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Abstracts of Papers for the Seventh Annual Meeting of the Society of Toxicology, Washington, D.C. March 4–6, 1968

1. *Chlorinated Hydrocarbon Insecticides in Plasma and Milk of Pregnant and Lactating Women.* A. CURLEY and R. KIMBROUGH, Toxicology Laboratory, Pesticides Program, National Communicable Disease Center, U.S. Public Health Service, Atlanta, Georgia. (Sponsor: W. J. Hayes, Jr.)

Five pregnant women were studied for the estimation of chlorinated hydrocarbon insecticide content in their blood and milk. Blood samples were obtained between days 30 to 150 and 240 to 263 of pregnancy, and between days 1 to 6, 35 to 55, and 90 to 115 postpartum. Milk samples were collected 3–6 days postpartum. Other milk samples were collected simultaneously with the other two postpartum blood samples. Extracts from these samples were analyzed by electron-capture gas-liquid chromatography.

Nine chlorinated insecticides were detected in plasma and milk. The concentrations in plasma were within the lower part of the range previously reported for healthy, nonpregnant women of the same socioeconomic group and city, who had no known special exposure to pesticides. However, the mean concentrations (ppm) of seven compounds—*p,p'*-DDT (0.0028), *o,p'*-DDT (0.0008), *p,p'*-DDE (0.0049), *o,p'*-DDE (0.0003), *p,p'*-DDD (0.0003) and total beta and gamma-BHC (0.0018)—in the plasma were significantly lower than those of the nonpregnant women. This finding suggests but does not prove that pregnancy leads to lower plasma levels of insecticides. The dieldrin concentration (0.0012 ppm) was not significantly different from that in nonpregnant women. Heptachlor epoxide was detected also, but no data are available for this compound in nonpregnant women. A pronounced decrease in the plasma concentration of *p,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD, total beta and gamma-BHC and dieldrin was observed 1–6 days postpartum in all the women. Subsequently, the concentration of these compounds increased to approximately the levels found during pregnancy. The concentration of *o,p'*-DDT did not show this trend. Although there was much individual variation, the mean excretion levels of *p,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, dieldrin, and total beta and gamma-BHC in the milk increased. Little correlation was found between the concentrations of total-DDT, total-BHC, heptachlor epoxide, and dieldrin in the plasma and milk.

2. *Toxicity and Enzyme Inducing Activity of Trifluoromethylbenzophenone Guanylhydrazone.* F. K. KINOSHITA, M. FLYNN, M. ROOT, and K. P. DUBOIS, Toxicity Laboratory, University of Chicago, Chicago, Illinois.

The present investigation was undertaken to obtain information on the toxicity of a candidate antimalarial drug, 4-fluoro-4'-trifluoromethylbenzophenone guanylhydrazone hydrochloride (WR 9792). Acute oral toxicity measurements gave the following LD_{50} values (mg/kg): male rats 199.4, male mice 114.2, and male guinea pigs 132.3. Deaths were usually delayed for several days. Subacute toxicity tests were conducted by administration of various doses orally for 14 days. Cumulative toxicity was observed, since the highest daily dose that could be tolerated with no mortality for 14 days was 20 mg/kg. Blood studies revealed a decrease in total leukocytes at daily dose levels above 5 mg/kg. Marked thymic atrophy and mild splenic atrophy occurred in rats that received 20 mg/kg/day. To ascertain whether WR 9792 alters the capacity of the liver to detoxify other chemicals, measurements were made of its effect on hepatic microsomal enzymes. The initial experiment consisted of administration of one-fifth of the acute LD_{50} (40 mg/kg) orally for 5 days. On day 6, assays for EPN detoxification, *N*-demethylase, and *O*-demethylase were conducted on the livers. There was no change in enzyme activity except in *O*-demethylase, which increased to 254% of normal.

The dose-response relationship for *O*-demethylase induction was measured by oral administration of daily doses from 1 to 20 mg/kg for 5 days. The amount of induction was dose-dependent up to 5 mg/kg/day. A single oral dose of 10 mg/kg caused maximal induction to 200% of normal in one day which was maintained for 3 days before reversal occurred.

3. *Toxicity and Anticholinesterase Action of Bis(diethylthio)chloromethyl Phosphonate.* M. ROOT, M. FLYNN, F. KINOSHITA, and K. P. DUBOIS, Toxicity Laboratory, University of Chicago, Chicago, Illinois.

A study was conducted to obtain information on the mammalian toxicity and anticholinesterase action of a new organic phosphate insecticide, bis(diethylthio)chloromethyl phosphonate (BCP). Measurements of the acute intraperitoneal toxicity gave the following LD_{50} values (mg/kg): female rats 22.8, male rats 27.5, female mice 42.6, male mice 45.7, and male guinea pigs 109.2. Orally the LD_{50} values were 40.1 mg/kg for female rats, 41.8 mg/kg for male rats, and 223.5 mg/kg for male guinea pigs. The dermal LD_{50} values for xylene solutions of BCP were 78.5 and 109.5 mg/kg for female and male rats, respectively. Atropine sulfate (100 mg/kg) and 2-PAM (100 mg/kg) given intraperitoneally immediately before BCP each increased the LD_{50} of BCP from 22.8 to 33 mg/kg. In subacute toxicity tests, female rats tolerated 2 mg/kg/day intraperitoneally for 60 days without mortality, but higher doses caused partial or complete mortality. Measurements of the effects of various dietary levels on rats are being conducted. BCP caused 50% inhibition of rat brain cholinesterase *in vitro* at a final molar concentration of 1.9×10^{-4} *M*. Cholinesterase assays were performed at intervals from 1 hour to 14 days after administration of a sublethal dose of BCP (14 mg/kg) on brain, submaxillary gland, and serum. Maximal inhibition of cholinesterase occurred at 3 hours after administration. Recovery was relatively slow during the 14-day observation period. One ppm was the minimum dietary level that caused significant aliesterase inhibition.

4. *Comparison of Enide (N,N-Dimethyl-2,2-diphenylacetamide) and Botran (2,6-Dichloro-4-nitroaniline) with DDT with Respect to Toxicity to Fish and Wildlife.* W. KNOTT and W. J. SCOTT, Woodard Research Corporation, Herndon, Virginia. (Sponsor: M. J. Bleiberg.)

Technical Enide (*N,N*-dimethyl-2,2-diphenylacetamide) an herbicide and Technical Botran (2,6-dichloro-4-nitroaniline) a fungicide, were toxicologically evaluated against *p,p'*-DDT on three species of fish. In addition, Enide 50W, Botran 75W, and DDT (77.2% *p,p'*-DDT) were evaluated in subacute toxicity studies using bobwhite quail and mallard ducks.

The mortality of all species tested were examined by the method of Litchfield and Wilcoxon (1949) in terms of LC_{50} values and 95% confidence limits. The LC_{50} values for the fish were based on 48- and 96-hour mortalities.

Species	LC_{50} (ppm active ingredient)			
	Enide	*p,p'*-DDT	Botran	*p,p'*-DDT
Bobwhite quail	>9,000	486	2,438	486
Mallard ducks	15,000	525	8,850	525
Rainbow trout[a]	8.6	0.0028	1.6	0.0032
Bluegill sunfish[a]	>32.0	0.0022	37.0	0.0020
Goldfish[a]	34.0	0.0025	>32.0	0.0049

[a] 96-Hour mortality.

The results obtained revealed that both grades of Enide and Botran had considerably higher LC_{50} values in all instances than DDT (77.2% *p,p'*-DDT).

5. *Diazinon Toxicity—Comparative Studies in Dogs and Miniature Swine.* F. L. EARL, B. E. MELVEGER, J. E. REINWALL, G. W. BIERBOWER, and J. M. CURTIS, Division of Pharmacology, Food and Drug Administration, U.S. Department of Health, Education, and Welfare, Washington, D.C. (Sponsor: E. J. Van Loon.)

Thirty purebred beagle dogs and thirty Hormel-Hanford miniature swine were distributed in 5 groups each. Diazinon was given to the dogs at levels of 20 mg/kg, 10 mg/kg, 5.0 mg/kg, and 2.5 mg/kg. Swine were given diazinon at levels of 10 mg/kg, 5 mg/kg, 2.5 mg/kg, and 1.25 mg/kg. Six animals of each species were retained as controls. The compound was given by capsule daily for a period of 8 months.

A myeloid:erythroid (M.E.) ratio in excess of 100:1 was found in some dogs receiving 20 mg/kg and dying in the first 30 days of the experiment. The M.E. ratio in pigs dying during the same period of time was found to be in excess of 3.4:1. A reticulocytopenia was found in these animals.

Eleven biochemical determinations were made at monthly intervals on each animal. Amylase was the only determination showing a dose-related elevation. Individual variations in biochemical responses were more marked in the lactic dehydrogenase and ornithine carbamyl transferase determinations than in the other tests conducted. Responses observed were not sex related.

6. *Dieldrin and DDT Retention in Dogs Fed Aldrin and DDT Individually and as a Mixture.* W. B. DEICHMANN and M. L. KEPLINGER, Department of Pharmacology and the Research and Teaching Center of Toxicology, University of Miami School of Medicine, Coral Gables, Florida.

Aldrin (0.6 mg/kg/day), DDT (24 mg/kg/day), and a combination of aldrin (0.3 mg/kg/day) plus DDT (12 mg/kg/day) were administered by capsule to beagle dogs for 10 months. Liver and adipose tissue were taken by biopsy during the feeding period and for one year thereafter for chemical analyses using gas-liquid chromatography. Blood was also analyzed.

Aldrin alone or aldrin plus DDT caused stimulation of the central nervous system, anorexia, and loss of body weight in some dogs.

Dieldrin levels in fat, blood, and liver were practically the same in dogs fed aldrin and in those fed aldrin at half the dose plus DDT. At 10 months, mean levels in both groups were: fat 80–100 ppm, liver 20 ppm, and blood 0.15 ppm.

DDT levels in the fat, blood, and liver were considerably higher from feeding DDT with aldrin than from feeding DDT alone at twice the dose. Feeding DDT alone, the mean levels of *p,p'*-DDT at 10 months were: fat 550 ppm, liver 50 ppm, and blood 0.4 ppm. Administration of DDT at half the dose plus aldrin led to the retention of the following concentrations: fat 1300 ppm, liver 70 ppm, and blood 1.6 ppm.

When administration of the pesticides was discontinued, dieldrin decreased slowly over the next 12 months in the fat, liver, and blood: in fat from 100 ppm to 20 ppm; in liver from 20 to 6 ppm; and in blood from 0.15 to 0.02. The DDT content in fat, liver, and blood of dogs fed DDT alone decreased rapidly: in fat from 550 to 25 ppm in 4 months; in liver from 50 to 4 ppm, and in blood from 0.4 to 0.05 ppm, in 1 month. Following discontinuation of treatment with DDT plus aldrin, DDT levels fell rapidly during the first month or two, but more slowly during the following months.

7. *Interactions of Lead and Dieldrin on the Nervous System of Mice.* C. R. CRESS, G. B. FINK, and R. E. LARSON, Department of Pharmacology, Oregon State University, Corvallis, Oregon. (Sponsor: G. L. Plaa.)

Intoxication by the chlorinated insecticides such as dieldrin produces effects on the nervous system of animals that resemble in many respects those produced by lead. Since both dieldrin and lead are causing some concern as environmental contaminants, the potential for toxic interaction between the two merits investigation.

The effects of exposure to lead and dieldrin singly and in combination were studied in adult CF1 mice. Acute doses of both agents were chosen on the basis of 72-hour LD_{50} curves. Lead acetate was administered ip in saline and dieldrin po in corn oil. In chronic studies lead was present in the drinking water (150–2400 ppm) and dieldrin in the food (0.5 and 1 ppm).

Alterations in susceptibility to chemically induced seizures were evaluated by the infusion of pentylenetetrazole (0.5% in saline) at a constant rate into the tail veins of the treated mice. Pretreatment with dieldrin alone, either chronically or acutely, reduced the amount of pentylenetetrazole required to produce an epileptiform seizure whereas lead given alone had no effect. However, with the combination of chronic lead and acute dieldrin, seizure susceptibility was greater than with dieldrin alone. Additional evidence of interaction was observed in that this combination impaired the ability of mice to maintain their equilibrium on a rotating bar (15, 20, or 25 rpm) whereas singly the agents had no effect. (Supported by P.H.S. Training Grant No. ES 00055-03.)

8. *The Effect of Several Pesticides and Drug Combinations on Some Behavioral and CNS Responses in Rats.* L. R. Weiss and R. Brodie, Division of Toxicological Evaluation, Food and Drug Administration, Washington, D.C. (Sponsor: K. J. Davis.)

The effects on the central nervous system (CNS) of several pesticides, alone and when combined with CNS-active drugs, were studied to develop further information on pesticide toxicity.

Four chlorinated hydrocarbon pesticides (dieldrin, chlordane, methoxychlor, and DDT), alone and when combined with chlorpromazine (CPZ), were studied for their effects on spontaneous motor activity (SMA) and also were tested for their ability to alter amphetamine toxicity and convulsive seizure thresholds. After 24-hour acute doses (1/2 LD_{50}), only dieldrin and chlordane caused highly significant depressant effects on SMA, increased the toxicity of amphetamine, and after 48 hours markedly sensitized the animals to pentylenetetrazole convulsions. Only dieldrin produced a behavioral interaction with CPZ characterized by a reversal of the depressant effects.

The effects of carbaryl (a carbamate anticholinesterase pesticide) in combination with amphetamine, atropine, CPZ, or Ditran (an experimental psychotogenic agent), were evaluated for possible drug interactions on SMA. Carbaryl in doses which show minimal inhibition of brain AChE (5–10 mg/kg, ip), caused depressant effects and counteracted the stimulation of amphetamine, atropine, and Ditran; it enhanced the depressant effects of CPZ on SMA. In behavioral studies using the conditioned avoidance response, Mobam (a carbamate anticholinesterase), 20 mg/kg orally, blocked the avoidance response as did CPZ (5 mg/kg, ip). Lower doses of each had no effect, whereas combinations of Mobam and CPZ in low doses significantly disrupted avoidance escape behavior.

9. *β-Glucuronidase Activity in Serum and Liver of Rats Administered Various Pesticides.* C. H. Williams, Division of Toxicological Evaluation, Food and Drug Administration, Washington, D.C. (Sponsor: K. H. Jacobson.)

β-Glucuronidase (β-Glu), an enzyme widely distributed in animal tissues, is involved in the conjugation of steroids, bile acids, hepatotoxic and carcinogenic agents with glucuronic acid. Because of its role in detoxification, the effect of various pesticides upon its activity in rat serum and liver was investigated.

Aldrin and chlordane administration at doses which increased serum and liver aliesterase levels had no effect upon β-Glu activity in serum and liver.

Paraoxon administration resulted in a marked increase in serum β-Glu activity after 2 hours with a steady decrease and return to control activity by 18 hours. No effect upon liver β-Glu was noted. A dose producing no cholinergic signs (1/8 LD_{50}) resulted in a 30-fold increase in serum β-Glu in 2 hours, while the erythrocyte and brain acetylcholine (AChE) esterase activity remained unchanged.

The carbamate Banol administered in subacute doses also increased serum β-Glu; the increase was lower than with paraoxon and was of shorter duration. The serum AChE levels were not changed. Serum β-Glu activity was unchanged in rats fed Banol in their diet for 24 months, although erythrocyte AChE was depressed at the highest feeding level.

Rats on diets of varying casein contents, and given acute doses of parathion, showed marked elevation of serum β-Glu activity at all protein levels; greatest activity appeared at 15% dietary level of casein.

10. *Effect of DDT on Parathion Toxicity in Mice.* S. W. BASS and A. J. TRIOLO, Department of Pharmacology, Jefferson Medical College, Philadelphia, Pennsylvania. (Sponsor: J. M. Coon.)

The chlorinated hydrocarbon insecticides are known to decrease hexobarbital sleeping time in most laboratory animals by stimulating hepatic microsomal enzyme activity. Hart and Fouts [*Proc. Soc. Exptl. Biol. Med.* **114**, 388 (1963)] reported that although the insecticide DDT decreased hexobarbital sleeping time in the rat, it failed to exert this effect in the mouse. Studies in our laboratory [Triolo and Coon, *J. Agr. Food Chem.* **14**, 549 (1966)] demonstrated that several insecticides of this class, such as aldrin, dieldrin, and chlordane, also protect mice against the toxicity of a number of the organophosphate insecticides, including parathion. The reported species difference in the effect of DDT on hexobarbital sleeping time prompted the present study to determine whether this insecticide can alter the toxicity of the organophosphate parathion in mice. A single oral dose of 75 mg/kg of DDT protected mice against an approximate LD_{80} of parathion administered orally or intraperitoneally. This effect of DDT reached its maximum about 2 days after treatment. DDT pretreatment did not alter the toxicity of paraoxon, the active metabolite of parathion. In animals treated 2 days previously with DDT, parathion produced less inhibition of whole blood and brain cholinesterase than in controls. DDT had no effect on the inhibition by paraoxon of either blood or brain cholinesterase. These results suggest that the protective effect of DDT against parathion toxicity in mice is due to an inhibition of the conversion of parathion to the potent cholinesterase inhibitor paraoxon.

11. *Responses to Cyclohexylamine in Animals.* I. ROSENBLUM, Department of Pharmacology and Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York.

Intravenous injection of 1.0, 3.0, or 10.0 mg/kg cyclohexylamine caused a pressor response and tachycardia of approximately 20 minutes' duration in anesthetized, bilaterally vagotomized cats. In cats under artificial respiration with opened chests, cyclohexylamine produced an increase in myocardial contractile force. This positive inotropic effect could also be demonstrated in isolated perfused rat and guinea pig hearts and in isolated atria of the rabbit. Higher doses of cyclohexylamine caused a vasodepressor effect accompanied by bradycardia and negative inotropism.

The vasopressor effect of cyclohexylamine was tachyphylactic and was blocked by cocaine or phenoxybenzamine. It was enhanced by iproniazid. Reserpine, mecamylamine, or propranolol did not prevent the vasopressor response to cyclohexylamine. The pressor response persisted in cats after bilateral adrenalectomy or bilateral nephrectomy and in rats after spinal section or decerebration.

Cyclohexylamine probably produces its cardiovascular effects through release of endogenous catecholamines as well as by direct stimulation of α-adrenergic receptors.

12. *The Sympathomimetic Action of Cyclohexylamine, a Metabolite of Cyclamate.* H. I. YAMAMURA and R. L. DIXON, Department of Pharmacology, School of Medicine, University of Washington, Seattle, Washington.

Cyclamate is widely used as a noncaloric sweetening agent for various foods including beverages. It has been reported recently that a portion of administered cyclamate is metabolized to cyclohexylamine. The structure of this metabolite suggested an investigation of

its possible sympathomimetic action and possible interaction with monoamine oxidase inhibitors.

The pharmacologic actions of cyclohexylamine on the autonomic nervous system were tested using the cat's blood pressure and nictitating membrane. Cyclohexylamine (5 mg/kg) had no effect upon the adrenergic, cholinergic, or histaminic responses but demonstrated a pressor action. This pressor activity was blocked by the α-receptor blocking agent phenoxybenzamine. Prior treatment of the animals with reserpine abolished the cyclohexylamine pressor response, which could then be restored with norepinephrine infusion. Thus, cyclohexylamine appeared to be an indirect acting sympathomimetic amine which closely resembles tyramine.

The interaction of cyclohexylamine and a monoamine oxidase inhibitor was studied because of the numerous reports of serious hypertensive crises in patients taking certain monoamine oxidase inhibitors and ingesting food high in tyramine. It is possible that significant blood levels of cyclohexylamine might be attained due to either the metabolism of ingested cyclamate or the presence of cyclohexylamine in the diet as an impurity or a spontaneous breakdown product. However, although pretreatment of animals with the monoamine oxidase inhibitor pargyline (25 mg/kg) resulted in a potentiation of the tyramine responses tested, this same treatment did not alter the pressor response of cyclohexylamine. This observed lack of interaction was most likely due to the fact that cyclohexylamine is not a substrate of monoamine oxidase as is tyramine.

13. *Nutritional Studies with Calcium Cyclamate in Rats.* M. S. WEINBERG and E. M. HORRINGTON, Biological Science Laboratories, Foster D. Snell, Inc., Bronx, New York.

Several studies have been conducted to evaluate the biologic effects of inclusion of sodium or calcium cyclamate or combinations of cyclamates and saccharin in rat diets. Recent reports [Nees and Derse, *Nature* **211**, 1191 (1967)] associated depression of food intake when calcium cyclamate was fed to laboratory rats. The authors described changes in utilization of nutrients in metabolism with administration of diets containing cyclamate.

In order to further investigate these findings, we established a littermate, paired-feeding study of this nonnutritive sweetener on nutrient conversion, growth, skeletal development, and carcass composition. Test levels used were 0.5, 1.0, or 2.0% calcium cyclamate. The findings have been significantly different from those previously reported.

Addition of calcium cyclamate to the diet appears to have been associated with increased food consumption by the rats. Growth and skeletal development were comparable among test and control groups. No gastrointestinal effects of the type previously associated with cyclamate administration have been noted.

The specific *in vivo* observations as well as the postmortem findings are reviewed in detail and discussed with reference to studies in other species and to other experiments which have not been previously reported. (These studies were supported by North Chicago, Illinois.)

14. *Growth and Reproduction Studies with Cyclamate-Saccharin (10:1) in Rats.* B. L. OSER, S. CARSON, and E. E. VOGIN, Food and Drug Research Laboratories, Inc., Maspeth, New York.

Chronic feeding studies are being performed with a 10:1 mixture of sodium cyclamate and sodium saccharin (C/S) in rats. One hundred forty male and 180 female weanling rats were divided into four groups, each consisting of 35 males and 45 females. The control group receives a nutritionally adequate diet and the test groups receive 500, 1120, and 2500 mg of C/S per kilogram per day, incorporated into the diet.

The protocols were designed to evaluate the effects of C/S on growth, food utilization, blood and urine composition, reproductive performance, and histopathology in these animals.

The only physical sign seen in these animals is occasional soft stools or diarrhea occurring primarily in male rats in the high dosage group. No significant physical or behavioral alterations have been found. The cyclamate-saccharin mixture does not produce an anorexigenic

effect such as has been reported elsewhere for cyclamate. Growth and food intake were similar in the control and test animals. Efficiency of food utilization was greater in male rats receiving 2500 mg/kg of this mixture than in control or low-dosage group animals. Paired feeding studies between rats receiving the highest dosage of C/S and control animals revealed that the food intake in the treated animals had to be reduced to the levels consumed by the control of each pair. This is in contrast to the usual experience in which control animals are restricted to match the treated pair mates. Hemograms and urine analyses performed at periodic intervals reveal no adverse responses attributable to the cyclamate-saccharin mixture.

The findings in the reproduction and teratologic studies with this mixture in both rats and rabbits will be reported.

15. *Chronic Studies with Sodium Saccharin and Sodium Cyclamate in Dogs.* O. E. FANCHER, R. J. PALAZZOLO, L. BLOCKUS, M. S. WEINBERG, and J. C. CALANDRA, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois, and Abbott Laboratories, North Chicago, Illinois.

With the increased production and consumption of food sweetened with so-called non-nutritive sweeteners, there has been a renewed interest in investigation of biologic effects of these dietary supplements. Since translation of animal data to man is facilitated by increasing the number of nonhuman species investigated, it was felt that a study in dogs was required. The most often used noncaloric sweetener is the 10:1 combination of cyclamates and sodium saccharin.

Growth, food consumption, behavior, general physical appearance, and hematologic and biochemical parameters have been compared among dogs receiving 0, 0.5, 1.0, or 1.5 g of the combination per kilogram of body weight per day and among their offspring receiving the same doses. In addition to examination of hepatic biopsy specimens and eye grounds, special attention was directed to appearance of stools and to criteria of endocrine function. No toxicologically significant changes were noted among the test animals in the parent generation or among their offspring which were continued on the test dosing regimen to the age of one year.

The F_1 generation dogs were sacrificed after one year of cyclamate dosing and submitted to extensive histomorphologic examination. No significant lesions were detected at necropsy or upon microscopic examination of the tissues of these animals.

It appears, therefore, that administration of as much as 1.5 g. sodium cyclamate-sodium saccharin (10:1) per kilogram body weight per day to dogs (the equivalent of 75 g per day in a 50-kg man) has no adverse effects on dogs.

These findings are discussed and compared with other studies of the same materials.

16. *Absorption and Excretion of Cyclamate in Animals and Man.* R. C. SONDERS and R. G. WIEGAND, Department of Pharmacology, Abbott Laboratories, North Chicago, Illinois. (Sponsor: E. T. Kimura.)

Previous studies on the distribution and excretion of ^{35}S-labeled sodium cyclamate have shown essentially complete recovery of cyclamate in the excreta. These results have been confirmed with cyclamate-^{14}C and the GLC method in rats and dogs. In rats, cyclamate has an average serum half-life of about 8 hours. About 32% of cyclamate present in plasma is not bound to plasma proteins. Studies in rats with ligated kidneys show distribution into all major tissues except brain. Similar results were also found in dogs. In addition, levels of cyclamate in the milk (0.001% of dose/ml) of lactating dogs were higher than the corresponding blood levels.

Plasma levels in two human subjects given a single 5-g dose of cyclamate-^{14}C peaked at 6–8 hours with levels up to 20 μg/ml. The plasma half-life was 8 hours. About 30% of the plasma cyclamate levels were not bound to plasma proteins. Urinary excretion of cyclamate-^{14}C was 51.8 and 73.5%, and the fecal elimination was 50.5 and 24.5%, with total recoveries of 102.3–98.0%.

Urinary levels of cyclamate were determined by GLC in samples from human subjects given unlabeled cyclamate both as single 5-g doses and daily 3-g doses (1 g t.i.d.). Preliminary studies on cyclohexylamine-^{14}C, a reported metabolite of cyclamate, will be discussed. Dicyclohexylamine was looked for as a metabolite of cyclamate, but was not found in either animals or man.

17. *A Three-Month Study of Daily Intake of Sodium Cyclamate by Man.* J. H. WILLS, E. JAMESON, G. STOEWSAND, and F. COULSTON, Institute of Experimental Pathology and Toxicology of Albany Medical College and New York State Department of Health, Albany, New York, and Medical Department of Clinton Prison, Dannemora, New York.

Groups of 8 men took by mouth t.i.d. capsules containing a placebo or Na cyclamate to furnish daily intakes of 5, 10, or 18 g of cyclamate. One man on the highest intake continued taking the original dose for 62 days without development of persistent diarrhea; the other 7 men on this intake all developed severe, persistent diarrhea. Two men taking 10 g/day of Na cyclamate developed severe, persistent diarrhea. No one taking 5 g/day of Na cyclamate has suffered from diarrhea. All men who developed significant diarrhea on their original intakes of Na cyclamate have been able to take 3 g/day without ill effect. No significant ill effects other than diarrhea have appeared. Of 92 samples of urine from men taking Na cyclamate, 24 have contained free cyclohexylamine. One urine sample from a man taking placebo also has contained free cyclohexylamine. Four men have excreted free cyclohexylamine in significant quantities while taking 3 g/day of Na cyclamate. Three men who received 5 g/day of Na cyclamate, among whom is the most consistent excretor of a high concentration of cyclohexylamine, have excreted free cyclohexylamine in their urines. The remaining three excretors of free cyclohexylamine are among the 6 men taking 10 g/day of Na cyclamate.

18. *Medicinal Iron Powder from 1681 to 1968.* M. N. SHANAS and E. M. BOYD, Department of Pharmacology, Queen's University, Kingston, Ontario, Canada.

Iron was originally believed to impart strength to the human body and was taken in various forms such as drinking the water in which swords had been steeped. In 1681, Sydenham introduced a form of powdered iron in the therapy of chlorosis with dramatic results because chlorosis was an iron deficiency anemia, although this was unknown to Sydenham. Powdered iron was subsequently proved to be absorbed when given by mouth and to be an effective hematinic in iron deficiency anemia. Nevertheless, the attitude persisted that powdered iron was a rather crude drug which should be replaced by more refined substitutes, and the elemental powder was deleted from the British Pharmacopoeia after the edition of 1932 and from the United States Pharmacopeia after the 12th edition (1942). Substitutes for elemental iron, including ferrous sulfate, may produce undesired side effects and occasionally have caused death. We therefore decided to study the acute oral toxicity of medicinal powdered iron and found that its LD_{50} was 25–200 times higher than those of other iron preparations in albino rats. Death was due mainly to bowel obstruction from oral doses of the order of one-tenth of body weight; this would correspond, on a body weight basis, to the ingestion of 35,000 capsules, each 0.2 g, by a man of 70 kg body weight. Toxicitywise, Sydenham's medicinal iron powder would appear to be the drug of choice for iron deficiency anemia. (Supported by a grant from the Medical Research Council of Canada.)

19. *The Toxicology of a Rubber Accelerator after Ethylene Oxide Sterilization.* W. L. GUESS and R. K. O'LEARY, Drug-Plastic Research and Toxicology Laboratories, College of Pharmacy, The University of Texas at Austin, Austin, Texas.

The increased use of ethylene oxide vapors as a potent sterilant has coincided with the introduction of many rubber and plastic medical devices which cannot be subjected to sterilization by heat. Although this sterilizing agent has a broad antimicrobial spectrum and the ability to penetrate relatively complex loads, it is unfortunate that there are certain difficulties

arising which must be made known if the real status and value of ethylene oxide sterilization is to be appreciated. In the particular case of sterilizing rubber and plastic materials, it has become known that residual amounts of the toxic gas have produced skin burns when the medical device was insufficiently aerated after sterilization. Of greater toxicologic significance is the fact that many of the additives incorporated into the formulations for such products as rubber bottle cap liners, anesthesia masks, catheters, and syringe hubs, react readily with ethylene oxide to produce compounds having toxicity potentials greater than the unsterilized material. In this study, 2-mercaptobenzothiazole (MBT), a vulcanization accelerator commonly found in rubber, was reacted with ethylene oxide under sterilization conditions to produce 2-(2-hydroxyethylmercapto)thiazole) (HMBT). A preliminary study utilizing cell culture techniques revealed that the HMBT exerted an enhanced toxic effect to this test system. As a consequence, a broad-based study including LD_{50} (orally and intraperitoneally) values, gross toxicology, and pathology was conducted. A comparison of the relative toxicity between the MBT and HMBT was made.

20. *The Toxicity of Cannabis sativa (Marihuana).* J. C. MUNCH, Advisory Committee, Federal Bureau of Narcotics, Miami Shores, Florida.

Information is presented regarding the acute and chronic toxicity in animals and in humans following ingestion or inhalation of combustion products of *Cannabis sativa* produced in various parts of the world. Central nervous system disturbances and deterioration have been confirmed following chronic usage. The need for additional pharmacodynamic research is indicated.

21. *Some of the Effects of Tetramethylthiuram Disulfide (TMTD) on Reproduction of the Bobwhite Quail (Colinus virginianus).* J. WEDIG, A. COWAN, and R. HARTUNG, The University of Michigan, Ann Arbor, Michigan. (Sponsor: H. H. Cornish.)

Tetramethylthiuramdisulfide (TMTD) is used mainly as an agricultural fungicide and repellent.

Poisoning which alters the pattern of reproduction by decreasing egg production has been reported in the domestic chicken (*Gallus domesticus*), pigeon (*Colomba livia*), and pheasant (*Phasianus colchicus torquatus*).

TMTD (99.9% pure) was effective in inhibiting ovulation in bobwhite quail. Recovery occurred approximately 14 days after its removal from the diet. A dose level of 8.8 mg kg^{-1} day^{-1} caused a 50% reduction in egg laying. This level of TMTD in the diet of bobwhites can be reached readily when many seed species are treated according to manufacturer's recommendations.

The general effects of TMTD poisoning observed during the reproductive period suggest an alteration of hormone levels. These effects include (a) significant weight loss of the ovary and oviduct, (b) decrease in serum calcium level, which is estrogen controlled, (c) lower egg production, (d) alteration in the normal maturation pattern of the ova in treated birds, and (e) an increase in relative activity.

TMTD also suppressed the ovarian development normally associated with the change from quiescent to breeding condition. This suppression of ovary development can be correlated with the biologic observations noted above.

22. *Teratology and Reproduction Studies with an Antinauseant.* J. P. GIBSON, R. E. STAPLES, E. J. LARSON, W. L. KUHN, D. E. HOLTKAMP, and J. W. NEWBERNE, Scientific Laboratories, The Wm. S. Merrell Company, Division of Richardson Merrell Inc., Cincinnati, Ohio.

Dicyclomine hydrochloride (Bentyl®) (B), doxylamine succinate (Decapryn®) (D), and the anti-nauseant (Benedectin®) (BDP), which contains 10 mg each of B, D, and pyridoxine hydrochloride (P) per tablet, were each administered orally to rabbits by intubation and to rats in the diet. The methods used were adaptations of those previously described [*Exptl. Mol. Pathol.*, Suppl. **2**, 81 (1963)]. Dosages of B and D were 10, 30, or 100 mg/kg/day, given

to rabbits on days 9 through 16 of gestation (day 1 = day of insemination) and to rats for a minimum of 80 days prior to breeding and continued through the breeding and raising of one or two successive litters. BDP was administered as the pharmaceutical tablet, but pulverized to provide controlled dosing. The dosage of BDP provided 1, 2, and 10 mg/kg/day of each component (B, D, and P) for rabbits given on days 9 through 16 of gestation, and 1, 2.5, and 20 mg/kg/day for rats given through two successive gestation and lactation cycles.

These compounds produced no adverse effects on breeding, conception, parturition, or nursing in rats. In rabbits receiving B and D, resorption rates were increased in the highest dose levels; however, this was attributed to maternal toxicity and/or intercurrent infection rather than to any direct drug effects. The type and incidence of the malformations observed in treated fetuses were similar to those of the control populations. The results of these tests in animals are in agreement with results obtained in a controlled survey of a group of BDP-treated humans [*Current Therap. Res.* **5**, 245 (1963)].

23. *Teratogenic Effects of Carbaryl and Other Pesticides in the Hamster, the Rabbit, and the Guinea Pig.* J. F. ROBENS. Division of Toxicological Evaluation, Food and Drug Administration, Washington, D.C. (Sponsor: J. M. Taylor.)

Teratogenic agents are known to be most potent when administered during organogenesis, a relatively short period during early gestation. At that time the most striking and easily recognizable changes in body structure can be effected.

Carbaryl, Diazinon®, Herban®, disulfiram, and thiram were administered to pregnant hamsters as single oral doses in this period and as multiple doses where no fetal toxicity could be produced by a single dose. Corn oil, carboxymethylcellulose, and/or dimethylsulfoxide were used as vehicles. Of the five pesticides, disulfiram and thiram in dimethylsulfoxide, and thiram in carboxymethylcellulose were embryotoxic and teratogenic, but only at doses toxic to some of the mothers; dimethylsulfoxide alone produced the same response. Varied skeletal and visceral malformations were produced.

Carbaryl and Diazinon® up to 200 and 30 mg/kg, respectively, were administered orally daily for 10-day periods to pregnant rabbits. No terata were produced.

Carbaryl up to 350 mg/kg was administered to pregnant guinea pigs for a 10-day period. Changes were produced in the axial skeleton, particularly the cervical vertebrae. These same defects again appeared after single doses of carbaryl.

24. *Teratologic Study in Rats and Rabbits Exposed to an Isoproterenol Aerosol Nebair®.* E. E. VOGIN, R. E. GOLDHAMER, and S. CARSON, Food and Drug Research Laboratories, Inc., Maspeth, New York.

Reproduction and teratology studies were performed in accordance with current FDA guidelines using W2348-17, an isoproterenol and thonzonium bromide[1] formulation (Nebair®). Doses were administered by inhalation to rats in whole-body exposure chambers and to rabbits by individual face-mask exposures.

The potential factor of psychic trauma due to aerosol exposure in rabbits was controlled by sham treatment with the propellant (Freon) for several days prior to the critical time periods in the experimental protocol.

Four test groups were exposed, viz. a Freon control, a placebo control lacking only the isoproterenol component of the formulation, and two dosage groups receiving the test formulation. The animals received two 15-minute doses with their respective formulations during the critical periods to provide daily dosage levels of isoproterenol 0.153 and 0.458 mg/kg.

The results indicated a reduction in the number of animals becoming pregnant in both species and in all groups (control and treated). There was no evidence of drug or methodological teratogenicity in rats or rabbits that came to term or were delivered by Cesarean section. Gestation, viability, and lactation performance of the rats were not adversely affected by the above concentrations of W2348-17 (Nebair®).

The details of the procedure are discussed.

[1] Supplied by Warner-Lambert Research Institute.

25. *Teratogenicity of Diphenylhydantoin in Swiss-Webster and A/Jax Mice.* J. E. GIBSON and B. A. BECKER, Department of Pharmacology, College of Medicine, University of Iowa, Iowa City, Iowa.

Diphenylhydantoin increases incidence of cleft palate in A/Jax mice, which are genetically prone to that malformation. The purpose of this study is to evaluate diphenylhydantoin-induced teratology including cleft palate in a nonprone mouse strain. Diphenylhydantoin, 50 mg/kg, sc, was administered to primigravid Swiss-Webster and A/Jax mice during early (days 7, 8, 9) and late (11, 12, 13) embryogenesis. Litters were delivered by cesarean section on gestational day 18, weighed, examined, and measured. Resorption rates were not altered in either strain treated during early embryogenesis. A/Jax, but not Swiss-Webster, mice treated during late embryogenesis had a significant ($P < 0.05$) increase in resorptions in treated *vs* A/Jax control mice. Diphenylhydantoin resulted in reduced fetal weight in both strains when given during late embryogenesis. Fetal long-bone length, measured in alizarin red-stained preparations was shortened only in late-treated Swiss-Webster mice. The incidence of cleft palate, corrected for cleft palate occurring with cleft lip, was significantly elevated in late, but not early, treated progeny of both strains: Swiss-Webster (7/46) and A/Jax (8/26) *vs* 0/37 and 1/24 for the respective controls. Cleft palate with cleft lip occurred only in A/Jax mice; the rate was not different from vehicle-treated controls. No other soft tissue malformations were found in either strain following Bouin's fixation and freehand cross-section examination. In summary, diphenylhydantoin produced micromelia and cleft palate in Swiss-Webster mice, which are not genetically prone to cleft palate.

26. *Effect of Antibiotics upon Pregnancy in the Rabbit.* D. M. BROWN, K. H. HARPER, A. K. PALMER, and S. A. TESH, Beecham Research Laboratories, Betchworth, Surrey, England, and Huntingdon Research Centre, Huntingdon, England.

Previous communications to this Society [Gray and Lewis, *Toxicol. Appl. Pharmacol.* **8**, 342 (1966); Madissoo *et al.*, *ibid.* **10**, 379 (1967)] dealt with the lethal and abortifacient actions of antibiotics in pregnant rabbits. Studies in our laboratories have confirmed and extended these observations with a number of antibiotics.

Tetracycline and penicillins in general proved the most toxic, therefore meaningful teratogenicity data could not be achieved with conventional tests. Chloramphenicol, polymyxin B, colomycin, and cycloserine, on the other hand, were well tolerated.

Because of the probable role of the gut flora in determining the effect of antibiotics it was suggested that the suckling rabbit, known to have an incomplete flora, might be conditioned to a particular antibiotic by gradually increasing the dosage, thus providing "antibiotic tolerant" adults for teratogenic studies.

To test this hypothesis, groups of weanling New Zealand White rabbits were treated with Pen G, Pen V, cloxacillin, dicloxacillin, methicillin, or tetracycline at an initial daily dosage of 10 mg/kg increasing weekly by 10 mg/kg until a dosage of 100 mg/kg was reached; this was maintained through mating and gestation.

Although some animals died before the dose reached 100 mg/kg, survivors were able to support pregnancy; there was no increased incidence of abortion and no evidence of a teratogenic action. There was some evidence of embryonic death related to maternal condition.

It is felt therefore that abortifacient and embryotoxic response to penicillins and tetracycline relates to the deterioration in maternal condition rather than to direct action on the embryo.

27. *Development of Mouse Populations for Use in Toxicology.* E. D. PALMES and J. DELPUP, Institute of Environmental Medicine, New York University Medical Center, New York, New York.

An attempt was made to develop mouse populations which could be used as test objects in toxicity studies. It was possible to breed and maintain four populations of 400 mice each living at a population density of about 1 mouse per 4 square inches. Females are removed from

the common cage just prior to delivery and are returned to the population after weaning of the litter. The population is maintained at a constant level by replacing dying animals with randomly selected weanlings. Each animal is marked and his vital statistics are recorded on IBM cards. Programs have been developed for characterization of the populations in common epidemiological terms.

The four populations have been at full strength for well over a year. They appear healthy and are producing weanlings at a great excess over the death rate.

28. *Salicylate Intoxication—Treatment with Potassium Citrate.* W. T. DOBBINS, Children's Hospital, Denver, Colorado (Sponsor: A. K. Done.)

The primary treatment of salicylate intoxication consists of the rapid removal of salicylate from the body. This is best accomplished by the diuresis of an alkaline urine. Use of sodium bicarbonate has not been entirely successful since patients with severe acute and chronic salicylate intoxication have shown a resistance to alkalization of the urine. In addition, the administration of large amounts of sodium bicarbonate has produced sodium poisoning with the development of hypernatremia, alkalosis, tetany, and hypokalemia. Based on the premise that these complications were due to a relative potassium deficit, a study was undertaken to determine the effectiveness of large amounts of oral potassium citrate in promoting the production of an alkaline urine in children with salicylate intoxication. Children ranging in age from 3 months to 8 years, with severe acute and chronic salicylate intoxication were treated with various fluid regimens, with and without potassium citrate. The combination of (1) intravenous fluids (44 meq $NaHCO_3$, 35 meq KCl in 1000 ml of 5% glucose) 2000 ml/M^2/8 hours, (2) $NaHCO_3$, 3 meq/kg by push and 1.5 meq/kg q15 minutes if needed to obtain an alkaline urine; and (3) potassium citrate, oral or nasogastric drip, 200 meq/M^2/8 hours produced the best results: (a) All patients had a rapid diuresis of an alkaline urine. (b) blood levels of salicylate decreased an average of 70% in 8 hours; (c) salicylate clearance averaged 20 mg/kg/hour, (d) none of the patients developed complications.

29. *Adsorption of Drugs and Poisons by Activated Charcoal.* W. J. DECKER, H. F. COMBS, and D. G. CORBY, Research and Development and Pediatric Services, William Beaumont General Hospital, El Paso, Texas. (Sponsor: J. F. Borzelleca.)

Activated charcoal has long been known as an antidote against some toxic agents. Little investigative work of recent date has been done to show its effectiveness as an adsorbent for a wide variety and concentration levels of poisons. The principal object of this *in vitro* study is to show the effectiveness of a given amount of activated charcoal (Norit A®) as an adsorbent for dangerous amounts of drugs and poisons at various concentration levels. Over 20 commonly available compounds were studied. In each case a low, medium, and high dose of the compound is incubated at 37°C in 100 ml of artificial gastric juice with a slurry of 5 g of Norit A in a metabolic shaker. After 20 minutes, a simulated elapsed time from ingestion until treatment, the charcoal is separated from the solution either by rapid filtering or centrifuging. The amount of compound remaining in the artificial gastric juice is then determined by appropriate analytical procedures, and the percent adsorbed by the activated charcoal calculated.

In those instances where the compound was of limited solubility in the artificial gastric juice, the amount dissolved in the media under like conditions was determined separately. Results showed that the adsorption by activated charcoal under these conditions ranged from 95% for strong tincture of iodine to 7% for iron tablets.

30. *Microcirculatory Effects of Ethanol.* R. C. MARTZ, P. D. HARRIS, E. K. GREENWALD, R. B. FORNEY, and F. W. HUGHES, Department of Toxicology and Pharmacology and Department of Physiology, Indiana University Medical School, Indianapolis, Indiana.

The microcirculatory effects of ethanol were studied in the subcutaneous vasculature of the wing of unanesthetized bats (*Myotis leucifugus*). A binocular compound microscope equipped

with a beam splitter in the optical path permitted a view of the microcirculation on the photocathode of a closed circuit television system.

The video information was recorded on a video tape recorder with subsequent playback on a 23-inch monitor for analysis (total magnification of 2000 ×). Venule and arteriole diameters were measured at 30-second intervals, and venule vasomotion (the number of venule contractions per minute) was counted over 1-minute intervals. Rectal temperatures were measured with a thermistor.

After a 25-minute control period, bats were injected intraperitoneally with ethanol (3.0 g/kg body weight) in a balanced salt solution buffered to pH 7.3. The vascular responses were recorded for an additional 30 minutes, since this period was considered sufficient to allow steady-state vascular responses to the ethanol injection. Two types of steady-state responses were observed for venules (approximately 60 μ mean diameter) and arterioles (20 μ mean diameter). In one type, venule vasomotion dropped to zero, and both the venule and arteriole significantly dilated. In the second type, there were no significant changes in either venule vasomotion or venule and arteriole diameters. In both types of responses, there were no significant changes in rectal temperature. (Supported in part by P.H.S. Fellowship 5-Fl-GM-20, 422 and by P.H.S. Grants HE 08618 and GM 1089-04.)

31. *Electrocardiographic Effects of Vasopressin, Epinephrine, or Ethanol in Dogs with Myocardial Lesions Induced by Isoproterenol or Coronary Ligation.* T. BALAZS, J. R. CUMMINGS, and J. F. NOBLE, Lederle Laboratories, American Cyanamid Co., Pearl River, New York.

In a group of normal conscious dogs, intravenous infusions of vasopressin (0.4 unit/kg), epinephrine (4 μg/kg), or 50% ethanol (1 ml/kg) failed to cause ventricular arrhythmias. In a second group of dogs, myocardial necrosis produced by the subcutaneous injection of 1 mg/kg isoproterenol or by ligation of the left anterior descending coronary artery evoked ventricular extrasystoles. This electrocardiographic abnormality disappeared within a few days, but thereafter the infusion of vasopressin elicited paroxysmal ventricular extrasystoles. Electrocardiographic changes were not seen in dogs dosed subcutaneously with 0.5 mg/kg isoproterenol, but paroxysmal extrasystoles following infusion of vasopressin revealed cardiac injury, thereby indicating the usefulness of vasopressin response for cardiotoxicity testing. Epinephrine and 50% ethanol given to dogs with myocardial lesions evoked a response which in several instances was similar to that elicited by vasopressin. These results further implied an alteration in drug sensitivity of the damaged myocardium, a finding of toxicologic interest.

32. *Electrocardiographic Findings in Dogs Inhaling the Vapors of Diverse Chlorinated Hydrocarbons.* H. E. JOHNSON and S. P. SHANOR, Duquesne University, Department of Pharmacology and Toxicology, Pittsburgh, Pennsylvania. (Sponsor: C. L. Winek.)

We investigated the possible cardiosensitizing effects of exogenous epinephrine, administered while vapors of a series of chlorinated hydrocarbons were being inhaled, to clarify whether cardiac arrhythmias may occur. In all cases electrocardiographic techniques were utilized. Primary attention was paid to fibrillation and tachycardia, while secondary notice was paid to bradycardia and cardiac arrest. These specific cardiac phenomena can be clearly ascertained while the more subtle electrocardiographic nuances such as EKG wave heights or depths are of uncertain significance.

The chemicals utilized, either of the practical or technical grade, were: 1,2-dichloroethane, 1,2-dichloropropane, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethane, tetrachloroethylene, and tetrachloromethane.

Two of the 7 chlorinated hydrocarbons (trichloroethylene and tetrachloroethylene) had a cardiotoxic effect following sensitization by epinephrine. Another (tetrachloromethane) had a cardiotoxic effect with or without exogenous epinephrine. The remaining 4 chlorinated hydrocarbons showed only bradycardia, due to a vagotonic effect, and cardiac arrest.

33. *The Selective Antagonism of Epinephrine Toxicity.* C. B. NASH, Department of Pharmacology, University of Tennessee Medical Units, Memphis, Tennessee.

Intravenous infusions of epinephrine or norepinephrine produce postinfusion shock, myocardial hemorrhage, and death. This study is an attempt to eliminate as many as of the toxic effects of epinephrine as possible while retaining some degree of pressor action. Mongrel dogs were anesthetized with sodium pentobarbital and arranged to record arterial blood pressure, respiration, right atrial pressure, and electrocardiograms. Blood samples were taken for measurement of pH and hematocrit at hourly intervals. Epinephrine was infused at a rate of 10 μg/kg/min for 2 hours, and recordings were made for an additional 2 hours, after which terminal autopsies were performed.

Epinephrine infusions alone produced cardiac hemorrhage, pericardial effusion, low voltage ECG, low blood pH, increased hematocrit, arrhythmia, tachycardia, severe hypertension followed by shock, and sometimes death. Propranolol pretreatment increased these toxic effects and resulted in 100% deaths. Phenoxybenzamine pretreatment resulted in 100% survival, but blood pressures were low and cardiac hemorrhage and arrhythmia were present. A combination of phenoxybenzamine and propranolol was found that virtually eliminated toxic effects and still permitted a moderate pressor response to epinephrine. These data support the recent concept of an anti-α-adrenergic blocking action by propranolol and suggest possible clinical applications for dual adrenergic blockade.

33a. *Endrin and Dieldrin: A Comparison of Hepatic Excretion Rates in the Rat.* J. F. COLE, L. M. KLEVAY, and M. R. ZAVON, Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, Ohio. (Sponsor: H. E. Stokinger.)

The rates of excretion of radioactivity from radiolabeled endrin and dieldrin, chlorinated hydrocarbon insecticide stereoisomers, have been compared in whole rats, bile-fistula rats, and isolated, perfused rat livers.

Male, Holtzman rats, with and without bile fistulas, were given intravenous doses (0.25 mg/kg) of endrin-^{14}C or dieldrin-^{14}C. Urine and feces were collected daily. Bile was collected at 1, 3, 6, 12, and 24 hours and daily thereafter. The animals were maintained for 5–7 days; then were sacrificed.

Isolated, perfused rat liver experiments [Miller *et al.*, *J. Exptl. Med.* **94**, 431-453 (1951)] were performed with radiolabeled insecticides in the perfusates (0.003 mg/ml or 0.0003 mg/ml). Bile and perfusate were sampled hourly. The liver was sampled at the end of the 4- to 6-hour experiment.

Radioactivity in all excreta, carcasses, bile, perfusates, and liver tissue was measured by liquid scintillation counting [Tye and Engel, *Anal. Chem.* **37**, 1225-1227 (1965)].

Over 90% of the excreted activity was found in the feces of the intact animals and in the bile of fistula animals. Fifty percent of the radioactivity administered as endrin was excreted within the first day, and 50% of the dieldrin during the third day by intact animals. Similar results were obtained with fistula animals. Fifty percent of the endrin radioactivity was excreted in the bile in approximately 1 hour in the perfusion experiments, whereas only 32% of the dieldrin had been excreted by the end of 6 hours.

The more rapid fecal elimination of endrin can be explained by its more rapid biliary excretion. Intestinal absorption and fatty tissue sequestration have been excluded as major factors, and the liver has been identified as the source of the difference. These results may explain the greater storage of dieldrin in the body fat of most mammals, including man.

34. *Effects of Chronic Inhalation of Vinyl Bromide in Laboratory Animals.* K. J. LEONG and T. R. TORKELSON, Dow Chemical Company, Midland, Michigan.

Inhalation exposures of monkeys, rabbits, and rats to vinyl bromide at 250 and 500 ppm for 6 months have been performed. No significant effects were noted with respect to body

weight changes, food consumption, a number of hematological variables, or the histologic appearance of the tissues. Special attention was given to the uptake of bromide in the blood. It was found that the relation between blood bromide concentration, y, and duration of exposure, x, had the form $y = a + b$ [log (log x)]. The rate of uptake and the final concentration of blood bromide attained were directly related to the chamber concentration of vinyl bromide.

35. *Effects from Repeated Short-Term Inhalation of Fluorine.* M. L. KEPLINGER, Research and Teaching Center of Toxicology, University of Miami School of Medicine, Coral Gables, Florida.

Mice, rats, and rabbits were exposed to fluorine for 5, 15, 30, or 60 minutes at weekly intervals. Exposures were used that caused marked effects, slight effects, or no effects after a single exposure. Pathology in the lungs was used as the most sensitive index of effect. Animals were sacrificed immediately after the last exposure, or at 7, 14, 21, or 45 days after the last exposure. A single exposure caused marked changes (grade 3) in the lungs and some changes in the liver and kidneys. After four exposures (at weekly intervals) to the same concentration, the lungs had slight changes (grade 1). The livers were normal. The kidneys showed slight changes at 7 and 14 days, but were normal at 21 and 45 days. Four exposures, at concentrations which had caused no effect after one exposure, caused no effect either. Therefore, four exposures to fluorine caused no more damage than a single exposure to the same concentration. Repeated exposures were also made with different concentrations during each exposure. A low, but apparently harmless, level was used first. Then 4, 24, and 96 hours later, the LC_{50} of fluorine to these animals was determined. The LC_{50}'s of preexposed animals were higher and there was less damage to the lungs than in control animals. Exposures to a low concentration were also repeated every third day (for 4 times). At 1, 3, and 7 days after the last exposure the LC_{50}'s were determined. Even at 7 days the preexposed animals had higher LC_{50}'s and showed less edema in the lungs than animals that were not pretreated by previous exposure to fluorine.

36. *Safety Evaluation of Enide and Desmethyl Enide in Cigarette Smoke by Repeated Inhalation Exposure of Rats and of Dogs.* M. J. BLEIBERG, W. J. SCOTT, and R. P. BELILES, Woodard Research Corporation Herndon, Virginia.

To evaluate the safety of residues of Enide (*N,N*-dimethyl-2,2-diphenylacetamide) and its degradation product desmethyl Enide on tobacco, an inhalation study was conducted with rats and dogs exposed to smoke from cigarettes prepared with tobacco containing these materials at the desired test levels. Two automated smoking devices used the principle of the falling water column to draw a 25-ml puff of smoke in 2 seconds once every minute. The puff was diluted 10-fold with air and pumped into one of two exposure chambers: (1) a manifold type for 10 rats, (2) a hexagonal chamber approximately 23 liters in capacity for the heads of six dogs. Each group received 10–35-ml puffs at the rate of one puff per minute four times daily, 7 days a week for 14 weeks. The five test levels were (1) air; (2) smoke from control cigarettes; smoke from cigarettes containing (3) Enide (DPA) 4 ppm + desmethyl Enide (DMPA) 20 ppm; or (4) DPA ppm; or (5) DMPA (100 ppm). Rats showed no remarkable evidence of toxicity based on weight gain, hemograms, and gross necropsy. Histopathologically there was evidence of pulmonary and CNS irregularities in all treatment groups. Dogs exposed to cigarette smoke showed toxic signs including emesis, injected conjunctival vessels, salivation, and relaxation of nictitating membrane. In dogs hemograms, biochemical determinations, blood pressure, heart rate measurements, gross necropsy, and histopathology showed no significant differences between treatment and control groups. (R. P. Beliles now at Lakeside Laboratories, Milwaukee, Wisconsin.)

37. *Respiratory Gas Tensions and pH of Arterial Blood in Cynomolgus Monkeys (Macaca irus).* C. M. BANERJEE, M. WOOLARD, and Y. ALARIE, Hazleton Laboratories, Inc., Falls Church, Virginia. (Sponsor: H. N. MacFarland.)

The arterial blood gas tension of oxygen ($P_{a(O_2)}$), carbon dioxide ($P_{a(CO_2)}$), and pH were measured by the Astrup analyzer (Radiometer) in 51 unanesthetized restrained cynomolgus monkeys (1.5–4.0 kg). The plasma bicarbonate values were calculated from the nomogram (Siggard-Andersen) and verified by direct measurements.

The mean values and standard deviations for $P_{a(O_2)}$, $P_{a(CO_2)}$, pH, and bicarbonate were 110 ± 12 mm Hg, 29 ± 5 mm Hg, 7.25 ± 0.079, and 13 ± 4 meq, respectively. Identical measurements were taken in 58 cynomolgus monkeys 15 minutes after 1.0–1.5 mg/kg intramuscularly of phencylidine hydrochloride (Sernylan, Parke-Davis Co.). The values obtained for $P_{a(O_2)}$, $P_{a(CO_2)}$, pH, and bicarbonate were 101 ± 7 mm Hg, 42 ± 4 mm Hg, 7.40 ± 0.169, and 25 ± 2 meq, respectively.

The above findings indicate that a stage of moderately severe acidosis existed in the first group of animals. This acidosis may be attributed to the struggling and stress imposed on the animals while catching them and sampling the blood.

38. *A Mechanistic Model for the Analysis of Upper Respiratory Tract Irritation.* Y. ALARIE and C. TIBBETTS, Hazleton Laboratories, Inc., Falls Church, Virginia.

It has been previously shown that the decrease in respiratory rate in mice can be related to the logarithm of airborne irritant concentration [Alarie, *Arch. Environ. Health* **13**, 433-449 (1966)]. A mechanistic model has been developed, based on drug-receptor theory [Beidler, *Progr. Biophys. Chem.* **12**, 109-151 (1962)], in order to obtain more meaningful parameters to analyze structurally-related chemicals possessing irritating properties. The predictions from the model were confirmed experimentally and the following parameters were found to be useful: maximum obtainable response (R_s), affinity constant (k_1), dissociation constant (k_2) and the concentration capable of evoking a 50% decrease in respiratory rate (RD_{50}). A series of chemicals has been tested and the results will be presented.

39. *Effects of Nitrogen Dioxide on Pulmonary Cell Population.* D. E. GARDNER, R. HOLZMAN, and D. L. COFFIN, Experimental Pathology Unit, HERP-NCAPC, Public Health Service, Cincinnati, Ohio. (Sponsor: H. E. Stokinger.)

Studies from this laboratory have shown that ozone has produced changes in the number and function of cells obtained by pulmonary lavage.

In similar experiments, rabbits exposed to levels of NO_2 from ambient to 30 ppm demonstrated increased numbers of polymorphonuclear leukocytes in the lung washings. This phenomenon persisted for more than 48 hours following a single 3-hour exposure.

When streptococci were instilled into the lungs of NO_2 exposed anesthetized rabbits, 30 minutes prior to lavage, a pronounced inhibition of phagocytic activity was observed.

Using these criteria, NO_2 appeared less effective than ozone as a pulmonary irritant.

40. *Dust Removal from the Lung Parenchyma: An Investigation of Clearance Simulants.* P. E. MORROW, F. R. GIBB, H. DAVIES, and M. FISHER, Department of Radiation Biology and Biophysics, University of Rochester, Rochester, New York.

The investigation described was concerned with the application of ultrafiltration tests and parenteral injections to a variety of radioactively tagged aerosol materials in order to better understand the behavior of these materials in the lungs after inhalation. The results indicate that both approaches were useful in determining the relative persistence of these materials in the lung parenchyma and clearly suggest that the physical-chemical properties of the aerosolized materials were the dominant factors in pulmonary clearance. The prevailing concept

that the major pulmonary clearance mechanism for "insoluble" particulate material is removal by macrophages is challenged. The study also indicated that many of the reported retention times (biological half-lives) for inhaled substances in the lungs are erroneous and probably pertain to extrapulmonary storage and removal processes.

The results and conclusions are based upon over 400 ultrafiltration tests on 19 different compounds, including cesium and strontium chlorides, zinc and tin phosphates and manganese and plutonium oxides; and upon animal studies which included lung clearance measurements in 77 dogs and 178 rats, intramuscular clearance data from 62 rats, together with many intravenous studies and excreta, whole body, and organ analyses.

41. *Fission Products: Transmutation, Translocation, and Retention of the Radionuclide Pair, Zirconium-95–Niobium-95 in the Rat.* B. V. RAMA SASTRY, Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee.

Zirconium-95 (half-life, 65 days) and its daughter, ^{95}Nb (half-life, 35 days) are components of atomic fallout. It is generally assumed that they are in transient equilibrium (half-life, $\doteq$ 65 days) during monitoring populations for fallout isotopes and in determining maximum permissible limits. To verify this assumption, the metabolic behavior of ^{95}Zr–^{95}Nb was studied in the rat for a period of 42 days after intraperitoneal administration. The transient equilibrium between the radionuclides retained in the tissues was judged by "effective physical half-times" ($ET_{1/2}$ in days). After day 41, the rats did not excrete any ^{95}Zr–^{95}Nb; the whole body ^{95}Zr–^{95}Nb was in transient equilibrium. The $ET_{1/2}$ of urine samples decreased from 98 on the first day to about 43 on days 3 and 4, and again increased to about 57 during 5–42 days. The $ET_{1/2}$ of ^{95}Zr–^{95}Nb in the blood was 66 during the first 2-day samples but decreased to 57 during 3–42 days. Even on day 42, the $ET_{1/2}$ of the radionuclides in the bone, liver, and kidney was longer, and the $ET_{1/2}$ in the gonads and spleen was shorter, than 65 days. These observations would indicate that the radionuclides were not in transient equilibrium in tissues. During the first 2 days, ^{95}Zr was preferentially accumulated by the bone and excreted by the kidney. During 3–42 days a part of ^{95}Zr (+ ^{95}Nb) was translocated from bone (and other tissues) and excreted in urine. (Supported by grants from US AEC and US PHS.)

42. *Fission Products: Enhancement of Cesium-137 Retention during Hypokalemia and Low Tissue Potassium Levels in the Rat.* B. V. RAMA SASTRY, Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee.

Among all mammals studied, muscle is the single organ which contains the largest amount of fall-out ^{137}Cs after oral ingestion. The reasons for this preferential accumulation of ^{137}Cs are not known. In view of the similar physicochemical properties of potassium and cesium, the retention of ^{137}Cs was investigated during physiological or pharmacologic situations that were known to produce low tissue potassium levels. The retention of ^{137}Cs in the rat was studied under the following experimental conditions: (1) treatment with deoxycorticosterone trimethylacetate (12.5 mg/kg/day; 7 doses, ip), (2) treatment with chlorothiazide (50 mg/kg/day; 7 doses, ip), (3) low potassium diet for 9–10 weeks, and (4) transection of sciatic nerve innervating the gastrocnemius and tibial muscles. The control and the experimental rats were injected with ^{137}Cs (11.35 μc/kg, ip). The animals were sacrificed at the end of day 8 ($BT_{1/2}$, biological half-time of ^{137}Cs: 7–8 days) in experiments 1–3. In experiment 4, the rats were sacrificed at the end of day 4, when peak concentrations of ^{137}Cs were found in the gastrocnemius muscles of the control rats. The gastrocnemius muscles of all animals were analyzed for ^{137}Cs concentrations by gamma scintillation spectrometric methods. The total retention of ^{137}Cs was determined by whole-body counting methods. In all cases, the gastrocnemius muscles of the experimental animals contained higher concentrations (1.3–2.3 ×) of ^{137}Cs than the controls. The low potassium diet retained all administered ^{137}Cs for more than 8 days. (Supported by grants from US AEC, US PHS, and American Cancer Society.)

43. *Metabolic Behavior of Inhaled Radionuclides of Varied Solubility in Beagle Dogs.* R. O. McClellan and B. B. Boecker, Fission Product Inhalation Program, Lovelace Foundation for Medical Education and Research, Albuquerque, New Mexico. (Sponsor: K. L. Gabriel.)

A knowledge of the metabolic behavior of inhaled radionuclides of varied solubility is necessary for correct establishment of the radiation dose to various portions of the body and meaningful interpretation of the radiation dose-effect relationship for inhaled radionuclides. To illustrate this, selected data obtained in our laboratory on the metabolism of inhaled radionuclides of varied solubility in the dog are reviewed with emphasis placed on studies conducted with two forms of ^{137}Cs. After inhalation of ^{137}Cs as CsCl, the ^{137}Cs was rapidly translocated from the lung to other tissues, the highest concentrations being observed in muscle. The long-term component of a multiexponential retention equation corresponded to a biological half-life of 43 days. Urinary excretion of ^{137}Cs predominated. In contrast, when ^{137}Cs was inhaled in fused clay particles, almost all the retained material (approximately 95%) remained in the lung, with small quantities in tracheobronchial lymph nodes, and even smaller quantities present in other tissues. The long-term component of retention corresponded to a biological half-life of 440 days. Fecal excretion predominated, presumably as a result of the insoluble ^{137}Cs being cleared from the upper respiratory tract, ingested, and passed through the gastrointestinal tract without absorption. Because of the difference in metabolic patterns, exposure resulting in equivalent initial body burdens would result in an infinite cumulative tissue radiation dose for lung that is over 500 times that for whole body, the critical organs, respectively, for the "insoluble" and soluble forms. (Work performed under Contract AT(29-2)-1013 with the U.S. Atomic Energy Commission.)

44. *Cutaneous and Parenteral Toxicologic Studies with Vehicles Containing Isopropyl Myristate and Peanut Oil.* J. E. Fitzgerald, J. L. Schardein, and D. H. Kaump, Research Laboratories, Parke, Davis & Company, Ann Arbor, Michigan.

Following cutaneous application, isopropyl myristate induced a prompt dermatosis in mice and rabbits. This was characterized grossly by erythema, lichenification, and fissure formation, and histologically by acanthosis, para- and hyperkeratosis, focal erosion, and focal hemorrhage. In rabbits, the skin lesion regressed slowly after cessation of treatment, whereas in mice the lesion regressed during continued treatment. Similar reactions occurred with combinations of isopropyl myristate and peanut oil, but the intensity of the dermatosis decreased as the proportion of isopropyl myristate was decreased in the mixture. Peanut oil, used alone, produced only minor gross and microscopic changes.

Single intramuscular injections in rabbits, and repeated intramuscular injections in rats, dogs, and monkeys, produced only minor local damage at injection sites, with no definitive systemic effects.

45. *Toxicity of p-n-Butylaniline, 2-Amino-4-n-butylaniline, and 2-Nitro-4-n-butylacetanilide after Topical Application to the Rabbit.* J. J. Pollock, B. J. Payne, E. Arena, and B. Wright, Pathology and Toxicology, Smith Kline & French Laboratories, Philadelphia, Pennsylvania. (Sponsor: J. F. Borzelleca.)

Topical application of *p-n*-butylaniline to the shaved skin of White Castle albino rabbits in doses ranging from 0.01 to 2.00 ml/day for 1–3 days produced some or all of the following changes:

I. Clinical: Decreased spontaneous motor activity, hypotonia, dyspnea, cyanosis, anemia, methemoglobinemia, Heinz body formation, leukocytosis, and death.

II. Postmortem: Icterus in the abdominal fat, congestion of spleen, toxic nephritis and tubular cast formation, toxic hepatitis, gastric hemorrhage, necrosis and hemorrhage of urinary bladder, and necrosis at site of application.

A single application of 0.1 or 0.5 ml of *p-n*-butylaniline to one eye of the rabbit caused swelling of the eyelid, nictitating membrane, and conjunctiva; purulent inflammation of the limbus; and necrosis of the cornea.

Application to the skin of 2-amino-4-*n*-butylaniline (6 ml/day for 3 days of a 1 or 10% solution) caused slight cutaneous hyperplasia and a moribund state in some rabbits. Eye lesions caused by 2-amino-4-*n*-butylaniline (0.1 or 0.5 ml of a 1 or 10% solution) were similar to those of *p-n*-butylaniline, but less severe. No drug-related lesion or effect occurred with 2-nitro-4-*n*-butylacetanilide applied to either the skin or the eye at concentrations comparable to those of 2-amino-4-*n*-butylaniline.

46. *Comparative Response of Rabbit Skin and the Nonglandular Mucosa of Rat Stomach to Certain Topical Agents.* J. E. GRAY and G. A. ELLIOTT, Research Laboratories, The Upjohn Company, Kalamazoo, Michigan.

During the conduct of dermal toxicity studies on the clipped back skin of the rabbit, a rather nonspecific proliferative response of the epidermis has been characterized.

An investigational topical drug, U-3243, 5% in Veriderm® ointment, was applied to weanlings for 2 weeks and to adults for 3 weeks. The treated area became wrinkled transversely. Histologically, micropustules appeared transiently at the epidermal surface by the third day. After 6 days, mild hyperplasia of the spinose cell layer and retention of the horny layer with parakeratotic scaling were present. These changes occurred, to a lesser degree, in the skin of rabbits given applications of the base ointment.

A more definitive dose-related response was produced by applications of a second topical agent, U-19,718, in a liquid vehicle, at 0.1% and 1% concentrations for as long as 31 days. In 24 hours, the treated area of approximately 7.5 × 10 cm showed mild erythema, increased sensitivity, and slight swelling. By 6 days it was wrinkled transversely and covered with a brownish crust. The thickening of the horny layer consisted chiefly of layered masses of degenerating pseudoeosinophils. The crust was thinner and did not recur after peeling off in rabbits treated continuously with the lower concentration.

In rats treated orally with 100 mg/kg of U-19,718 for 11 days, encrustations of horny tissue were formed on the nonglandular mucosa of the stomach. A recurring vesiculopustular sequence initiated in the granular layer of the stratified squamous epithelium.

47. *The Detection of Contact Allergens by Means of a Guinea Pig Basophil Leukocyte Test.* Y. GRESSEL, Avon Products, Inc., Suffern, New York. (Sponsor: J. F. Borzelleca.)

The conventional animal screening techniques designed to predict the contact sensitization potential of materials applied to the skin, rely primarily on the subjective evaluation of skin changes, e.g., erythema. Where weak skin reactions are elicited, or where the test material is itself a primary irritant, it is often quite difficult to classify a material as a contact allergen or as a primary irritant.

This paper describes a simple, rapid testing procedure designed to overcome the above difficulties by the objective assessment of the sensitization potential. The test is based on reports by others that the concentration of basophil leukocytes in the peripheral blood of the guinea pig undergoes a change during the course of treatment with various allergens.

A variety of materials have been tested, and it has been found that treatment with contact sensitizers, e.g., dinitrochlorobenzene, formalin, and *p*-phenylenediamine produces a significant rise in the blood basophil count during the course of the sensitization induction period and also following challenge. In contrast, treatment with nonsensitizing irritants, e.g., sodium hydroxide and aluminum chloride, does not result in an increased basophil count. The effect of the above treatments on other parameters, e.g., the total leukocyte count and the eosinophil count, was also investigated and is discussed.

48. *Comparative Studies of Known Sensitizers.* D. T. VON SUMPTER, C. G. GERBIG, and W. D. FISHER, Pharmacology Laboratory, Pesticides Regulation Division, ARS, U.S. Department of Agriculture, Beltsville, Maryland. (Sponsor: H. W. Hays.)

The intact skin of the organism presents a relatively effective barrier for the penetration of many substances. However, studies in the field of cutaneous absorption indicate that the skin is permeable in some degree to a great variety of compounds.

It has been adequately shown that eczematous sensitization or contact dermatitis to simple compounds can occur in human beings. The human skin is easily sensitized to such simple compounds as 2,4-dinitrochlorobenzene and nitrodimethylaniline and less easily to certain coal-tar colors.

The ideal test subject for the determination of sensitization to a compound is man, but the use of such subject is usually neither wise nor expedient. The white male guinea pig has been shown by Landsteiner and Jacobs to be a reasonably satisfactory test animal. A modified procedure of this test as well as two other procedures have been studied in this laboratory.

Preliminary results with dinitrochlorobenzene and phenylmercuric acetate indicate that the closed patch method is more reliable and more sensitive when compared with topical applications and intradermal injection methods. This appears to be true even when lower concentrations of the given substance are used. Several other known sensitizers under study in this laboratory have produced similar results.

49. *Patterns of Photoreactivity and Crossreactivity in Persons Sensitized to Tetrachlorosalicylanilide.* J. F. GRIFFITH and R. O. CARTER, The Procter & Gamble Company, Miami Valley Laboratories and Ivorydale Technical Center, Cincinnati, Ohio.

Ten individuals sensitized to 3,3′,4′,5-tetrachlorosalicylanilide (TCSA) by patch testing have been studied for photoreactivity and for cross-reactivity to other halogenated salicylanilides and related compounds. These subjects still retained a high degree of hyperreactivity to TCSA nine to eleven years after they had become sensitized. This hyperreactivity was neither mediated nor enhanced by ultraviolet light of wavelength greater than 3100 Å. The incidence and intensity of reactions to the related antibacterial agents were less than those to TCSA and ultraviolet light had little or no effect on the level of reactivity. 4′,5-Dibromosalicylanilide elicited the largest number of cross-sensitization reactions, followed by 3,4-′,5-tribromosalicylanilide, 3,5-dibromosalicylanilide, bithionol, and hexachlorophene when these antibacterial agents were tested simultaneously. With the exception of one subject, however, cross-reactivity to 3,4′,5-tribromosalicylanilide did not occur when this material was tested alone, but it was likely to occur in subjects challenged simultaneously with 4,5′-dibromosalicylanilide.

It is concluded that simultaneous patch testing of multiple agents in diagnosing causes of contact dermatitis may result in artifactitious reactions that would not occur if the compounds were tested singly. Thus 4′,5-dibromosalicylanilide is capable, at least in some TCSA-sensitive individuals, of inducing hyperreactivity to 3,4′,5-tribromosalicylanilide that would not occur of itself.

50. *The Dermal Absorption of Four Solid Formulations of Carbaryl in Rabbits.* H. J. TROCHIMOWICZ and W. E. RINEHART, Department of Occupational Health, Graduate School of Public Health, The University of Pittsburgh, Pittsburgh, Pennsylvania.

Four solid formulations of carbaryl were investigated to determine whether a difference in formulation caused a difference in absorption through the skin of rabbits. Each formulation was applied at 2 g of carbaryl per kilogram body weight and was held in contact with the skin for 4 hours, using both saline and xylene as moistening agents. Urine was then collected at 24- and 48-hour intervals and analyzed colorimetrically for 1-naphthol. The excess of this principal carbaryl metabolite over control values was taken as an index of absorption.

All formulations of carbaryl showed measurable absorption with both saline and xylene as moistening agents, but two formulations showed greater absorption with xylene. However, greatest absorption occurred with a wettable powder preparation when either moistening agent was used. It was not clear why the wettable formulation should penetrate to the greatest extent.

51. *Steroid-Induced Elevated Intraocular Pressure in Rabbits.* O. J. LORENZETTI. Therapeutics Research Laboratory, Miles Laboratories, Inc., Elkhart, Indiana. (Sponsor: R. K. S. Lim.)

Humans treated with ophthalmic corticosteroids have shown a slow development of elevated intraocular pressure (IOP) which is believed to be genetically determined. Our studies have shown that the elevation of IOP occurs also in rabbits. Dexamethasone 21-phosphate was instilled topically (50 μl twice daily) in the rabbit eye for 3 months at 0.01, 0.1, and 1.0% concentration. Total body weight and physical changes were observed daily, and IOP was recorded weekly. Organ weights were determined at the end of the experiment. Only one eye received steroid while the other received vehicle. In addition, a group of rabbits received vehicle in both eyes. All determinations were made under blind conditions.

Rabbits receiving 0.01% and 0.1% dexamethasone displayed minimal changes in organ weights and slight depression of growth curve over the 3-month period. There were no significant changes in the IOP for rabbits receiving 0.01% dexamethasone. Some rabbits (13/20) receiving 0.1% dexamethasone displayed significant elevation by the fifth week. Rabbits receiving 1.0% dexamethasone had a severely depressed growth curve, significantly lower adrenal, spleen, and lens weight, and significantly elevated kidney weight. The IOP response was variable in this group and thus, it was divided into three groups—those displaying an elevation greater than 100% (14/72), those displaying an elevation of 50–100% (20/72), and those showing less than 50% response (38/72). The 1.0% dexamethasone-treated rabbits displayed loss of muscle mass, diarrhea, polydipsia, polyuria, exophthalmus, and frequent deaths (15/72). Cessation of dosing with 1% and 0.1% dexamethasone resulted in a return of IOP to normal and an increase in slope of the growth curve. Rabbits exhibiting elevated IOP responded to topical l-epinephrine, pilocarpine, or physostigmine with a lowering of IOP.

The results show that chronic instillation of a corticosteroid will induce elevation of IOP in rabbits as well as related toxic manifestations.

52. *Corneal and Lens Opacities in Dogs Treated with 2,6-dichloro-4-nitroaniline.* J. M. CURTIS, H. BERNSTEIN, F. L. EARL, and H. E, SMALLEY, JR., Special Pharmacological Animal Laboratory and the Bureau of Medicine, Food and Drug Administration, U.S. Department of Health, Education, and Welfare, Washington, D.C. (Sponsor: E. J. Van Loon.)

In the course of experiments designed to confirm the reported toxicity of the pesticide 2,6-dichloro-4-nitroaniline, opacity in the eyes of dogs was observed grossly. More detailed observation of these opacities by slit-lamp microscopy showed that the cornea was affected, and this change was in the anterior layers of the supporting connective tissue. In addition opacities developed on the anterior surface of the lens. These changes are dose dependent in that they developed at dosages of 48 mg/kg/day, more slowly at 24 mg/kg/day, but cannot be observed clinically at 6 mg/kg/day. The changes are observable, using the slit-lamp microscope, in approximately 50 days at the higher doses, but take somewhat longer to develop at lower doses. Histologically, it is observed that lipid droplets, stained in frozen section with Oil Red O, formed in the anterior portion of the cornea, just beneath the epithelial layer and were associated with the nuclei of stromal fibroblasts. On electron microscopic examination these droplets were shown to be intracellular in the cytoplasm. Experiments in which the eyes of treated animals are protected from daylight suggest that the development of the above described eye pathology may be related to the exposure of the animal's eye to sunlight.

53. *Hair Dye Ophthalmia as Evaluated in the Monkey and Rabbit: A Comparative Study.* T. WERNICK and J. L. MAGNANI, Clairol Incorporated, Stamford, Connecticut. (Sponsor: S. Carson.)

Much has been made in the past about the hazard potential of oxidation type hair dyes in the area of the eye. When these products are evaluated using the standard Draize Rabbit Eye Test, it is often impossible to predict with any reliability their effects on the human eye. Faced

with this problem, we have turned to rhesus monkeys as the test animal. The use of the monkey eye to evaluate eye irritants is not new, as evidenced by the work of Beckley and his colleagues.

Various hair dye formulations have been instilled into the lower conjunctival sac of rhesus monkeys and New Zealand White rabbits. The eyes were examined at various intervals after instillation of the test materials. The examination included, in sequential fashion, gross observation, slit lamp examination, fundus examination, fluorescin staining, photography, and histologic examination.

Scoring of the eye utilized the standard Draize readings. The eyes were examined through the irritation and recovery phases. When irritation occurred, the recovery rate was an important factor in the overall evaluation.

None of the hair dye formulations tested have shown any permanent damage when instilled into the monkey eye. Special emphasis will be placed on *p*-phenylenediamine (PPD), and some substituted nitro-1,4-diaminobenzene containing formulas.

54. *Chromatographic Identification of 4,4′-Dinitrobiphenyl Metabolites.* S. LAHAM, and J.-P. FARANT, Environmental Toxicology Programme, Environmental Health Centre, Department of National Health and Welfare, Ottawa, Canada.

During studies on the comparative metabolism of a new bladder carcinogen, 4,4′-dinitrobiphenyl (4,4′-DNB), it was found necessary to develop a chromatographic method for the identification of biotransformation products present in the urine of various animal species. The metabolites considered in this report include free nitro and amino derivatives (substituted in para-para position), *O*-hydroxyl derivatives as well as *N*-acetyl and sulfates (*O*- and *N*-) of the same compounds.

The standards are spotted on Whatman paper No. 1 in amounts ranging from 0.5 to 5 μg. The chromatograms are run in both cylindrical and "chromatocabs" for various times in 20 different solvents. Of these 20 solvents, only 10 were found suitable and gave a wide variety of R_f's for a large number of these compounds. The chromatograms are then examined in day and ultraviolet light (2537 and 3600 Å) and sprayed with 15 different reagents. After spraying, they were examined again for development of colors characteristic of specific structures.

The results of these investigations is discussed in relation to the metabolism of 4,4′-DNB.

55. *Comparison of in Vitro Assay Methods for Studying Metabolic Mechanisms That Affect Paraoxon Toxicity.* R. R. LAUWERYS and S. D. MURPHY, Department of Physiology, Harvard University School of Public Health, Boston, Massachusetts.

The metabolic degradation of paraoxon [*O*,*O*-diethyl, *O*-(*p*-nitrophenyl)phosphate] *in vitro* has frequently been investigated either by direct measurements of the hydrolysis product, *p*-nitrophenol (PNP) or by measuring the loss of anticholinesterase activity of tissue-paraoxon incubates (bioassay). Usually relatively high concentrations (10^{-4} to 10^{-2} *M*) of paraoxon are used for the PNP methods, and low concentrations (10^{-7} to 10^{-6} *M*) for the bioassay techniques. In an attempt to evaluate the relevance of these *in vitro* methods to studies of the metabolic regulation of paraoxon toxicity, we used both techniques to compare the effects of various factors on paraoxon degradation by rat plasma and liver homogenates. Kinetic studies showed that the PNP method obeyed the principles of an enzyme-catalyzed reaction while the bioassay technique appeared to measure a nonenzymatic or binding reaction. EDTA and Mg^{++} inhibited and Ca^{++} activated PNP production *in vitro*, but they did not affect the loss of anticholinesterase activity of low concentrations of paraoxon in the presence of tissues. Livers and plasma taken from rats 16 hours after intraperitoneal injection of 125 mg/kg of triorthotolyl phosphate (TOTP) failed to degrade paraoxon as measured by the bioassay procedure, but degradation measured by PNP production was unaffected. The intraperitoneal LD_{50} of paraoxon in TOTP-pretreated rats was 0.52 mg/kg compared to 1.33 mg/kg for corn oil-pretreated controls. Thirty minutes after 0.25 mg/kg of paraoxon, brain cholinesterase activities of TOTP and corn oil-pretreated rats were 14.4 ± 6.3 and $91.7 \pm 4.0\%$ of controls, respectively. TOTP alone did not affect brain cholinesterase activity. The results suggest that

a nonenzymatic (tissue binding) reaction is an important detoxication mechanism for paraoxon *in vivo*. (Supported by Research and Training grants ES 00084 and ES 45 from the U.S.P.H.S.)

56. *Effect of Feeding and Fasting on Excretion of Phenobarbital in the Rabbit.* J. M. FUJIMOTO and R. A. DONNELLY, Department of Pharmacology, School of Medicine, Tulane University, New Orleans, Louisiana.

The circadian nature of the excretion of phenobarbital and its metabolite, *p*-hydroxyphenobarbital glucuronide, was studied. Phenobarbital-2-^{14}C, 9.7 μc/kg, was administered intravenously to 6 rabbits, 3 rabbits each in groups A and B. The excretion of the two radioactive products was followed by countercurrent distribution analysis. Urine was collected every 6 hours by catheterization of the bladder except in the few cases where it was obtained from the metabolism pan when micturition had occurred. Group A which was fed during each alternate 6-hour period showed a circadian type excretion of phenobarbital when excretion was expressed as the percent phenobarbital per total ^{14}C in each 6-hour urine sample. Since this percentage was correlated with pH changes in the urine, the circadian rhythm in excretion is due to circadian changes in pH. Group B which was fasted had a uniformly low pH and showed no such rhythm. Recovery of total ^{14}C was greater in group B. This was due to significantly greater excretion of the metabolite. Reversing the feeding-fasting treatment during the experiment reversed the urinary pH but did not alter this difference. Therefore the difference in amount of metabolite excreted is dependent on other than pH of the urine. Polydipsia and polyuria caused by fasting seemed to have little effect per se on phenobarbital and metabolite excretion. (Supported by U.S. Public Health Service Research Grant GM05514.)

57. *Enhanced Toxicity of Desipramine (DMI) in Aggregated Mice: Possible involvement of Catecholamine Release.* H. LAL and R. M. BROWN, Department of Pharmacology, University of Rhode Island, Kingston, Rhode Island, and Department of Pharmacology, University of Chicago, Chicago, Illinois.

Studies of percentage mortality, onset of convulsions, and onset of death showed that imipramine and DMI were more toxic in aggregated mice than in isolated mice. DMI was more toxic than imipramine. Pretreatment with reserpine (3 mg/kg), but not with chlorpromazine (0.5, 1, 2, 5, 10, or 20 mg/kg), disulfiram (400 mg/kg), or *p*-chlorophenylalanine (315 mg/kg daily for 3 days), protected the mice against DMI toxicity. Reserpine protection was reversed by 3,4-dihydroxyphenylalanine (DOPA, 400 mg/kg) alone or DOPA and disulfiram, but not methyl-DOPA (400 mg/kg) or 5-hydroxytryptophan (400 mg/kg). These data suggest a relationship between tissue norepinephrine or dopamine, but not tissue serotonin, and acute toxicity of DMI in aggregated mice. Amphetamine [Cohen and Lal, *Nature* **201**, 1037 (1964)] and cocaine [Lal and Chessick, *Nature* **208**, 295 (1965)] were also more toxic to aggregated mice in similar experiments. All these drugs inhibit tissue re-uptake of catecholamines. Intense sensory stimulation during aggregation of animals may cause catecholamine release. Inhibition of catecholamine re-uptake and thus prolongation of the receptor stimulation may enhance the drug toxicity. (Supported by University of Kansas General Research Fund and work done at that university.)

58. *Adrenergic Responses in the Shark.* S. L. SCHWARTZ and J. F. BORZELLECA, U.S. Naval Medical Research Institute, Bethesda, Maryland, and Medical College of Virginia, Richmond, Virginia.

Very little information exists regarding the responses of the shark to chemical stimuli under controlled conditions. These data are required for such applications as pharmacologically based antishark devices. In addition, the characterization of such responses to pharmacologic agents may also yield valuable information about the physiology of these animals.

d-Tubocurarine (3 mg/kg) was administered intravenously to lemon (*Negaprion brevirostris*) and nurse (*Ginglymostoma cirratum*) sharks, 1–6 kg. These were then anesthetized with

tricaine methanesulfonate (MS-222), 1:10,000, administered by gill perfusion. The sharks were restrained in a supine position and the caudal artery and vein were cannulated with polyethylene tubing. Blood pressure and electrocardiograms were recorded by means of standard procedures. Adequate oxygenation was assured by means of continuous gill perfusion with seawater. All drugs were dissolved in balanced salt solution, and these were administered into the caudal vein.

Pressor responses to epinephrine were noted with doses of 1–1000 μg/kg. Subsequent doses of epinephrine resulted in attenuated or abolished responses even when the doses were increased a hundredfold. Depressor responses were noted with a dose of 0.5 μg/kg. Norepinephrine elicited a pressor response resulting in an attenuation of subsequent doses. Dibenzyline resulted in a decrease in blood pressure and an attenuation of the pressor responses to epinephrine. Isopropylarterenol elicited only depressor responses. Nicotine, in doses up to 1000 mg/kg, elicited a prolonged depressor response and no subsequent pressor response. Fasciculations were noted at the higher doses, and these doses were not always fatal. The fasciculations were blocked by previous administration of *d*-tubocurarine. These data suggest that there are similarities in the responses to adrenergic agents between sharks and mammals, but some qualitative and quantitative differences do exist.

59. *Some Chemical and Pharmacologic Properties of a Novel Anti-inflammatory Protein.* W. HUBER, T. L. SCHULTE, S. CARSON, R. E. GOLDHAMER, and E. E. VOGIN, Beta Laboratories, Inc. Palo Alto, California, Food and Drug Research Laboratories, Inc. Maspeth, New York 11378.

Extensive physicochemical, biochemical, pharmacologic, and toxicologic studies have been performed with Ontosein®, an anti-inflammatory protein derived from bovine liver. Emphasis has been placed on its characterization by several anti-inflammatory test procedures in rats, guinea pigs, and rabbits. Available data indicate that Ontosein is not an enzyme.

The protein has been compared at levels of 0.01–5 mg/kg with prednisone and phenylbutazone and found to be equal to or more potent than these drugs on a weight for weight basis. Antiphlogistic potency data are correlated with *in vitro* latex agglutination.

Some data are discussed which indicate the relative action mechanism of Ontosein to DNA.

60. *Studies on Cobalt Toxicity.* G. S. WIBERG, Pathology and Toxicology Section, Research Laboratories, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada.

Earlier studies from this laboratory indicated that cobalt ions depressed pyruvate, octanoate, and stearate metabolism in rat heart mitochondria [Wiberg, Munro, and Morrison, *Can. J. Biochem.* **45**, 1223 (1967)]. It was postulated that the cobalt ions complexed with α-lipoic acid and thus inhibited the pyruvate dehydrogenase and α-ketoglutarate dehydrogenase systems. Further studies will be presented on the effect of cobalt ions on myocardial metabolism with particular reference to the α-ketoglutarate dehydrogenase complex and the reversal of the "metabolic block" with various thiol groups. Also the effects of cobalt ions on the energy metabolism of skeletal muscle will be discussed and compared to changes found in cardiac muscle.

61. *The Interaction Between Various Drugs and Methoxytrexate.* R. L. DIXON, Department of Pharmacology, School of Medicine, University of Washington, Seattle, Washington.

Methotrexate (4-amino-N^{10}-methylpteroylglutamic acid, MTX) is a folic acid antagonist used widely in cancer chemotherapy. Methotrexate is bound to plasma proteins [*Federation Proc.* **24**, 454 (1965)]. Plasma protein binding determines to a large extent the amount of free (unbound) drug available for membrane penetration and drug action, and also for metabolism and excretion. However, the phenomenon of plasma protein binding becomes even more important when considering agents such as MTX which have steep dose response slopes and

are administered in doses which result in blood and tissue concentrations that approach very closely levels associated with toxicity. In this study we have attempted to investigate the *in vivo* effect of various clinically useful drugs on the subacute toxicity of MTX. The addition of any one of a large variety of drugs *in vitro* appears to compete with MTX for plasma protein binding sites and result in an increase in the unbound concentration of MTX and a decreased amount of drug bound per mole of protein. A number of these agents have been tested for *in vivo* effect on MTX lethality.

Male mice were treated for 5 days with each of four doses of MTX (1.53–12.25 mg/kg) administered intravenously. Immediately after the MTX treatment, each group of animals received either the test drug or an equal volume of saline administered intraperitoneally. Each treatment dose level had 12 mice, and the animals were observed daily for 15 days after the first injection. Sulfamethoxypyridazine (Kynex), sodium salicylate, *p*-aminosalicylic acid, diphenylhydantoin, tolbutamide, chloromycetin, and tetracycline, at doses which produced no toxic effect, each appeared to be capable of decreasing the lethal dose and/or decreasing the median survival time of MTX-treated mice.

62. *The Effect of Uncoupling Agents on the Transport, Toxicity, and Effectiveness of Methotrexate.* I. P. LEE and R. L. DIXON, Department of Pharmacology, School of Medicine, University of Washington, Seattle, Washington.

Methotrexate (4-amino-N^{10}-methylpteroyglutamic acid, MTX) is a potent anticancer agent used especially in the treatment of childhood leukemia. The clinical effectiveness of MTX is eventually limited by the development of cellular resistance to the antifolate. This resistance has been frequently correlated with an increased level of the target enzyme dihydrofolate reductase. However, more recently it has been reported that in certain systems resistance to methotrexate can be better correlated to the cellular ability to actively transport MTX. Preliminary studies of the transport of MTX by human white cells in this laboratory have been performed. It was noted that the rate of MTX influx was not inhibited by 2,4-dinitrophenol; rather, this uncoupling agent resulted in a cellular accumulation of MTX. It appeared that MTX might be the substrate for an active efflux mechanism rather than an active influx system. These data correlate with the observations of Goldman and Oliverio [*Federation Proc.* **26**, 206 (1967)].

In vivo studies designed to investigate the effect of various uncoupling agents on the lethality and effectiveness of MTX have been conducted. Uncoupling agents (2,4-dinitrophenol, amobarbital, and thyroxine) administered concomitantly with MTX (LD_{10}) significantly increased the lethality of MTX to male DBA/2 mice. Studies with transplantable leukemia L-1210 have demonstrated that uncoupling agents administered at the same time as effective doses of MTX will alter antitumor effectiveness. The effect of uncoupling agents on the effectiveness of MTX against MTX-resistant L-1210 leukemia has also been investigated. It appears that the efflux of MTX from human white cells and murine L-1210 cells plays a role in the therapeutic effectiveness of MTX.

63. *Prevention of Acetamide Hepatocarcinogenicity by Arginine Glutamate.* R. S. YAMAMOTO, R. M. GLASS, and J. H. WEISBURGER, Biology Branch, National Cancer Institute, Bethesda, Maryland.

Jackson and Dessau [*Lab. Invest.* **10**, 909 (1961)] discovered that dietary acetamide caused liver cancer in rats. The mechanism is completely unknown. On the theory that acetamide might give rise to a chronic cellular ammonia intoxication, experiments were designed to protect against this event by joint administration of acetamide and arginine glutamate [Greenstein *et al.*, *Arch. Biochem.* **64**, 342 (1956)].

Weanling male Wistar rats were fed 2.5% acetamide or 2.5% acetamide plus 5.6% arginine glutamate in the diet for 12 months. There were negative untreated controls and controls given arginine glutamate, or 4.8% ammonium citrate. Some of the rats were necropsied after one year, the remainder held an additional three months on control diet. In confirmation of the

findings of Jackson and Dessau, approximately half of the rats on acetamide exhibited liver cancers and the livers of most of the animals at risk had evidence of hyperplasia. Significantly, however, hepatoma was absent in the animals on acetamide plus arginine glutamate. In this group areas of hyperplasia were the most advanced hepatic lesions, and a sizable proportion of rats showed no precancerous lesions. The various control groups likewise had no cancer.

64. *Potentiating Effects of Low-Protein Diets on Effect of Aflatoxin in Rats.* P. M. NEWBERNE and G. N. WOGAN, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts.

A number of investigators have reported a dietary effect on the response of animals to carcinogenic agents [R. W. Engle and D. H. Copeland, *Cancer Res.* **12**, 211 (1952); B. H. Ershoff, *Exptl. Med. Surg.* **22**, 28 (1964)]. Madhavan and Gopalan [*Arch. Pathol.* **80**, 123 (1965)] observed increased susceptibility in rats to the hepatotoxic effects of aflatoxin in short-term trials, and we have found that low-protein diet or choline deficiency cirrhosis sensitizes the liver of rats to the carcinogenic effects of aflatoxin [Newberne *et al.*, *Lab. Invest.* **15**, 962 (1966)]. Recent studies have confirmed and extended previous observations. Diets containing 9% protein resulted in a higher incidence of liver tumors in a shorter period of time than a diet containing 22% protein when both groups were intubated with 375 μg of aflatoxin B_1 (11/15 after 8 months and 7/14 after 10 months, respectively). Both early and late lesions are illustrated and discussed. (This research was supported by NIH Research Grant CA 08870 and a grant from the William S. Merrell Co.)

65. *Utilization of Newborn Mice in the Bioassay of Chemical Carcinogens.* J. L. GARGUS, O. E. PAYNTER, and W. H. REESE, JR., Hazleton Laboratories, Inc., Falls Church, Virginia.

Newborn mice have been utilized in studies to establish parameters of a meaningful bioassay procedure for screening candidate chemicals for carcinogenic potential. Mice were injected subcutaneously at 48 hours of age with the maximum tolerated dose of the chemicals [Pietra *et al.*, *Nature* **183**, 1689 (1959)]. Animals were sacrificed 6 months later, and the number of lung adenomas were counted. Results following treatment with test chemicals were compared to control values. A mean number of adenomas double the incidence observed in controls were considered a positive carcinogenic response [Shimkin *et al.*, *J. Natl. Cancer Inst.* **36**, 915-935 (1966)].

Control values following vehicle injection, saline or peanut oil, varied from 2 to 16%, and the mean number of adenomas per animal varied from 0.02 to 0.18.

The positive control chemical, urethan, at 1000–1500 mg/kg induced adenomas in 89–100% of the animals, and the mean number varied from 9.55 to 26.8. The strong carcinogen, *N*-nitrosodiethylamine, was positive, whereas mesidine dye, a relatively weak carcinogen, was negative.

Adenoma induction by nitrogen mustard, dissolved in three different solvents, water, peanut oil, and tricaprylin was investigated. At 3 months the means were 2.9, 0.9, and 0.2, respectively, for the three vehicles. The selectivity of the procedure was evaluated with various "promotor" chemicals, including croton oil (0.5 ml/kg) and dimethylsulfoxide (12.5 ml/kg), which were negative.

66. *The Role of Trace Elements in Newer Theories of Metal Carcinogenesis.* J. R. DIXON, D. B. LOWE, D. E. RICHARDS, and H. E. STOKINGER, U.S. Department of Health, Education, and Welfare, Public Health Service, Bureau of Disease Prevention and Environmental Control, Cincinnati, Ohio.

This presentation reports on the inhibition of benzpyrene hydroxylase by beryllium, copper, and other trace metals of critical biologic importance. A current theory of metal

carcinogenesis postulates that metals may act as carcinogens only indirectly by inhibiting the metabolism of polyaromatic hydrocarbons, thus prolonging the residence time of the true carcinogen in an organ.

When trace metals in the range of 0.5 μg/ml to 3 μg/ml were added to supernatant fraction of a lung or liver homogenate, it was found that an inhibition of 10–65% of benzpyrene hydroxylase activity occurred. The amount of inhibition of the enzyme was dependent on the type and concentration of the metal added to the tissue homogenate. At metal-homogenate concentrations greater than 3 μg/ml, some metals stimulated hydroxylase activity.

The action of metals on hydroxylase activity in combination, both *in vitro* and *in vivo*, is presented.

The study suggests that the body is capable of maintaining a homeostatic level of trace metals sufficient for normal enzymatic activity and metabolism of the carcinogen. When this level becomes unbalanced, there is a resulting abnormal enzymatic activity favoring carcinogenicity.

67. *Chemical Carcinogenesis in the Nonhuman Primate.* W. H. REESE, JR., M. G. KELLY, and R. W. O'GARA, Hazleton Laboratories, Inc., Falls Church, Virginia, Laboratory of Chemical Pharmacology, National Cancer Institute, and Laboratory of Pathology, National Cancer Institute, Bethesda, Maryland. (Sponsor: T. W. Tusing.)

Historically, many chemical, biological, and physical agents have readily induced tumors in rodents; therefore, studies were undertaken to (1) study the effects of such agents in the newborn nonhuman primate to determine whether the nonhuman primate, like the rodent, would be highly susceptible to carcinogensis, and (2) compare the response of monkeys and rodents to materials suspected or known to be carcinogenic to man, thereby evaluating the relative merits of rodents and primates in predicting for man.

The following chemical agents are being evaluated by the oral, subcutaneous, or dermal route of administration: 3,4,9,10-benzpyrene, 3-methylcholanthrene, urethane, *p*-dimethylaminoazobenzene, *N,N*-dimethyl-*p*-(*m*-tolylazo)aniline, fluoranylacetamine, 2,7,2-diacetamidofluorene, cupric chelate of *N*-hydroxy-2-fluoranylacetamide, *N*-isopropyl-γ-(2-methylhydrazino)-*p*-toluamide, 1-nitrosopiperidine, *N*-nitrosodiethylamine, aflatoxin, cycasin, and methylnitrosourea. In addition, several human and plant substances have also been studied.

Frequent granulomatous reactions have occurred at the intradermal or subcutaneous site of compound administration. One compound, *N*-nitrosodiethylamine, has induced hepatocellular carcinoma in 42 of 62 monkeys which had received a total dose of 1.35–57.8 g by the oral or peritoneal route. Induction time ranged between 13 and 40 months. (Supported by Contract No. PH43-66-84.)

68. *The Hepatotoxicity of the Carcinostatic Compound 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU, NSC 409962) in Rats.* G. R. THOMPSON and R. E. LARSON, Department of Pharmacology, Oregon State University, Corvallis, Oregon. (Sponsor: G. L. Plaa.)

Long-term hepatotoxic effects of single oral doses of BCNU were studied in male rats. Doses were based on a 30-day LD_{50}. Liver function was assessed at 3 days, 1, 2, 4, 9, 12, and 17 weeks. Bromosulfalein (BSP) retention, pentobarbital sleeptime (ST), and plasma levels of bilirubin (BR), pseudocholinesterase, SGPT, and alkaline phosphatase were measured. Liver samples were taken for histopathologic study (HP). Significant alterations in these parameters (delayed 4–9 weeks at the lower doses) were seen; their time of appearance and degree were dose dependent. BSP retention and ST were affected first. BR elevations appeared later. With time BSP retention approached control values as did total BR, but the pattern shifted from direct to indirect-reacting BR. HP revealed early pericholangitis and necrosis of bile ductules. Later proliferation of the ductules and biliary cirrhosis were seen. The delayed appearance of functional changes correlated with the time course for histopathologic change. (Supported by PHS Grant No. CA 08794-02.)

69. *Derivative Characteristics of Evoked Brain Responses.* C. XINTARAS, A. A. STRONG, J. V. SETZER, and M. F. SOBECKI, Behavioral Toxicology Unit, National Center for Air Pollution Control, U.S. Department of Health, Education, and Welfare, Cincinnati, Ohio. (Sponsor: E. J. Fairchild II.)

One aspect of current-evoked response research is the promise it offers of bridging the gap between neurophysiologic and behavioral events. A study was initiated to furnish additional information about the derivative characteristics (high frequency components) of evoked brain responses, particularly as the derivative may vary during different levels of consciousness. It was anticipated that this method might provide some insight into the nature of the mechanisms of CNS information processing.

EEG signals were recorded from rats with chronically implanted electrodes in response to periodic stimulation with paired flashes of light. These waveforms (fundamental and derivatives) were processed on-line by a four-channel computer, and the "time-locked" average response waveforms to the stimuli were computed and graphed on an *x-y* plotter.

High frequency activity (20–40 cps) occurring 50–80 msec after onset of stimulation was attenuated during periods of slow-wave sleep and early segments of REMS epochs. A phase-locked relationship was noted between the background EEG during REMS (6–8 cps) and the amplitude and temporal characteristics of components III and IV of the fundamental evoked response. During behavioral arousal and late segments of REMS, the second derivative showed an increase in high frequency activity time-locked to the incidence of photic stimulation.

The findings suggest that the derivative characteristics of evoked brain responses may be related to a central mechanism for processing incoming sensory messages.

70. *The Use of Squirrel Monkeys in Behavioral Studies for the Evaluation of Drug Effects.* T. R. A. DAVIS and C. J. KENSLER, Life Sciences Division, Arthur D. Little, Inc., Cambridge, Massachusetts.

Five operant behavior schedules have been used to study the psychopharmacologic effects of compounds in squirrel monkeys. Two of these schedules, Sidman avoidance and multiple fixed interval/fixed ratio schedules, are standard and well known. Two are physical work schedules with one using positive reinforcement and the other negative reinforcement. The positive reinforcement work schedule consists of a pole with a lever at the bottom and a lever at the top approximately 2.5 meters apart. In order to obtain food the animal climbs up and down the pole and presses the levers on a variable interval schedule. In the negative reinforcement work schedule the animal operates two levers 2.5 meters apart across a grid to which shock impulses can be delivered. A warning buzzer sounds when a shock is about to be given. The interval between warning sounds can be varied and the animals paced by sound only. The rate of work can be varied both by tilting the operant chamber to any desired angle and by varying the interval between sounds. The fifth schedule is an adaptation of the human steadiness test. The animal is required to operate a lever passing through a 2 cm hole and keeping it free of the sides of the hole, thereby accumulating time toward a positive reinforcement. Studies on nicotine, caffeine, amphetamine, a tetrahydrocannabinol derivative, pentobarbital, and alcohol show that the effect of each compound is highly dependent upon the type of schedule used and that, in squirrel monkeys, the doses required to produce a psychopharmacologic effect in most instances were similar to those commonly used by man.

71. *Relationships between the Activity and Structure of Methylenedioxyphenyl and Related Compounds: Effects on Hexobarbital Sleeping and Zoxazolamine Paralysis in Mice.* K. FUJII, H. JAFFE, and S. S. EPSTEIN, Laboratories of Carcinogenesis and Toxicology, Children's Cancer Research Foundation, Inc., Boston, Massachusetts. (Sponsor: J. H. Weisburger.)

Methylenedioxyphenyl (MDO) compounds, viz., piperonyl butoxide (PB), potentiate the action of certain insecticides by inhibiting detoxifying microsomal enzymes. PB also produces

similar effects on mammalian microsomal liver enzymes. Combined administration of PB and Freons® to neonatal mice produces synergistic acute toxicity and hepatocarcinogenicity [Epstein *et al.*, *Nature* **214**, 526 (1967)]. Acute toxicity was also produced by combinations of PB with benzo[*a*]pyrene and with griseofulvin (Epstein *et al.*, *Toxicol. Appl. Pharmacol.* in press).

In an attempt to elucidate the mechanism of action of PB, a structure activity study was undertaken on a series of 56 MDO and related compounds, using prolongation of hexobarbital sleeping and zoxazolamine paralysis times as measures of inhibitory effects. The compounds were administered intraperitoneally to adult male mice at 4-fold concentrations from 2.5 to 640 mg/kg in conjunction with 75 mg/kg hexobarbital or 80 mg/kg zoxazolamine.

Approximately one-third of the compounds showed moderate to high activity in both assays; activity was dose related. Results of both assays were generally parallel.

The following structure-activity correlations were found: (1) Inhibitory activity is not specifically associated with the MDO-ring, in view of the high activity of allylbenzene, *p*-allylanisole, methyl eugenol, and methyl isoeugenol. (2) MDO compounds were, however, more active than corresponding 3-hydroxy-4-methoxy open-ring analogs. (3) Allyl derivatives of both open-ring and MDO compounds were more active than corresponding propenyl derivatives. (4) Methoxylation of the phenyl ring markedly enhances the activity of open-ring compounds, but reduces activity of the MDO structure. (Supported by National Cancer Institute Grant C-6516.)

72. *Toxicologic Studies on Repeated Intravenous Dosages of N,N-Bis-(2-methanesulfonoxyethyl)-p-nitrosoaniline (NAMS) in Dogs and Monkeys.* P. E. PALM, M. S. NICK, E. P. DENINE, and C. J. KENSLER, Life Sciences Division, Arthur D. Little, Inc., Cambridge, Massachusetts.

The compound *N,N*-bis-(2-methanesulfonoxyethyl)-*p*-nitrosoaniline (NAMS) has strong antitumor activity against Dunning leukemia and Walker 256 carcinosarcoma in mice and is of interest as a possible cancer chemotherapeutic agent.

Data from repeated-dose intravenous toxicity studies of NAMS administered in 10% polyethylene glycol in dogs and monkeys indicate that dosages of 0.063 mg/kg × 14 produce only occasional transitory changes. Dosages $\geq$ 0.125 mg/kg × 14 frequently resulted in body weight loss, leukopenia, hyperglycemia, elevated serum glutamic-pyruvic and glutamic-oxaloacetic-transaminase, blood urea nitrogen, and lactate dehydrogenase values. A few of the test animals at the 0.25 and 0.50 mg/kg dosage levels developed a clinical picture suggestive of severe congestive heart failure. The course of the syndrome in these animals was generally characterized by the development of hypotension, rales, pleural effusion, ECG abnormalities, and death by cardiac arrest. Subsequent microscopic examination of tissues from dogs and monkeys which received the higher dosages revealed hypoplastic bone marrow and lymphoid tissues, pulmonary congestion and edema, congestion, and focal degeneration of kidney glomeruli and convoluted tubules, pericentral fatty infiltration of the liver, and occasional cardiac lesions including myocardial hemorrhage and infarcation and ventricular adenosine triphosphatase inhibition observed histochemically. (This study was supported by Contract No. PH-43-65-61 with the Cancer Chemotherapy National Service Center, National Cancer Institute, Public Health Service.)

73. *The Toxicity of Bromacil, A Substituted Uracil.* H. SHERMAN, Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, Newark, Delaware.

Bromacil [5-bromo-3-sec-butyl-6-methyluracil] is used as an industrial herbicide and, at lower application rates, for weed control in citrus and pineapple.

Bromacil has low acute oral toxicity; its LD_{50} for rats is 5200 mg/kg body weight, for dogs > 5000 mg/kg. When administered to rats at doses of 670 mg/kg/day for 10 days, it produced transient histologic changes in the liver (focal cell hypertrophy and hyperplasia).

Bromacil fed to pregnant New Zealand white rabbits from day 8 to day 16 of gestation at dietary levels of 50 and 250 ppm was not teratogenic. A three-generation, six-litter reproduction study with rats fed a dietary level of 250 ppm was without adverse effects upon reproduction and lactation performance; no pathologic changes were observed in weanling pups of the F_{3B} generation.

Male and female rats have been fed nutritionally complete diets containing 50, 250, and 1250 ppm bromacil for two years. Except for a lower rate of weight gain, a slightly decreased food intake, and a slightly lower food efficiency among the female rats that received 1250 ppm of bromacil, there was no nutritional, clinical, hematologic, urinary, or biochemical evidence of toxicity in the test groups; there was, however, a suggestion of slight hyperplasia in the thyroids of animals that received the highest dietary level.

Male and female beagle dogs, 1–2 years of age, fed nutritionally complete diets containing 50, 250, and 1250 ppm bromacil for two years, showed no evidence of toxicity.

74. *Safety Evaluation of 2-Aminobutane.* H. M. WORTH and R. C. ANDERSON, The Lilly Toxicology Laboratories, Eli Lilly and Company, Greenfield, Indiana.

Two-aminobutane (2-AB) controls postharvest rots of fruits and vegetables. Our studies have shown the compound is safe for this use.

Two-AB is a mono-alkyl substitution product of ammonia; like ammonia, its toxicity and irritating characteristic is lessened as the pH is reduced.

Single-dose toxicity studies have been made in a number of species with the base and several salts. Feeding and multiple dose experiments with rats and dogs were made using the acetate at pH 8 to avoid most of the acutely irritating effects.

Subacute and chronic feeding studies incorporating relatively high levels of 2-AB as the acetate in the mash fed to rats produced no evidence of effect other than growth retardation. Reproductive performance of treated rats through 2 litters from F_3 parents equaled or bettered the controls. Dogs were given daily oral high doses of 2-AB as the acetate without the appearance of any significant changes in hematology, chemistry, or histopathology. Metabolites of the fungistat have been isolated from the urine of the treated dogs. The pharmacologic properties noted were those of a weak sympathomimetic amine, and included only slight mydriasis seen in the dogs.

Infrequent occurrences of untoward effects in workers handling 2-AB formulations indicated there were relatively few hazards associated with use of the material.

75. *Toxicology of d-Propoxyphene.* J. L. EMMERSON, W. R. GIBSON, P. N. HARRIS, and R. C. ANDERSON, The Lilly Toxicology Laboratories, Eli Lilly and Company, Greenfield, Indiana.

Two salts of *d*-propoxyphene (P) were used in toxicologic evaluation in animals: P HCl and P 2-naphthalene sulfonate (napsylate). Although the latter was practically insoluble in water, in contrast to the freely soluble HCl salt, the two forms differed toxicologically only in acute toxicity. In single oral doses P napsylate was less toxic than P HCl in 4 species (e.g., LD_{50} mouse, P HCl 288 mg-0.76 m*M*/kg, P napsylate 941 mg-1.71 m*M*/kg). In subacute studies in rats and dogs, the effects produced by daily administration of equimolar doses of the two P salts were indistinguishable.

Large daily doses of P (0.345% P HCl or 0.5% P napsylate in diet for one year) were tolerated by rats with no apparent effects other than terminal liver hypertrophy and a reduction in growth rate. In the dog, unequivocal signs of toxicity were produced within 90 days by oral doses of 30 or 40 mg/kg/day P HCl and equivalent doses of P napsylate. The following effects were dose-related in incidence or degree: loss of body weight, elevation of serum alkaline phosphatase activity, increased liver weight, and slight fatty metamorphosis of the liver. Lower doses were not toxic in the dog.

Both salts of P were tested in reproductive-teratogenic studies. Reduced fertility in rats and toxicity to the newborn were encountered, but only at doses that were toxic to the dam. In neither rats nor rabbits were there any signs of drug-induced teratogenicity.

76. *Safety Evaluation of Botran® (2,6-dichloro-4-nitroaniline) in Laboratory Animals.* C. D. JOHNSTON, G. WOODARD, and M. T. I. CRONIN, Woodard Research Corporation, Herndon, Virginia, and Yale University, New Haven, Connecticut.

Albino rats and purebred beagle dogs of both sexes were given Botran in their respective diets for 104 weeks. Dietary levels were 0, 20, 100, and 3000 ppm for both species.

Survival at 104 weeks (adjusted for an interim sacrifice) in the Botran-fed rats was 67–80%, 50–67%, 43–57%, and 52–70% in the high, middle, low, and control groups, respectively. Most deaths occurred after 70 weeks. Mean body weights of the high-level rats were 72–75% those of respective controls at 104 weeks; the other groups weighed at least 95% as much as controls. Increased organ-to-body weight ratios were seen (3000 ppm only) for liver, kidney, testis, and thyroid. Histopathologic findings were confined to liver changes at 3000 ppm; incidences and types of tumors were comparable among all groups.

One dog receiving Botran-3000 ppm died (74 weeks) on study; all other survived until scheduled sacrifice (including interim). Body weight gains and hemograms were comparable among dogs in all groups. Clinical chemistry values were normal, except for some increases in serum transaminases among a few dogs at 3000 ppm (including the one that died).

Changes seen, chiefly in dogs at 3000 ppm, included yellowing of skin and mucosa, and histologic liver changes. Eyes, examined both with H & E stain and with Oil Red O, showed no histopathology.

A three-generation rat reproduction study was also conducted (diet level, 100 ppm). To judge by number of pups per litter, number of litters per group, stillbirth rates, mean birth and weaning weights and weanling survival, Botran had no adverse effects on rat reproduction.

77. *Two-Year Feeding Study in Rats with Linear Alkylbenzene Sulfonate (LAS).* E. V. BUEHLER, E. A. NEWMANN, and W. R. KING, The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio.

Groups of 50 male and 50 female rats were fed the test material for two years at levels of 0.5%, 0.1%, 0.02%, and 0% of their total diets. Food and water were offered *ad libitum.* Values for food consumption, body weight gain, and feed efficiency were recorded weekly for 12 weeks, and monthly thereafter. Necropsies were performed after the 8th and 16th month in which 5 males and 5 females were examined grossly and major tissues were obtained for microscopic evaluation. All animals that died during the study were autopsied, when possible, and all surviving animals were sacrificed at 24 months. All tumors observed during the study were noted, examined and identified to determine any carcinogenic effect. Hematologic values were determined from tail-vein blood at 4, 8, 11, 16, 21, and 24 months.

The above diets were used also in a three-generation study to determine any effects of LAS on fertility, reproduction, gestation, parturition, and lactation.

LAS had no significant biologic effect on the rats in either study.

78. *Toxicity and Metabolism of 2-Hydroxy-4-n-octoxybenzophenone (CYASORB® UV 531 Light Absorber).* Y. M. PATEL and G. J. LEVINSKAS, Environmental Health Laboratory. American Cyanamid Company, Princeton, New Jersey.

UV 531 is a stabilizer for plastics. Toxicity and metabolism studies were conducted to evaluate its safety as a component of plastics in contact with foods.

On the basis of acute toxicity and irritation tests, UV 531 is regarded as a relatively harmless material.

Albino rats of both sexes tolerated dietary levels of 18,000 ppm of UV 531 for 90 days with no adverse effect except for a significant increase ($P < 0.05$) in mean kidney weight for males. Rats fed 6000 ppm, or less, of the product did not differ from their controls.

Diets containing 18,000 ppm of UV 531 were poorly accepted by beagles. A dietary level of 6000 ppm, or less, had no adverse effect on dogs during a 90-day trial period.

Preliminary metabolic studies with male rats indicated that most of the orally administered UV 531 could be recovered unchanged from the feces. The small portion that was absorbed was excreted as a glucuronide in the urine. Consequently, groups of male rats were fed diets containing 12,500 ppm or 50,000 ppm of UV 531 for periods up to 30 days. Daily records were kept of UV 531 intake and urine and feces were collected separately each day.

The metabolic pattern was the same at each dietary level. The mean daily recovery of UV 531 from the feces was 90%, and the mean daily excretion of UV 531, conjugated as a glucuronide, was 10%. Since most of the ingested UV 531 was recovered from the feces, and since only a small portion of it was absorbed, conjugated, and excreted in the urine, UV 531 appears to be quite inert. The inertness to absorption may be due to the presence of the hydroxyl and octoxy groups in the aromatic ring.

79. *Toxicological Studies with 3α-Dimethyltyrosine Methylester-HCl, an Inhibitor of Tyrosine Hydroxylase.* E. HANSSON, G. MAGNUSSON, and H. CORRODI, Toxicology Laboratories, AB Astra, Sodertalje, Sweden, and Research Laboratories, AB Hassle, Gothenburg, Sweden.

3α-Dimethyltyrosine methylester-HCl is a potent inhibitor of tyrosine hydroxylase *in vivo*. The 3α-dimethyltyrosine is not converted to displacing amines, and might be a more convenient tool for studying the pure functional significance of the inhibition of tyrosine hydroxylase than the related compound, α-methyltyrosine.

During the subacute toxicologic investigation of this compound, renal lesions with formation of stones in the urinary tract was observed in rats. In these experiments the 3-α-dimethyltyrosine methylester-HCl was given to 6 groups of rats by gastric tube with levels of 200, 500, and 1000 mg/kg orally, and 25–50, 100–200, and 300–500 mg/kg intraperitoneally every day during 4 weeks. An increase in the urea nitrogen of the blood serum was observed in the groups receiving 500 mg/kg and 1000 mg/kg orally, and 300–500 mg/kg ip. The kidneys of these animals were enlarged and had yellow spots. The renal tubules contained crystals which sometimes occluded the lumen. The tubular epithelium had regressive changes. Inflammatory cells were observed around the crystals and in the interstitial tissue. The renal pelvis, ureters, and urinary bladder contained calculi.

Chemical investigation showed that the calculi consisted of 3α-dimethyltyrosine. The 3α-dimethyltyrosine methylester-HCl is hydrolyzed in the body to 3α-dimethyltyrosine which is excreted via the kidney. Due to its low water solubility at physiological pH, crystals are precipitated in the kidney, ureters, and urinary bladder.

80. *Effect of NaF and NF_3O on Growth and Thyroid Function in the Rat.* E. W. VAN STEE, Toxicology Branch, Toxic Hazards Division, 6570th Aerospace Medical Research Laboratories (MRBTT), Wright-Patterson Air Force Base, Ohio. (Sponsor: K. C. Back.)

This study determined the effects on thyroid function after exposure to a strong oxidizing agent, NF_3O. A total of 128 rats (Sprague-Dawley strain) were used. Surgical thyroidectomy was performed on 32 rats of each sex at 28 days of age, with a similar group left intact. The rats were divided into 16 treatment groups containing 8 rats of each sex. These groups were given daily equimolar doses ip for 60 days of either 10.0 ml/kg 5% dextrose (control), 0.5 μg/rat thyroxine, 65 mg/kg KI, 17 mg/kg NaF, 12 mg/kg NF_3O, 0.5 μg thyroxine plus 17 mg/kg NaF, or 0.5 μg thyroxine plus 12 mg/kg NF_3O. Exposure to NF_3O caused significant growth retardation in the absence, but not in the presence, of the thyroid gland. NF_3O caused a slight reduction of the ^{131}I uptake, but the effect was much less pronounced than that caused by an equivalent dose of iodide. The long exposure caused no effect on adrenal weight. The presence of the thyroid gland (or thyroxine injection in the thyroidectomized animal) has a sparing effect on NF_3O-induced growth retardation.

The compound has no appreciable effect on thyroid function even though the thyroid preferentially holds significantly large amounts of either fluorine or NF_3O after exposure.

81. *Comparison of Mortality Rates of Some Barbiturates in Normal and Triiodothyronine-Induced Hyperthyroid Rats.* R. D. HARBISON and B. A. BECKER, Department of Pharmacology, University of Iowa, Iowa City, Iowa.

Many body processes, including drug metabolism may be markedly altered by a hyperthyroid condition. Certain metabolic pathways, e.g. metabolism of zoxazolamine, are reported to be accelerated after treatment with thyroxine; however, thyroxine treatment decreased the metabolism of hexobarbital. The purpose of this investigation is to study the mortality of selected barbiturates under hyperthyroidism induced by L-3,3',5-triiodothyronine (T_3). Groups of 20 male Sprague-Dawley rats, weighing 180–220 g were pretreated with T_3, 2 mg/kg, ip, for 5 days. The barbiturates were injected intraperitoneally on day 6. The number of dead rats were recorded 24 hours after the barbiturate administration. The mortality rate was significantly ($P < 0.05$) increased in the T_3-pretreated rats as compared to those pretreated with the vehicle:

MORTALITY (% ± SE) OF SOME BARBITURATES IN T_3 RATS

Drug	Dose (mg/kg)	T_3 Treated	Control
Hexobarbital	305	95 ± 8	15 ± 5
Thiopental	88	90 ± 9	20 ± 7
Amobarbital	185	75 ± 9	20 ± 9
Pentobarbital	106	90 ± 7	35 ± 10
Phenobarbital	218	65 ± 10	20 ± 9

T_3 pretreatment, like thyroxine pretreatment, resulted in a lengthened hexobarbital sleeping time: 26 ± 3 minutes in the vehicle controls to 71 ± 6 minutes in the T_3-pretreated animals. The increase in mortality rates may be associated with altered microsomal metabolism of the barbiturates.

82. *The Effect of Flavins on Purified and Microsomal Azoreductase.* L. SHARGEL and P. MAZEL, Department of Pharmacology, The George Washington University School of Medicine, Washington, D.C.

Flavins affect the *in vitro* metabolism of neoprontosil by stimulating the liver microsomal as well as the purified azoreductase. The effect of flavins on azoreductase has been utilized to further elucidate the pathways of the reduction of azo compounds by liver microsomal enzymes.

The effect of phenobarbital on the *in vitro* activity of microsomal azoreductase from riboflavin-deficient rats was investigated. Microsomes from riboflavin-deficient animals displayed a decrease in azoreductase (77%), NADPH cytochrome *c* reductase (35%), flavin content (50%), and protein (21%). However, microsomal demethylation of aminopyrine was unaffected by flavin deficiency. Phenobarbital pretreatment (80 mg/kg) ip daily for 3 days of deficient animals increased microsomal azoreductase (212%), microsomal protein (32%), NADPH cytochrome *c* reductase (45%), and cytochrome P-450 (183%). Moreover, flavin content was unaltered. Pretreatment of deficient animals with riboflavin (10 mg/kg) ip daily for 3 days increased azoreductase (45%), NADPH cytochrome *c* reductase (18%) without affecting aminopyrine demethylase, whereas cytochrome P-450 was slightly reduced. When deficient animals were pretreated with both riboflavin and phenobarbital, increases were found in azoreductase (107%), NADPH cytochrome *c* reductase (94%), aminopyrine demethylase (192%), and cytochrome P-450 (108%).

CO decreased microsomal azoreductase by 35% in normal rats and in deficient animals by 67%, indicating that most of the microsomal azoreductase activity in deficient rats involves a

CO-sensitive pathway. *In vitro* addition of FMN stimulated azoreductase activity in all groups (N_2 and CO) to a greater extent than that seen in nondeficient animals. Interpretation of the data indicates that riboflavin deficiency results in a decrease of a microsomal CO-insensitive azoreductase pathway, whereas phenobarbital pretreatment stimulates a CO-sensitive azoreductase pathway in both controls and riboflavin-deficient animals which correlates directly with the increased microsomal cytochrome P-450. (Supported by grants 5-T1-GM-26 and GM-13749 of USPHS.)

83. *Stress-Inducible Liver Enzymes in Rats Given Hepatotoxic Chemicals.* S. D. MURPHY and S. MALLEY, Department of Physiology, Harvard University School of Public Health, Boston, Massachusetts.

We have previously reported that toxic doses of irritants, organophosphate insecticides, and acute cold exposure increase the activity of the glucocorticoid-inducible rat liver enzymes: tyrosine-α-ketoglutarate transaminase (TKT), tryptophan pyrrolase (TP), and alkaline phosphatase (LAP). It was hypothesized that hepatotoxic chemicals might prevent these enzyme inductions. Initial experiments revealed, however, that after single oral doses of allyl alcohol or carbon tetrachloride (CCl_4) marked increases in liver AP and TKT occurred in fasted male rats in spite of concomitant liver damage as evidenced by increased serum alkaline phosphatase (SAP) and serum alanine-α-ketoglutarate transaminase (SAKT) activities and gross and histologic changes. Twenty hours after 1.0 ml/kg of CCl_4 total liver TKT and LAP activities were 3 to 4 times control values, liver TP decreased slightly, SAP was increased about 2-fold, and SAKT increased by 10-fold. Time-response studies showed that plasma corticosterone levels increased to a maximum at 60 minutes after 1 ml/kg CCl_4 and then declined. Increased liver TKT was detected at 2 hours after CCl_4, before SAKT increased. Increases in liver AP activities were delayed for several hours but, unlike liver TKT, persisted for several days. Adrenalectomy before CCl_4 prevented the increased liver TKT, reduced the elevation of SAKT and SAP, but did not affect the increase in LAP. Rats given 1 ml/kg CCl_4 twice weekly for 4 weeks and sacrificed 3 days after the last dose had elevated SAP and SAKT but about normal liver TKT and TP activities. Cold stress or exogenous corticosterone induced liver TKT and TP in these animals as well as in controls. The results indicate that increased liver AP activity after CCl_4 is adrenal independent, and that hepatotoxic doses of CCl_4 do not prevent induction of liver TKT or TP activities by exogenous or endogenous glucocorticoids. (Supported by Research Grants UI 00424 and ES 00002 from the U.S.P.H.S.)

84. *Effect of Carbon Tetrachloride on the Metabolism, Storage, and Excretion of Sulfobromophthalein.* C. D. KLAASSEN and G. L. PLAA, Oakdale Toxicology Center, Department of Pharmacology, College of Medicine, University of Iowa, Iowa City, Iowa.

The general abnormal hepatic functional effects produced by CCl_4 are demonstrable by the plasma retention of sulfobromophthalein (BSP). Since BSP undergoes storage, metabolism, and excretion by the liver, these parameters were examined to determine which processes are altered by CCl_4.

Significant plasma dye retention (4–32 minutes after 60 mg/kg, iv, BSP) was observed 24 hours after CCl_4 administration (1.0 ml/kg, ip). No change in hepatic storage was demonstrated (0.64 *vs* 0.58 mg/mg%/kg; controls *vs* CCl_4, respectively), but significant decreases in *in vitro* metabolism (13.2 *vs* 8.5 mg BSP/5 min/g liver), and in biliary transport maximum (1.09 *vs* 0.68 mg/min/mg) were observed.

These parameters were also measured using an analog of BSP which is not metabolized, phenol-3,6-dibromophthalein disulfonate (DBSP). CCl_4 did delay the plasma disappearance of DBSP (8–32 minutes after 49 mg/kg, iv, DBSP), did not affect its hepatic storage (0.58 *vs* 0.58 mg/mg%/kg), but did depress its biliary transport maximum (0.80 *vs* 0.59 mg/min/kg). During infusions of BSP and DBSP at rates below the maximal biliary excretory rate, decreased dye excretion was observed in the CCl_4-treated rats.

It appears that CCl_4 exerts an important effect on hepatic excretory function, and perhaps that this effect is the one which contributes the most toward plasma BSP retention. (Supported by USPHS Grant GM-12675.)

85. *Stimulation of Hepatic Processing Enzymes and Liver Growth by a Series of Pyridyl Carbinol Fungicides.* D. G. HOFFMAN, H. M. WORTH, and R. C. ANDERSON, The Lilly Toxicology Laboratories, Eli Lilly and Company, Greenfield, Indiana.

Numerous reports concerning the stimulation of hepatic processing enzymes by drugs and other chemicals have appeared during the past decade, but only recently have attempts been made to quantify the inducing properties of these compounds. The no-effect levels (NEL) for stimulation of hepatic *p*-nitroanisole (*p*-NA) *O*-demethylation, and increased liver weight have been determined for a series of pyridyl carbinol fungicides after administration via the diet to weanling male rats for 14 days. Bis-(4-chlorophenyl)-3-pyridyl methanol was the most potent of the compounds tested. The NEL for stimulation of p-NA metabolism was 26.8 ± 3.3 ppm and for stimulation of liver growth 36.8 ± 7.6 ppm. The least potent compound was biscyclohexyl-3-pyridyl methanol with a NEL for stimulation of p-NA metabolism of 499 ± 74 ppm. Although measurements of the NEL for stimulation of liver growth lack precision, there was a close agreement between these values and the NEL for stimulation of metabolism for each compound.

86. *The Effects of Chronic Nitroglycol Poisoning on Soluble Monoamine Oxidase (MAO) and Urinary Vanillyl Mandelic Acid (VMA) in Rabbits.* F. C. PHIPPS and J. T. MOUNTAIN, U.S. Department of Health, Education, and Welfare, Public Health Service, Bureau of Disease Prevention and Environmental Control, Cincinnati, Ohio.

The study of the toxicity of ethylene glycol dinitrate (EGDN) has to a large degree been prompted by interest in the "Monday morning fatalities" experienced by workers in the dynamite industry. Since this has been observed to be a chronic effect, the experiment was set up accordingly.

Male rabbits were divided into two groups. Group A was injected (sc) daily with EGDN (25 mg/kg) Monday through Friday for a period of 6 weeks. Group B was sham injected.

Urine was collected from Sunday AM to Monday AM, and VMA excretion was determined. MAO and methemoglobin were determined in arterial blood Monday and Friday, respectively.

Changes in these biochemical and hematologic parameters will be discussed in relation to newer theories of the mechanism of nitroglycol poisoning.

87. *The Red Cell Fragility Test As A Measure of Local Tissue Toxicity.* J. O. HOPPE and L. P. DUPREY, Pharmacology Section, Sterling-Winthrop Research Institute, Rensselaer, New York.

The red cell fragility test offers a simple, rapid, *in vitro* method for the estimation of local tissue toxicity. A suspension of washed rabbit red cells was added to graded concentrations of test drug in distilled water or distilled water to which sodium chloride was added to approximate isotonicity. Percent hemolysis was determined colorimetrically and compared with the same volume of red cells added to distilled water as a positive control representing complete hemolysis. The highest concentration of drug tested, expressed in percent, which produced less than 25% hemolysis was defined as the threshold hemolytic concentration (THC). Drugs studied included phenol, quinine HCl, benzalkonium chloride, PEG 600 MO, tyloxapol, chlorpromazine HCl, propoxycaine HCl, dibucaine HCl, tetracaine HCl, mepivacaine HCl, morphine sulfate, meperidine HCl, thenyldiamine, and histamine. Results were compared with an *in vivo* estimate of irritancy by the threshold irritant concentration (TIC) [*J. Am. Pharm.*

Assoc. Sci. Ed. **39**, 147 (1950)]. The ratio TIC:THC, ranged from 1 to 4 for 11 of the 14 compounds. Irritancy by the THC exceeded the estimate by the TIC by a ratio of 10 with PEG 600 MO and 16 with chlorpromazine HCl and underestimated with histamine by a ratio of 0.13. It was concluded that the red cell fragility test can be used as a preliminary estimate of irritancy. The results, however, should be compared with an *in vivo* test for irritancy in the final estimation of local tissue toxicity.

88. *Barbiturate–Salicylate Interaction in the Rat.* B. B. COLDWELL and G. SOLOMONRAJ, Pathology and Toxicology Section, Research Laboratories, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Ontario, Canada. (Sponsor: G. S. Wiberg.)

Previously, we reported that acetylsalicylic acid (ASA) produced a dose-dependent increase in the sleeping time of rats subsequently administered barbiturates [*Proc. Can. Federation Biol. Soc.* **10**, 50 (1967)]. Our continuing study of the interaction of these drugs has revealed significant enhancement of free and total salicylic acid (FSA and TSA) levels in the plasma and brain of rats given ASA (orally) with pentobarbital (ip) compared to those receiving ASA alone. Sodium salicylate (orally or ip) reacted similarly. The rate of detoxification of pentobarbital was not altered by ASA. Ethanol, urethane, or codeine did not affect plasma salicylate levels.

The plasma TSA:TFSA ratio was lower in the barbiturate-treated rats than in those given ASA alone. Further, urinary excretion of TSA, FSA and salicyluric acid by the former rats significantly exceeded that of the latter group during the period from 3 to 7 hours after barbiturate injection. Salicyl glucuronide excretion was higher in the barbiturate-treated animals after 7 hours, becoming significant after 24 hours. The implications of these finding is discussed.

89. *Development of Tolerance to Pentobarbital and Thiopental in Albino Rats.* J. SINGH, Division of Biological Science, College of Pharmacy, Xavier University, New Orleans, Louisiana. (Sponsor: J. Fujimoto.)

Tolerance to barbiturates is expressed as tolerance index (TI), and this is computed as follows: hypnotic effect of the first dose is divided by the hypnotic effect of the second dose. When the TI is unity or less than unity, it signifies no tolerance to the drug in question, however, a TI significantly greater than unity signifies tolerance to the drug. TI to pentobarbital is close to unity at 2 hours after the first injection. However, TI becomes significantly greater than unity at 24 hours after the first injection of pentobarbital. TI to thiopental is always less than unity at 2 and 24 hours. In contrast to normal animals the TI to thiopental and pentobarbital can be increased by feeding the animals a 3% thiouracil diet. In animals, with about 70% of the liver removed, TI to pentobarbital depends on regeneration of the liver cells.

90. *Studies of Hydrazine Hepatotoxicity.* W. L. BANKS, JR., Department of Biochemistry, Medical College of Virginia, Richmond, Virginia. (Sponsor: J. F. Borzelleca.)

A single subconvulsive dose of neutralized hydrazine (40 mg per kilogram body weight) produces increased contents of liver DNA, RNA, and protein in rats after 24 hours. The uptake of leucine-^{14}C into liver protein was increased following hydrazine treatment *in vivo*. These data taken together suggest that liver protein biosynthesis was stimulated by hydrazine treatment. The free amino acid patterns of the skeletal muscle and liver suggest that the amino acids required for the increased liver protein biosynthesis might have been made available through skeletal muscle protein catabolism. Preliminary *in vitro* studies indicate that liver DNA biosynthesis may be stimulated by hydrazine treatment. Supported by USPHS Grant MH 12602.

91. *Morphine and Etorphine (M99) on the Oxygen Consumption of Mice.* M. W. WILLIAMS, C. S. WILLIAMS, and G. R. DEWITT, Veterans Administration Hospital, Tucson, Arizona.

The investigational drug etorphine (M99) 6:14-endoetheno-12-(2-hydroxy-2-pentyl-tetrahydro-oriparine, a thebane derivative, is related chemically to morphine and, like morphine is considered to be a narcotic.

Classically, morphine is understood to induce respiratory depression at a medullary level. The studies reported here involve the measurement of the oxygen consumption of Swiss-Webster mice given both drugs at several levels. A control run was followed by intraperitoneal injection of the drug, and the oxygen consumption was measured periodically for 2 hours and 45 minutes.

Dosages of M99 from 39 μg/kg upward resulted in narcosis. This dose was approximately one fifteen-hundredth of the LD_{50}. Dosages up to 60% of the LD_{50} of morphine failed to produce similar inactivity.

The oxygen consumption of mice dosed with 1.5 mg of morphine (56–88 mg/kg) sulfate failed to show significant change in O_2 uptake at 30 and 60 minutes but did show significant depression at 105 and 165 minutes, $P < 0.05$ and $P < 0.02$, respectively.

The oxygen consumption of mice on M99 at a total dose of 25 μg, i.e., 890–1250 μg/kg, showed significant depression at 30 minutes and again at 165 minutes with no depression at 60 or 105 minutes.

Mice given a total ip dose of M99 of 50 μg, i.e., 1900–2600 μg/kg, showed significant depressions at all times except 105 minutes.

92. *Turpentine-Induced Histological Changes in Isolated Rat and Guinea Pig Lungs.* F. SPERLING and W. L. MARCUS, Department of Pharmacology, College of Medicine, Howard University, Washington, D.C.

Perfused excised lungs of rats and guinea pigs were placed in the Delaunois isolated lung apparatus [*Arch. Intern. Pharmacodyn. Therap.* **148**, 598 (1964)]. The lungs breathed turpentine vapor or aerosol; or had the substance intratracheally instilled; or had it injected into the pulmonary artery. Turpentine introduced by airway (vapor, aerosol, or intratracheal) in guinea pigs damaged large scattered areas of alveoli and reduced the height of the convolutions of the bronchiolar epithelium. Intraarterial injection induced widespread and extensive alveolar damage but did not affect the bronchiolar epithelium. In rats, alveolar damage resulting from vapor inhalation was not apparent. Aerosol inhalation resulted in areas of alveolar damage. Intratracheal instillation induced extensive alveolar damage. The bronchiolar epithelium was unaffected by vapor, showed questionable effects after aerosol, and showed enlargement of the lining convolutions after intratracheal instillation. Intraarterial injection induced extensive alveolar damage but had no effect on the bronchiolar epithelium. The histologic effects on the two portions of the respiratory system, of the two species, correlated with the polygraph records of respiration amplitude and/or apneusis. The degree of histologic damage was greater in the guinea pig than in the rat. The absence of histologic effect in vapor-exposed rat lungs and the damage in both species after vascular administration correlated with *in vivo* observations previously reported [Sperling, Marcus, and Collins, *Toxicol. Appl. Pharmacol.* **10**, 8 (1967)].

93. *Influence of Age on the Reserpine-Induced Gastric Lesions in Rats.* J. REILLY, P. CASSIDY, J. S. WATTS, L. M. LUSKY and S. KROP, Division of Pharmacology, Bureau of Science, Food and Drug Administration, Washington, D.C.

Lusky *et al.* previously reported [*Federation Proc.* **24**, 715 (1965)] that the incidence and degree of hemorrhagic gastric lesions in reserpine-treated rats was increased by electrostress. The present experiments show that reserpine-induced gastric lesions in Osborne-Mendel rats are also influenced by age, sex, and cold stress. Male rats (6–7 weeks old) receiving reserpine show higher lesion scores than female rats of a comparable age. Lesion scores in both males and females rise with dose (1–3 mg/kg/im). Also the score rises with time after administration

of reserpine, i.e., it is higher after 7 hours than after 4 hours. Weanling rats are less uniform in their response to reserpine-induced gastric lesions and do not show the sex difference observed in older rats. Both reserpine-treated and control weanling rats have higher mortality rates when subjected to cold stress than older animals; also control weanling rats may survive for 4 hours at 4°C whereas reserpine-treated weanlings (1–3 mg/kg/im) do not survive these conditions. The high mortality rate of the reserpine-treated animals subjected to cold stress limits the use of weanlings in studying cold enhancement of reserpine-induced gastric lesions.

94. *Preliminary Observations on the Histochemical Changes of the Gastrointestinal Tract of Monkeys Due to Various Treatments.* R. M. DIENER and B. M. SPARANO, Division of Toxicology and Pathology, Ciba Pharmaceutical Company, Summit, New Jersey.

Information regarding the mucosal enzymes of the gastrointestinal tract is scanty, especially as far as subhuman primates are concerned. The effects of various environmental, bacterial, and drug treatments have not been investigated in any detail.

Rhesus monkeys (*Macaca mulatta*) were used in the present study in an effort to determine the effects of starvation, bacterial enteritis, oral tetracycline medication, and castor oil purgation on the gastrointestinal mucosa. Histochemical techniques were employed to demonstrate aberrations in acid and alkaline phosphatase activity and variations in the mucopolysaccharide and fat content of cell lining the gastrointestinal tract. The meaning of these changes, as well as their correlation to normal findings, is discussed in detail.

95. *Nephrotoxic Effects of Monomethylhydrazine in Monkeys.* M. E. GEORGE, W. MAUTNER, and K. C. BACK, Toxicology Branch, Toxic Hazards Division, 6570th Aerospace Medical Research Laboratories (MRBTT), Wright-Patterson Air Force Base, Ohio, and Mt. Sinai Hospital, New York, New York.

With the increased emphasis on the use of monomethylhydrazine (MMH) as a propellant, it has become necessary to determine the toxic effects of this compound. Previous studies indicated there was a nephrotoxic effect in dogs after MMH exposure. This study was designed to determine possible effects on kidney function in monkeys following single and repeated injections of MMH. Renal function tests and needle biopsies for electron microscopic examination of kidney tissue were performed on monkeys exposed to MMH. The left kidney of each animal was surgically translocated to a subcutaneous pocket and baseline needle biopsy samples taken; baseline renal function tests, i.e., glomerular filtration rate, renal plasma flow, and maximum tubular excretion rate, were also determined. One group of monkeys was exposed to a single injection of 7.5 mg/kg-MMH, one group to 2.5 mg/kg daily for 14 days, one group to 5.0 mg/kg every other day for 14 days, and one group to 5.0 mg/kg daily for 5–10 days. The renal function tests were repeated 24 hours after the final exposure and renal biopsy samples taken 48 hours after exposure. There was no change in the renal function tests in any group. However, examination of the renal biopsy samples revealed major changes in subcellular morphology in all groups of monkeys following MMH exposure.

96. *Histopathologic Changes Associated with the Short-Term Oral Administration of a Resin in Dogs.* W. M. BUSEY, M. B. POWERS, and L. M. CREWS, Hazleton Laboratories, Inc., Falls Church, Viriginia. (Sponsor: D. C. Jessup.)

Microscopic examination of tissues from dogs which received 90 daily oral doses of an amine compound, incorporated in a granular resin substrate, revealed unusual vascular histopathologic alterations. These degenerative changes were found particularly in the larger vessels or stroma, or both, in the thyroid, lung, heart, liver, spleen, kidney, stomach, and ovary. Tissues from rats which were fed the amine without the resin did not show these changes, strongly suggesting the resin as the causal agent.

A study is currently in progress to determine whether the resin alone could evoke these microscopic changes when administered orally to dogs for 90 days.

97. *Histologic Reaction to Polyurethane Dust.* R. H. RIGDON and D. J. KILIAN, University of Texas Medical Branch, Galveston, Texas, and Industrial Medicine and Toxicology, Texas Division, Dow Chemical Company, Freeport, Texas. (Sponsor: V. K. Rowe.)

The reaction to polyurethane dust has been observed in different tissue of experimental animals. It was given intratracheally to ducks, intramuscularly to chickens, and subcutaneously and intraperitoneally to mice and rats. The histologic reaction to these "dust particles" was similar. Leukocytes and mononuclear and giant cells infiltrated the stroma around the particles of polyurethane. Subsequently the area was infiltrated by fibrous tissue. A progressive accumulation of a brown-staining granular pigment accompanied this cellular reaction. This pigment stained positive by the periodic acid-Schiff and potassium ferrocyanide techniques. Granules of the pigment were located in the cytoplasm of the mononuclear and giant cells. It is suggested that the pigment results from a degeneration of polyurethane.

98. *Light and Electron Microscopy Studies of Dog Adrenal Cortex after Treatment with o,p'-DDD.* M. L. VERNON, E. R. HOMAN, and T. W. TUSING, Hazleton Laboratories, Inc., Falls Church, Virginia, and National Cancer Institute, Bethesda, Maryland.

Dogs given *o,p'*-DDD at dosage levels of 5, 25, 50, 75, and 100 mg/kg for 14 or 15 days were biopsied or sacrificed at various intervals after dosing. Pregnant dogs were given the drug at 25 mg/kg for 14 days during mid-gestation. The 5 mg/kg dosage level had no apparent effect on the adrenal clinically or morphologically. At the higher levels there were severe alterations in the zona reticularis often accompanied by fibrosis. The zona fasciculata was affected in a dose-related fashion.

By electron microscopy surviving cells of the reticularis contained large quantities of very dense material distorting an otherwise intact nucleus. Greatly reduced numbers of apparently normal mitochondria could be found in the reticularis. There were striking changes in mitochondria of the zona fasciculata in an animal sacrificed immediately after dosing at 50 mg/kg, but not in an animal administered 75 mg/kg which had returned to clinical normalcy before sacrificing.

Grossly and clinically the progeny of the pregnant dogs were normal although the drug was recovered from fetal and neonatal carcasses as well as from maternal tissues. Morphologically there was evidence of necrobiosis accompanied by active mitoses. Severely altered mitochondria were often seen in the principal cells of the cortex. (Supported by Contract No. PH 43-63-1168.)

99. *Percutaneous Absorption of Some Narcotic Drugs in Dimethylsulfoxide (DMSO).* W. D. COLLOM, C. L. WINEK, and S. P. SHANOR, Duquesne University Department of Pharmacology and Toxicology, Pittsburgh, Pennsylvania.

The percutaneous absorption of morphine sulfate, meperidine hydrochloride, cocaine hydrochloride, codeine sulfate, and codeine base was studied from DMSO solutions using albino rabbits. Rate of absorption studies were made with codeine sulfate and codeine base in DMSO solution. The distribution of codeine in various tissues after percutaneous absorption from DMSO was compared to the distribution after subcutaneous injection in saline. The 24-hour excretion of codeine after percutaneous absorption in various dilutions of DMSO was also studied.

Cocaine was not detected in liver samples of animals after application of the hydrochloride in DMSO. This was apparently due to vasoconstriction. The other salts were detected in the liver at 1, 2, and 3 hours after application, with codeine sulfate producing the highest level. Both codeine and codeine sulfate produced maximum blood levels one-half hour after administration, with codeine producing the higher level. Four hours after administration the tissue distribution of codeine was not significantly different between animals which were dosed by topical application in DMSO or subcutaneously injected in saline. Twenty-four-hour excretion of codeine was greater with 90% DMSO in water than in any other concentration

tested. The excretion after application of codeine in undilated DMSO was not significantly greater than that after application in water.

The maximum excretion of codeine was only 3% of the topically applied dose, compared to 40–60% for most other routes. This indicates that there would be little effect associated with contamination of the skin by DMSO and one of the narcotic compounds. The inefficiency of absorption would also preclude topical application of DMSO solutions of these narcotic compounds as a preferred route of administration.

100. *Neuromuscular Effects of Carbaryl Insecticide in Swine.* H. E. SMALLEY, P. J. O'HARA, C. H. BRIDGES, and R. D. RADELEFF, Southwestern Veterinary Toxicology and Livestock Insects Research Laboratory, and Texas Agricultural Experiment Station, College Station, Texas.

Carbaryl (Sevin®, 1-naphthyl-*n*-methyl carbamate, technical grade) was mixed with a commercial swine feed and fed to weanling S.P.F. pigs at 150 and 300 mg/kg levels. Carbaryl at high levels may induce vomition and anorexia, therefore it was decided to initiate dosing at the lower level. After all test animals become accustomed to the mixture, the toxicant level was increased to 300 mg/kg in one group while the other group continued to receive carbaryl at the lower level.

Signs of intoxication were first observed in high dose males 9 days after dose-escalation and in the female after 16 days. In the low dose group, the male showed signs after 62 daily doses whereas the female was not affected until after 72 doses.

The patterns of intoxication appeared to be progressive and were similar in all test animals. Signs appeared in this order: tendency to lie down, tendency to walk with rear legs well forward, a string-halt gait, extreme awkwardness when sitting, ataxia, incoordination, intermittent prostration, complete prostration with spastic paresis, and finally death.

Microscopic lesions were found in the central nervous system and skeletal muscles. The muscular lesions consisted of three morphologically distinct forms: a discrete myodegeneration of a traumatic or ischemic type, an acute hyaline and vacuolar myodegeneration healing by continuous regeneration, and an acute degenerative process associated with dystrophic calcification apparently centered on mitochondria of the sarcotubular system. Myelinated tracts of the cerebellum, brain stem, and upper spinal cord were edematous and were associated with vascular degenerative changes consistent with a vasogenic type of edema.

101. *Teratogenic and Toxic Effects of Locoweeds.* L. F. JAMES, R. F. KEELER, W. BINNS, and K. L. BENNETT, ARS, USDA, Animal Disease and Parasite Division, Logan, Utah.

Locoweed is one of the most widely distributed poisonous plants of the western United States, and is one of the most common causes of livestock poisoning. It affects all classes of range livestock. Locoweeds are members of the *Oxytropis* and *Astragalus* genera.

Observations suggested that locoweeds are somewhat unpalatable to all classes of livestock; however, controlled experiments with sheep grazing desert ranges infested with *Astragalus pubentissimus* demonstrated that this particular locoweed was readily grazed in the winter, even when ample forage of good variety and quality was available. Sheep dug through snow to obtain it, and grazed it in preference to other desert range plants.

Locoweeds caused abortion and congenital malformations in offspring when consumed by sheep and cattle during pregnancy. Malformations included lateral rotation of forelimbs, arthrogryphosis, contracted tendons, anterior flexure and hypermobility of stifle and hock joints. Field observations indicated that permanent skeletal malformations occurred in 1 of every 50 lambs.

Contracted tendons, anterior flexure and hypermobility of the stifle and hock joint occurred in about 1 of every 5 lambs born to locoed ewes. Most recovered spontaneously, except lambs lost from the band. The incidence of abortion varied greatly, being highest among ewes severely locoed. Lambs that seemed anatomically normal were small and weak at birth and, from range observations, grew slowly.

The period of insult to the fetus was very nonspecific.

The incidence of abortions and anterior flexure, hypermobility, and contracted tendons was approximately the same in field and experimental conditions.

102. *Clinical, Toxicologic and Pathologic Aspects of Arsanilic Acid Poisoning in Swine.* A. E. LEDET, J. R. DUNCAN, and W. B. BUCK, Iowa State University, Ames, Iowa. (Sponsor: K. L. Gabriel.)

Organic arsenicals are commonly added to swine rations for the prevention and treatment of enteric diseases and for increased weight gains. Arsanilic acid is the most commonly used form.

Untoward reactions occur with accidental or inadvertent overdosage of this drug. Numerous field cases of toxicity due to overdosage with arsanilic acid have been investigated and confirmed by the Iowa Veterinary Diagnostic Laboratory, Ames, Iowa. In these cases, a syndrome of paraparesis and quadraplegia was observed. Histopathologic studies indicated that clinical signs were due in part to demyelination of peripheral nerves.

This paper deals with the clinical syndrome, drug levels in selected tissues, and description of histopathologic changes in nervous tissue after experimental high-level feeding of arsanilic acid.

The clinical syndrome of arsanilic acid toxicity at 1000 ppm is described. Cutaneous hyperemia, mild transient diarrhea, rough hair coat, and hypersensitivity to touch were typical early signs. Incoordination and a diminished sense of balance occurred as early as day 6. Paraparesis with inability to rise on the pelvic limbs occurred by day 15.

Early removal of the drug resulted in recovery. Delayed removal of the drug until paraparesis developed resulted in a progressive paralysis, although animals could be kept alive with good nursing care. Continued feeding of the drug at 1000 ppm eventually resulted in death due to interference with prehension of feed and water.

Chemical findings of arsenic levels in the nervous system, blood, and other tissues were included.

Histopathologic changes consisted principally of demyelination occurring primarily in peripheral and cranial nerves. Special strains were used to characterize the changes.

103. *Mycotoxins in Animal Feeds.* G. H. NELSON, C. M. CHRISTENSEN, and C. J. MIROCHA, University of Minnesota, St. Paul, Minnesota. (Sponsor: K. L. Gabriel.)

The poisoning of livestock and poultry resulting from consumption of feeds invaded by toxin-producing fungi concerns veterinary diagnosticians because herein may lie the solution to many common and important, but hitherto unexplained, diseases of domestic animals.

When mycotoxicoses are suspected, we then obtain samples of feeds suspected of harboring the toxic fungi, isolate fungi from these grains or feeds, grow them in moist autoclaved corn, dry them, grind them, and feed to experimental animals. The isolates that are found to be toxic are grown in larger amounts, and the chemist undertakes to isolate, purify, and identify the toxins.

In this manner, we have tested over 1500 isolates over the past four years, and have shown that a number of common fungi are capable of producing potent toxins.

In our own work in 1965–1967, of 573 isolates of fungi from corn, feed, and food which were tested, 331 isolates, or 58%, were lethal to laboratory animals. *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Fusarium*, and *Penicillium* made up 481 of the 573 isolates, or 83.9%, and of these 481 isolates, 265, or 55%, were lethal within 7 days to the rats, turkey poults, chicks, or ducklings to which they were fed.

The problem is complex, because the production of toxin is influenced by many factors such as: (1) the strain or isolate of the fungus, (2) the substrate on which the fungus grows, (3) the temperature or temperatures at which it grows, (4) the length of time it grows, (5) the complex of other microflora with it. Therefore, the presence of fungi in a sample of feed, even of species known to produce toxin, is not in itself evidence that the feed is toxic.

104. *Integrated Studies of the Effects of Dieldrin on Behavior, Electroencephalograms, and Body Chemistry in Sheep.* G. A. Van GELDER, W. B. BUCK, G. C. KARAS, and D. SUSSMAN, Iowa State University, Ames, Iowa. (Sponsor: K. L. Gabriel.)

The effects of oral exposure to dieldrin, a chlorinated hydrocarbon insecticide, on behavior, electroencephalographic and electrocardiographic activity, hematology, and body chemistry are being studied.

An avoidance shuttle box and a "Y" maze discrimination apparatus have been utilized in the behavioral studies. The behavioral testing techniques employed concurrently with other clinical and experimental determinations broaden the scope of the investigations into the functional state and capacity of the total animal.

Electrocardiograms and electroencephalograms are being recorded with the aid of radio telemetry in order to obtain recordings from free ranging animals.

Results of several preliminary studies will be reported. In the first study 10 sheep received 15 mg of dieldrin/per kilogram body weight daily and 10 sheep served as nonexposed controls. The extinction of a shuttle box avoidance response, conditioned heart rate, and body weight were investigated. No differences were noted between exposed and nonexposed animals.

In a second study, the electroencephalographic (EEG) activity of 20 sheep was studied prior to, during, and following oral administration of dieldrin at 25, 15, and 10 mg/kg body weight. The EEG changes were compared to blood and brain levels of dieldrin.

In the third study to be reported, the effects of dieldrin exposure on "Y" maze discrimination performance was determined in 10 sheep.

The use of a multicriterial or interdisciplinary approach to this research problem offers the advantage of an in-depth study of the reaction of the organism to its environment.

105. *Bone Composition as Deleteriously Influenced by Prolonged Administration of K_2EDTA and an Aldosterone Inhibitor to Cattle.* D. E. BAILEY, J. T. BLAKE, and E. J. FISHER, College of Agriculture, Utah State University, Logan, Utah. (Sponsor: J. L. Shupe.)

To determine effects of the combined stresses of hypocalcemia, hyperkalemia, and hypoxia on bovine tissues, forty 4-month-old Hereford calves were distributed into four groups, with treated and control groups at elevations of 9000 and 4500 feet, respectively. Treatment consisted of intraperitoneal injections of K_2EDTA, SC-14266 (aldosterone inhibitor), KCl, and a diet radically imbalanced in Ca:K. This paper deals with effects of the electrolyte imbalance on bone composition.

Metatarsal sections were ground, ether extracted, dried, and dry ashed. Calcium and K concentrations were determined by atomic absorption spectrophotometry. For additional information, Mg and Na were similarly determined and P was determined colorimetrically.

The drugs had marked influences on bone composition. Statistically highly significant changes were as follows: Ca decreased to 25.27% from a control level of 32.54%; P decreased to 16.47% from 19.00%; the Ca:P ratio decreased to 1.53 from 1.71; and K increased to 0.161% from 0.080%. In addition, Mg content significantly decreased to 0.508% from 0.828%. The drugs did not significantly influence Na content or total percent of ash on a fat-free, dry matter basis.

Although less marked than changes caused by the drugs, the high altitude influenced bone composition. Phosphorus increased to 18.06% from a control level of 17.46%, and K, Na, and Mg decreased to 0.112% from 0.129%, to 1.090% from 1.167%, and to 0.640% from 0.696%, respectively. Ca, Ca:P ratio, and total percent ash were not influenced.

The only interaction between drug and altitude effects occurred with Mg.

106. *Glomerular Lesions Induced in Pekin Ducks by Dietary Dimethylnitrosamine.* W. W. CARLTON and J. R. WELSER, Department of Verterinary Physiology and Pharmacology and Department of Veterinary Anatomy, School of Veterinary Science and Medicine, Purdue University, Lafayette, Indiana. (Sponsor: T. S. Miya.)

Male white Pekin ducks were placed on a complete mash containing 0.005% dimethylnitrosamine when approximately 36 hours of age. Test and control ducks (fed only mash)

were killed by decapitation at weekly intervals (1–8 weeks) for necropsy and collection of tissue for histopathology. Tissues were obtained for electron microscopy after 5 and 6 weeks, were fixed in Palade's solution, and embedded in Epon. Necropsy findings included ascites and hydropericardium, splenomegaly and hepatomegaly, and swelling of the kidneys. Obvious glomerular lesions were observed after 4 weeks of feeding and consisted of a reduced cellularity of the glomerular tufts with diffuse thickening of the capillary loops, but, early, the basal stalk region was spared. In severely affected glomeruli, the normal structure was completely destroyed and replaced by a solid, hyaline, eosinophilic material. The glomerular deposits stained variably with Gomori's trichrome, were negative for amyloid by Congo Red staining, and were PAS positive. The deposits took the silver component of the Humason and Lushbaugh stain [Stain *Technol.* 35, 209 (1960)]. The PAS staining response was not removed by diastase or hyaluronidase digestion, and the material was not metachromatic with toluidine blue stain. Ultrastructural alterations included thickening of the basement membrane of the glomerular capillaries by "deposits" of a proteinaceous material, and degenerative changes in the foot processes. The material was distributed through the various divisions of the basement membrane and "stained" more darkly than the adjoining cytoplasms. It was not osmiophilic and generally appeared structureless; there were no recognizable molecules such as collagen, fibrin, or amyloid in the deposits. The glomerular lesions resemble those described in diabetes and in experimental toxicoses characterized by severe hepatopathy. (Supported in part by NIH Grant HE 10015.)

107. *The Teratogenicity of Some Industrial Chemicals.* S. M. ROCHE and C. H. HINE, Department of Pharmacology and Experimental Therapeutics (Toxicology Activity), School of Medicine, University of California at San Francisco.

Eleven compounds representing different chemical classes were screened for possible teratogenic effects. The agents were tested in two species: the rat fetus and the chick embryo. Teratogenicity was defined as the production of either a gross abnormality or a significant change in body weight and certain bone lengths.

Two butyl alcohols, *p-tert*-butyltoluene, four epoxy compounds, and an imine were teratogenic. Benzene and toluene were not active in either species. The chick embryo is highly susceptible and presents problems of interpretation. (Supported in part by Public Health Service Grant No. 5 TO1 GMO1304.)

108. *Effects of Trace Amounts of Toxic Metals on Hepatic Microsomal Enzymes.* H. A. RIBEIRO and C. H. HINE, Department of Pharmacology and Experimental Therapeutics (Toxicology Activity), School of Medicine, University of California at San Francisco.

A variety of environmental toxicants have been shown to affect the liver microsomal enzymes. This study was designed to investigate whether trace amounts of toxic metals present in the environment could stimulate and/or inhibit several of these microsomal enzymes.

The effect of arsenic, beryllium, lead, and mercury has been investigated in both *in vivo* and *in vitro* systems. Data are presented which show the effects of these metals on hexobarbital-induced sleep times and the activity of certain liver microsomal enzymes which are involved in drug metabolism. (Supported in part by Public Health Service Grant No. 5 TO1 GMO1304.)

109. *Predicted and Measured Uptake of Three Solvents in Human Subjects.* J. P. AITCHISON and C. H. HINE, Department of Pharmacology and Experimental Therapeutics (Toxicology Activity), School of Medicine, University of California at San Francisco.

Exposure of industrial workers to organic vapors for long intervals is usually expressed in terms of the product of concentration and time. In such expressions the quantity of compound absorbed by the exposed subject is not known. A method is presented for estimating the dose absorbed by a standard man when exposed to concentrations approximating threshold limit values. The method utilizes an electrical analog of the body to describe uptake of solvent

vapors into several body compartments which differ in terms of blood flow and solubility characteristics.

Prediction of uptake derived from the analog is presented for three solvents which are not metabolized and have low irritating potency. These predictions are compared with values obtained in experimentally exposed subjects.

The possible application of the analog to estimation of the body burden of solvents is discussed in terms of analysis of expired air and blood. (Supported in part by Public Health Service Grant No. 5 TO1 GMO1304.)

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