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CENTRAL NERVOUS SYSTEM TOXICITY OF ALPHA-CHACONINE IN RATS

by

Charles Newell Aldous III

A thesis submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

in

Toxicology

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1979

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Charles N Aldous

Charles Newell Aldous III

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ABSTRACT

Central Nervous System Toxicity of Alpha-Chaconine in Rats

by

Charles Newell Aldous III, Master of Science

Utah State University, 1979

Major Professor: Dr. R. P. Sharma
Department: Toxicology

Effects of alpha-chaconine were examined in the central nervous system on the basis of electrophysiological tests and by assaying for changes in the levels of several known neurotransmitters of the brain. The possibility that brain acetylcholine levels might increase corresponding to acetylcholinesterase inhibition in treated rats was investigated, where an increase would suggest that the anti-acetylcholinesterase activity of alpha-chaconine was physiologically significant.

Various doses of alpha-chaconine were given to rats prepared for physiological tests. Some symptoms were observed at relatively low doses (8 to 10 milligrams per kilogram). These included constriction of abdominal muscles, respiratory impairment, and sedation. At the same dosage the electroencephalogram pattern was altered by a notable increase in the low-frequency component. Tachycardia was observed at both low (10 milligrams per kilogram) and at high doses (40 milligrams per kilogram), whereas intermediate doses (20 to 30 milligrams per kilogram) were associated with bradycardia.

Although other physiological tests implicated the brain as the target organ, analyses of brain neurotransmitter chemicals failed to

show significant trends. Compounds assayed were acetylcholine, norepinephrine, dopamine, serotonin, and the serotonin metabolite, 5-hydroxyindoleacetic acid. The observation that acetylcholine levels were unchanged after alpha-chaconine administration did not support the hypothesis that the major toxic effect of the test compound was caused by alterations in the levels of this neurotransmitter.

(76 pages)

INTRODUCTION

Human mortality due to the ingestion of the tubers or other parts of the domestic potato, *Solanum tuberosum* L., is a rare event. Most human poisonings involved the use of potato shoots (Willimott, 1933), potatoes which had turned green upon exposure to the sun (Hansen, 1925), or tubers unusually high in solanidine glycoalkaloids.

About 95 percent of the glycoalkaloids in *Solanum tuberosum* L. consist of α-chaconine and α-solanine, the balance being sugar hydrolysis products of the two compounds (Wood, 1976). Alpha-solanine consists of a solanidine molecule to which galactose, glucose and rhamnose are attached as shown in Figure 1. The same figure shows α-chaconine, which is identical to α-solanine except that the attached sugars are one glucose unit and two rhamnose units per aglycon molecule. Because important studies have been made on α-chaconine, α-solanine and on mixtures of the two compounds (usually under the generic name "solanine" or under "total glycoalkaloid"), cross-comparisons would be useful if they could be justified. Nishie et al. (1975) found no essential differences between α-chaconine and α-solanine effects on rabbit electroencephalogram (EEG), electrocardiogram (ECG), respiration or blood pressure recordings. Because α-chaconine is the more abundant glycoalkaloid in tubers (Herb et al., 1975) and because fecal and urinary excretion are more severely impaired by α-chaconine than by α-solanine (Nishie et al., 1975), α-chaconine was selected as the test compound for central nervous system (CNS) toxicity studies.

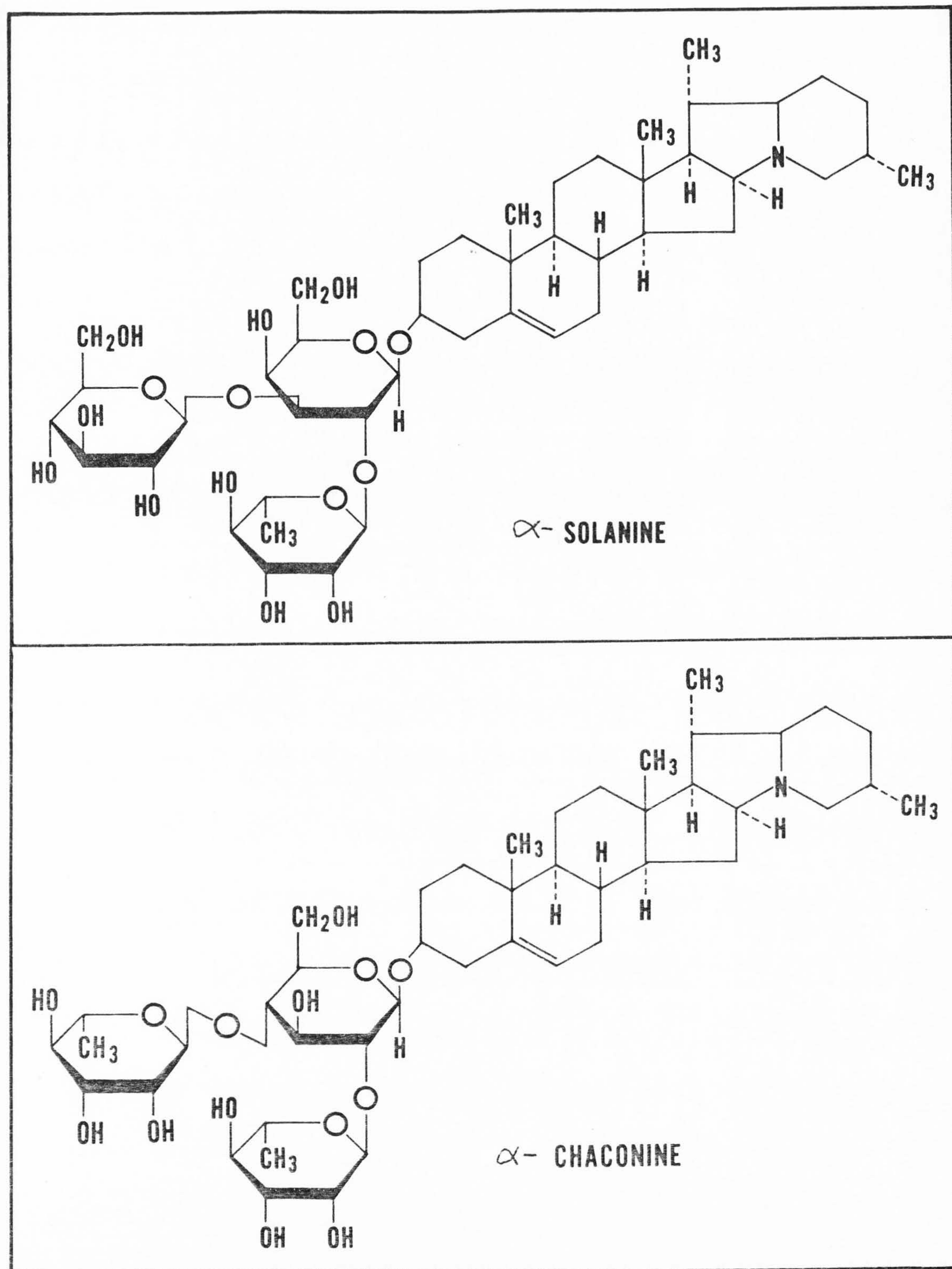


Figure 1. Structural formulas of α-solanine and α-chaconine.

Concern that solanidine glycoalkaloids might affect cholinergic function originated when Orgell et al. (1958) analyzed potato plants for cholinesterase (ChE) inhibition associated with pesticide residues, and were surprised to find such inhibition in the absence of agricultural chemicals. Evidence that CNS anti-acetylcholinesterase effects were attributable to solanidine glycoalkaloids was obtained by Nishie et al. (1971) and by Patil et al. (1972). Subsequent proof of brain acetylcholinesterase (AChE) inhibition *in vitro* as well as *in vivo* was reported by Alozie (1977).

The solanidine glycoalkaloids cause a complex of symptoms. A primary organ affected is presumably the heart. Alpha-chaconine and α-solanine are among the more potent cardiac glycosides derived from plants of *Solanaceae* species in terms of the amount required to elicit a positive inotropic effect in frog heart preparations (Nishie et al., 1976). Lethal doses of solanidine glycoalkaloids are often associated with tachycardia in man and animals (Willimott, 1933; Nishie et al., 1971). Inflammation of the alimentary tract has been reported in man (Rühl, 1950; Willimott, 1933). Hemorrhagic ascites were reported in rats given intraperitoneal (ip) injections of α-solanine (Nishie et al., 1971; Chaube and Swinyard, 1976).

Considerable interest in the solanidine compounds arose from the hypothesis of Renwick (1972), in which he implicated blighted potatoes and the associated solanidine to be the cause of an unusual increase in the incidence of the congenital defects of the brain and spinal column, anencephaly and spina bifida. Subsequent experiments have not found solanidine to be teratogenic (Brown et al., 1978).

The major toxic effects of the solanidine glycoalkaloids after ip injection are associated with the heart and the CNS. The studies reported here investigated the CNS as the primary target for α-chaconine toxicity. Special emphasis was given to effects on the concentrations of brain neurotransmitter chemicals, particularly acetylcholine (ACh), since changes in the levels of these compounds are often associated with abnormal synthesis, storage, release, or metabolism of neurotransmitters. Rat brain ACh levels were measured to determine whether or not the AChE inhibition reported by Alozie (1977) was sufficient to alter ACh levels in the brain. Concentrations of other neurotransmitter amines such as norepinephrine (NE), dopamine (DA), and serotonin (5-HT) have been observed to change in response to organochlorine pesticides (Hrdina, 1973; Sharma, 1973) which also affect ACh levels. Assays of these biogenic amines and of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), were performed to find possible effects on non-cholinergic systems. Electroencephalograms (EEGs) were recorded to observe excitatory or depressant effects as manifested in the electrical activity of the cortex. The EEGs were contrasted with those of organophosphate AChE inhibitors, which cause EEG excitement at low levels and depression at high levels of the drugs (Rieger, 1975; Hyde et al., 1978). Alpha-chaconine doses were chosen which were below levels that markedly compromise cardiac function so that statistically significant findings or unusual observations would not be attributable to cardiovascular embarrassment.

REVIEW OF LITERATURE

Toxic constituents of potatoes

Recent reviews on stress metabolites of the Irish potato (*Solanum tuberosum* L.) have been written by Wood (1976) and Kuć (1973). They considered normal constituents of tubers such as chlorogenic and caffeic acids, scopolin and scopoletin, as well as some compounds elicited by fungal infection, including rishitin, phytuberin, and related compounds. These non-glycoalkaloidal compounds appear or increase upon infection of the tubers with such fungi as *Phytophthora infestans*. About 95 percent of the glycoalkaloids in potatoes are α-solanine and α-chaconine, the balance being partially hydrolyzed products of those two compounds (Wood, 1976). Glycoalkaloid synthesis is inhibited by infections, but increases in response to mechanical injury (Wood, 1976), light, and temperature (Salunkhe et al., 1972). The glycoalkaloids offer some protection to the plants and plant parts of *Solanaceae* species against microbial and insect pests. The fungitoxicity of solanine was the basis of a bioassay by Patil et al. (1972) employing *Trichoderma viride*. The wild potato plant, *Solanum chacoense*, has an acetate group attached to the solanidine moiety of α-solanine and α-chaconine molecules, constituting leptine II and leptine I, respectively (Zitnak, 1968). Leptine I effectively protects plant parts from being eaten by potato beetle larvae (Kuhn and Löw, 1961). The leptines are apparently not found in appreciable amounts in *Solanum tuberosum* L. tubers, as they were not reported by Herb et al. (1975) in gas chromatographic analyses of potato glycoalkaloids.

Alpha-solanine and α-chaconine are the compounds of normal potatoes which constitute a significant hazard to human health (Wood, 1976). Alpha-chaconine is the more abundant of the two glycoalkaloids in potatoes (Herb et al., 1975). These compounds can be extracted into dilute acetic acid (Gull and Isenberg, 1960) or into polar solvent systems such as methanol-chloroform (2:1) (Herb et al., 1975). If an acetic acid extract is heated after raising the pH to 9 with ammonium hydroxide, the solanidine glycoalkaloids precipitate and can be removed by centrifugation (Gull and Isenberg, 1960). The pellet can be recrystallized in ethanol as a mixture of α-solanine and α-chaconine. When separation is desired, it is accomplished chromatographically with alumina or silica gel (Kuhn and Löw, 1961; Rönsch and Schreiber, 1966; Zitnak, 1968).

Human toxicity

Incidents of potato toxicity to humans are few because people avoid potatoes which are green or bitter tasting due to high glycoalkaloid content. Willimott (1933) reported an unusual practice of eating young potato shoots in certain districts of Cyprus. One family partook of a meal including potato shoots, and all members of the family suffered to some extent. Symptoms were delayed for about 12 hours. They included gastroenteritis, headache, severe colic-like pains in the stomach and abdomen, fever (38-40°C), tachycardia (100 to 110 beats per minute), vomiting, nausea, diarrhea with blood and mucus, great weakness and depression. Victims experienced breathing difficulties, drowsiness and general apathy. One girl suffered convulsions. Of the family, one died 44 hours after the meal. All

others were recovering by the fourth day. The deceased was a man 52 years of age, who had become semiconscious, somewhat cyanotic, and was in great pain shortly before his death, which was attributed to syncope. On autopsy the stomach and intestinal tract were found to be inflamed, and his blood was more fluid than normal.

A similar report was given by Rühl (1951) concerning a fatal poisoning of a two-year-old girl who had eaten potato blossoms. She survived for 13 days and showed many of the signs described by Willimott (1933), such as minor dyspnea, cyanosis, fever, vomiting, and diarrhea interrupted by temporary constipation. Death followed a final state of unconsciousness. Histological examination indicated substantial bleeding and inflammation in several areas of the body. Superficial ulcerations and considerable destruction of the mucous membranes occurred, especially in the lips, tongue, mouth, and lower esophagous. Bleeding occurred in mediastinal tissues. Hemorrhage and inflammation were seen in the mucous membrance of the intestinal tract, especially in the cecum and ascending colon. Subcutaneous fat tissues underwent multiple hemorrhages, especially over the shins. Diffuse fatty liver degeneration was observed microscopally.

Similar symptoms were reported by Hansen (1925). Patients were dull and apathetic. All were constipated except for one who had diarrhea. Two were restless before the onset of exhaustion prior to death.

Absorption, distribution, and metabolic fate of α-solanine and α-chaconine

The studies of Nishie et al. (1971) and of Norred et al. (1976) found that α-solanine and α-chaconine undergo similar distribution and

fate in the rat. Oral doses of radioactive glycoalkaloids were excreted predominantly in the feces. Most of the glycoalkaloid content was excreted during the first 24 hours by this route. After 48 hours, 80 percent of the dose was excreted in the feces. Urinary excretion accounted for 10 percent of the dose. Fecal material contained largely solanidine, but about 25 percent of fecal radioactivity after oral dosing of α-chaconine was excreted unchanged. A small fraction of radioactivity consisted of compounds with chromatographic mobilities intermediate between the α-glycoalkaloids and solanidine. These were presumably products of partial hydrolysis of the sugar units. Intraperitoneal (ip) doses led to greater distribution to tissues and reduced excretion rates of the glycoalkaloids. With α-solanine, 15 to 20 percent of the dose was excreted in feces, and a similar amount in urine. The pattern with ip injections of α-chaconine was slightly different in that the degree of urinary excretion (14%) was approximately double the fecal excretion in a 24-hour period. A notable impairment of excretory mechanisms was observed with both glycoalkaloids. While α-solanine fecal excretion was significantly reduced only at the 25 mg/kg level, fecal elimination was virtually halted at α-chaconine levels of 15 mg/kg. Urinary excretion of α-chaconine was dramatically impaired at 25 mg/kg. Excretory impairment may account for much of the enhancement of toxicity of these compounds at high doses.

Acute toxicity in experimental animals

The median lethal dose (LD_{50}) of the mixed potato glycoalkaloid given ip to rats was reported as 75 mg/kg (Gull et al., 1970). Chaube

and Swinyard (1976) found LD_{50} values for female rats to be 84 mg/kg for α-chaconine, 67 mg/kg for α-solanine, and 60 mg/kg for "total glycoalkaloid extract." Mice were more sensitive than rats. Nishie et al. (1975) found the LD_{50} values of α-solanine and α-chaconine to be 34.5 and 32.3 mg/kg (statistically equal) in mice. Rabbit LD_{50} values were not computed, but appear to be intermediate between those for the two rodents. Due to the poor absorption of the glycoalkaloids, the oral LD_{50} values are much higher than for the ip route. Gull et al. (1970) determined the LD_{50} in rats to be 590 mg/kg after oral dosing. Nishie et al. (1971) reported a 1000 mg/kg oral dose of α-solanine to be nonlethal to mice. A summary of acute toxicity data related to *Solanum tuberosum* L. and its glycoalkaloids is given by Jadhav and Salunkhe (1975).

Pharmacological effects

Nishie et al. (1971) performed a series of pharmacological tests with α-solanine on laboratory animals. A 30 mg/kg dose given ip to a rabbit markedly increased both respiratory rate and heart rate. Increased heart rate is contrary to the anti-ChE effect and suggests either a direct or CNS-mediated effect of this organ. The heart continued to beat rapidly until the animal became cyanotic a few minutes before death. Cyanosis was concommitant with low frequency (delta) activity in all EEG leads. The investigators concluded that death resulted from CNS depression, since the EEG signals disappeared before respiration ceased. Respiration and EEG tracings suggested the possibility that brain failure was due to respiratory failure, since delta waves did not appear until cyanosis was apparent. They surmised

that the delta activity was caused by high intracranial pressure, but they did not report any measurements of pressure in their study.

Other results of the study suggested a CNS effect of α-solanine. The spontaneous motor activity of mice was reduced by ip doses as low as 10 mg/kg. Twenty mg/kg increased the pentobarbital sleping time of mice. Alpha-solanine caused contraction of guinea pig ileum strips at levels of 50 to 100 micrograms/ml, suggestive of anti-ChE activity.

Anti-acetylcholinesterase activity

Orgell et al. (1958) found anti-AChE activity in potato plants which were free of pesticide residues. Later Orgell (1963) reported marked ChE inhibition by such *Solanum* glycoalkaloids as α-solanine, tomatine, and leptine I. Harris and Whittaker (1962) found that α-solanine and solanidine inhibited differentially the serum cholinesterases of three human phenotypes in the same manner as the local anesthetic, dibucaine. Inhibition of ChE in the three serum types was 80, 63, and 20 percent in response to 10^{-5} M dibucaine. Alpha-solanine was about four times more potent than dibucaine, and solanidine was slightly less potent than α-solanine. Such phenotypic differences were not observed with the organophosphate AChE inhibitors used in research and agriculture.

Patil et al. (1972) found weak to moderate inhibition of serum and erythrocyte ChE activity *in vivo* after ip injections of α-solanine into rabbits. Pre-injection of mice with atropine sulfate reduced the mortality due to 40 mg/kg α-solanine from 9/10 (controls) to 5/10. Pargyline and amphetamine had no prophylactic effects. Atropine has been used as a rapid and effective means to counter the

effects of AChE inhibition in the brain. Intravenous injections of atropine restored the activity of the brain's respiratory center in 10 to 30 seconds in cats which had been given lethal doses of the centrally-acting AChE inhibitor, tetraethylpyrophosphate (Douglas and Matthews, 1952). When cats were maintained on artificial respiration long enough to compensate for neuromuscular block of the diaphragm, they could tolerate well over the lethal dose of the organophosphate when atropinized. Atropine is ineffective at countering the effects of AChE inhibition at nicotinic sites such as the neuromuscular junction. Atropine is generally indicated for protection against the CNS effects of AChE inhibitors. It also offers some protection against AChE inhibitors which do not reach the brain due to their inability to cross the blood-brain barrier. Thus DuBois et al. (1952) found a twofold increase in the LD_{50} of two diethyl *bis* (dimethylamido-) pyrophosphate compounds which had been shown to be virtually excluded from the CNS.

There is no reason to assume that the anti-AChE effects of the solanidine glycoalkaloids and the organophosphate AChE inhibitors operate by the same mechanism, but there are similar effects and similar responses to prophylactic measures. Metcalf (1971) described AChE as having a "hydrophobic patch" around an anionic site, which is located about 0.5 nm from an esteratic site. Nearly irreversible inactivation requires the formation of a covalent bond between the serine hydroxyl oxygen and the esteratic carbon or phosphorus atom of the interacting molecule. Solanidine glycoalkaloids have a tertiary amine in an aliphatic environment, but lack ester linkages, hence covalent bonding in the manner of the classical AChE inhibitors appears unlikely.

Alozie (1977) found brain acetylcholinesterase activities 79, 55, and 19 percent of controls in male rates injected with 10, 30, and 60 mg/kg α-chaconine. He separated rat brain homogenates into three zones of AChE activity electrophoretically, and found one zone to be completely inhibited at the 30 mg/kg level, one was partially inhibited at the same dosage, and one was not affected. *In vitro* studies of brain homogenates found six ChE or AChE isoenzyme bands to be inhibited from 15.6 to 100 percent when the gels in which they were separated were incubated in 10^{-4} M α-chaconine. No attempts were made to examine differential inhibition of AChE between different brain structures. Differences between AChE inhibition due to α-chaconine and α-solanine were not reported.

Alozie (1977) found α-chaconine to be a partially noncompetitive mixed-type inhibitor of specific and nonspecific cholinesterases. He considered α-chaconine to be a "specific" cholinesterase inhibitor, since it caused greater inhibition of true AChE than it did of butyrylcholinesterase. There are numerous natural compounds which can nonspecifically inhibit cholinesterases. Some of the *Solanaceae* derivatives listed by Orgell (1963) were mentioned. He also found some of the digitalis alkaloids to be moderate inhibitors. Some soybean saponins have anti-ChE activity. Their genins are non-alkaloidal triterpenoid compounds resembling the sterols in form as well as in origin. A soybean saponin extract was found to inhibit chymotrypsin, trypsin, papain, an insect larval midgut enzyme, and ram blood cell ChE (Ishaaya and Birk, 1965). Several proteins, when individually added to the medium containing the saponin extract, diminished AChE

inhibition, apparently by diluting the affected enzymes. It is likely that the solanidine glycoalkaloids are similarly nonspecific.

Cardiac effects

Doses of solanidine glycoalkaloids high enough to elicit marked CNS impairment also have marked effect on cardiac function. A lethal dose of α-solanine given to a rabbit caused the heart rate to increase from 190 to 320 beats per minute, which rate persisted until the animal became cyanotic (Nishie et al., 1971). Alpha-chaconine elicited a similar response (Nishie et al., 1975). Although cardiac slowing is generally the more common effect in therapeutic as well as in toxic doses of digitalis drugs, atrial or ventricular tachycardias are observed in clinical practice (Moe and Farah, 1975).

Alpha-chaconine was about 23 percent as potent as the digitalis glycoside, K-strophanthoside, in its ability to increase the contractility of frog heart preparations by 50 percent (Nishie et al., 1976). In the same tests, α-solanine appeared to be less potent than α-chaconine, but differences were not significant. Solanidine was 28 percent as potent as α-chaconine, and α-chaconine lacking one rhamnose unit, β-chaconine, was only 18 percent as potent as α-chaconine on a g/ml basis. These tests showed that the nature of the aglycon and the number, but not the type, of sugar moieties accounted for most of the differences in potency between compounds. Tomatine, another solanum glycoalkaloid, was twice as potent as α-chaconine in increasing the contractility of the heart. Tests of cardiotonic drugs have shown that the therapeutic/lethal dose ratios are similar from one drug to the other (Farah and Maresh, 1948). If cardiac failure were the cause

of death due to *Solanaceae* glycoalkaloids and if their mechanism were the same as the digitalis glycosides, one might expect the acute toxicity of the *Solanum* glycoalkaloids to reflect their cardiac potentency. Such does not appear to be the case. Tomatine was equivalent to α-solanine and α-chaconine in LD_{50} values in the mouse, but much less toxic in the rabbit (Nishie et al., 1975). Although one must be cautious in extrapolating from one animal species to another in comparing cardiac glycosides, these experiments suggest that the major toxic effect of the solanidine glycoalkaloids probably is not a digitalis-like cardiac effect.

Digitalis drugs frequently produce neurological effects in humans. Batterman and Gutner (1948) reported visual blurring and color distortion, headache, drowsiness, restlessness, irritability, hallucinations, illusions, epileptiform convulsions, stupor, and coma as CNS effects of digitalis toxicity. Shear and Sacks (1978) reported delirium, forgetfulness, neuroses, as well as other symptoms mentioned above, and noted that symptoms could be precipitated by any clinical digitalis preparation.

Other acute toxic manifestations

Nishie et al. (1971) injected a rabbit with a lethal dose of α-solanine, and observed delta activity in the EEG concomitant with marked slowing of the respiratory rate. The ECG continued to be rapid but regular. They concluded that respiration failed due to CNS depression. They speculated that CNS depression was associated with high intracranial pressure.

Studies by Patil et al. (1972) and by Alozie (1977) implicated an impaired cholinergic function as the cause of respiratory failure. Some anti-AChE compounds do not penetrate into the CNS, so that brain AChE is not measurably inhibited and all toxic manifestations are peripheral (Sharma et al., 1973; DuBois et al., 1952). Other organophosphate AChE inhibitors penetrate the blood-brain barrier and cause failure of the respiratory center (Nachmansohn and Feld, 1947; Douglas and Matthews, 1952). Some neuromuscular block is inevitable, and may be the cause of death even with compounds which penetrate the brain freely. Douglas and Matthews (1952) found tetraethylpyrophosphate to cause a rapid onset of profound neuromuscular block so that the diaphragm could not be stimulated via the phrenic nerve for 20 minutes. The respiratory center failed, as evidenced by complete stoppage of the phrenic nerve activity even in an artificially ventilated animal. The great importance of functional brain AChE activity in diisopropylfluorophosphate poisoning was illustrated by Nachmansohn and Feld (1947), who administered approximately the LD_{50} to rabbits and observed at least 10 percent brain AChE activity in survivors, but invariably found near zero activity in those which died.

The solanidine glycoalkaloids are structurally related to steroidal hormones, and thus it is not surprising to find some overlap with hormonal function. Satoh (1967) found that α-solanine dramatically increased blood sugar levels in intact rats in doses as small as 5 mg/kg. The effect was blocked by adrenalectomy or by administration of various adrenergic blocking agents or reserpine. Neither the mechanism of action nor the consequences of α-solanine-caused hyperglycemia were determined, but the process apparently entails the mediation of

epinephrine and gluconeogenesis in the liver. Darόczy and Hernadi (1971) found other *Solanum* glycoalkaloids of the solasodine and tomatidenol groups to induce tryptophan pyrrolase activity in the livers of rats. The enzyme activation, an indicator of increased gluconeogenesis, did not occur in adrenalectomized rats.

Teratogenic effects

Much of the current interest in toxicity associated with potato constituents stems from the hypothesis of Renwick (1972), in which he linked blighted potatoes temporally and geographically with increased incidence of spina bifida and anencephaly among newborns. Solanidine and its glycoalkaloids were suspect, and numerous tests were conducted to prove or disprove the hypothesis. Knox (1972) and Emanual and Sever (1972) performed retrospective studies and found no correlations to link potatoes with the abnormalities. Some laboratory tests appeared to be positive. Mun et al. (1975) observed rumpless and trunkless chick embryos after injection of α-solanine or extracts of potatoes infected with *Phytophthora infestans*. Sharma et al. (1978) found an anencephalic fetus in one of two litters of miniature swine fed *Phytophthora infestans*-blighted potato concentrate. They also found incomplete closure of the vertebral column in rabbit fetuses born to does fed extracts of potatoes infected with either *Alternaria solani* or *P. infestans*. Keeler et al. (1975) had previously tested four species including the rabbit with extracts of blighted potatoes and found no incidence of anencaphaly or spina bifida. Systematic studies by Brown and Keeler (1978) of various solanidan epimers suggested that the conformational requirements for teratogenicity were not met by solanidine,

and that the causes for positive findings of teratogenesis would have to be sought among other compounds.

Multiple changes in brain amine levels after administration of toxins

Only ACh has been considered thus far as a brain amine whose concentration would be expected to change in response to solanidine glyalkaloids. There are, however, instances where other neurotransmitter concentrations or turnover rates change concurrently with ACh. Among causative chemicals are some chlorinated hydrocarbons. Hrdina et al. (1973) found DDT to cause a decrease in ACh in the cerebral cortex and striatum to 72 percent and 62 percent of normal, respectively. Norepinephrine in brain stem dropped to 63 percent of normal. Pretreatment with the monoamine oxidase inhibitor, pargyline, caused a substantial increase in the 5-HT content of the brains of DDT-treated animals as compared with controls. Thus, serotonin was shown to have a more rapid turnover without a change in its concentration. Chronic dieldrin administration to mallard ducks caused depletion of 5-HT, NE, and DA (Sharma, 1973).

Multiple changes in biogenic amines are not routinely expected in AChE inhibitor studies. The solanidine glycoalkaloids, however, are not specific AChE inhibitors, and may well have unanticipated actions upon the brain. There is an ever-increasing body of evidence for a complex intersystem communication between neurons associated with different neurotransmitters. McGeer et al. (1977) reviewed experiments in which cholinergic striatal interneurons stained with a marker for choline acetyltransferase were contacted by dopaminergic nerve endings which were traced after their degeneration caused by 6-hydroxydopamine.

Similarly, gamma-aminoburyric acid (GABA) neurons were traced by the axoplasmic flow of tritium-labeled protein or ^{3}H-GABA from the pallidal cell bodies to the *substantia nigra,* where they met degenerating dopaminergic neurons.

Physiological testing procedures

Toxins associated with a complex of symptoms are ideally studied when all effects except for the one of primary interest are either held constant or are under steady observation. Numerous animal "preparations" are used to hold constant or eliminate confounding symptoms. Typical of these are decerebrate animal, heart-lung, and nerve-muscle preparations. A step further are a host of *in vitro* tests using isolated organs, either intact, homogenized, or prepared otherwise, to allow the investigator to monitor a response apart from the living animal. Since the value of such experiments must eventually relate to the intact animal, many investigators choose to forego the convenience of simplified preparations. Such a choice requires monitoring of several parameters, but animal species can occasionally be selected to minimize confounding effects. In the case of solanidine glycoalkaloids, if one wishes to measure CNS effects with minimal interference of digitalis-like effects, the rat is a good choice because of its relative immunity to the cardiac effects of digitalis drugs (Okita, 1967).

EEG effects and their measurements

Nishie et al. (1971) reported an initial activation of the EEG characterized by high frequency waves after ip administration of a lethal dose of α-solanine. Delta waves were observed minutes before

death and may have reflected anoxia due to respiratory impairment. Alpha-chaconine had a similar effect (Nishie et al., 1975).

Some anti-AChE toxins have effects similar to the solanidine glycoalkaloids. Organophosphate AChE inhibitors have also shown an unstable high frequency pattern in stages in which cerebral anoxia had not set in (Toman and Davis, 1949; Burchfiel, 1975; Rieger and Okonek, 1975). Toman and Davis (1949) found increasing levels of such poisons to be associated with high amplitude delta activity. In the most severe cases, delta activity disappeared and only low amplitude waves of relatively high frequency remained. An irregular high frequency activity superimposed upon slow delta waves was associated with AChE inhibition. These symptoms could be abolished or their onset could be delayed by atropine.

Methods for computerized analysis of random wave forms such as EEGs have been available for some years, but only in recent years has the cost of computer time decreased and the efficiency of wave analysis programs increased sufficiently to make such methods practicable. Probably the most popular and useful form of output is the power spectrum, which is described in detail in Koopmans (1974). The "power" of a time function is the integral of the squared value of its amplitude over time. A spectral representation requires that the waves be segregated by frequency, so that the power values can be summed for waves belonging to respective frequency ranges. Segregation can be conceptually visualized as a matching of each wave of a record with the best fitting sine or cosine wave. Computers fit curves by using complex arithmetic functions which are equivalent to these trigonometric forms.

The power spectrum of a time series yields the power at each frequency as a function of frequency.

Power spectra lend themselves to statistical analysis, and programs and suggestions are abundant in recent literature for their applications (Fails and Verlander, 1977; Rosadini et al., 1977). Power spectra are rather sensitive indicators of behavioral states (Coenen, 1975). Behavioral or awareness states must be stabilized as much as possible in order to perform meaningful pharmacological tests (Gramsbergen, 1976). Recordings of the visual cortex of the alert rat have a peak in the 6 Hertz (Hz) range (Gramsbergen, 1976), which becomes less predominant and may be obliterated after administration of compounds with pronounced excitatory or depressant effects.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley derived rats (Simonson Laboratories, Gilroy, California) averaging 283 g body weight were used in all tests. All were acclimatized to the surroundings for 7 to 10 days before testing. They were fed a commercial laboratory chow and had free access to feed and water except during experiments.

Preparation of α-chaconine

The test compound, α-chaconine, was prepared from freeze dried potato sprouts. These were extracted into acetic acid and the glycoalkaloids were precipitated with ammonium hydroxide (Gull and Isenberg, 1960). Silica Gel G (Applied Science Laboratories, State College, Pennsylvania) was used to separate the compounds by thin layer chromatography using the solvent system 1-butanol:acetic acid:water (10:3:1) (Zitnak, 1968). Alpha-chaconine was identified as the second band from the origin (Zitnak, 1968). It had an R_f identical to a commercial standard of α-chaconine. The only other prominent band corresponded to commercial α-solanine. Alpha-chaconine was dissolved in dimethyl sulfoxide-propylene glycol (3:7) (Nishie et al., 1971), and concentrations were adjusted to make injection volumes of approximately 0.5 ml.

Electrophysiological tests and other observations of live animals

Instrumentation for electrophysiological experiments. All EEG, ECG, respiration, and blood pressure readings were made on a

Physiograph-Six recording unit (Narco Biosystems, Inc., Houston, Texas). The EEGs were additionally recorded on an analog/digital computer (EAI 590 System, Electronics Associates, Inc., West Longbranch, New Jersey), after signal amplification on the Physiograph unit. The amplifiers and transducers with their accessories were Narco products. Channel Emplifiers, Type 7070, processed signals from a variety of couplers. Hi-Gain Couplers, Type 7171 received EEG and ECG signals. Respiration measurements utilized an Impedance Pneumograph Coupler, Type 7212. An Electrosphygmograph Coupler, Type 7211, measured blood pressure with the aid of a Pneumatic Pulse Transducer and a 7/16 inch metal tubular tail cuff. Instrumental settings and testing procedures were made according to general suggestions of the Narco Physiograph manual, and amplifications were adjusted to produce convenient trace amplitudes on the strip chart recorder. A small heat lamp was used to warm animals before taking blood pressure measurements with the tail cuff. Shielded cables were employed to reduce electrical noise in the EEG records.

As the EEG records were being recorded on the strip chart recorder of the Physiograph unit, the preamplified signals were carried from the Physiograph to the analog/digital computer. When actuated, the computer recorded voltages of two channels simultaneously. Voltage differences were recorded in digital form at intervals of 0.0171 seconds for 600 counts (10.26 seconds elapsed time). When recordings were complete, the digital tape was sent to the Burroughs Corporation in Salt Lake City to be transcribed into a format compatible with a Burroughs 6700 digital computer, which performed the EEG data reduction. Power spectra were performed by the IMSL subroutine "FT FREQ" (International Mathematical and Statistical Library, Houston, Texas). Plots and

associated data manipulations utilized the computing system "Minitab II" (Professor Thomas A. Ryan, Jr., The Pennsylvania State University, University Park, Pennsylvania).

Animal preparation prior to electrophysiological tests. Small stainless steel machine screws 1.6 mm in diameter served as leads to regions of the cerebral cortex, and also formed the leads for reference electrodes and for electrical grounding of the animals (Schiff, 1974). Screws had previously been soldered to 5-cm lengths of insulated speaker cable, which were stripped of insulation at both ends for making connections. Wires containing a large single strand of metal were more satisfactory than multifilament types.

Surgery and lead arrangements followed the method of Gohd et al. (1974) except for the placement of reference electrodes and ground leads. When a rat was sufficiently anesthetized under 40 mg/kg of pentobarbital, it was mounted on a stereotaxic instrument. The skull was bared and dried, and holes were made with a dental drill fitted with a bit of proper size to allow the electrodes to be screwed tightly until the tips rested on the *dura mater*. Frontal, parietal, and occipital leads were placed in a line 3 mm lateral to the saggital suture. Frontal and parietal electrodes were placed 3 mm rostral and 3 mm caudal to the coronal suture, respectively. The occipital lead was 1 mm rostral to the lambdoid suture. The left side of the brain was arbitrarily chosen for the primary electrodes. The parietal location on the right side served to ground the animal for EEG, ECG, and respiratory measurements, and to help anchor the headgear. A reference electrode was located 3 mm caudal to the intersection of the saggital and lamdoid sutures (D. E. Shearer and E. Snyder, personal communication). A diagrammatic

representation of the lead configuration is given in Figure 2. A generous application of dental cement anchored the leads in place and insulated the extracranial portion of the leads. The incision was then sutured with surgical gut. Lead wires were wrapped with strapping tape to protect the headgear and to prevent the rats from becoming snagged in the wire cage tops. All rats received 0.1 ml of Combiotic® after surgery. A minimum of 10 days passed before testing began.

On the day of testing, rats were temporarily anesthetized with ethyl ether. A pair of sterling silver wire electrodes was implanted for EEG and respiratory measurements. Placement was in the right side of the chest and on the left portion of the groin, essentially the "Position II" of classical electrocardiography. Wires were conveniently placed by first inserting a large-gauge needle under about 1 cm of skin and allowing the tip to protrude, then inserting the wire into the needle before withdrawing the needle. Tape wrapped around the wires prevented short circuits. Rats were restrained by tying all limbs with strapping tape, and placing animals into cylinders made of copper screen. Thus secured, they had some whole-body flexibility and yet were sufficiently immobilized to maintain a favorable ECG axis and to permit tail cuff blood pressure recordings. A rat prepared for testing is shown in Figure 3.

Electrophysiological testing procedures. Measurements of EEG, ECG, and respiration in response to sublethal doses of α-chaconine were recorded in a single session. Technical difficulties prevented concurrent recordings of blood pressures, therefore records of preliminary tests were used for blood pressure data. An additional rat was given a lethal dose of α-chaconine (40 mg/kg) and EEG, ECG, blood pressure, and

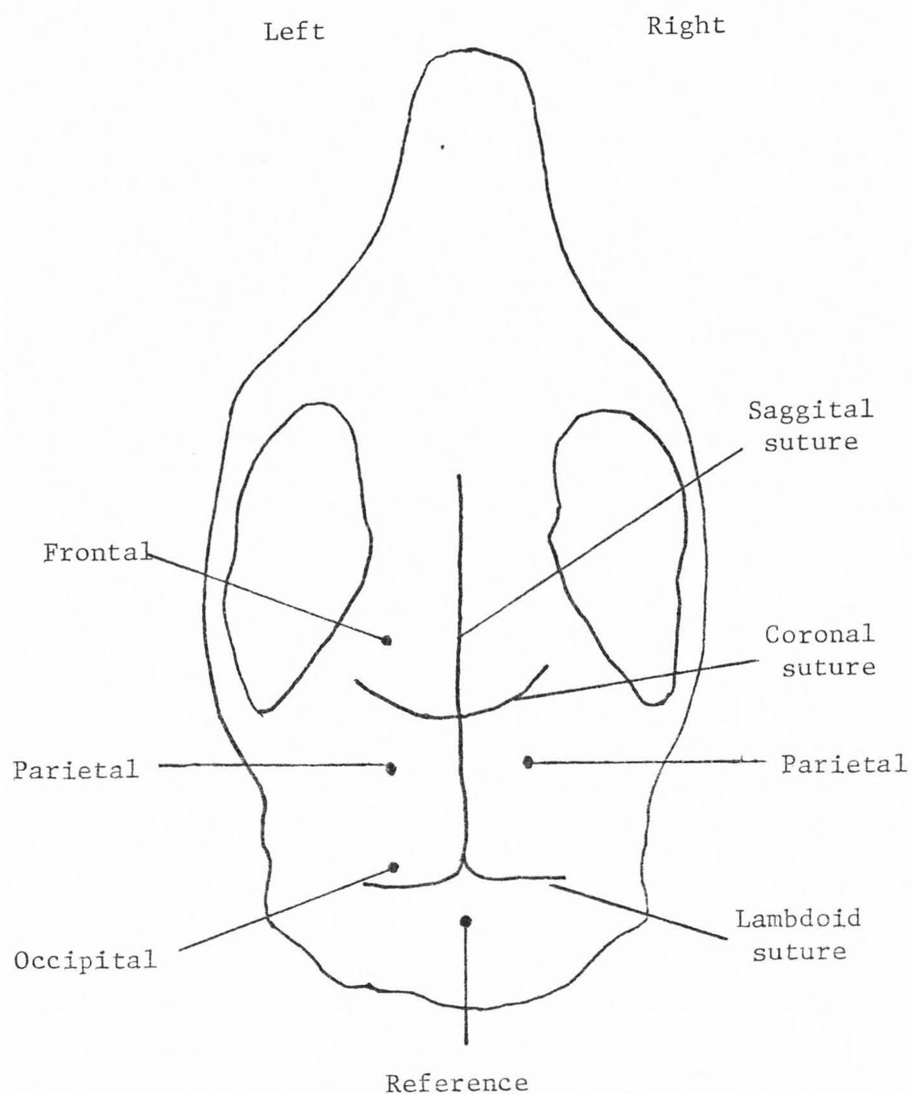
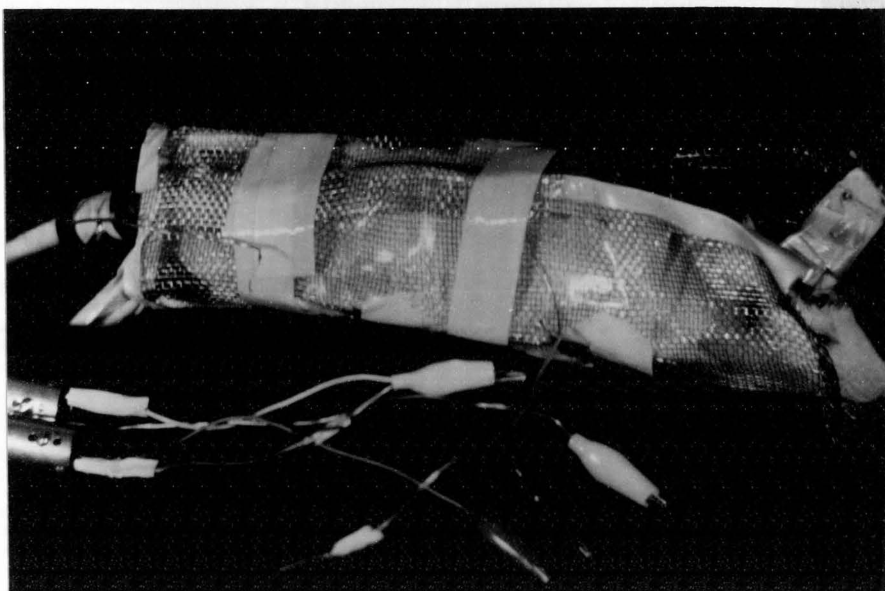


Figure 2. Illustration depicting locations of electrodes for EEG studies. (Left frontal, parietal, and occipital leads used cerebellar reference electrode. Right parietal lead for grounding of animal. Magnification 3X.)

A



B

Figure 3. Rat implanted with electrodes for physiological testing.

A. Showing tail cuff, ECG leads emerging from restraining cage, and alligator clips for electrophysiological recordings.
B. Close-up of headgear. The five leads correspond to the five positions shown in Figure 2.

respiration data were monitored on the Physiograph until the death of the animal.

The main experimental session involved 12 rats, 3 per dosage level and 0, 10, 20, and 30 mg/kg α-chaconine. Immediately before injection, each rat was monitored to give a baseline record to serve as its own control. In addition to samples of ECG and respiration activity, two 10.26-second EEG periods were recorded digitally for each of the three cortical locations, using the cerebellar lead as an indifferent electrode. The first and second post-injection recordings followed 3 and 7 hours later.

Analysis of power spectra. Power spectra for EEG analysis were calculated in steps of 1.01 Hz, thus the "6 Hz" band refers to the power in the frequency range 6.05 ± 0.50 Hz. Records vitiated in part by muscle movement were "edited" by removing from consideration whole 10.26-second recordings of affected leads, the more objectionable halves (5.13 second) of records, or a combination of the two. In all cases, a minimum of one 5.13-second recording was used for each lead on each rat at each time interval, and the contributions from each animal to group-averaged power spectra were weighed equally.

Power spectra were summed for rats treated alike and were subjected to statistical comparisons. Because of large differences in the amplitudes of EEG signals between animals, and because the total power varied in a non-Gaussian fashion (see Appendix A), the nonparametric Wilcoxon rank-sum test (Lapin, 1975) was used to compare dose-related differences in total power in the range 3-29 Hz.

For studies of shifts of predominant frequencies, the raw power spectra were first "normalized" in the range from 3 to 29 Hz by dividing

the power of each frequency band by the total power in that range. An apparent shift of the 6 Hz peak to lower frequencies was investigated by examining the changes in the ratios of power at 6 Hz over power at 5 Hz in response to α-chaconine. A two-tailed t test was used for analysis (Lapin, 1975).

Manifestations of toxicity by visual inspection. Rats treated with α-chaconine for the neurotransmitter determinations as described in the next section were observed visually for signs of sedation, sluggishness, constriction of abdominal muscles, or breathing impairment. Findings were reported in a tabular form.

Neurotransmitter determinations

Forty-eight rats were divided into four dosage level groups, and each level was further divided into two groups based upon the time interval between dosing and sacrifice. Extracts of each brain sample were split into fractions for all neurotransmitter determinations. Neurotransmitter concentrations were calculated in terms of nanomoles per gram fresh weight of brain. Differences between controls and α-chaconine-dosed rats were analyzed by a two-tailed t test with a 95% confidence interval.

Chemicals and chemical assay equipment. All biogenic amine standards were purchased from Sigma Chemical Company (St. Louis, Missouri). They were formulated as the following salts: acetylcholine iodide, norepinephrine HCl, dopamine HCl, serotonin creatinine sulfate, and 5-hydroxyindole-3-acetic acid dicyclohexylammonium salt. Adenosine 5'-triphosphate, tetra (triethylammonium) salt (γ - ^{32}P) was purchased from New England Nuclear (Boston, Massachusetts). Yeast choline kinase

and AChE (from *Electrophorus electricus*) were purchased from Worthington Biochemical Corporation (Freehold, New Jersey). Other chemicals were analytical grade reagents and solvents. The latter were redistilled and acid washed when used for fluorometric analyses. Distilled, deionized water was employed for all chemical tests.

Fluorometric determinations were made on an Aminco-Bowman Spectrophotofluorometer (American Instrument Company, Inc., Silver Spring, Maryland). Radioactivity measurements in the method for acetylcholine were made using a Packard Tri-Carb Liquid Scintillation Spectrophotometer (Packard Instrument Company, Inc., Downers Grove, Illinois). (Details will be discussed later in the paper.)

Animal sacrifice and brain tissue extraction. Animals were accommodated to a regular daylength schedule and familiarized with a rat dipping cage in advance of the time of sacrifice (Smith et al., 1975). A 12-hour light period beginning at 6:00 A.M. was established 2 weeks before testing. Rats were exposed to a sham dipping apparatus four times during the week before sacrifice, and daily during the week in which the rats were killed. Each rat was made to run through the cylindrical chicken wire cage during the accommodation sessions.

Twelve rats, three at each of four dosages, were sacrificed on each of four consecutive mornings. In each case the rats were transported on the evening before sacrifice to a facility convenient to a large refrigerated room for processing of tissues. Rats to be killed 3 hours after dosing were injected between 6:00 and 7:00 A.M. Those to be killed after 12 hours were injected between 9:00 and 10:00 P.M. of the evening before. Doses of α-chaconine were 0, 3, 8, and 20 mg/kg. Rats were caged individually after injection.

Rats were killed by dipping for 10 seconds in liquid nitrogen according to the "near freezing" method of Takahashi and Aprison (1964). Brains were removed after saggital section of the head, and were immediately frozen in liquid nitrogen. Extraction and cleanup followed the method of Smith et al. (1975). Frozen brains were ground in liquid nitrogen in a porcelain mortar. About 1 gram of the powder was weighed and extracted into 1 M formic acid/acetone (v/v:15/85). Four additional tubes of pooled brain matter were extracted and fortified with 0, 2, or 4 nanomoles of NE, DA, 5-HT, and 5-HIAA. These were handled in parallel with the rest of the samples. Extracts were washed with two volumes of heptane/chloroform (8:1). The aqueous phase was adjusted to 5 ml by distilled water addition or by blowing with nitrogen gas. Each sample was split into three fractions. A 2-ml portion was transferred to a vial for analysis of NE, DA, and 5-HT. Another 2-ml portion was measured for ACh, and a 0.5-ml fraction was prepared for 5-HIAA determination. All vials were frozen and then freeze dried. They were then capped and stored at -30°C.

Acetylcholine assay. The method of Goldberg and McCaman (1973) was used for ACh determination. Lyophilized samples were reconstituted in water of pH 6.6. Quaternary amines were extracted into a solution of tetraphenylboron in 3-heptanone. The amines were re-extracted into acidic solution and lyophilized. Samples were reconstituted with a buffer solution containing ATP, $MgCl_2$, and choline kinase. After a brief incubation to phosphorylate choline and interfering compounds, an aliquot of ATP (γ - ^{32}P) and AChE was added to hydrolyze the ACh and to permit a portion of the label to appear in the newly phosphorylated choline. Removal of unused labeled ATP was accomplished by precipitation

with barium acetate and by passage of an aliquot through an anion exchange resin. Samples were washed directly into a scintillation cocktail and counted. Quantiative estimates were based on simultaneously run standard samples.

Assays of norepinephrine, dopamine, and serotonin. Norepinephrine, DA, and 5-HT were determined by the method of Karasawa et al. (1975). Samples were reconstituted in a buffer of pH 8, and layered onto small columns of alumina, which were superimposed over similar columns of a weak cation exchange resin. Columns were washed with water, first in tandem and then separately. Alumina columns were eluted with 0.2 M HCl for determinations of NE and DA. The resin columns were eluted with 0.5 M HCl for 5-HT estimation. One notable exception was made to the Karasawa procedure: the "heated tissue blank" suggested by Smith et al. (1975) was used in 5-HT estimations in favor of the tissue blank method of Karasawa et al. (1975), since the latter gave excessively high blank values.

Determination of 5-hydroxyindoleacetic acid. The method of Anton and Sayre (1971) was employed for 5-HIAA assays. The cleanup entailed an initial precipitation of protein contaminants with perchloric acid. The supernatant was made acidic and washed with chloroform, then extracted into ethyl ether, and finally re-extracted into water adjusted to pH 8. An aliquot was made strongly basic with K_3PO_4 and heated to form the fluorophore.

RESULTS

Live animal experiments

Effects of a lethal dose. A rat injected ip with 40 mg/kg α-chaconine displayed a steady increase in heart rate until death (Figure 4). Respiratory rate was steady for the first half hour, then began to increase markedly. Breathing became much slower and more labored during the last few minutes of life. Blood pressure could not be measured after one-half hour following injection because of reduced peripheral blood flow, but appeared to be rising slightly during the first 30 minutes.

Effects of sublethal doses on heart and respiratory functions. Rats given α-chaconine doses of 20 to 30 mg/kg ip underwent marked bradycardia (Figure 5). Ten mg/kg doses caused significant tachycardia. Blood pressure records of eight rats injected with 0 to 30 mg/kg showed no consistent pattern (Figure 6). Transient high blood pressure was observed in two rats given 20 and 30 mg/kg, respectively. These peak pressures were neither sustained by the affected rats, nor were they observed generally at any dose level.

Respiratory rates did not vary significantly with sublethal doses (Figure 7). A trend seemed to be toward a lower rate 3 hours after injection and toward a higher rate 7 hours after dosing with higher amounts of α-chaconine.

Effects of sublethal doses on EEG. The parietal and occipital EEG patterns showed increased slow wave activity at doses of 20 to 30 mg/kg (Figure 8). Frontal EEGs were not noticeably affected by the toxin.

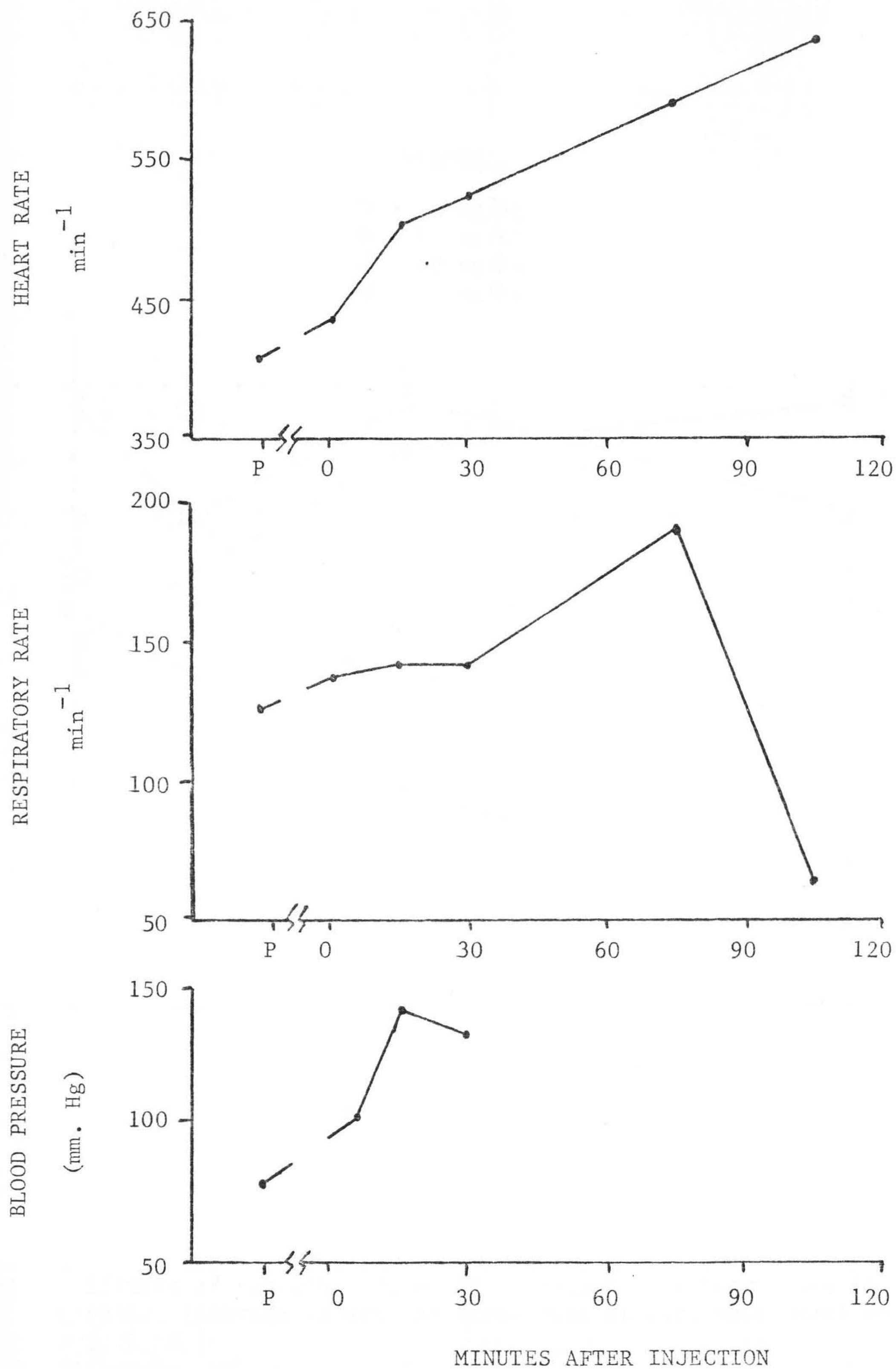


Figure 4. Effects of intraperitoneal injection of 40 mg/kg α-chaconine on heart rate, respiratory rate, and blood pressure of a rat. (P indicates preinjection measurement.)

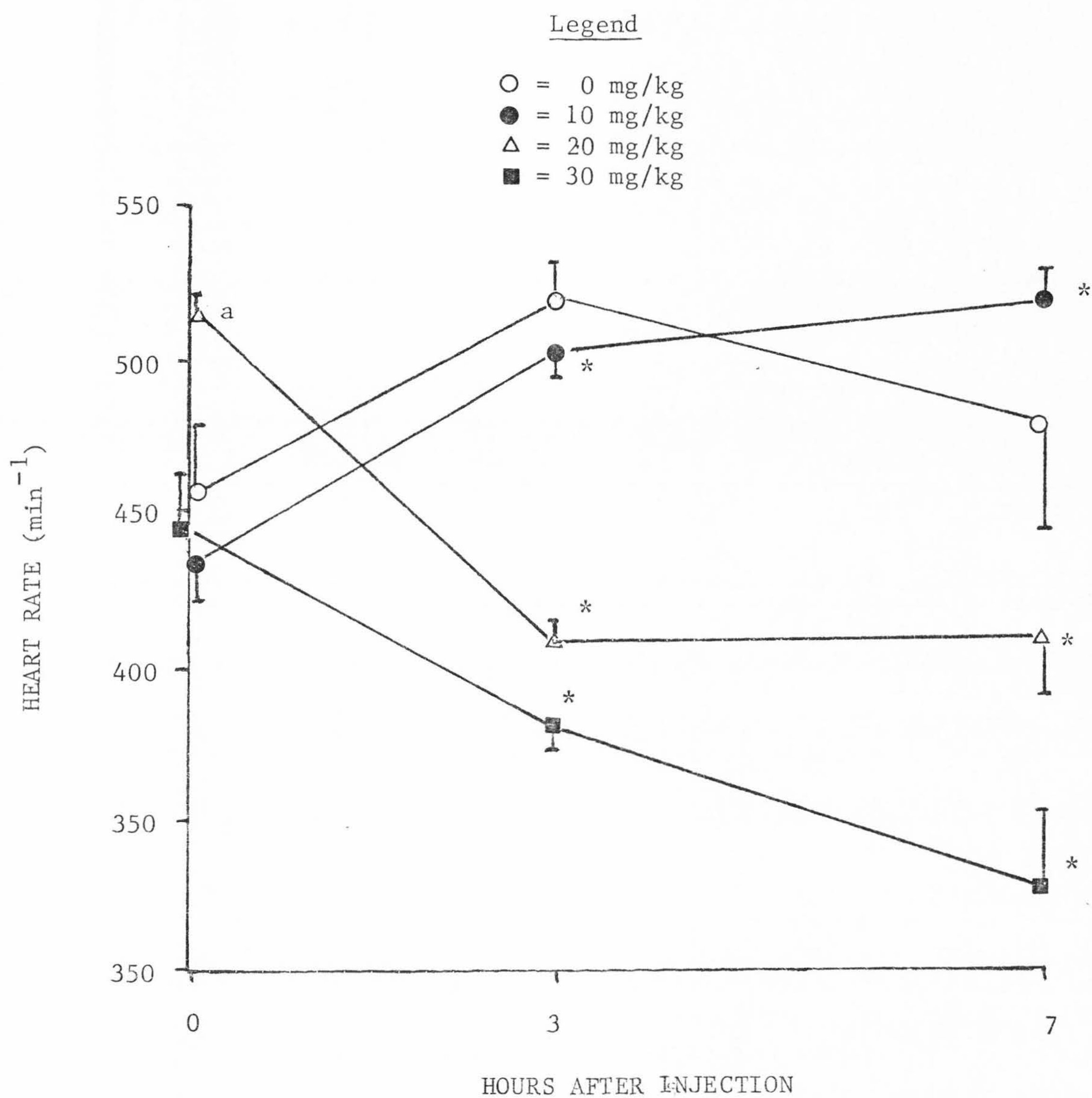


Figure 5. Effects of sublethal doses of α-chaconine on heart rate in rats. (Average values for three rats at each dose level ± S. E. M.)

*Indicates significantly different from pre-injection values ($p < 0.05$).

[a]Indicates significantly different from other pre-injection values ($p < 0.05$).

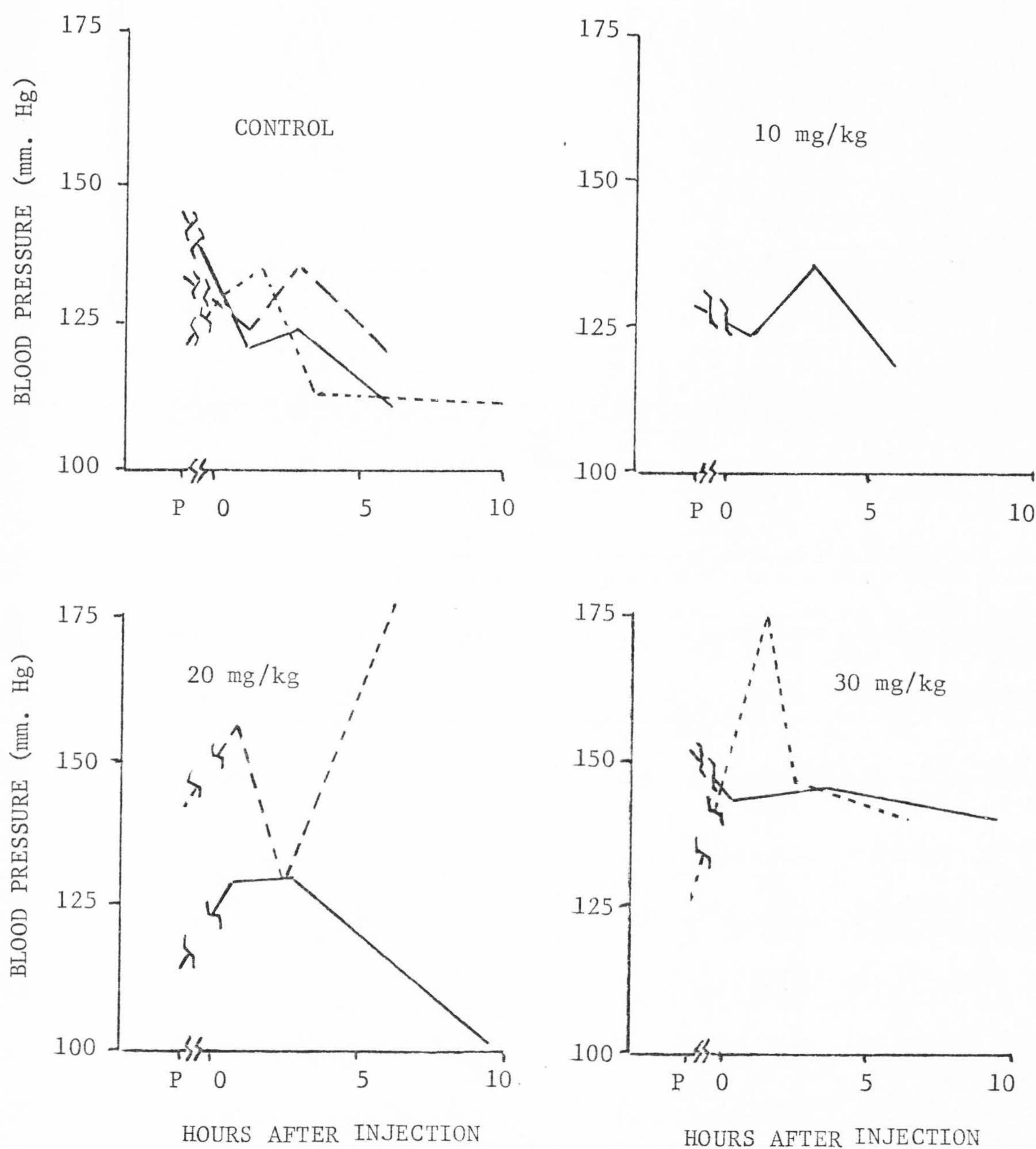


Figure 6. Effects of sublethal doses of α-chaconine on blood pressure in rats. (One to three rats per dose level, each indicated by a different line.)

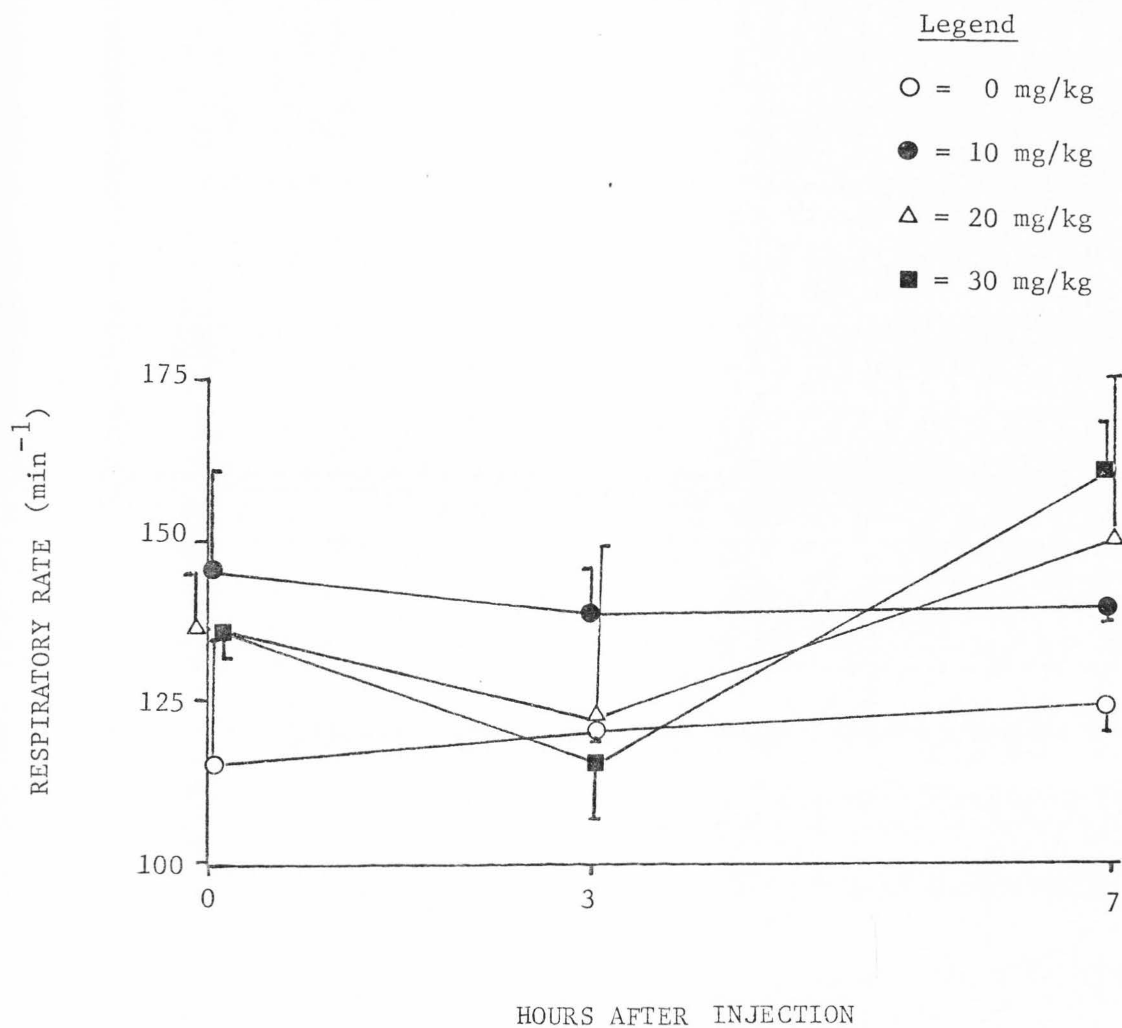


Figure 7. Effects of sublethal doses of α-chaconine on respiratory rate in rats. (Average values for three rats at each dose level ± S.E.M.)

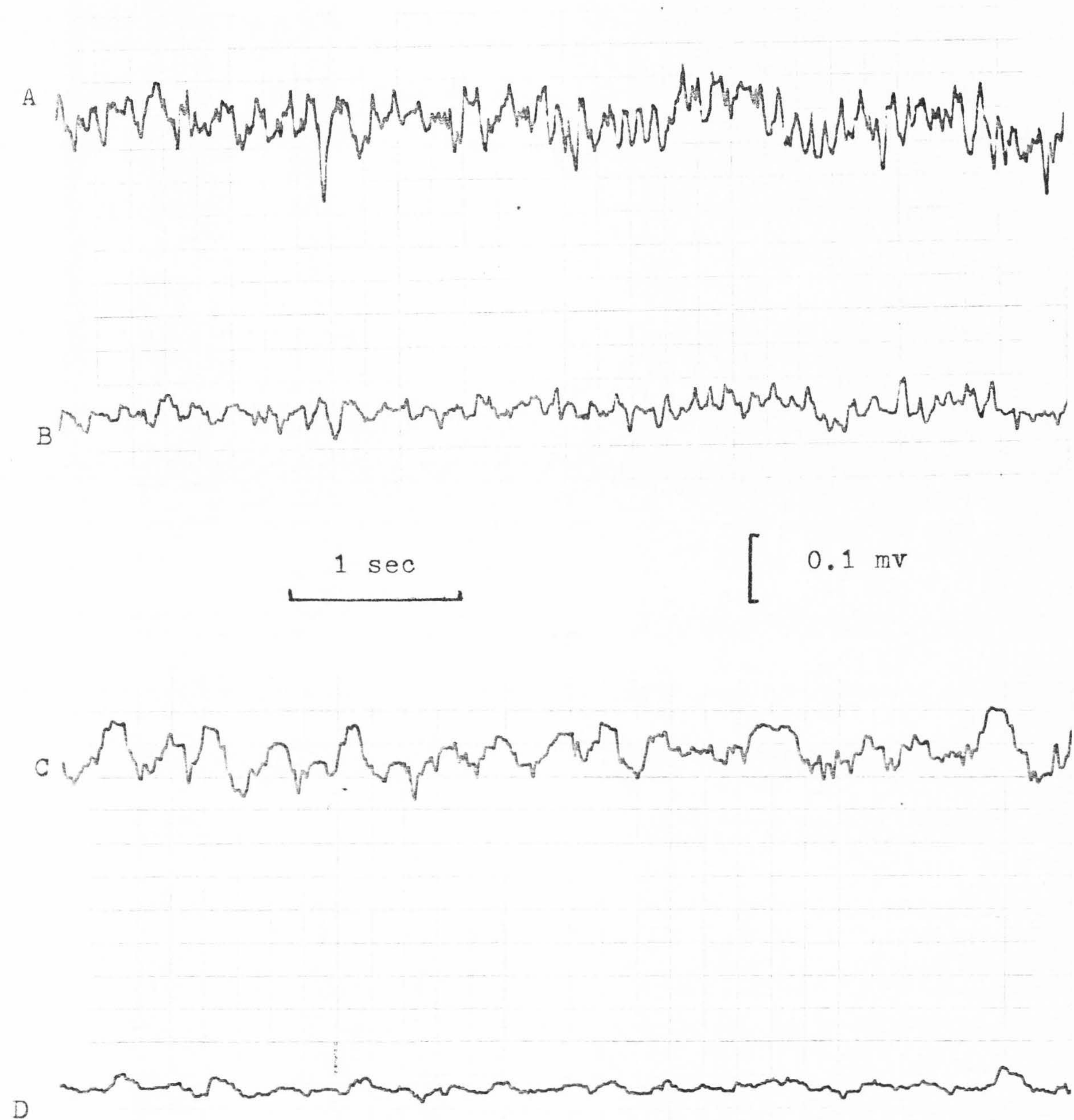


Figure 8. Appearance of slow wave EEG activity after injection of 30 mg/kg α-chaconine in a rat.

A. Parietal lead before injection.
B. Occipital lead before injection.
C. Parietal lead 3 hours after injection.
D. Occipital lead 3 hours after injection.

There was no difference in total power of any brain region (data shown in Appendix A). The averaged occipital power spectra for rats treated with 30 mg/kg show features which were observed in most cases (Figure 9). The reduction in overall power which was observed 7 hours after injection in this figure was general for controls and for all dosage levels, and was not related to α-chaconine levels. The disappearance of the 6 Hz peak after injection was common.

The relative predominance of the 6 Hz peak was measured by taking the ratios of the power at 6 Hz over the power at 5 Hz. This was done for individual rats in all cortical locations at each time interval. Table 1 scores the number of rats in which the 6 Hz/5 Hz ratio exceeded unity in each category out of three rats per dose level. Frontal records showed no pattern. Parietal records showed essentially uniform disappearance of the 6 Hz peak at all dose levels. The occipital record showed a marked response to α-chaconine with a dose-related disappearance of the 6 Hz peak. When the ratios were averaged and subjected to analysis of variance (Lapin, 1975), significant reduction in the 6 Hz/5 Hz power ratio appeared at the 10 mg/kg level (Table 2).

Clinical effects of α-chaconine. Results of visual observations of rats treated with sublethal doses are summarized in Table 3. One case of respiratory impairment was associated with a 3 mg/kg dose. Generalized symptoms were first seen at the 8 mg/kg level. Sedation, sluggishness, constriction of abdominal muscles, and impaired breathing were typical at the 20 mg/kg level. These symptoms were transitory. Only 3 of the 20 mg/kg rats showed symptoms after 4 hours: these displayed marked constriction of the abdominal muscles or mild sedation in the fifth hour after dosing.

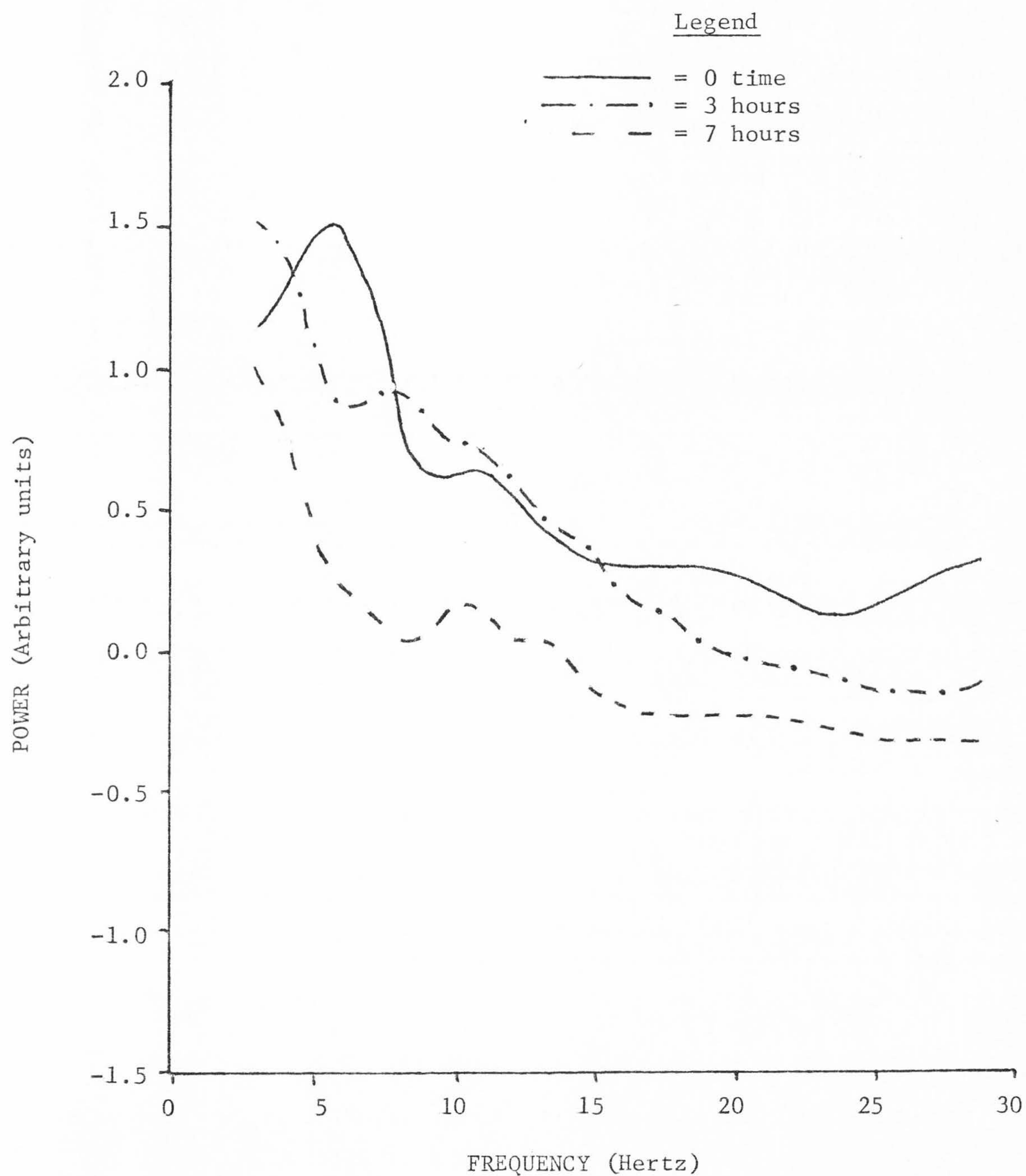


Figure 9. Averaged occipital power spectra for rats injected intraperitoneally with 30 mg/kg α-chaconine (n = 3).

Table 1. Changes in EEG power spectra after α-chaconine administration: number of rats in which power at 6 Hertz exceeded power at 5 Hertz at different dosages as a function of time[a]

Dose (mg/kg)	Frontal			Parietal			Occipital		
	Hours after injection								
	P[b]	3	7	P	3	7	P	3	7
0	0	0	0	1	0	1	1	1	2
10	1	0	0	3	0	0	3	2	0
20	0	0	1	1	0	0	2	0	0
30	0	0	1	3	0	0	3	0	0

[a]Three rats per dosage group.
[b]P = preinjection. Record taken immediately prior to injection.

Table 2. Effect of α-chaconine on power spectra of EEGs of the occipital cortex of rats[a]

Dose (mg/kg)	Hours after injection		
	Preinjection	3	7
0	0.985 ± 0.069	0.998 ± 0.115	1.070 ± 0.125
10	1.321 ± 0.090	0.894 ± 0.141	0.878* ± 0.028
20	1.156 ± 0.116	0.399* ± 0.041	0.572* ± 0.100
30	1.190 ± 0.072	0.607* ± 0.133	0.629* ± 0.069

[a]The ratio of power at 6 Hertz over power at 5 Hertz is given as the average for rats treated alike ± S.E.M., n = 3.
*Indicates values significantly different from preinjection levels ($p < 0.025$).

Table 3. Visually observed signs of toxicity of α-chaconine in rats[a]

Dose (mg/kg)	Symptoms			
	Sedation	Sluggishness	Abdominal muscle constriction	Impaired breathing
0	0	0	0	0
3	0	0	0	1
8	6	0	4	4
20	7	10	10	6

[a]Twelve rats per intraperitoneal dose level.

Neurotransmitter Assays

The influence of α-chaconine on whole rat brain ACh is presented in Table 4. No changes were associated with the toxin. An unexpected difference between the ACh levels in rats associated with the two preinjection intervals was significant according to the t test ($p < 0.025$). No systematic changes associated with dosage were observed in either preinjection group.

Table 4. Acetylcholine contents of rat brain following intraperitoneal administration of α-chaconine

Dose (mg/kg)	Hours after treatment[a]	
	3	12
0	16.1 ± 3.4	31.2 ± 5.8
3	20.0 ± 5.3	32.3 ± 8.0
8	25.2 ± 6.4	29.7 ± 7.0
20	16.3 ± 3.2	24.4 ± 3.1

[a]The average concentration in nanomoles/gram ± S.E.M. (n = 6).

The catecholamine levels did not vary significantly after doses of up to 20 mg/kg (Table 5); however, NE showed a downward trend in the 3-hour group. Serotonin and 5-HIAA were unaffected by α-chaconine treatment (Table 6).

Table 5. Catecholamine contents of rat brain following intraperitoneal administration of α-chaconine

Brain catecholamine	Dose (mg/kg)	Hours after treatment[a]	
		3	12
Norepinephrine	0	2.56 ± 0.10	2.95 ± 0.24
	3	2.71 ± 0.15	2.45 ± 0.08
	8	2.46 ± 0.10	2.97 ± 0.19
	20	2.27 ± 0.17	2.57 ± 0.19
Dopamine	0	4.13 ± 0.31	4.48 ± 0.45
	3	5.25 ± 0.30	4.38 ± 0.59
	8	4.94 ± 0.38	6.75 ± 1.10
	20	4.67 ± 0.82	5.07 ± 1.01

[a]The average concentration in nanomoles/gram ± S.E.M. (n = 6).

Table 6. Serotonin and 5-hydroxyindoleacetic acid contents of rat brain following intraperitoneal administration of α-chaconine

Brain amine	Dose (mg/kg)	Hours after treatment[a]	
		3	12
Serotonin	0	2.00 ± 0.16	1.78 ± 0.17
	3	2.09 ± 0.17	1.92 ± 0.22
	8	2.16 ± 0.04	1.87 ± 0.34
	20	1.94 ± 0.09	2.12 ± 0.13
5-Hydroxyindole-acetic acid	0	1.68 ± 0.54	1.80 ± 0.83
	3	2.30 ± 0.93	1.63 ± 0.51
	8	1.82 ± 0.29	2.01 ± 0.79
	20	1.67 ± 0.49	1.88 ± 0.82

[a]The average concentration in nanomoles/gram ± S.E.M. (n = 6).

DISCUSSION

Visually and electrophysiologically observed effects

A lethal dose of α-chaconine elicited symptoms in rats similar to those reported for α-solanine (Nishie et al., 1971) and for α-chaconine (Nishie et al., 1975) in rabbits. Heart rate and respiratory rate increased initially, after which the respiratory rate fell concurrently with the appearance of slow wave EEG activity. The heart continued to function after EEG and respiratory activity had ceased.

Signs of α-chaconine poisoning were apparent with doses as low as 8 to 10 mg/kg. They included a shift of the predominant EEG frequencies toward slow wave activity, especially apparent in the occipital cortex. The slow wave predominance associated with α-chaconine was apparent even at low doses, in contrast to typical effects of low levels of organophosphate AChE inhibitors, which cause an increase in high frequency activity (Hyde et al., 1978; Rieger and Okonek, 1975). Abdominal muscle constriction, tachycardia, impaired breathing and sedation were observed at the same level. Higher doses caused marked sluggishness. Of these symptoms, only abnormal EEGs and tachycardia persisted for more than 4 to 5 hours.

The heart was measurably slowed at doses of 20 to 30 mg/kg, suggestive of digitalis drugs at therapeutic levels (Rosen et al., 1975). This was a stark contrast to the effect of a lethal dose, 40 mg/kg, which increased the heart rate to 60 percent above normal. Blood pressure changes showed no consistent pattern at α-chaconine levels up

to 30 mg/kg. The lack of adverse cardiac effects at doses higher than sufficient to cause central and peripheral effects is evidence that CNS depression was not associated with heart effects. Slow wave activity appeared in the EEG at dose levels to small to be associated with anoxia. It is apparent that CNS depression is a primary effect of α-chaconine.

Neurotransmitter effects

Acetylcholine levels for rats sacrificed 3 hours after dosing were within the range reported for rats sacrificed by the near-freezing technique (Smith et al., 1975). Rats dosed 12 hours before sacrifice had ACh levels about 30 percent higher than reported for this sacrifice method. It is possible that the comparatively low ACh values of the 3-hour group compared to the 12-hour group was because the effects of the excitement of being handled and injected had not worn off before sacrifice. Tissues from the two groups were processed separately, so that differences may have resulted from batch-to-batch variation. There was no systematic dosage response within either group.

Brain concentration of α-chaconine has been shown to be near maximal during the period between 3 and 12 hours after dosing (Alozie, 1977). Since he showed that AChE was measurably inhibited in the range of 20 mg/kg α-chaconine, it would not have been surprising to have found a response reflected in ACh levels. This was not observed. It is possible that brain ACh levels had undergone a rapid rise and fall before 3 hours had elapsed. Studies by Trabucchi et al. (1975) showed marked reduction in ACh turnover after injection of physostigmine or the anti-muscarinic drug, oxytremorine, both of which cause initial

increases in brain ACh content. Such feedback inhibition could account for a return to normal ACh content in rat brain observed 24 hours after injection of the potent AChE inhibitor, diisopropylfluorophosphate (DFP), at which time brain AChE was still 91 percent inhibited (Kewitz et al., 1977). The turnover time of ACh varies from about 0.9 to 5.6 minutes in different brain parts (Stavinoha et al., 1976). It is not surprising that these and other investigators found brain ACh levels to reach a plateau within a few minutes after the administration of anti-AChE drugs: sufficient ACh to signal a need for a production slowdown was available within minutes. In view of the rapid turnover time of ACh and of the capacity of the brain to return to normal ACh levels even when over 90 percent of AChE is inhibited, it is not surprising that no alteration in ACh was observed 3 hours after injection. Whether or not the more transient symptoms associated with α-chaconine administration resulted from a brief increase in ACh can be resolved by measuring ACh levels at shorter intervals. The persistent CNS effects such as slow wave EEG patterns were found not to relate to alterations in ACh levels of whole brain.

It has been suggested that ACh content might not always change significantly in whole brain in response to a drug or toxin even when sufficient alteration of ACh might occur in key brain parts to have a pharmacological effect (Watanabe and Sharma, 1972; Modak et al., 1975). Sensitive radiochemical methods such as that of Goldberg and McCaman (1973) offer sufficient sensitivity to allow measurement of the ACh content of specific brain parts. A recent method for separating ACh into "free," "labile bound," and "stable bound" fractions has been reported (Kewitz et al., 1977), which could be used to detect changes in

ACh content within the cell in response to α-chaconine. Such a study could distinguish changes in the ACh pools considered to be more biologically significant (bound forms) from the "free" pool. It would also be useful to follow changes in the subcellular distribution of α-chaconine over time (0.5 to 12 hours) and the rates of recovery of AChE isoenzymes in the brain over time. Alozie (1977) reported both items in rats sacrificed 3 hours after dosing.

The apparent failure of other neurotransmitter levels to respond to α-chaconine injections suggests that persistent CNS changes were not associated with alterations in NE, DA or 5-HT content. The lack of change in 5-HIAA levels is evidence that 5-HT turnover was not greatly affected. A slight decrease in NE levels in the 20 mg/kg group, although not statistically significant, implied that alterations in levels of this biogenic amine may have been associated with α-chaconine toxicity at high dosages.

It is necessary to determine the relative importance of the central and peripheral effects of α-chaconine. It is possible to show pharmacological effects on the CNS when mortality results from a peripheral failure, typically neuromuscular block at the diaphragm. Subsequent investigations might well begin with physiological preparations designed to distinguish between the modes of toxicity. A good model for such experimentation is the test series of Douglas and Matthews (1952). They monitored the impulses of the phrenic nerve to follow the activity of the respiratory center. In other experiments they stimulated the same nerve to test the responsiveness of the diaphragm to nerve impulses. The vagus nerve activity could also be monitored in view of α-chaconine effects upon the heart. These investigations should precede any extensive continuance of studies of α-chaconine effects upon the brain.

SUMMARY AND CONCLUSIONS

Central nervous system effects of α-chaconine were investigated electrophysiologically and by determination of brain biogenic amines in response to acute ip injections of the glycoalkaloid. Visually observable manifestations of sedation and respiratory difficulties were noted.

A lethal dose of 40 mg/kg α-chaconine caused profound tachycardia, which persisted until the death of the animal. Respiration rate accelerated to a point, then fell simultaneously with the appearance of high-amplitude delta activity in the EEG, signaling anoxia. It was not clear whether peripheral respiratory failure, failure of the brain respiratory center, or heart effects were responsible for the death of the animal.

Sublethal doses caused marked but generally short-lived symptoms in rats. Eight mg/kg α-chaconine caused sedation, constriction of abdominal muscles, and respiratory difficulty. These symptoms were more pronounced and were accompanied by general motor sluggishness at the 20 mg/kg level. These effects did not persist for more than 4 hours except for a few of the rats administered 20 mg/kg.

Electrophysiological tests found cardiac and brain function to be significantly altered by sublethal doses of α-chaconine. Heart function was affected at doses as low as 10 mg/kg. Twenty to 30 mg/kg slowed the heart rate significantly, while 10 mg/kg caused tachycardia. Cardiac alterations at these dosages did not appear to be pathological. Respiration rate and blood pressure did not vary significantly in

response to α-chaconine. The EEG changed significantly at doses as low as 10 mg/kg. The major effect on EEG was a disappearance of the prominent 6-Hertz peak and a predominance of slow wave activity. The EEG and cardiac effects persisted longer than other observed physiological effects.

The effects of α-chaconine on brain biogenic amine levels were studied. Doses up to 20 mg/kg failed to significantly change levels of ACh, NE, DA, 5-HT, or of the serotonin metabolite 5-HIAA in animals sacrificed 3 or 12 hours after injection. The lack of alterations in ACh levels suggested that CNS effects were not associated with changes in brain ACh content. It was suggested that subsequent experimentation should involve physiological preparations capable of determining the major site of toxicity of α-chaconine. Whereas electrophysiological tests suggested that the CNS was the primary target of α-chaconine toxicity, brain amine assays offered few clues about the mechanisms of toxic effects.

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APPENDICES

Appendix A

Power Spectra of Rats at Three Cortical Locations

Table 7. Total power in power spectra of rats in the frequency range 3 to 29 Hertz at three cortical locations[a]

Dose (mg/kg)	Frontal			Parietal			Occipital		
	Hours after injection								
	P[b]	3	7	P	3	7	P	3	7[c]
0	715	1,499	425	1,618	1,735	224	213	150	22
	171	508	423	554	608	867	271	91	30
	72,979	714	374	1,178	870	1,066	205	180	40
10	424	386	371	608	612	559	206	211	22
	195	604	788	1,034	926	830	196	72	12
	143	195	252	311	285	732	102	38	6
20	416	399	409	661	415	516	203	84	18
	141	222	223	727	127	357	240	54	57
	1,602	236	373	360	307	564	133	49	2
30	631	88	467	791	968	581	187	254	71
	485	247	303	245	184	219	74	24	33
	469	454	297	369	906	488	243	144	13

[a]Values associated with three dosages of α-chaconine and controls are compared as a function of time. Spectral power of individual animals; three rats per group.
[b]P = preinjection measurement.
[c]Occipital values were consistently low in rats after 7 hours; not dose-related.

Appendix B

Program Used by Analog/Digital Computer to Digitize Voltage Recordings[1]

```
C       PROGRAM TO DIGITIZE THROUGH 3 A-D CHANNELS
        SCALED FRACTION A(3)
        DIMENSION ICDA(150),ICDB(150)
        DEFINE ICDA('30000),ICDB('31000)
        CALL QSHYIN(IERR,580)
        CALL QSC(1,IERR)
C       SELECT TAPE DRIVE '15
        CALL QMON(22,'15)
      1 TYPE 250
    250 FORMAT(60H ENTER SENSE CHANNEL AND FIRST INPUT CHANNEL'/)
        ACCEPT 251,ISEN,IN
    251 FORMAT(2I5)
     17 TYPE 830
    830 FORMAT(60H LOAD TAPE'/)
C       WRITE END OF FILE AT BEGINNING OF TAPE
        OCT 25000
        CALL QMON(15)
        IFILE=0
     10 TYPE 260
    260 FORMAT(60H ENTER RUN NO. AND NO. OF SAMPLES TO DIGITIZE'/)
C       IF RUN NO = 0 CLOSE TAPE AND REWIND
        ACCEPT 251,IRUNA,ISAMPL
        IF(IRUNA.GT.9999) GOTO 10
        IF (IRUNA.EQ.0) GO TO 501
        NREC=ISAMPL/50
        TYPE 205
    205 FORMAT(10HENTER DATA/)
      2 OCT 25000
        IFILE=IFILE+1
        IREC=0
C       SET UP RECORD PACKET 0 AND 1 FOR WRITING OUT ON TAPE '15
        LA  /'20010
        OCT 160760
        LA  /'30225
        OCT 160761
        LA  /'30000
        OCT 160762
        LA  /'20010
        OCT 160764
        LA  /'31225
        OCT 160765
        LA  /'31000
        OCT 160766
C       START THE SAMPLING LOOP
C       LOAD BUFFER ICDA
C        SET N=1
    203 OCT 20000
        STA N
C       SET INDEX REGISTER TO ZERO
        OCT 26740
        OCT 26500
C
C        READ SENSE LINE 0 AND TEST FOR HIGH ' IF HIGH SAMPLE, IF LOW LOOP
C       UNTIL HIGH IS SENSED
C
    201 OCT 2042
        AND ISEN
        OCT 27407
        J   .201
C        STORE INDEX REGISTER AS IR
        OCT 26500
        STA IR
```

[1]Credit for program belongs to Dr. A. Leon Huber, UWRL 123C, UMC 82, Utah State University. Computer type: EAI 590 System, Electronics Associates, Inc., West Longbranch, New Jersey.

```
C
C      SAMPLE DATA AND A-D CONVERT
C
       CALL ORBADS(A,IN,3,IERR)
C      RESTORE INDEX REGISTER
       LA  IR
       OCT 26500
       LA  A
       STA ICDA,1
       LA  A+1
       STA ICDA+1,1
       LA  A+2
       STA ICDA+2,1
C      INCREMENT INDEX REGISTER BY 3
       OCT 22003
C      ADD ONE TO N AND CHECK FOR FULL BUFFER ' IF FULL WRITE IT OUT AND
C      START FILLING BUFFER ICDB
       07  N
       LA  /51
       11  N
       OCT 27410
       J   .201
C
C      BUFFER ICDA IS FULL
C      CHECK STATUS OF DMAC --- ERROR MESSAGE IF NOT READY
C
       OCT 4037
       OCT 24100
       J   .150
  170  TYPE 280,IREC
  280  FORMAT(60H DATA TOO FAST FOR TAPE TRANSFER RATE'/
      $I5,60H  RECORD(S) WERE SUCCESSFULLY TRANSFERRED TO TAPE'/)
       PAUSE
       J   .401
C
C      WRITE ON TAPE '15 BUFFER ICDA
C
C      SELECT TAPE UNIT '15
  150  LA  /'30001
       OCT 5414
       OCT 1414
C      INCREMENT IREC BY 1 AND TEST TO SEE IF DONE
       07  IREC
       LA  NREC
       11  IREC
       OCT 27407
       J   .401
C      LOAD BUFFER ICDB
       OCT 20000
       STA N
C      SET INDEX REGISTER TO ZERO
       OCT 26740
       OCT 26500
  301  OCT 2042
       AND ISEN
       OCT 27407
       J   .301
       OCT 26500
C      STORE INDEX REGISTER AS IR
       STA IR
C
C      SAMPLE DATA AND A-D CONVERT
C
```

```
      CALL QPBADS(A,IN,3,IERR)
C
C     RESTORE INDEX REGISTER
      LA  IR
      OCT 26500
      LA  A
      STA ICDB,1
      LA  A+1
      STA ICDB+1,1
      LA  A+2
      STA ICDB+2,1
C     INCREMENT INDEX REGISTER BY 3
      OCT 22003
C     ADD ONE TO N AND CHECK FOR FULL BUFFER ! IF FULL WRITE IT OUT AND
C     START FILLING BUFFER ICDA
      07  N
      LA  /51
      11  N
      OCT 27410
      J    .301
C
C     BUFFER ICDB IS FULL
C     CHECK STATUS OF DMAC !!! ERROR MESSAGE IF NOT READY
C
      OCT 4037
      OCT 24100
      J    .248
      J    .170
C
C     WRITE ON TAPE '15 BUFFER ICDB
C
C      SELECT TAPE UNIT '15
  248 LA  /'30001
      OCT 5414
      OCT 1514
C     INCREMENT IREC BY 1 AND TEST TO SEE IF DONE
      07  IREC
      LA  NREC
      11  IREC
      OCT 27410
      J    .203
  401 DO 407 KK=1,10000
  407 OCT 27400
      CALL QMON(15)
      IPTS=IREC*50
C
C     PRINT RUN INFORMATION ON LINE PRINTER
C
      WRITE(6,402)IFILE,IRUNA,IPTS,IREC
  402 FORMAT(9H FILE NO.I4,5X3HRUNI5,I8,8H SAMPLESI8,8H RECORDS/)
      J    .10
  501 CALL QMON(15)
      CALL QMON(14)
      STOP
      END
```

Appendix C

Programs to Access IMSL Program, FTFREQ, to Print Results, and to Take Averages of Several Spectra[1]

```
MAR 22, 1978
17:10:11 LOGON5554             C335.  ORIGINATING LSN: 6  MCS: 1  SIGN ON BY NEW LOG ON

17:10:22 BOT   5555            "CANDE WRITER". (PROCESS)
17:10:22       5555 FIND       F.(CHUCKPROCESS.,KIND=PK64,MYUSE=IN)
17:10:23       5555 OPEN       OUTFILE.(OUTFILE.,KIND=LP,MYUSE=OUT)
17:10:24       5555 BLKEXIT    OUTFILE.(OUTFILE.,SF=0,BLKSZ=22,MRECSZ=22,UNITS=WDS)
17:10:24       5555 BLKEXIT    F.(CHUCKPROCESS.,SF=30,BLKSZ=420,MRECSZ=14,UNITS=WDS)
17:10:24 EOT   5555            0.488 SEC CPU, 1.018 SEC IO, KWSEC MEM: DATA = 3.784 CODE = 17.761
                               AVERAGE MEM: DATA = 2513 CODE = 11794 WORDS

17:10:26 BOT   5557            "CANDE WRITER". (PROCESS)
17:10:27       5557 FIND       F.(SPECTOUT.,KIND=PK64,MYUSE=IN)
17:10:28       5557 OPEN       OUTFILE.(OUTFILE.,KIND=LP,MYUSE=OUT)
17:10:29       5557 BLKEXIT    OUTFILE.(OUTFILE.,SF=0,BLKSZ=22,MRECSZ=22,UNITS=WDS)
17:10:29       5557 BLKEXIT    F.(SPECTOUT.,SF=30,BLKSZ=420,MRECSZ=14,UNITS=WDS)
17:10:29 EOT   5557            0.467 SEC CPU, 0.911 SEC IO, KWSEC MEM: DATA = 3.654 CODE = 16.255
                               AVERAGE MEM: DATA = 2651 CODE = 11795 WORDS

17:10:34 BOT   5558            "CANDE WRITER". (PROCESS)
17:10:34       5558 FIND       F.(SPECTCOMB.,KIND=PK64,MYUSE=IN)
17:10:35       5558 OPEN       OUTFILE.(OUTFILE.,KIND=LP,MYUSE=OUT)
17:10:35       5558 BLKEXIT    OUTFILE.(OUTFILE.,SF=0,BLKSZ=22,MRECSZ=22,UNITS=WDS)
17:10:35       5558 BLKEXIT    F.(SPECTCOMB.,SF=30,BLKSZ=420,MRECSZ=14,UNITS=WDS)
17:10:36 EOT   5558            0.448 SEC CPU, 0.768 SEC IO, KWSEC MEM: DATA = 3.099 CODE = 14.346
                               AVERAGE MEM: DATA = 2548 CODE = 11796 WORDS

17:10:39 LOGOF5554             PROC. TIME = 0.000 SEC, MEM: VIR. = 0.000 KW/SEC  SIGN OFF BY NORMAL LOG-OFF

ESTIMATED CHARGE FOR B6700 USAGE
CPU TIME                1.40 SECS   $      0.07 MEMORY               58.90 KW-SEC  $      0.07
I/O TIME                2.70 SECS   $      0.11 LINES PRINTED          187         $      0.11
CARDS READ              0           $      0.00 CARDS PUNCHED            0         $      0.00

*** TOTAL COST = $0.36
```

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[1] Raw data had previously been transformed from octal to decimal numbers and had been translated into a format compatible with the Burroughs 6700 Computer. Programmer was Mr. Michael R. Stephenson, Utah State University Computer Center.

```
100     $SET AUTOBIND                                                        00000100
200     $BIND = FROM IMSL/=                                                  00000200
300     FILE  13(KIND=DISK,MAXRECSIZE=151,BLOCKSIZE=151,AREASIZE=1,AREAS=2,  00000300
400     -        TITLE="SPECTRUMOUT.")                                       00000400
500     FILE  10(KIND=DISK,FILETYPE=8)                                       00000500
600     FILE  11(KIND=DISK,TITLE="RTITLES.",FILETYPE=8)                      00000600
700     FILE  12(KIND=DISK,TITLE="STITLES.",FILETYPE=8)                      00000700
800     FILE  6(KIND=PRINTER)                                                00000800
900     FILE  5(KIND=DISK,TITLE="PARAMETERS.",FILETYPE=8)                    00000900
1000    DIMENSION X1(600),X2(600),IND(6),XIND(2),XYMV(6),ACV(58),            00001000
1010    -  XFER(60),COHER(30),XYMV2(6),PS2(30),                              00001010
1100    -  FREQ(30),PS(60),XCOV(59),XSPECT(60),AMPHAS(60),                   00001100
1200    -  RTITLS(290),STITLS(290),RTITL(2),STITL(2)                         00001200
1300    DATA IND/0,600,0,29,0,0/,XIND/.0171,0/                               00001300
1400    READ(11,30)RTITLS                                                    00001400
1500    READ(12,30)STITLS                                                    00001500
1600    30 FORMAT(10A6)                                                      00001600
1700    10 READ(5,100,END=99)ICHAN,IRUN,LIMIT                                00001700
1800    100 FORMAT(I1,2I3)                                                   00001800
1900    ILOW=ICHAN                                                           00001900
2000    IHIGH=ICHAN                                                          00002000
2100    IF((ICHAN.LT.1).OR.(ICHAN.GT.3))GO TO 999                            00002100
2200    IF(ICHAN.NE.3)GO TO 15                                               00002200
2210    ILOW=1                                                               00002210
2220    IHIGH=2                                                              00002220
2300    15 DO 11 I=1,2                                                       00002300
2400    RTITL(I)=RTITLS(2*IRUN+I-2)                                          00002400
2500    11 STITL(I)=STITLS(2*IRUN+I-2)                                       00002500
2600    CHANGE(13,TITLE=STITL)                                               00002600
2700    CHANGE(10,TITLE=RTITL)                                               00002700
2800    READ(10,101,END=98)(X1(I),X2(I),I=1,LIMIT)                           00002800
2900    101 FORMAT(2I6)                                                      00002900
3000    98 NUMBR=I-1                                                         00003000
3100    DO 12 I=ILOW,IHIGH                                                   00003100
3200    IND(2)=NUMBR                                                         00003200
3300    IF(I.EQ.2)GO TO 13                                                   00003300
3500    CALL FTFREQ(X1,IND,XIND,XYMV,ACV,FREQ,PS,XCOV,XSPECT,                00003500
3600    -    AMPHAS,XFER,COHER,IER)                                          00003600
3700    GO TO 12                                                             00003700
3900    13 CALL FTFREQ(X2,IND,XIND,XYMV2,ACV,FREQ,PS2,XCOV,XSPECT,           00003900
4000    -    AMPHAS,XFER,COHER,IER)                                          00004000
4100    12 CONTINUE                                                          00004100
4200    WRITE(13,102)ICHAN,IRUN,LIMIT,NUMBR,XYMV(1),XYMV2(1),XYMV(2),        00004200
4210    -  XYMV2(2),(PS(J),PS2(J),J=1,30)                                    00004210
4300    102 FORMAT(I1,3I3,64E14.7)                                           00004300
4400    LOCK 13                                                              00004400
4410    CLOSE(10)                                                            00004410
4500    WRITE(6,103)ICHAN,IRUN,LIMIT,NUMBR                                   00004500
4600     103 FORMAT(///' PARAMETERS SPECIFIED WERE:'/' CHANNEL = ',I1/       00004600
4700    -  ' RUN NUMBER ',I3/,' NUMBER OF POINTS REQUESTED = ',I3/           00004700
4800    -  ' NUMBER OF POINTS USED = ',I3)                                   00004800
4900    DO 14 I=ILOW,IHIGH                                                   00004900
4910    IF(I.EQ.1)GO TO 14                                                   00004910
4914    DO 16 J=1,30                                                         00004914
4918    16 PS(J)=PS2(J)                                                      00004918
4922    DO 17 J=1,2                                                          00004922
4926    17 XYMV(J)=XYMV2(J)                                                  00004926
5000     14 WRITE(6,104)I,XYMV(1),XYMV(2),                                   00005000
```

```
5100      -  ( (J-1)/(2.0*IND(4)*XIND(1)),PS(J),J=1,30)                      00005100
5200       104 FORMAT(//' CHANNEL ',I1,':'//                                  00005200
5300      -  ' MEAN = ',E14.7,'     VARIANCE = ',E14.7/                       00005300
5400      -  ' POWER SPECTRA : '/'  FREQ     POWER'/                          00005400
5500      -(1X,F5.2,E14.7))                                                   00005500
5600      GO TO 10                                                            00005600
5700      999 WRITE(6,105)                                                    00005700
5800      105 FORMAT(' CHANNEL NUMBER IS OUT OF BOUNDS'/)                     00005800
5900      GO TO 10                                                            00005900
6000      99 STOP                                                             00006000
6100      END                                                                 00006100
```

```
 300   FILE  13(KIND=DISK,MAXRECSIZE=151,BLOCKSIZE=151,AREASIZE=1,AREAS=2,   00000300
 400   -          TITLE="SPECTRUMOUT.")                                       00000400
 700   FILE  12(KIND=DISK,TITLE="STITLES.",FILETYPE=8)                        00000700
 800   FILE  6(KIND=PRINTER)                                                  00000800
 900   FILE  5(KIND=DISK,TITLE="SPARAM.",FILETYPE=8)                          00000900
1000   DIMENSION FS(30),PS2(30),XYMV(2),XYMV2(2),STITLS(290),STITL(2)         00001000
1500   READ(12,30)STITLS                                                      00001500
1600   30 FORMAT(10A6)                                                        00001600
1700   10 READ(5,100,END=99)ICHAN,IRUN,ISLOW,ISHIGH                           00001700
1800   100 FORMAT(I1,I3,2I2)                                                  00001800
1900   ILOW=ICHAN                                                             00001900
2000   IHIGH=ICHAN                                                            00002000
2100   IF((ICHAN.LT.1).OR.(ICHAN.GT.3))GO TO 999                              00002100
2200   IF(ICHAN.NE.3)GO TO 15                                                 00002200
2210   ILOW=1                                                                 00002210
2220   IHIGH=2                                                                00002220
2300   15 DO 11 I=1,2                                                         00002300
2500   11 STITL(I)=STITLS(2*IRUN+I-2)                                         00002500
2600   CHANGE(13,TITLE=STITL)                                                 00002600
4200   READ(13,102)ICHAN,IRUN,LIMIT,NUMBR,XYMV(1),XYMV2(1),XYMV(2),           00004200
4210   -  XYMV2(2),(PS(J),PS2(J),J=1,30)                                      00004210
4300   102 FORMAT(I1,3I3,64E14.7)                                             00004300
4400   CLOSE(13)                                                              00004400
4500   WRITE(6,103)ICHAN,IRUN,ISLOW,ISHIGH,NUMBR                              00004500
4600    103 FORMAT(///' PARAMETERS SPECIFIED WERE:'/' CHANNEL = ',I1/         00004600
4700   -  ' RUN NUMBER 'I3,/' NORMALIZATION STEP RANGE:',I2,' TO ',I2/        00004700
4800   -  ' NUMBER OF POINTS USED = ',I3)                                     00004800
4802   SUM1=0.                                                                00004802
4804   SUM2=0.                                                                00004804
4806   DO 21 I=ISLOW,ISHIGH                                                   00004806
4808   SUM1=SUM1+PS(I)                                                        00004808
4810   21 SUM2=SUM2+PS2(I)                                                    00004810
4812   IF(SUM1.EQ.0)SUM1=1.                                                   00004812
4814   IF(SUM2.EQ.0)SUM2=1.                                                   00004814
4816   DO 22 I=ISLOW,ISHIGH                                                   00004816
4818   PS(I)=PS(I)/SUM1                                                       00004818
4820   22 PS2(I)=PS2(I)/SUM2                                                  00004820
4900   DO 14 I=ILOW,IHIGH                                                     00004900
4910   IF(I.EQ.1)GO TO 14                                                     00004910
4914   DO 16 J=1,30                                                           00004914
4918   16 PS(J)=PS2(J)                                                        00004918
4922   DO 17 J=1,2                                                            00004922
4926   17 XYMV(J)=XYMV2(J)                                                    00004926
5000    14 WRITE(6,104)I,XYMV(1),XYMV(2),                                     00005000
5100   -  ( (J-1)/(2.0*29*.0171),PS(J),J=ISLOW,ISHIGH)                        00005100
5200    104 FORMAT(//' CHANNEL ',I1,':'//                                     00005200
5300   -  ' MEAN = ',E14.7,'    VARIANCE = ',E14.7/                           00005300
5400   -  ' POWER SPECTRA : '/'  FREQ     POWER'/                             00005400
5500   -(1X,F5.2,2X,F7.6))                                                    00005500
5600   GO TO 10                                                               00005600
5700   999 WRITE(6,105)                                                       00005700
5800   105 FORMAT(' CHANNEL NUMBER IS OUT OF BOUNDS'/)                        00005800
5900   GO TO 10                                                               00005900
6000   99 STOP                                                                00006000
6100   END                                                                    00006100
```

```
 100    FILE  13(KIND=DISK,MAXRECSIZE=151,BLOCKSIZE=151,AREASIZE=1,AREAS=2,    00000100
 200    *         TITLE="SPECTRUMOUT.")                                        00000200
 300    FILE  12(KIND=DISK,TITLE="STITLES.",FILETYPE=8)                        00000300
 400    FILE  6(KIND=PRINTER)                                                  00000400
 600    FREQ(K)=(K-1)/(2.0*29.0*.0171)                                         00000600
 700    DIMENSION STITLS(290),PSIN(30),STITL(2),PS(60),XY(4)                   00000700
 900    DATA ISLOW/4/ISHIGH/30/                                                00000900
1000    READ(12,30)STITLS                                                      00001000
1100    30 FORMAT(10A6)                                                        00001100
1200    10 READ(5,110,END=99)NUMBER                                            00001200
1300    110 FORMAT(I2)                                                         00001300
1400    WRITE(6,112)NUMBER                                                     00001400
1500    112 FORMAT(///' THE NUMBER OF RUNS COMBINED IS ',I2/)                  00001500
1600    DO 61 L=1,30                                                           00001600
1700    61 PSIN(L)=0.0                                                         00001700
1900    DO 60 M=1,NUMBER                                                       00001900
2000    READ(5,100,END=99)ICHAN,IRUN                                           00002000
2100    WRITE(6,113)M,ICHAN,IRUN                                               00002100
2200    113 FORMAT(1X,I2,': CHANNEL ',I1,3X,'RUN # ',I3)                       00002200
2300    100 FORMAT(I1,I3)                                                      00002300
2900    15 DO 11 I=1,2                                                         00002900
3000    11 STITL(I)=STITLS(2*IRUN+I-2)                                         00003000
3100    CHANGE(13,TITLE=STITL)                                                 00003100
3200     READ(13,102)IC,IR,LIMIT,NUMBR,XY,(PS(K),PS(K+30),K=1,30)              00003200
3400    102 FORMAT(I1,3I3,64E14.7)                                             00003400
3500    CLOSE(13)                                                              00003500
3600    SUM1=0.                                                                00003600
3700    J=(ICHAN-1)*30                                                         00003700
3800    DO 21 I=ISLOW,ISHIGH                                                   00003800
3900    21 SUM1=SUM1+PS(I+J)                                                   00003900
4100    IF(SUM1.EQ.0)SUM1=1.                                                   00004100
4300    DO 22 I=ISLOW,ISHIGH                                                   00004300
4400     22 PS(I+J)=PS(I+J)/SUM1                                               00004400
4600    DO 50 K=1,30                                                           00004600
4700    50 PSIN(K)=PSIN(K)+PS(K+J)                                             00004700
4900    60 CONTINUE                                                            00004900
5000    WRITE(6,117)                                                           00005000
5100     117 FORMAT(/'  FREQ',3X,'NORM. P.S.')                                 00005100
5200    SUM1=0.0                                                               00005200
5400    DO 62 K=ISLOW,ISHIGH                                                   00005400
5500    SUM1=SUM1+PSIN(K)                                                      00005500
5600     62 CONTINUE                                                           00005600
5700    IF (SUM1.EQ.0)SUM1=1.0                                                 00005700
5800    IF(SUM2.EQ.0)SUM2=1.0                                                  00005800
5900    AVG1=0.0                                                               00005900
6000    AVG2=0.0                                                               00006000
6100    DO 63 K=ISLOW,ISHIGH                                                   00006100
6200    PSIN(K)=PSIN(K)/SUM1                                                   00006200
6400    63 AVG1=AVG1+PSIN(K)*FREQ(K)                                           00006400
6600    WRITE(6,115)( FREQ(K),PSIN(K),K=ISLOW,ISHIGH)                          00006600
6700    115 FORMAT(F6.2,E14.7)                                                 00006700
6800    WRITE(6,116)AVG1                                                       00006800
6900     116 FORMAT(/' AVERAGE =',E14.7)                                       00006900
7000    GO TO 10                                                               00007000
7100     99 WRITE(6,/)" I QUIT"                                                00007100
7200    END                                                                    00007200
```

Appendix D

Typical Output of Power Spectra[1]

```
THE NUMBER OF RUNS COMBINED IS  6

 1: CHANNEL 2    RUN # 113
 2: CHANNEL 1    RUN # 115
 3: CHANNEL 2    RUN # 125
 4: CHANNEL 1    RUN # 127
 5: CHANNEL 2    RUN # 137
 6: CHANNEL 1    RUN # 139

FREQ   NORM. P.S.
 3.02  .1554984E+00
 4.03  .1398009E+00
 5.04  .1288278E+00
 6.05  .1114247E+00
 7.06  .7480361E-01
 8.07  .5237145E-01
 9.07  .4088602E-01
10.08  .3817709E-01
11.09  .3412081E-01
12.10  .2809261E-01
13.11  .2276824E-01
14.12  .2089760E-01
15.12  .1893564E-01
16.13  .1585517E-01
17.14  .1436732E-01
18.15  .1357068E-01
19.16  .1212896E-01
20.17  .1182477E-01
21.17  .1086811E-01
22.18  .9347332E-02
23.19  .7609276E-02
24.20  .7118785E-02
25.21  .6853973E-02
26.21  .6542724E-02
27.22  .5770704E-02
28.23  .5830718E-02
29.24  .5706617E-02

AVERAGE =  .8500361E+01
```

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[1] In this case the column under the heading NORM. P.S. represents the averaged values for six power spectra which had previously been "normalized" so that total power in the range from 3.02 Hertz to 29.24 Hertz was scaled to unity.

VITA

Charles Newell Aldous III

Candidate for the Degree of

Master of Science

Thesis: Central Nervous System Toxicity of Alpha-Chaconine in Rats

Major Field: Toxicology

Biographical Information:

Personal Data: Born in Camden, New Jersey, on November 18, 1949, son of C. Newell and Verna G. Aldous.

Education: Graduated from Woodside High School, Woodside, California, in June, 1967; received the Bachelor of Science degree from University of California, Davis, in December, 1973, in Biological Sciences; completed requirements for the Master of Science degree at Utah State University in 1979.

Professional Experience: 1975-1977, Research Assistant, Toxicology curriculum, Utah State University, Logan, Utah; summers of 1974, 1975, laboratory aide, California State Pesticide Residue Laboratories.