports the theory that the primary toxicity of fluoroacetate stems from the inhibition of aconitase by fluorocitrate. One consequence of this inhibition is an accumulation of citrate which can be measured and which serves as a basis for the detection of fluoroacetate intoxication. In our studies, rats and mice were given

single doses of sodium fluoroacetate or CFNU and sacrificed at intervals afterwards. Kidney homogenates from animals sacrificed 1 or 6 hours after fluoroacetate treatment showed marked increases in citrate levels. Similar changes in citrate concentrations were observed in homogenates from CFNU-treated animals sacrificed 6 or 12 hours, but not 1 hour, after dosing. Homogenates from animals treated with the chloroethyl analog of CFNU exhibited no changes in citrate levels.

54. *Early Acute Aflatoxicosis Stimulates Rainbow Trout Hepatomagenesis.* J. E. HALVER, L. M. ASHLEY, C. E. SMITH, and G. N. WOGAN, Western Fish Nutrition Laboratory, Bureau of Sport Fisheries and Wildlife, U.S. Department of the Interior, Cook, Washington, and Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Crystalline aflatoxin B₁ was dissolved in acetone, adsorbed on alphacellulose flour, dried at 60°, and mixed into a test diet (CTD) of casein, 35; gelatin, 15; dextrin, 29; cod liver oil, 2; corn oil, 6; minerals, 4; vitamins, 1; and alphacellulose, 8. Rainbow trout (*Salmo gairdneri*) with an initial average weight of 0.5 g were fed diet to contain 0.1 or 0.4 µg of aflatoxin B₁ per fish in duplicate lots of 100 fish each during 2, 4, 8, 12, 16, 20, or 24 weeks, respectively; then continued on diet without aflatoxin B₁ for the remainder of one year on test. Other groups were fed diets with 20 ppb of aflatoxin B₁ before or after 2, 4, 8, 12, 16, or 20 weeks of CTD. Micro nodules of trabecular hepatoma appeared at 6 months after each fish ate only 0.1 µg of aflatoxin B₁ during the first 2 weeks of feeding, and then ate CTD for the remaining 24 weeks. Gross liver tumors appeared by nine months on test in all lots receiving 0.1 or 0.4 µg of aflatoxin B₁ during the initial challenge period. A tumor-dose-time relationship was evident.

55. *Effect of Acetanilide on Toxicity and Metabolism of N-2-Fluorenylacетamide.* R. S. YAMAMOTO, P. H. GRANTHAM, L. MOHAN, E. K. WEISBURGER, and J. H. WEISBURGER, Biology Branch, National Cancer Institute, Bethesda, Maryland.

Although 0.03% of the carcinogen N-2-fluorenylacетamide (FAA) in the diet is toxic to Fischer rats, causing death within 3 weeks, addition of 0.8% of acetanilide, the highest tolerated level, reduced the toxicity of FAA, as evidenced by the body-weight curves and the continued survival of the rats.

To investigate this finding, the metabolism of a single intraperitoneal dose of 2-FAA-9-¹⁴C was followed in groups of rats which had been fed the following in the diet for 6 weeks: (1) 0.02% of FAA, (2) 0.02% of FAA and 0.8% of acetanilide, (3) 0.8% of acetanilide, and (4) control diet. The animals were autopsied 24 hours after the dose of labeled carcinogen. The results demonstrated that feeding 0.02% of FAA reduced the body weight significantly. Body weights of rats fed the combination of acetanilide and FAA were slightly less than those of rats on control diets. The level of radioactivity in the plasma of FAA-fed rats was only half of that from rats fed acetanilide, acetanilide and FAA, or control diet. The level of isotope from FAA combined with liver protein was highest in control rats, about 30% less in acetanilide or acetanilide-FAA rats, and only one-third of the control value in those prefed FAA. In rats prefed FAA, a greater proportion of the radioactivity was excreted in the urine than in the other groups. The radioactivity in the urinary glucosiduronic acid fraction from rats prefed FAA was twice or three times the level in the corresponding fraction from the control, acetanilide, or acetanilide-FAA groups. The implications of these findings and the long-term results will be discussed.

56. *Metabolism of Nickel-63 Carbonyl.* F. W. SUNDERMAN, JR., C. E. SELIN, G. L. CASAL, H. L. WHEELER, and H. L. CROMROY, Departments of Pathology and Radiation Biology, University of Florida College of Medicine, Gainesville, Florida.

The distribution and excretion of ⁶³Ni was studied in rats following intravenous injection of an LD₅₀ of ⁶³Ni(CO)₄ (22 mg/kg as Ni). Nickel-63 was detected in exhaled air for 2 hours

after injection (mean excretion equal to about 5% of the administered dose) indicating that vapor of $\text{Ni}(\text{CO})_4$ crosses the alveolar wall. During 4 days after injection, urinary excretion of ^{63}Ni averaged 31% of the administered dose, and fecal excretion averaged 2%. Retained ^{63}Ni was present in the lung, liver and, to lesser degree, in other organs. Subcellular fractionations of lung and liver homogenates demonstrated that ^{63}Ni was located primarily in the supernatant fraction, and was bound predominantly to RNA and phospholipids. Under the described conditions, $\text{Ni}(\text{Co})_4$ inhibited the induction of benzpyrene hydroxylase and aminopyrine demethylase in lung and liver. The possible relationship of the binding of nickel to RNA to the inhibition of enzyme induction will be considered. [Supported by U.S. Atomic Energy Commission Grant AT-(40-1)-3461; by American Cancer Society Grant E-374A; and by U.S. Public Health Service Research Grant (National Cancer Institute) CA-08783-01.]

57. *Urinary Excretion of Primidone and Its Metabolites in Rabbits.* J. M. FUJIMOTO, W. H. MASON, and J. G. HAMILTON, Department of Pharmacology, Tulane Medical School, New Orleans, Louisiana.

A thin-layer chromatographic method was developed for estimating primidone and a metabolite, phenylethylmalondiamide. One to 2 ml of urine was extracted with an equal volume of chloroform after the addition of 0.5 g of potassium carbonate. Five- to 20- μl samples of the chloroform phase were chromatographed on silica gel thin-layer glass-fiber sheets using 2,2,4-trimethylpentane and acetic acid (100/20, v/v). After drying, the chromatograms were sprayed with concentrated sulfuric acid. The compounds appeared as dark spots when charred. Quantitation was done by comparing the spots visually with the standards run along with the unknowns. A second chromatogram was run, using an appropriate amount of the chloroform extract to fall within the 3–10 μg range of the standard. In this range, 1- μg differences in intensity could be detected. Phenobarbital was determined by the method of Bush [*Life Sci.* 4, 1403 (1965)]. Excretion of primidone and phenylethylmalondiamide occurred appreciably before phenobarbital. The excretion of the latter rose slowly but persisted for a longer time. Phenobarbital is formed slowly from primidone but has a greater tendency to accumulate than primidone. With 400 mg/kg, p.o., about 50% of the dose was accounted for as primidone and metabolites.

58. *Toxicity Studies of Folate Deficiency States.* R. E. BAGDON, F. HALL, F. HEMRICK, and H. EISENBEIS, Department of Pharmacology, Research Division, Hoffmann-La Roche, Inc., Nutley, New Jersey.

To characterize toxic manifestations produced by folate deficiency, groups of rats were scheduled for a 13-week experiment as follows: group I, controls; group II, methotrexate, 1.5–3.0 mg/kg, p.o.; group III, methotrexate, 0.75 mg/kg, p.o.; group IV, folic acid (FA) deficient diet; group V, FA-deficient diet with 1% succinylsulfathiazole; group VI, FA-deficient diet supplemented weekly with 0.33 mg of FA, p.o. These results were compared with those obtained in rat studies of trimethoprim, an inhibitor of bacterial folate reductase, administered alone and in combination with sulfisoxazole. After 9 weeks, rats in group II exhibited retardation of growth and food consumption and, in males, a 40% mortality. The earliest signs were observed after 2–4 weeks and consisted of a slight anemia and relative neurotropenia; during weeks 6–8, the anemia was more marked and the mean corpuscular hemoglobin concentration and the mean corpuscular volume were decreased; the mean corpuscular hemoglobin, total white blood cell count, reticulocyte count, platelet count, and segmentation of neutrophils were normal. However, urinary forminoglutamic acid excretion after a histidine load was not elevated, a result indicating that tissue stores of folate coenzymes other than tetrahydrofolate were depleted in methotrexate-poisoned animals. In the nutritionally FA-deficient rats, growth retardation and an elevation of urinary forminoglutamic acid after histidine were detected after 2 weeks; however, hematologic abnormalities were not observed after 8 weeks. In contrast, rats given 200 mg/kg of trimethoprim alone or in combination with 800 mg/kg of sulfisoxazole exhibited normal growth, hemograms, and urinary forminoglutamic acid excretion.

59. *Tris (1-Aziridinyl) Phosphine Oxide: Studies of Distribution and Metabolism in the Rat Using Carbon-14.* G. J. WRIGHT and V. K. ROWE, Biochemical Research Laboratory, The Dow Chemical Company, Midland, Michigan.

Tris (1-aziridinyl) phosphine oxide (APO) labeled with carbon-14 in the ethylenimine moiety, was administered intraperitoneally to rats of the Dow-Wistar strain. Urine, feces, and expired air were monitored for excretion of radioactivity, and tissues were examined for residual activity.

The major route of elimination was via the urine, and minor amounts were excreted as $^{14}\text{CO}_2$ in exhaled air. Traces of activity were found in the feces, which could have been the result of cross-contamination. Unchanged APO and ethylenimine were identified in the urine within 4 hours after dosing. Between 65 and 90% of the radioactivity was excreted within 24 hours after dosing. The residual activity was firmly bound to components of the body fluids and cells, primarily proteins. The half-life of the residual activity was calculated to be 25 days.

Subcellular radioactivity in the liver at 24 hours posttreatment was distributed rather uniformly between mitochondrial, microsomal, and supernatant fractions. Isolated, intact nuclei contained very little radioactivity. No radioactivity could be detected associated with DNA isolated from these nuclei.

60. *Ethylenimine: Studies of the Distribution and Metabolism in the Rat Using Carbon-14.* G. J. WRIGHT and V. K. ROWE, Biochemical Research Laboratory, The Dow Chemical Company, Midland, Michigan.

Ethylenimine labeled with carbon-14 was administered intraperitoneally to rats of the Dow-Wistar strain. Since the material is chemically unstable, the purity of the radioactive ethylenimine was determined prior to every experiment. Urine, feces, carbon dioxide, and acid-soluble volatile materials were monitored for excretion of radioactivity. The rats were killed at the end of the experiment and tissues were examined for residual radioactivity.

The major route of excretion of radioactivity was via the kidneys with minor amounts found in the CO_2 , feces, and acid-soluble volatile material. The radioactivity found in the urine was contained in a mixture of products including ethylenimine and at least six other unidentified materials.

Although residual radioactivity was widely distributed among the tissues, the liver and spleen had the highest activity per gram of tissue. Adipose tissue contained only trace amounts of radioactivity indicating that either ethylenimine or some other highly water-soluble derivative is distributed generally before reaction with the tissues.

The rates at which ethylenimine is excreted or metabolized were examined. Evidence was found for at least three metabolic pools: (1) a material with a short biological half-life; (2) formation of material which can be degraded to CO_2 ; and (3) material which is firmly bound.

61. *Fate of 2,6-bis-(1'-Methylheptadecyl)-p-Cresol (DOPC) in Rats.* B. D. ASTILI and D. W. FASSETT, Laboratory of Industrial Medicine, Eastman Kodak Company, Rochester, New York.

DOPC is a hindered phenol intended for use as a stabilizer in food packaging. Ingestion by rats of 1.0 g/kg of DOPC is not attended by a significant increase in urinary conjugate output, and there is evidence for a large proportion of the dose in the feces. The ingestion of single, undiluted 190-860 mg/kg doses of DOPC, ^{14}C -labeled on both 1'-methyl carbon atoms, is followed by the rapid excretion of almost the entire dose in the feces, 96-99% of the dose being thus accounted for. Less than 1.0% is accounted for in the urine, and none is detectable in the expired air. The fate of DOPC- ^{14}C incorporated in the diet at 0.01% and ingested for 15 days is identical with that of large single doses. Traces of radioactivity are disseminated to brain, fat, kidney, and liver at higher single-dose levels, to liver at lower single-dose levels, and to brain and liver after ingestion in the diet. This radioactivity is not retained by the organism but is released at varying rates from the respective tissues.

62. *Physiologic Effects of 2,6-Dioctadecyl-p-Cresol (DOPC) in the Rat and Dog.* C. J. TERHAAR, R. L. ROUDABUSH, D. W. FASSETT, and M. S. WEINBERG, Laboratory of Industrial Medicine, Eastman Kodak Company, Rochester, New York, and Food and Drug Research Laboratories, Inc., Maspeth, New York.

The toxicity of 2,6-dioctadecyl-*p*-cresol (DOPC), an antioxidant that has found use in certain packaging films, has been investigated. Intubation of DOPC at 6400 mg/kg and 3200 mg/kg failed to kill rats and mice, respectively, nor were any significant signs noted. Twenty ml/kg under an occluded patch for 24 hours causes only slight skin irritation on guinea pigs. Rats fed up to 1.0% in the diet for 103 days showed no toxic effects except for a slight reduction in average weight gain in the highest-dosed male group only. All animals showed normal utilization of food, appearance, behavior, and stools. No toxicologically significant changes were seen in organ weights, hematology, or histology. Dogs tolerated similar levels in the diet for 90 days except for slight food rejection and weight loss during the early weeks of the study. No effects of the treatment were noted on behavior or physical development, hemograms, blood chemistry, or urine composition. No dose-related effects were seen in organ-to-body-weight ratios, nor were any significant lesions detected at necropsy or upon microscopic examination of tissues. It appears, therefore, that administration of as much as 1.0% of DOPC in the diet over a 90-day period had no adverse effects on dogs.

63. *Species Variation in the Plasma Disappearance, Metabolism, Storage, and Excretion of Sulfobromophthalein.* C. D. KLAASSEN and G. L. PLAA, Department of Pharmacology, College of Medicine, The University of Iowa, Iowa City, Iowa.

The ability of different species to remove sulfobromophthalein (BSP) from the plasma varies. However, comparable rates of plasma disappearance are seen when 60 mg/kg is given intravenously to rats, 60 mg/kg to rabbits, and 15 mg/kg to dogs. Since BSP is metabolized, stored, and actively secreted, these parameters were examined to determine the limiting factor in the elimination of BSP from the plasma of these three species.

The *in vitro* conjugation of BSP with glutathione, in terms of milligrams of BSP per gram of liver per 5 minutes, is 14 in rats, 2.1 in rabbits, and 0.18 in dogs. The relative storage capacity (defined as milligrams of BSP in the liver divided by the plasma concentration in mg/100 ml and the body weight in kilograms) is 0.51 in rats, 0.57 in rabbits, and 1.1 in dogs. The transport maximum (T_m) for biliary secretion of BSP is 0.95 mg/min/kg in rats, 1.13 in rabbits, and 0.19 in dogs. The maximal bile volume (V) during BSP infusion is 64 μ l/min/kg in rats, 60 in rabbits, and 16 in dogs. The maximal BSP concentration in bile is 15.6 mg/ml in rats, 15.6 in rabbits, and 12.5 in dogs.

Since the T_m and V values best parallel the amount of BSP needed to obtain comparable rates of plasma disappearance in the three species, biliary secretion appears to be the limiting factor in the elimination of BSP from the plasma. (Supported by U.S. Public Health Service Research Grant AM-5802.)

64. *Distribution and Excretion of 14 C-Monomethylhydrazine.* M. K. PINKERTON, E. A. HAGAN, and K. C. BACK, Toxicology Branch, Toxic Hazards Division, 6570th Aerospace Medical Research Laboratories (MRBTT), Wright-Patterson Air Force Base, Ohio.

A total of 20 mice, 20 rats, 17 dogs, and 16 monkeys received intraperitoneal injections of 14 C-monomethylhydrazine (MMH) at doses of 22 mg/kg (mice), 15 mg/kg (rats), and 10 mg/kg (monkeys and dogs). At 2, 4, 8, and 24 hours after exposure, representative samples of approximately 20 tissues from each animal were processed for 14 C assay using liquid scintillation counting techniques. Both blood and urine samples were simultaneously analyzed by a chemical colorimetric method for unchanged MMH, and the results were correlated with total 14 C content. Results of the 14 C assays indicated that the mouse, rat, and monkey excreted twice as much as the dog in the first 2 hours, and that all 4 species excreted 25–40%

of the total dose by 24 hours after injection. Approximately 50% of the total ^{14}C excretion, at all experimental times, was apparently unchanged MMH as implied by the colorimetric results. Tissue distribution of ^{14}C showed the highest concentrations in liver, kidney, bladder, pancreas, and blood serum. Both clinically and pathologically, the dog was apparently much more susceptible than the other species tested to the toxic effects of MMH and to severe kidney damage.

65. *Kidney Damage and the Rates of Metabolism of Organomercurials*. T. W. CLARKSON, Department of Radiation Biology, University of Rochester, Rochester, New York. (Sponsor: H. C. Hodge.)

Certain organomercurial compounds, e.g., chlormerodin, not only are given to patients as diuretics but are also used, when labeled with ^{203}Hg , in scintillation scanning of the brain and kidneys for suspected defects. The organomercurial molecules are known to be rapidly excreted in urine. However, the possibility exists that these compounds might be metabolized in the tissues, resulting in the release of inorganic mercury which is slowly excreted.

When chlormerodin labeled with ^{203}Hg was injected intramuscularly in chickens, dogs, rabbits, and rats, inorganic mercury was detected in the liver and kidneys of these animals. For example, 4 hours after injection of chlormerodin at a dose of 4 mg/kg, as Hg, 4.3% of the injected dose was present in the kidneys of rats as inorganic mercury and 68% as the unchanged organomercurial. Two days later, the quantity of inorganic mercury in kidney had risen to 10% of the injected dose whereas the amount of intact chlormerodin had fallen to zero. Thereafter, the kidney level of inorganic mercury slowly declined. The amount of inorganic mercury found in the tissues of other animals varied with the species. Other mercurial compounds also released inorganic mercury in the tissues. The impairment of kidney function produced by injection of organomercurials was related to the tissue level of inorganic mercury.

66. *Toxicology of Vibramycin*. C. S. DELAHUNT, R. T. JACOBS, R. B. STEBBINS, and N. RIESER, Department of Pharmacology, Chas. Pfizer & Co., Inc., Groton, Connecticut.

The new antibiotic Vibramycin, α -6-deoxyoxytetracycline, has been shown experimentally [English, *Proc. Soc. Exptl. Biol. Med.* 112, 1107 (1966)] and clinically [Barrett *et al.*, 6th Interscience Conference on Antimicrobial Agents and Chemotherapy, October 1966, Philadelphia] to be the most potent tetracycline yet available. Its high lipophilicity was indicative of its greater oral absorption [von Schach and Delahunt, *J. Pharmacol. Exptl. Therap.* 152, 164 (1966)]. This increase in absorption was reflected in low oral LD_{50} 's. In the mouse, rat, and dog, they were 1900, > 2000, and > 500 mg/kg, respectively. A rodent pediatric study yielded no evidence of increased toxicity in newborn animals compared to adults. In a 90-day pre-clinical canine toxicity investigation, there was hepatic dysfunction of the biliary type when animals were given 250 mg/kg, orally, for more than 2 weeks. This condition was reversible in 21 days and was considered to be due to unusually large amounts of the drug in the bile. Chronic toxicity studies in the rat, dog, and monkey revealed the characteristic tetracycline effects of bone, teeth, and thyroid staining. These phenomena are not reversible, and they are not deleterious. Reproductive trials in the rat, rabbit, and monkey showed the antibiotic to be nonteratogenic.

67. *Effect of Chlorpromazine, Phenobarbital, and Iproniazid on the Polyphasic Mortality Curve of Aggregate Amphetamine*. D. E. SMITH, C. M. FISHER, and C. H. HINE, Department of Pharmacology and Experimental Therapeutics (Toxicology Activity), School of Medicine, University of California at San Francisco, San Francisco, California.

The LD_{50} of *d*-amphetamine given to mice housed separately is 100 mg/kg. When amphetamine is given to animals grouped together, a lower LD_{50} in the range of 25 mg/kg is found. The term "aggregate amphetamine toxicity" is used to describe this phenomenon.

Chlorpromazine has been reported to antagonize aggregate amphetamine mortality in the 25 mg/kg range. Phenobarbital in sedative doses (50 mg/kg) was reported to have no effect on single or aggregate amphetamine toxicity. Gardocki *et al.* [*Toxicol. Appl. Pharmacol.* 8, 550 (1966)], however, recently described the mortality curve of aggregate amphetamine toxicity as polyphasic in nature, with 2 LD₅₀'s, one at 25 mg/kg and the second at 100 mg/kg.

Our study analyzed the effects of chlorpromazine, phenobarbital, and iproniazid on this polyphasic aggregate amphetamine mortality curve. Pretreatment with chlorpromazine at 1, 5, 25, and 50 mg/kg had equal effects in that they abolished the first mortality peak at 25 mg/kg and converted it to a "single animal" amphetamine mortality curve. No dose of chlorpromazine influenced the second LD₅₀ of 100 mg/kg despite the anticonvulsant effects of higher doses of chlorpromazine. Specific behavioral parameters were analyzed in an attempt to define the mechanism of polyphasic amphetamine toxicity and its partial abolition by chlorpromazine.

Iproniazid, at 10 mg/kg, potentiated aggregate amphetamine toxicity, and phenobarbital, at 35 mg/kg, had no effect.

Currently this aggregate phenomenon is being studied with other central nervous system stimulants such as mescaline, and urine excretion studies are being performed to determine whether alterations in metabolic products correlate with these behavioral parameters. (Supported in part by U.S. Public Health Service Toxicology Training Grant No. 5 T01 GM01304-02.)

68. *Acute Toxicity of Chlorinated Pyridines.* P. J. GEHRING, T. R. TORKELESON, and F. OYEN, Biochemical Research Laboratory, The Dow Chemical Company, Midland, Michigan. (Sponsor: V. K. Rowe.)

The single-dose intraperitoneal toxicity of the chlorinated pyridines was determined in mice using corn oil solutions at a dosage of 10 μ l of solution per gram of body weight. The LD₅₀ values expressed in meq/kg were as follows: 2,3,6-trichloro-, 0.82; 2,6-dichloro-, 0.90; 2,3-dichloro-, 0.91; 2,3,4,5,6-pentachloro-, 0.94; 2-chloro-, 1.14; 2,4,6-trichloro-, 1.54; 3-chloro-, 2.05; 2,3,5-trichloro-, 2.36; 4-chloro-, 2.46; 2,3,5,6-tetrachloro-, 5.30; 3,5-dichloro-, 7.98; and 2,5-dichloro-, 11.42.

The oral LD₅₀ of 2-chloropyridine given to mice in a corn-oil solution was 0.97 meq/kg. In studies using the rabbit, this compound was essentially as toxic when applied to the skin as when given by intraperitoneal injection.

The single-dose inhalation toxicity of 2-chloropyridine was determined using rats. Minimum exposures causing 100% mortality and maximum exposures causing no mortality as well as maximum exposures that cause minimum histologic changes are tabulated as follows:

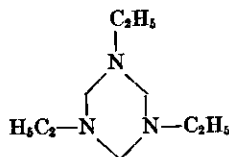
100% Mortality		0% Mortality		Minimal histopathologic alteration ^a	
Concentration (ppm)	Duration (min)	Concentration (ppm)	Duration (min)	Concentration (ppm)	Duration (min)
1000	60	1000	6	500	3
500	120	500	12	250	6
250	420	250	30	100	12
—	—	100	120	50	30
—	—	50	240	—	—

^a Some perivascular cuffing persisted in the liver at these exposure levels.

In this study, the primary organic damage was centrilobular necrosis, hemorrhage, and fatty degeneration, as well as perivascular cuffing in the liver.

69. *Toxicology of Hexahydro-1,3,5-Triethyl-s-Triazine, a Bacteriostatic and Fungistatic Agent.*
R. M. McCLAIN, C. L. WINEK, and S. P. SHANOR, Department of Pharmacology and Toxicology, Duquesne University, Pittsburgh, Pennsylvania.

The compound hexahydro-1,3,5-triethyl-s-triazine (Vancide-TH) is an experimental industrial bacteriostatic and fungistatic agent with the following structure:



The acute oral LD₅₀ in rats is 0.46 (0.38–0.55) ml/kg and in mice 0.37 (0.30–0.46) ml/kg. The compound was found to be hepatotoxic, and death was due to severe focal necrosis of the liver.

A 90-day feeding study with levels of 200, 600, 1800, and 5400 ppm was conducted. Parameters studied on 50% of the animals included hemoglobin concentration, hematocrit, white blood cell count, differential white blood cell count, serum glutamic-oxalacetic transaminase, and growth. At the conclusion of the study these same 50 animals were autopsied and the major organs were taken for histologic examination. Results will be reported.

Vancide-TH is readily absorbed through the skin of rabbits, inducing toxic manifestations. No animals survived after the application of 2 ml/kg or more in a single, 24-hour exposure. Death occurs within 2 to 4 hours at higher dosages. The compound penetrates the skin leaving a well defined, bluish-gray line of demarcation that continues into the deeper muscle layers. The exposed skin rapidly mummifies.

Results will be reported concerning an acute percutaneous study on intact and abraded skin and a subacute percutaneous study using as parameters, urinalysis, hematology, serum glutamic-oxalacetic transaminase, liver function, kidney function, skin irritation, eye irritation, aspiration toxicity, and histopathologic responses.

70. *Comparative Toxicology of 2,6-Dichloro-4-Nitroaniline in Rats and Rhesus Monkeys.*
D. M. SERRONE, P. PAKDAMAN, A. A. STEIN, and F. COULSTON, Institute of Experimental Pathology and Toxicology, Albany Medical College of Union University, Albany, New York.

2,6-Dichloro-4-nitroaniline (Botran®) is used as an agricultural fungicide against certain soil and foliar pathogens. The acute oral LD₅₀ in rats was estimated to be 8000 mg/kg. Doses up to 400 mg/kg orally were well tolerated by rats for 3 months; some mortality was observed at 1000 mg/kg orally. Daily oral administration of 160 mg/kg proved lethal to monkeys within less than 3 months. Botran appeared to be more toxic to female monkeys than to male monkeys. Rats produced an orange-colored urine following administration of Botran; coloration of monkey urine was not observed, possibly indicating different metabolic pathways in the two species. Oral administration to rats produced significant liver enlargement and was found to increase the activities of hepatic demethylase and desulfurase after either single or multiple administrations of Botran. Liver mitochondrial oxygen utilization was also increased at the time of hepatic enzyme induction. No alteration in heart or kidney mitochondrial oxygen consumption was observed. The activity of the hepatic processing enzymes in monkeys was not enhanced following oral administration of Botran.

Structural alterations in both liver and kidney of rats and monkeys were observed by light and electron microscopy. Centriolobular fatty infiltration of the liver was seen in sections of monkey liver. Swelling of mitochondria, with distortion of the cristae, was observed in electron micrographs of liver and kidney sections.

71. *Toxicity of Musk Ambrette*. D. A. DAVIS, J. M. TAYLOR, W. I. JONES, and J. B. BROUWER, Division of Toxicological Evaluation, Bureau of Science, Food and Drug Administration, U.S. Department of Health, Education, and Welfare, Washington, D.C.

Musk Ambrette (2,4-dinitro-3-methyl-6-*tert*-butylanisole) is used extensively in flavoring and perfumes as a blending agent. It has an oral LD₅₀ of 339 mg/kg in rats at a concentration of 25% (w/v) in corn oil.

It was fed to rats at 500, 1500, 2500, and 4000 ppm. Males were fed for 50 weeks and females (because of their greater susceptibility) for 20 weeks. Signs of toxicity were pronounced weight loss and progressive weakening of the hind quarters. Changes in the blood included a decreased erythrocyte count in females (and in the early stages of feeding in the males), icteric plasma, and decreased clotting time. Icteric plasma was observed at all levels, but the other blood changes and signs of toxicity appeared only at 1500 ppm and higher.

The weakness of the hind quarters was evidenced by poor locomotion and, after 16-40 weeks, complete lack of use of the legs. Microscopic examination revealed changes attributable to muscular atrophy, e.g., variations in size of and degenerative changes in muscle fibers, loss of cross striation, vacuolation of the sarcoplasm, and marked proliferation of sarcolemmal nuclei.

72. *Toxicity of Oil of Calamus (Jammu Variety)*. J. M. TAYLOR, W. I. JONES, E. C. HAGAN, M. A. GROSS, D. A. DAVIS, and E. L. COOK, Division of Toxicological Evaluation, Bureau of Science, Food and Drug Administration, U.S. Department of Health, Education, and Welfare, Washington, D.C.

Oil of Calamus is steam distilled from the rhizomes of *Acorus calamus* grown in North America, Europe, and Asia. It is a flavoring additive used in foods, beverages, and drugs.

The Jammu, India, variety of the oil consists of the following compounds: β -asarone, 75.8%; calamene, 3.84%; calamol, 3.2%; asarone, 1.32%; camphene, 0.92%; β -pinene, 0.56%; and asaronaldehyde, 0.2% [Vashist and Handa, *Soap Perfumery Cosmetics* 37, 135 (1964)].

The oral LD₅₀ of Oil of Calamus in rats is 777 mg/kg. An 18-week feeding study in rats produced mortality, growth depression, liver and heart abnormalities, and a serous effusion in the abdominal and/or peritoneal cavities.

A 2-year study in rats (25 males and 25 females at each level) was carried out at dietary levels of Oil of Calamus of 0, 500, 1000, 2500, and 5000 ppm. Moderate to marked growth depression (dose-related) was noted at all levels. Macroscopic liver alterations consisted of discoloration, leathery texture, and blunted edges. Microscopic examination showed degenerative and regenerative changes in the liver. Heart changes consisted of slight to moderate focal or diffuse myocardial degeneration.

Malignant tumors, noted initially after 59 weeks, were found in the duodenal regions of rats at all levels. Tumors of the same type were not seen in the controls.

73. *Toxicity of Plants of the Genus Dieffenbachia*. J. E. MANNO, F. W. FOCHTMAN, C. L. WINEK, and S. P. SHANOR, Department of Pharmacology and Toxicology, Duquesne University, Pittsburgh, Pennsylvania.

Certain species of the plant *Dieffenbachia* have gained scientific interest through the years because of their ability to produce severe irritation to the oral cavity upon ingestion.

Earlier investigators have attributed the irritation to the presence of crystals of calcium oxalate that can be readily identified under the microscope. Later studies eliminated saponins as the possible irritating factor. It has been suggested by several workers that the toxicity may be due to a proteinaceous substance.

Studies have been carried out on the expressed juice of *D. picta* and *D. exotica*. A determination of the oxalic acid content of the juice indicated a concentration of 0.15%, far below that of many edible plants. Eye irritation studies in rabbits showed that the fresh juice is a mild to severe irritant for *D. exotica* and *D. picta*, respectively. Juice stored at room temperature for not more than 24 hours was devoid of all irritating properties as was a 0.15% sus-

pension of calcium oxalate. Characteristic signs of *Dieffenbachia* poisoning, e.g., swelling of the tongue and irritation of the buccal mucosa, can be produced by placing 0.1 ml of the fresh juice of *D. picta* into the oral cavity. The juice of *D. exotica* did not elicit this response. However, if the juice of *D. exotica* was centrifuged and the insoluble portion reconstituted with buffer to a more concentrated volume, typical signs of *Dieffenbachia* poisoning were produced in the rat mouth. A 5% suspension of calcium oxalate did not produce any noticeable effect.

The presence of a protein has been determined and will be reported. Pharmacologic experiments designed to determine the mechanism of toxicity will be indicated.

74. *Analgesic and Narcotic Antagonist Properties of Noroxymorphone Derivatives.* H. BLUMBERG, H. B. DAYTON, and P. S. WOLF, Endo Laboratories, Carden City, New York.

Oxymorphone, or 14-hydroxydihydromorphinone, is a potent narcotic analgesic. However, the *N*-allyl derivative, naloxone, is a potent narcotic antagonist that is about 15–30 times as active as the widely used nalorphine in both animals and man. Three newly synthesized derivatives of noroxymorphone have now been investigated for analgesic activity by the phenylquinone writhing test in rats and for narcotic antagonist activity by the counteraction of oxymorphone narcosis in rats. The compounds were injected subcutaneously as the water-soluble hydrochlorides. *N*-3',3'-Dimethylallylnoroxymorphone was a moderately active analgesic, and about one-half as active as nalorphine as a narcotic antagonist. *N*-Cyclopropylmethylnoroxymorphone had limited effectiveness as an analgesic, but was an extremely potent narcotic antagonist, about 39 times as active as nalorphine. *N*-Cyclobutylmethylnoroxymorphone was a potent analgesic and at the same time a potent narcotic antagonist, being about 5 times as active as nalorphine. It is interesting that the analgesic activity of the three new narcotic antagonists could be counteracted by the other narcotic antagonist, naloxone. *N*-Cyclopropylmethylnoroxymorphone appears to be one of the most potent narcotic antagonists derived from the morphine and morphinone chemical series.

75. *The 90-Dose LD₅₀ and a Chronicity Factor as Measures of Toxicity.* W. J. HAYES, JR., Office of Pesticides, Communicable Disease Center, U.S. Public Health Service, Atlanta, Georgia.

A 90-dose LD₅₀ (or ED₅₀) and a chronicity factor are proposed to help communicate the results of tests involving repeated doses of compounds. To determine the oral 90-dose LD₅₀, groups of animals are fed appropriate dietary levels of a compound for 90 days and then held long enough for any sick survivors to die or recover. Food consumption is recorded and dosages are expressed as milligrams of compound per kilogram of body weight per day. Statistically, the 90-dose LD₅₀ is determined in the same way as the 1-dose LD₅₀ using logarithms of dosages and percent mortality expressed as probits. A ratio of the 1-dose LD₅₀ and 90-dose LD₅₀ of a compound is a measure of its cumulative effects and is termed the chronicity factor. The largest one found so far is more than 500 times the smallest found. These factors permit objective comparison of different classes of compounds but whether the smaller distinctions within a class are significant, remains to be learned.

By plotting the logarithm of time in days necessary to kill half of each group of animals against the logarithms of the daily dosages necessary to produce this effect, one may predict the 90-dose LD₅₀ of some compounds with acceptable accuracy, but only a much smaller, limiting value for other compounds.

76. *Toxicologic Studies on Repeated Intravenous Dosages of 5-Trifluoromethyl-2'-Deoxyuridine (F₃TDR) in Dogs and Monkeys.* P. E. PALM, M. S. NICK, J. T. FUNKHOUSER, and C. J. KENSLE, Life Sciences and Research and Development Divisions, Arthur D. Little, Inc., Cambridge, Massachusetts.

Data from repeated-dose intravenous studies of F₃TDR in dogs and monkeys indicate that dosages of 6.25 and 50.0 mg/kg × 14, respectively, produce only occasional transitory

changes. Dosages ≥ 12.5 mg/kg $\times 14$ in the dog frequently resulted in leukopenia and occasionally hypoglycemia. Serum calcium or phosphate levels were slightly depressed in 2/2 dogs at 25 mg/kg only. Dosages of 100 mg/kg $\times 14$ in the monkey characteristically produced leukopenia, transitory erythropenia, decreased hematocrit and hemoglobin, elevated serum glutamic-pyruvic transaminase and blood urea nitrogen values, and hypoglycemia. Serum calcium in one and phosphate levels in several monkeys were slightly depressed. Subsequent microscopic examination of tissues from animals which received the higher dosages revealed hypoplastic bone marrow (and lymphoid tissues in dogs) and focal degeneration of kidney proximal tubules and liver parenchyma. Intestinal hemorrhage was observed in 2/11 monkeys and 3/12 dogs.

Recovery data of total and inorganic fluoride in the urine suggest that (1) about 70% of the fluorine administered daily as F_3TDR may remain in the body, and (2) about 5-10% of the fluorine pool in the body may be converted to 5-COOH-U. Toxicity noted with F_3TDR in the mouse, dog, and monkey is compared with that reported for 5-fluorouracil and 5-fluorodeoxyuridine.

77. *Toxicology and Pathology of Repeated Doses of Monomethylhydrazine in Monkeys.* K. C. BACK and M. K. PINKERTON, Toxicology Branch, Toxic Hazards Division, 6570th Aerospace Medical Research Laboratories (MRBTT), Wright-Patterson Air Force Base, Ohio.

The effects of daily repeated doses of monomethylhydrazine (MMH) were studied in monkeys. Groups of monkeys were given from 2.5 to 5 mg/kg of MMH, i.p., for a total of 23 doses. Other monkeys were given from 7 to 10 mg/kg of MMH, i.p., for up to 4 days. Baseline and weekly clinical laboratory parameters studied were complete blood count, serum glucose, alkaline phosphatase, and glutamic-oxalacetic transaminase. At the end of the exposures, necropsies were performed on all animals. Special studies included fat stains of fresh cryostat sections of heart and liver, and luxol fast blue stains of pons, cerebellum, basal ganglia, and insular cortex. Results of the experiments have delineated the limits of toxicity for MMH in primates, as evaluated by clinical chemistry, symptomatology, and pathologic examination. Repeated doses of 5 mg/kg caused emesis and some convulsions when a total of 15 mg/kg was reached. Animals tolerated doses of 2.5 mg/kg/day for a total of 23 injections with no significant effects. Other animals tolerated 12 doses of 5 mg/kg/day after having received 5 mg/kg/day for 3 days and 2.5 mg/kg/day for 8 days or 95 mg/kg for a 4-week period. This experiment tends to negate a tolerance phenomenon. The most significant conclusions from the experiments are the relative lack of pathologic (either anatomical or clinical) alterations seen in the acute intoxications, and the extremely narrow limits between a no-effect and lethal dosage. Of extreme interest is the absence of kidney malfunction or renal pathology in these studies as contrasted with the results seen in dogs at these dosages. Monomethylhydrazine causes marked renal damage in dogs.

78. *Chronic Toxicologic Study with Estrogens in Rats.* J. P. GIBSON, J. W. NEWBERNE, and J. R. ELSEA, The Scientific Laboratories, The Wm. S. Merrell Co., Division of Richardson-Merrell, Inc., Cincinnati, Ohio, and Hill Top Research, Inc., Miami, Ohio.

The purpose of this study was to evaluate the effects of prolonged oral estrogen therapy in rats. Chlorotrianisene, a synthetic estrogen with unique anti-estrogen properties, was compared with another synthetic estrogen, diethylstilbestrol (DES), and with a substance composed of natural conjugated equine estrogens (CEE).

Weanling Sprague-Dawley rats were divided into one control and seven treated groups containing 20 males and 20 females each. Treated groups were administered one of these three estrogens in the diet continuously for two years at the following levels: chlorotrianisene at 0.05, 0.20, and 2.0 mg/kg/day; DES at 0.02 and 0.20 mg/kg/day; and CEE at 0.07 and 0.70 mg/kg/day.

In all treated groups, a dose-dependent depression in body weight gain was apparent, and the mortality was greatly increased in the high-level DES group. The incidence of pituitary

tumors was significantly higher than in controls in both the DES and CEE groups, and significantly lower in the chlorotrianisene groups. There was a slight dose-related increase in the incidence of mammary tumors in male rats receiving DES and CEE, and a slight decrease in females on these same estrogens. Mammary tumors were rare in rats receiving chlorotrianisene. These and other findings will be presented in detail and their significance discussed.

79. *Comparison of Isoproterenol and Metaproterenol Administered by Mouth Daily for Six Months to the Rhesus Monkey.* G. WOODARD and M. W. WOODARD, Woodard Research Corporation, Herndon, Virginia.

Isoproterenol or metaproterenol, as the sulfate, was administered by stomach tube to groups of 3 male and 3 female rhesus monkeys each, at daily dosages of 0 (control), 10, 30, and 100 mg/kg/day for 6 months. Hematologic, clinical, biochemical, and histologic observations revealed no effects of compound administration with the exception of (1) slightly reduced blood glucose for each treated group, (2) increased heart weight for isoproterenol at the two higher levels, and (3) cardiovascular changes occurring 1-3 hours after each day's dose roughly proportional to dosage as indicated by reddening of the oral mucosa and facial skin, heart sounds, and electrocardiograms. Qualitative and quantitative differences between the two compounds were slight or inconsistent.

80. *Placental Transport and Distribution of Thallium-204 Sulfate in Newborn Rats and Mice.* J. E. GIBSON, C. P. SIGDESTAD, and B. A. BECKER, Department of Pharmacology, College of Medicine, The University of Iowa, Iowa City, Iowa.

Groups of timed pregnant Swiss-Webster type mice or Sprague-Dawley rats were injected subcutaneously with 12.5 μC of $^{204}\text{Tl}_2\text{SO}_4$ at 1, 2, 4, 8, 16, or 24 hours before delivery by cesarean section on gestational day 18 and 19, respectively. The biological half-life of thallium in the pregnant mouse and rat was found to be 24 hours. Thallium-204 activity levels of females and individual fetuses of their litters were determined by crystal scintillation (X-ray) counting. The thallium-204 concentration in the litter equaled maternal concentration (cpm/g) after 1.5 hours in the mouse and 2.5 hours in the rat (maternal/fetal ratio = 1.0). Fetal concentrations of thallium gradually increased in both species until the fourth hour when a steady-state equilibrium was maintained through the twenty-fourth hour between mother and fetus (maternal/fetal ratio: rat, 0.34 ± 0.01 ; and mouse, 0.40 ± 0.02).

Distribution of $^{204}\text{Tl}_2\text{SO}_4$, 1.25 μC , i.p. or s.c., was studied in the 1-day and 7-day neonatal rat and mouse heart, kidney, spleen, intestines, brain, liver, stomach, lung, skin, and carcass. A diffuse distribution similar to that reported for adults was found with relatively large quantities in the kidneys.

In conclusion, thallium-204 was found to have a biological half-life of 24 hours in adult gravid rats and mice, to pass the placenta of near-term animals, and to be distributed in neonates as in adults.

81. *Factors Influencing Measurement of Ethyl Alcohol in the Expired Air.* J. P. ARCHISON, K. D. PARKER, and C. H. HINE, Department of Pharmacology and Experimental Therapeutics (Toxicology Activity), School of Medicine, University of California at San Francisco, San Francisco, California.

Estimation of the body burden of substances with a significant vapor pressure at body temperature can be made by analysis of the expired air. This principle has been used for some years in determination of the blood level of ethyl alcohol. Both mixed expired and alveolar air have been used. Determinations can be made immediately or samples may be taken to the laboratory for subsequent analysis. Most methods depend on the oxidation of alcohol by acid dichromate and are generally performed by the police. Factors which influence the validity of the result include the time since the last drink, the presence of saliva in the sample,

the nature of the collecting system, the temperature of collection, the depth of expiration, the stability of the reagents, and the correct functioning of the apparatus. A new method is described which differs from currently employed techniques in that a sample of alveolar air is collected in a glass cylinder for later analysis in the laboratory. Conditions necessary for corrected analysis of the alveolar air are discussed. The determination of ethyl alcohol content is accomplished by gas-liquid chromatography using a flame ionization detector. The sensitivity of the method is 0.1 μg . (Supported in part by U.S. Public Health Service Training Grant No. 5 T01 GM01304-02.)

82. *Inhibitory Effect of Cytoxan and Some Other Alkylating Agents on Hepatic Microsomal Enzymes.* R. G. TARDIFF and K. P. DUBOIS, Toxicity Laboratory, University of Chicago, Chicago, Illinois.

Previous studies in this laboratory demonstrated that sublethal doses of X-irradiation inhibit synthesis of hepatic microsomal drug metabolizing enzymes in young male rats. The present investigation was conducted to ascertain whether radiomimetic drugs exert a similar effect. Young, male rats (23 days old) were given cyclophosphoramide (Cytoxan), mechlorethamine (HN2), or *N',N'*-bis(3-bromopropionyl)piperazine (A-1803) intraperitoneally at sublethal doses of 150 mg/kg, 1.25 mg/kg, and 40 mg/kg, respectively. At intervals from the time of injection until 40 days of age, groups of at least 3 animals were sacrificed for assays of hepatic microsomal enzyme activity using the EPN detoxification system of Neal and DuBois [*J. Pharmacol. Exptl. Therap.* 148, 185 (1965)]. All the drugs inhibited development of this system. Maximal inhibition amounting to 60% occurred at 7 days after Cytoxan, at 7 days after HN2 (35%), and at 10 days after A-1803 (35%). In adult male rats the same doses, except for A-1803 (60 mg/kg), caused delayed inhibition of EPN detoxification and *O*-demethylase activity measured by a modification of the method of Netter and Seidel [*J. Pharmacol. Exptl. Therap.* 146, 61 (1964)]. Cytoxan produced maximal inhibition of EPN detoxification (83%) and *O*-demethylase activity (73%) in 14 days. A-1803 and HN2 produced maximal inhibition at 5 and 7 days, respectively. The drugs were not effective inhibitors of these enzymes *in vitro*. They did not inhibit phenobarbital induction of enzyme activity. The results suggest that the drugs inhibit the normal synthesis of hepatic microsomal enzymes.

83. *Comparison of Microsomal Drug-Metabolizing Enzyme Systems in Grouped and Individually Caged Rats.* T. BALAZS and W. DAIRMAN, Toxicology Evaluation Department, Toxicology Research Section, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

Individual caging of rats for a period of 3 months (isolation stress) has been reported to alter certain pharmacologic and toxicologic responses to a variety of agents [Wiberg and Grice, *Food Cosmetic Toxicol.* 3, 597 (1965)]. Increased sensitivity to the toxic effects of isoproterenol and a shortening of pentobarbital sleep time have been shown to occur in individually caged rats in comparison with group-caged controls. Since the latter effect might be linked to an altered rate of drug metabolism, the activities of hepatic microsomal drug-metabolizing enzyme systems were investigated in our study. Sherman male rats were caged individually or in groups of five for a period of 3 months; then they were examined.

In the individually caged rats, no increase in hepatic enzyme activities was detected with respect to hexobarbital, pentobarbital, and cortisol metabolism nor was the rate of *N*-demethylation of *p*-chloro-*N*-methylaniline increased. No differences were found in the urinary ascorbic acid contents nor in hexobarbital and pentobarbital sleep times. The liver-to-body weight ratio was similar for individually or group-caged rats.

The effect of prolonged individual caging, manifested by an increased sensitivity to isoproterenol, was observed under our experimental conditions. This indicated the development of the isolation stress phenomena [Balazs *et al.*, *J. Pharm. Pharmacol.* 14, 750 (1962)].

The result of our experiments implies that the activity of hepatic, microsomal drug-metabolizing enzyme systems is not affected by long-term individual caging of rats.

84. *Effects of Substituted-Urea Herbicides on Activity of Hepatic Microsomal Enzymes.* F. K. KINOSHITA and K. P. DuBois, Toxicity Laboratory, University of Chicago, Chicago, Illinois.

The ability of various chemical agents, including chlorinated hydrocarbon insecticides, to induce synthesis of hepatic microsomal enzymes has been well established. Recent studies in this laboratory [Kinoshita *et al.*, *Toxicol. Appl. Pharmacol.* **8**, 345 (1966)] demonstrated that two substituted-urea herbicides (Herban and Diuron) cause induction of microsomal enzyme activity. The present study was conducted to obtain quantitative data on enzyme induction caused by various dietary levels of these compounds and to ascertain whether other substituted-urea herbicides cause enzyme induction. Assay systems utilizing whole-liver homogenates were the most suitable. The systems used were the EPN detoxification system of Neal and DuBois [*J. Pharmacol. Exptl. Therap.* **148**, 185 (1965)], the *O*-demethylase system of Netter and Seidel [*J. Pharmacol. Exptl. Therap.* **146**, 61 (1964)] as modified by Kinoshita *et al.* [*Toxicol. Appl. Pharmacol.* **9**, 505 (1966)] and the *N*-demethylase system of La Du *et al.* [*J. Biol. Chem.* **214**, 741 (1955)]. Herban and Diuron were fed to weanling male and female rats at levels of 100–2000 ppm for 13 weeks. Enzyme assays were performed at intervals. Maximal stimulation occurred between 1 and 3 weeks followed by a gradual return to normal. Herban produced minimal stimulation of *N*-demethylase and EPN detoxification at 100 ppm while the lowest dose of Diuron that stimulated at least one enzyme was 250 ppm. Females were more sensitive to the stimulatory effects than males except for the *N*-demethylase system. Similar measurements were made on other substituted ureas fed for 1 week.

85. *Some in Vitro Studies on the Kinetics of Phagocytosis of Inorganic Particulate Materials.* G. V. METZGER and L. J. CASARETT, Department of Radiation Biology and Biophysics, University of Rochester Medical Center, Rochester, New York.

The *in vitro* phagocytic response of mononuclear cells from rat peritoneum and lung has been investigated for several metal oxide and carbon particles. The phagocytic index (PI, percentage of cells with ingested particles) is shown to reflect the rate of phagocytosis for a given period of incubation. For specified cell and particle concentrations, the PI has been shown to increase with increasing incubation time up to about 60 minutes, beyond which there is little further increase. This limiting value of the PI with time is believed to represent a gradual loss of function of the cells, since, although an increase in the particle concentration brings about an increase in the maximal PI, it is again reached by about 60 minutes.

The PI is found to increase linearly as a function of increasing particle concentration over a range of particle-to-cell ratios up to about 10, beyond which there is a gradual leveling off until no further increase occurs. As a function of cell concentration, again expressed as a particle-to-cell ratio, the PI gives similar results, increasing linearly and then leveling off. The final maximal PI, attained with either increasing particle concentration or decreasing cell concentration, is not believed to be related to a lack of function of the remaining fraction, because an increase in the incubation period appears to result in an increase in the final maximal PI. The significance of the gradual loss of function of the cells is discussed, and a collision model is proposed to explain the data.

86. *Morphology of Lung Disease in Rats Poisoned with Hexamethylphosphoramide.* R. D. KIMBROUGH and V. A. SEDLAK, Office of Pesticides, Communicable Disease Center, U.S. Public Health Service, Atlanta, Georgia. (Sponsor: W. J. Hayes, Jr.)

In previous experiments, severe respiratory disease was observed in male Sherman-strain rats given hexamethylphosphoramide (HEMPA) in their diet for 63–103 days at the rate of 750 ppm leading to a dosage that decreased from 80 to 40 mg/kg/day as the rats grew older [Kimbrough and Caines, *Nature* **211**, 146 (1966)]. In a long-term experiment, now in progress for 9 months, even dosages as low as 6.25 mg/kg/day affect the lungs.

In order to study the respiratory disease in detail, male Sherman-strain rats were given HEMPA in their diet for 52-72 days at the rate of 2000 ppm leading to a dosage that decreased from 127 to 106 mg/kg/day as the rats grew older. The morphology of the lung disease in this group of rats consisted essentially of severe bronchiectasis, bronchopneumonia with areas of squamous metaplasia, and fibrosis. The possibility that the lesions in the lungs of animals fed HEMPA are caused by a metabolite was considered.

87. *Fission Products: Localization of the Transport of Cesium-137 in the Nephron of the Dog.* B. V. R. SASTRY, C. T. SPALDING, and L. K. OWENS, Department of Pharmacology, Vanderbilt University, Nashville, Tennessee.

Cesium-137 is a component of thermal neutron or fast neutron fission of uranium, plutonium, or thorium. It is one of the three chief isotopes in atomic fallout which constitutes the principal health hazard to humans and animals. The chief route of its elimination is urine. Therefore, we have studied the sites of its transport in the nephron of the anesthetized dog using classical clearance techniques and stop-flow methods.

The ratio of excreted to filtered ^{137}Cs was always less than unity (0.13-0.99). Acetazolamide (50-250 mg/kg, i.v.) enhanced its excretion and this enhancement could be blocked with meralluride (6.8 mg/kg, s.c.) The stop-flow pattern (the clearance ratio of ^{137}Cs : creatinine vs. accumulative urine volume) under mannitol diuresis indicated that the clearance ratios between ^{137}Cs and creatinine were less than unity in the proximal tubule. A distinct peak, where the clearance ratio between ^{137}Cs and creatinine was greater than 2.0, was observed in the distal segment of the nephron. These results have suggested that ^{137}Cs was reabsorbed in the proximal tubule and was actively secreted in the distal tubule. The sites of reabsorption and secretion were similar to those for potassium.

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