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Modification of Acrylamide Neuropathy in Rats by Selected Factors[1,2]

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Modification of Acrylamide Neuropathy in Rats by Selected Factors. KAPLAN, M. L., MURPHY, S. D. AND GILLES, F. H. (1973). *Toxicol. Appl. Pharmacol.* **24**, 564–579. A modified rotarod technique was used to determine whether dietary deficiencies in pyridoxine or thiamine, bilateral adrenalectomy or cortisol treatment and pretreatment with microsomal enzyme inducers (DDT or phenobarbital) would modify the course of onset and recovery from functional acrylamide neuropathy in rats. Neither pyridoxine or thiamine deficiency nor daily injections of cortisol had any measurable effect on the cumulative dose of acrylamide required to produce functional impairment. Although adrenalectomized animals were more susceptible to acrylamide, the effect seemed nonspecific. The total cumulative doses of acrylamide required to produce neurologic deficit in DDT- and phenobarbital-pretreated rats were 520 and 600 mg/kg, respectively, compared to 360 mg/kg for the controls. Hepatic in vitro metabolism of acrylamide was studied to determine whether the observed delay could be explained by an increased capacity of the liver to detoxify acrylamide. There was a greater loss of acrylamide from incubation mixtures containing 9000 *g* supernatants from phenobarbital-pretreated rat livers than from control livers. Histologic studies of peripheral nerves from acrylamide-treated rats revealed that at the time of onset of functional impairment young and phenobarbital-pretreated adult rats had severe peripheral nerve damage, while no discernible peripheral nerve injury was seen in unpretreated adult rats.

Acrylamide is an industrial chemical widely used in the production of high molecular weight, water-insoluble polymers. The monomeric form is water soluble and can be neurotoxic in man (Fujita et al., 1960; Auld and Bedwell, 1967; Garland and Patterson, 1967). The neurotoxic effects are cumulative and can be produced by any route of administration (Hamblin, 1956). In laboratory animals acrylamide causes a "dying back" type of peripheral neuropathy (Fullerton and Barnes, 1966; Leswing and Ribelin, 1969; Prineas, 1969; Hopkins, 1970). There is a propensity for the distal parts of long and large-diameter peripheral nerve fibers to be affected first. The anatomical changes

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seen in the peripheral nerves are similar to Wallerian degeneration in that both axons and myelin degenerate (Fullerton and Barnes, 1966; Fullerton, 1969; Prineas, 1969; Hopkins, 1970).

In a previous investigation a modified rotarod apparatus (electrorod) was used to objectively measure the onset of neurologic deficit and functional recovery in rats treated with acrylamide (Kaplan and Murphy, 1972). The loss of ability of rats to maintain their balance on a rotating rod was the criterion for functional impairment. The present study was concerned with determining the influence of selected factors on the course of development and recovery from acrylamide poisoning. It was felt that factors which altered the rate of onset or recovery from neurologic deficit might indirectly lead to a better understanding of acrylamide neurotoxicity.

Glees (1961) reported that cortisone acetate had a therapeutic effect when given to chickens poisoned with triorthocresyl phosphate (TOCP). Since acrylamide and TOCP both selectively damage the distal ends of the longest peripheral nerve fibers first, it seemed that they might share a common mechanism of neurotoxicity. Both thiamine deficiency and isonicotinic acid hydrazide (isoniazid) administration have resulted in the distal degeneration of long peripheral nerve fibers (North and Sinclair, 1956; Cavanagh, 1967). Isoniazid interferes with pyridoxine metabolism, and decreased levels of pyridoxal phosphate result (Cavanagh, 1969; Thomas, 1970). Thus, it was considered possible that acrylamide could produce its neurotoxic effects by interference with vitamins essential for the integrity of nervous system function. Many foreign organic chemicals are known to be metabolized by enzymes of the smooth endoplasmic reticulum of hepatic cells (Conney, 1967; Mannering, 1968; Kuntzmann, 1969). The biotransformation and metabolic fate of acrylamide have not been extensively studied (Barnes, 1969; Hashimoto and Aldridge, 1970); however, if acrylamide was metabolized by the liver, it seemed possible that the intensity or duration of its neurotoxic effects might be influenced by compounds capable of increasing liver microsomal enzyme activity. It was for these reasons that the following factors were selected and tested for their effects on the time course of functional impairment produced by the cumulative administration of acrylamide: bilateral adrenalectomy, cortisol treatment, pyridoxine or thiamine deficiency, and pretreatment with DDT or phenobarbital (microsomal enzyme inducers).

A significant delay in the onset of neurologic deficit produced by acrylamide was observed in rats pretreated with microsomal enzyme inducers. Therefore, the hepatic in vitro metabolism of acrylamide was studied to determine whether this delay could be explained on the basis of an increased capacity of the liver to metabolize acrylamide. A histologic study was also carried out in an attempt to establish the degree of peripheral nerve injury at the time of onset and recovery from the functional impairment produced by acrylamide poisoning.

METHODS

Animals and measurements of neurologic deficit. For most experiments, male Holtzman rats (200–300 g) were used. They were housed in air-conditioned rooms and supplied with food and water ad libitum.

The rotarod apparatus used to measure neurologic deficit has been described previously (Kaplan and Murphy, 1972). The procedure involved the use of a partitioned

enclosure containing an electrode floor and a rod which turned at 8 rpm. Rats were trained to maintain their balance on the rod for three trial periods each lasting 2 min. During the administration of acrylamide, a rat was considered to have failed if he fell during any two of the three trials. When a fallen rat regained his ability to stay on the rod in subsequent trials, after acrylamide was discontinued, it was considered an index of functional recovery. The mean days until failure and recovery were tested for statistical significance using Student's *t* test or an analysis of variance.

Treatments. Aqueous solutions of acrylamide[4] were administered daily by ip injection until all the rats in a group had failed. Cortisol[5] was suspended in corn oil and given by sc injection. A single ip dose of 200 mg/kg of DDT[6] in corn oil was given 5 days prior to acrylamide administration. Aqueous ip injections of 50 mg/kg of phenobarbital[7] were given daily for 5 days prior to acrylamide and for the cumulative experiments were also continued simultaneously with acrylamide administration. A single ip injection of 20 mg/kg of SKF-525A[8] in water was given 1 hr before the challenge dose of acrylamide. All injections were given in concentrations which provided the appropriate dose in an injection volume equivalent to 0.1% of the body weight.

Bilateral adrenalectomies and sham operations were performed on previously trained rats 10 days prior to the administration of acrylamide. Adrenalectomized animals were maintained on drinking water containing 0.9% NaCl. Three days after surgery training was resumed, and prior to acrylamide administration no failures were observed in either adrenalectomized or sham-operated rats.

For vitamin deficiency experiments rats were fed either a complete high-protein diet[9] or diets with either pyridoxine or thiamine omitted. A decrease in the rate of growth in rats fed the incomplete diets was the criterion used for evidence of vitamin deficiency.

In vitro metabolism. Rat liver homogenates (20% w/v) were prepared in cold, isotonic (1.15%) KCl and centrifuged at 9000 *g* for 20 min at 0°C; the supernatant fraction was saved. The incubation mixture consisted of 1 ml supernatant, 1 ml 0.05 M phosphate buffer, pH 7.4, 200 μg acrylamide and 1 ml of a cofactor mixture containing 60 μmol nicotinamide, 15 μmol $MgCl_2$, 30 μmol glucose-6-phosphate and 0.9 μmol NADP. The preparation of supernatant, buffer and concentration of cofactors used has been previously described for other substrates (Kato and Takanaka, 1967). Incubations were carried out in 25-ml Erlenmeyer flasks shaken in a water bath at 37°C. Tissue and substrate blank incubations were included, omitting either the acrylamide or supernatant fraction, respectively. After 0 and 60 min the reaction was stopped by the addition of 3 ml methanol and the mixtures were centrifuged at 800 *g* for 15 min. The clear supernatants were decanted into calibrated centrifuge tubes and reduced to a final volume of

[4] Eastman Special grade for electrophoresis, purchased from Eastman Organic Chemical Co., Rochester, New York.

[5] Solu-cortef (hydrocortisone sodium succinate), purchased from the Upjohn Co., Kalamazoo, Michigan.

[6] Technical grade, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (77.2% *p,p'*-isomer), purchased from Nutritional Biochemical Corp., Cleveland, Ohio.

[7] USP crystalline powder, purchased from Merck Co., Rahway, New Jersey.

[8] 2-Diethylaminoethyl-2,2-diphenylvalerate HCl, supplied by Smith, Kline and French Laboratories, Philadelphia, Pennsylvania.

[9] Each kilogram of diet consisted of 25% vitamin-free casein, 60.7% sucrose, 9% corn oil, 1% cod liver oil, 4% Hegsted salt IV (Hegsted et al., 1941), 0.3% choline and a complete vitamin mix containing 4 mg thiamine HCl, 8 mg riboflavin, 40 mg niacin, 20 mg Ca pantothenate, 1 mg folic acid, 1 mg menadione, 0.2 mg biotin, 0.05 mg vitamin B_{12} and 4 mg pyridoxine HCl.

0.2 ml using an Evapo-mix[10] at 50°C and 25 mm Hg. Ten microliters of these concentrated supernatants were applied to silica gel thin-layer chromatographic plates (0.25 mm with fluorescent indicator)[11] and eluted in an ethanol–ammonia (5:1) solvent system. Acrylamide and nicotinamide were found to migrate to the same spot on the plate and could not be distinguished by their abilities to quench fluorescence. However, in reference spots containing acrylamide–nicotinamide mixtures, only the acrylamide has the ability to reduce the potassium permanganate spray reagent (0.01% solution in acetone) described by Croll (1971), and as little as 0.25 μg could be detected by spraying the spot and observing the appearance of a white area in a light purple background. The located spots (quenched areas containing both acrylamide and nicotinamide) were scraped off the glass plate into centrifuge tubes and eluted with 1 ml of an aqueous methanol solution (80%). The tubes were centrifuged at 800 *g* for 5 min, and clear 0.5-ml aliquots were removed and transferred to graduated centrifuge tubes; 1 ml of a 0.1% solution of tris(hydroxymethyl)aminomethane in methanol was added. The mixtures were placed in a fume hood, and 2 ml of a freshly prepared 1.5% ethereal–alcoholic solution of diazomethane[12] was added. Color was developed with 1 ml of a 0.1% solution of dimethylaminocinnamaldehyde in absolute ethanol and HCl. A Gilford spectrophotometer (240) set at 538 nm was used to determine the absorbance of each tube according to the method of Mattocks (1968). Acrylamide absorbance could be obtained by subtracting the appropriate tissue blank, and because nicotinamide did not produce color in this system it caused no interference. The quantity of acrylamide lost during 60 min of incubation was calculated by subtracting the corrected 0-time from the corrected 60-min absorbance. A standard curve for acrylamide in absolute ethanol was used to convert absorbance to micrograms.

Hexobarbital oxidase activity for each 9000 *g* supernatant used in the in vitro experiment was determined as described by Furner *et al.* (1969).

Histology. Acrylamide-treated and control rats used for histologic examinations were sacrificed by decapitation and exsanguination. Sections (1 cm) of right and left posterior tibial nerves were taken proximally from the tarsocrural joint and either fixed in 10% neutral buffered formalin or embedded in gum tragacanth and rapidly frozen in isopentane at −70°C. Axons were demonstrated by Bielschowsky's silver stain for neurofibrils (McManus and Mowry, 1960) and the frozen sections were stained with Oil Red O dye for neutral lipids (Lillie and Ashburn, 1943). The samples were mounted on slides, masked so that their source could not be identified and independently graded by two observers as negative, one plus or two plus according to the degree of peripheral nerve damage present.

RESULTS

Effect of Bilateral Adrenalectomy and Treatment with Cortisol on Functional Deficit Induced by Acrylamide

The cumulative dose responses for 7 adrenalectomized and 5 sham-operated rats given 50 mg/kg/day of acrylamide are shown in Fig. 1. The mean times for failure were

[10] Buchler Instrument, Fort Lee, New Jersey.

[11] Sil-G-25 UV_{254}, Brinkmann Instrument, Westbury, New York.

[12] This compound is explosive (Stecher et al., 1968) and carcinogenic (Hueper and Conway, 1964; Arcos et al., 1968). Therefore all work with it was carried out behind a safety shield in a fume hood.

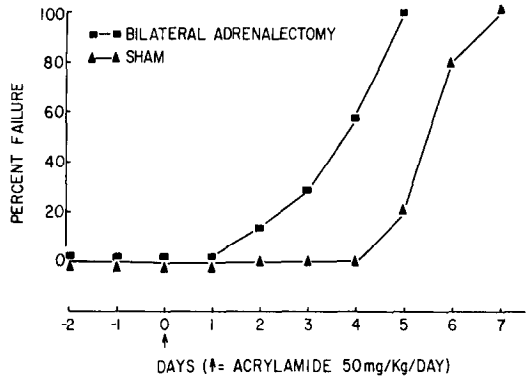

FIG. 1. Onset of acrylamide toxicity in adrenalectomized and sham-operated rats as measured by failure to maintain balance on a rotarod. Acrylamide was started at arrow (day 0) and continued daily in 7 adrenalectomized and 5 sham-operated rats until the 100% response in each group.

4.0 ± 0.42 and 6.0 ± 0.31 days, respectively ($t = 3.5$, $p < 0.01$). Adrenalectomized rats were more susceptible to acrylamide, and the entire group became cachectic and died 3 days after receiving a total cumulative dose of 300 mg/kg. No mortalities were observed in the sham-operated rats although they received an additional 100 mg/kg of acrylamide. The cumulative dose response (not shown) for another group of 6 adrenalectomized rats that were given 0.1 mg of cortisol twice daily postoperatively (maintenance dose of Barnes and Eltherington, 1966) in addition to 50 mg/kg/day of acrylamide, was essentially the same as for the adrenalectomized rats not treated with cortisol.

In Figure 2 the effect of acrylamide in 9 rats given 25 mg/kg of cortisol daily is compared to 8 corn oil-treated controls. Neither the time for onset nor the recovery from the neurologic deficit produced by 50 mg/kg/day of acrylamide was influenced by cortisol administration.

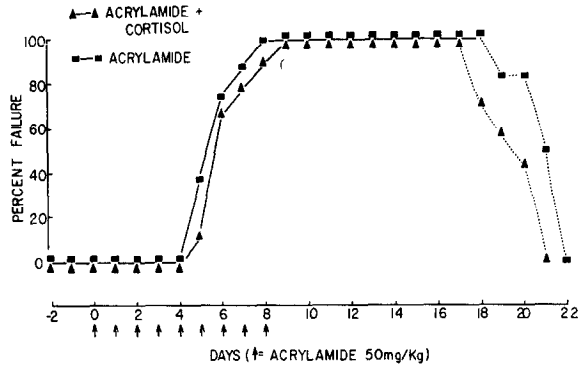

FIG. 2. Effect of cortisol treatment on acrylamide toxicity. Cortisol (25 mg/kg) and acrylamide were administered daily to 9 rats. Eight corn oil-treated controls were also given acrylamide. All injections of acrylamide were given until the 100% response in each group. Recovery curves are indicated by broken lines.

Effect of Vitamin Deficiency on Functional Deficit Induced by Acrylamide

Three groups of 14 young male rats (70–80 g) were placed on either a complete high protein diet or diets that were deficient in thiamine or pyridoxine. When apparent vitamin deficiency was observed, 8 animals in each vitamin-deficient group and controls

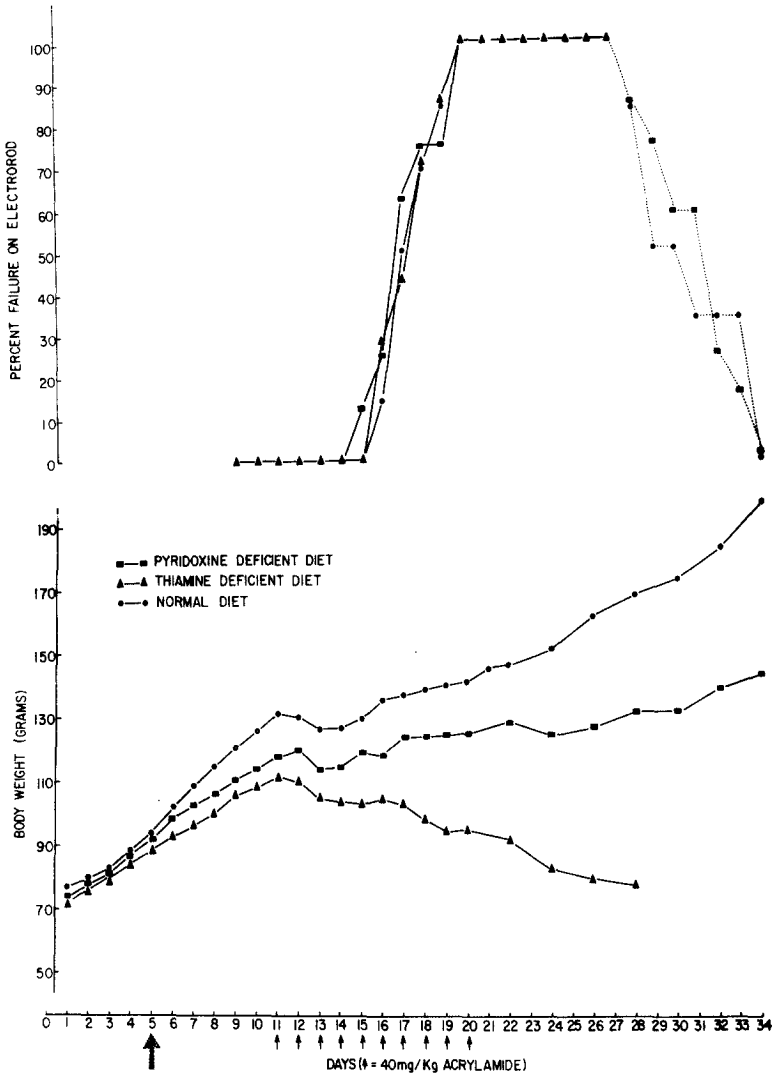

FIG. 3. Effect of pyridoxine or thiamine deficiency on acrylamide toxicity. At large arrow 2 groups of 8 five-week-old male rats were placed on vitamin-deficient diets while another group of 14 continued to receive the complete diet. Recovery curves for pyridoxine deficient and control rats are indicated by broken lines.

were given 40 mg/kg of acrylamide daily. Six vitamin-deficient rats in each group were not treated with acrylamide. As shown in Fig. 3, the response curves for all acrylamide-treated rats were almost identical. No failures were observed in the vitamin-deficient rats that were not treated with acrylamide. The thiamine-deficient rats in both treated

and untreated groups became moribund and died during the last 10 days of the experiment.

Effect of Hepatic Microsomal Enzyme Inducers on Functional Deficit Induced by Acrylamide

Phenobarbital sodium was given to 9 rats at the rate of 50 mg/kg/day for 20 days. These rats were also injected with 40 mg/kg of acrylamide daily starting on day 6 of phenobarbital pretreatment. Another group of 10 rats was given a single dose of 200 mg/kg of DDT 5 days prior to acrylamide administration. Ten control rats received either a single injection of corn oil or daily injections of distilled water in addition to acrylamide.

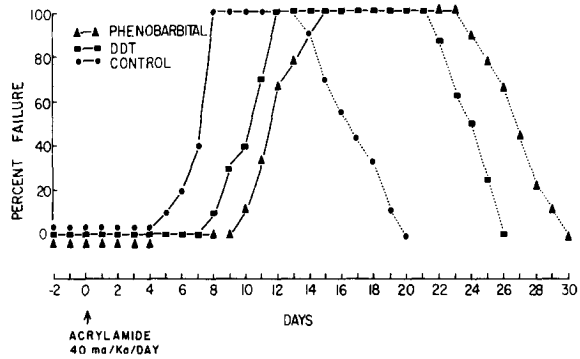

FIG. 4. Effect of microsomal enzyme inducers on acrylamide toxicity. Acrylamide was started at arrow (day 0) and continued daily in 9 phenobarbital pretreated, 10 DDT pretreated and 10 control rats until the 100% response in each group. Recovery curves are indicated by broken lines.

Figure 4 shows that pretreatment with DDT or phenobarbital delayed the onset of neurologic deficit significantly ($F = 9.2$, $p < 0.01$). The total cumulative dose of acrylamide required for the 100% failure of control rats was 360 mg/kg compared to 520 and 600 mg/kg for the DDT and phenobarbital-pretreated animals, respectively. No failures were observed in rats given only DDT or phenobarbital.

Reduced hexobarbital sleep times can be used to provide indirect evidence for hepatic microsomal enzyme induction in rats (Mannering, 1968). Twelve additional pretreated

TABLE 1

PHENOBARBITAL PROTECTION IN ACRYLAMIDE-TREATED RATS

Acrylamide (mg/kg/day)	Phenobarbital[a] (50 mg/kg/day)	Mean day for		Total cumulative dose (mg/kg)
		Onset	Recovery	
30	–	10.7 ± 0.36	23.4 ± 0.69	390
40	–	6.7 ± 0.33	18.0 ± 0.57	360
40	+	12.3 ± 0.47	28.1 ± 0.67	600
60	+	6.0 ± 0.40	17.6 ± 0.63	480

[a] Phenobarbital started 5 days before and continued simultaneously with acrylamide.

rats from each group and their respective controls were not treated with acrylamide but were given 100 mg/kg of hexobarbital[13] ip on the first experimental day (day 0 in Fig. 4), and their sleep times were compared. Phenobarbital-pretreated and control rats slept 6.4 ± 1.3 and 19.8 ± 1.8 min, respectively ($t = 5.5$, $p < 0.01$) while DDT-pretreated and control rats slept 7.0 ± 1.1 and 18.2 ± 1.7 min, respectively ($t = 5.6$, $p < 0.01$).

To determine the extent of phenobarbital protection, two groups of 9 adult rats were given daily injections of either 30 or 40 mg/kg of acrylamide and their rotarod performance was compared to two groups of 9 phenobarbital-pretreated adult rats given daily injections of 40 or 60 mg/kg of acrylamide. Table 1 shows that the times for onset and recovery from neurologic deficit produced by acrylamide were the same for adult rats given 40 mg/kg of acrylamide daily and for phenobarbital-pretreated adult rats given acrylamide at the rate of 60 mg/kg/day. The phenobarbital-pretreated adult rats given 40 mg/kg of acrylamide daily took almost twice as long for onset of functional deficit when compared to unpretreated adult rats receiving the same daily dose of acrylamide. On this basis the extent of protection by phenobarbital in acrylamide poisoning was estimated to be on the order of 30–50%.

TABLE 2

IN VITRO METABOLISM OF ACRYLAMIDE AND HEXOBARBITAL BY 900 *g* SUPERNATANTS FROM PHENOBARBITAL-PRETREATED AND CONTROL RAT LIVER HOMOGENATES

Pair	Acrylamide lost (μmol/g/60 min)	Hexobarbital lost (μmol/g/60 min)	Liver/body weight (g/100 g)
1 Phenobarb.	3.38[a]	8.73[b]	5.3
Control	0.62	4.03	4.6
2 Phenobarb.	3.38	8.80	5.9
Control	2.48	4.53	4.5
3 Phenobarb.	3.60	8.22	5.3
Control	2.36	3.74	4.2
4 Phenobarb.	2.82	—	5.4
Control	0.34	—	4.2
5 Phenobarb.	3.38	9.16	5.3
Control	2.08	3.93	4.5
Mean difference (phenobarbital—Control)	+1.74	+4.67	+1.04
Paired *t*	4.8, $p < 0.01$	27.7, $p < 0.01$	8.3, $p < 0.01$

[a] Each value represents the mean of 4 replicate determinations.
[b] Each value represents the mean of 2 replicate determinations.

In Vitro Metabolism of Acrylamide

The finding that rats pretreated with microsomal enzyme inducers were able to tolerate higher total cumulative doses of acrylamide before functional impairment became evident suggested that acrylamide might be metabolized by the liver and that these animals were better able to detoxify each daily dose of acrylamide compared to

[13] Purchased from K and K Laboratories, Plainview, New York.

controls. A similar delay was observed in 5-week-old compared to 11-week-old rats during earlier experiments (Kaplan and Murphy, 1972), and since increased microsomal enzyme activity had also been reported for 5-week-old rats (Kato *et al.*, 1964), it

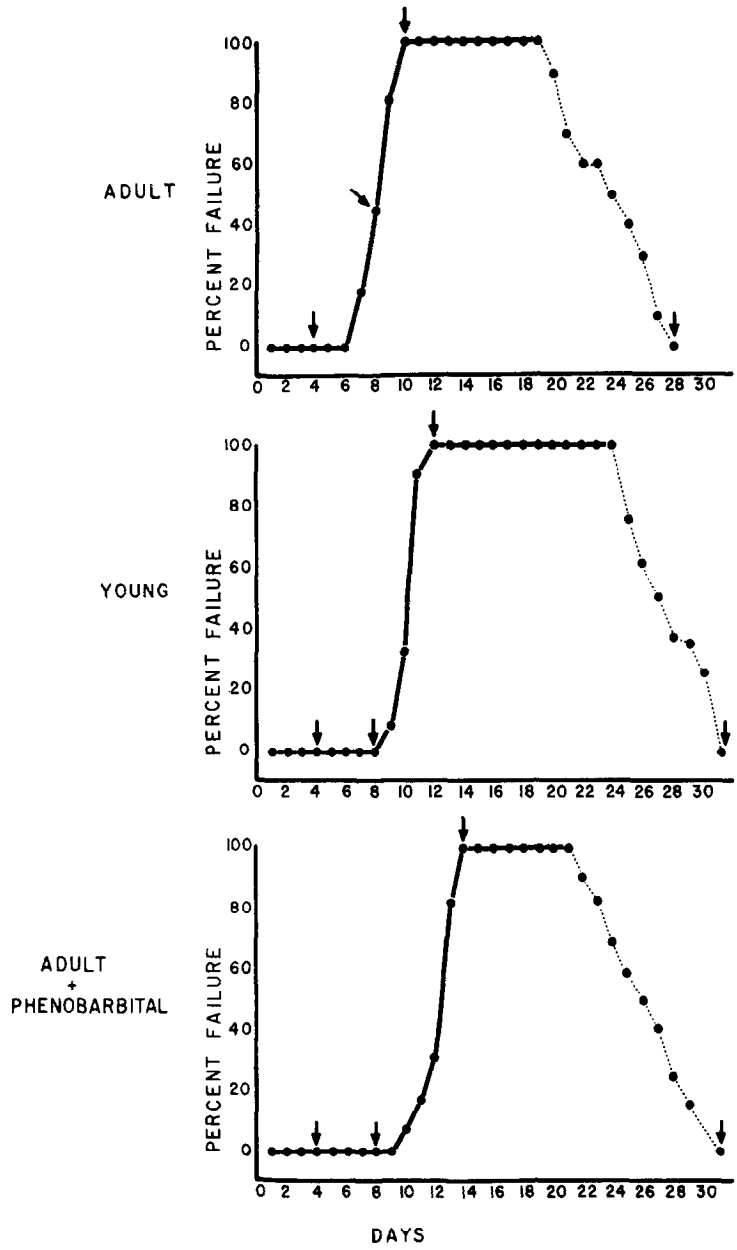

FIG. 5. Onset and recovery from acrylamide toxicity in adult, young and phenobarbital-pretreated adult rats. Acrylamide (40 mg/kg) was started at day 0 and continued daily for each group of 12 young, adult and phenobarbital pretreated adult rats until the 100% response. Recovery times are indicated by broken lines. Peripheral nerve samples were taken at arrows from 2 acrylamide treated and 1 control rat in each group.

seemed possible that the delay in functional impairment seen in these young rats also resulted from an increased capacity to detoxify acrylamide. Preliminary support for the hypothesis that acrylamide was metabolized by hepatic microsomal enzymes was

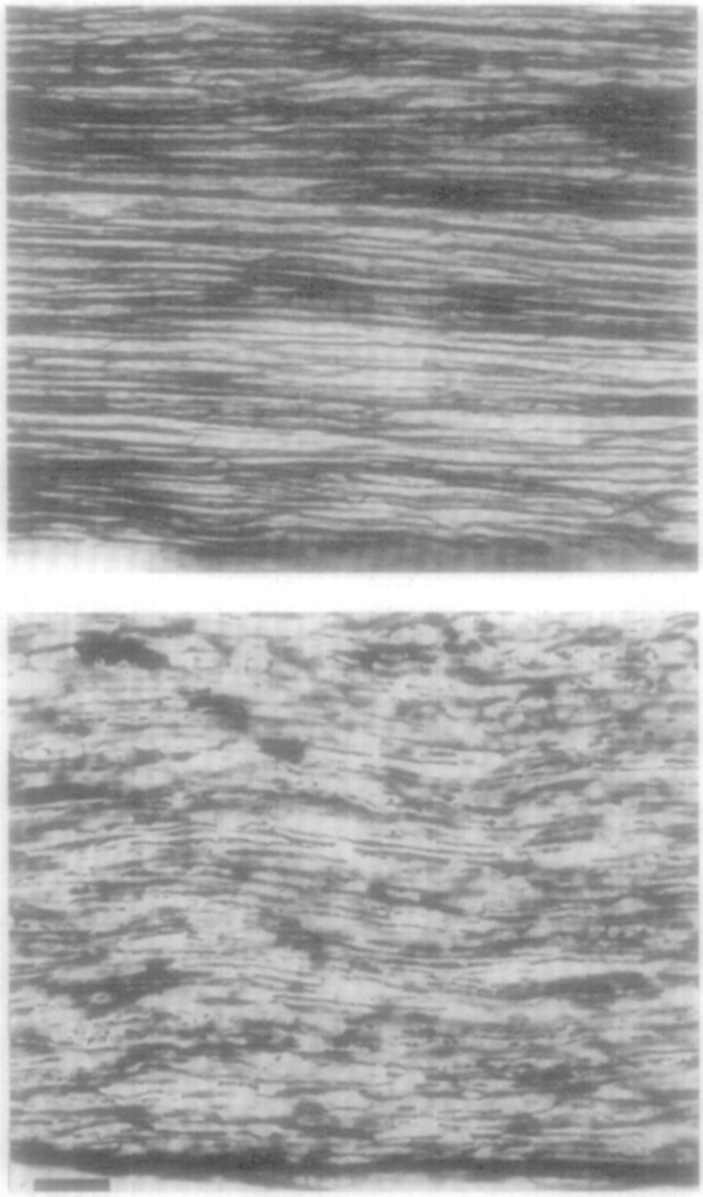

FIG. 6. Bielschowsky's silver stain for axons. The continuity of axons from control nerves (top) are compared to the fragmented and swollen axons of treated nerves (bottom). Marker = 25 μ.

obtained when we were able to increase or decrease the single dose toxicity of acrylamide in rats by pretreatment with SKF-525A or phenobarbital, respectively. Rats were given 158 mg/kg of acrylamide and within 24 hr 5/7 controls died compared to 7/7 and 3/7 for SKF-525A and phenobarbital pretreated rats, respectively.

In order to obtain more direct evidence that phenobarbital altered the metabolism of acrylamide, the following experiment was conducted. Five pairs of rats were selected at random and 50 mg/kg/day of phenobarbital was given to one rat from each pair for 5

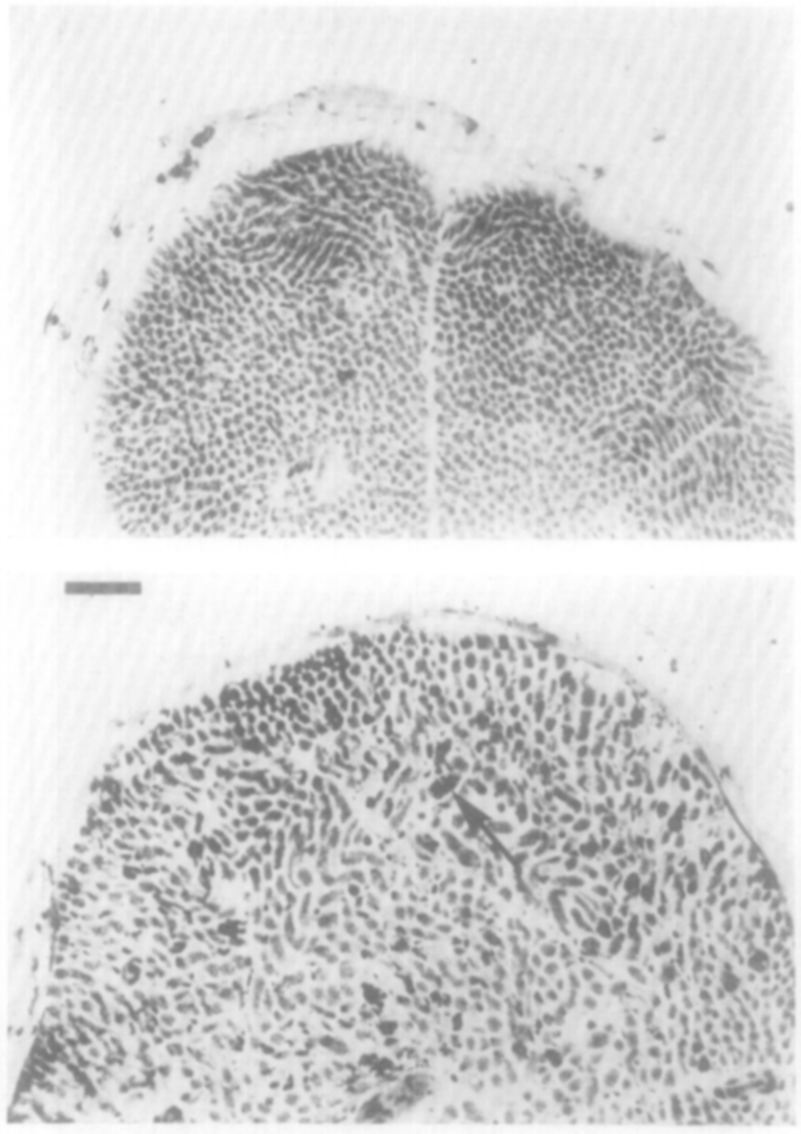

FIG. 7. Oil Red O stain for degenerating myelin. Cross section of normal myelinated nerve (top). Normal myelin phospholipid is hydrophilic and does not stain with the oil-soluble dye. Degenerating myelin in treated nerve (bottom) is hydrophobic because of cholesterol esters and neutral fats and stains red. Arrow indicates the presence of red material in macrophage. Marker = 50 μ.

days. On the sixth day, the rats were weighed and sacrificed by decapitation and exsanguination. Livers were removed, weighed and prepared for incubation with acrylamide as described in Methods. Table 2 shows that there was a greater loss of acryl-

amide from incubation mixtures containing 9000 *g* supernatants from phenobarbital-pretreated rat livers as compared with control rat liver preparations. The micromoles of acrylamide lost for each liver incubate was determined after 60 min. Incubations were run in duplicate and chromatographed in duplicate so that each value represents the mean of 4 absorbance readings. The increased liver to body weight ratios and the increase in hexobarbital oxidase activity in livers of phenobarbital-pretreated rats are indicative of microsomal enzyme induction (Mannering, 1968; Furner *et al.*, 1969).

Peripheral Nerve Changes in Acrylamide-Treated Rats

As shown in Fig. 4, the onset of neurologic deficit was delayed in DDT and phenobarbital pretreated rats but once impairment was produced in these animals they required a longer time to recover functionally. A similar delayed impairment followed by prolonged recovery had also been observed for young (5 week) as compared to older (11 week) rats (Kaplan and Murphy, 1972). In order to determine whether the differences in onset and recovery times were associated with differences in peripheral nerve damage we compared histologic sections of peripheral nerves from young, adult and phenobarbital-pretreated adult rats given 40 mg/kg of acrylamide daily.

The sampling days were determined using 3 additional groups of 12 cohort rats also given 40 mg/kg of acrylamide daily and tested on the rotarod. Untested rats used for histologic examinations were previously marked for one of four sampling days. Animals were sacrificed and peripheral nerve samples taken at the times indicated in Fig. 5. Peripheral nerve samples from two acrylamide-treated and one control rat from each group were taken on every sample day.

Photomicrographs of typical normal and severely damaged nerves are shown in Figs. 6 and 7. The grades for the degree of axon and myelin damage in 2 treated rats from each group are shown in Tables 3 and 4. The onset of functional impairment produced by acrylamide in unpretreated adult rats was not associated with discernible peripheral nerve damage, but in phenobarbital-pretreated adult rats and in young rats functional impairment was associated with severe peripheral nerve injury. In all animals the duration of neurologic deficit seemed to reflect the total cumulative dose of acrylamide received. Degeneration of myelin seemed to follow injury to the axon and was not observed in the absence of axonal damage.

TABLE 3

GRADES FOR THE DEGREE OF AXON DAMAGE IN TWO RATS TREATED WITH 40 mg/kg OF ACRYLAMIDE DAILY

Sample taken	Adult	Young	Adult + phenobarbital
Day 4 (160 mg/kg)[a]	−,−	−,−	−,−
Day 8 (320 mg/kg)	−,−	−,+	−,−
Day of 100% failure	−,−	++,++	++,[b]
	(440 mg/kg)	(520 mg/kg)	(600 mg/kg)
Day of functional recovery	++,+	++,++	++,++

[a] Figures in parentheses = cumulative dose of acrylamide received.
[b] Was not graded because of stain artifact.

TABLE 4

GRADES FOR THE DEGREE OF MYELIN DAMAGE IN TWO RATS TREATED WITH 40 mg/kg OF ACRYLAMIDE DAILY

Sample taken	Adult	Young	Adult + phenobarbital
Day 4 (160 mg/kg)[a]	−,−	−,−	−,−
Day 8 (320 mg/kg)	−,−	−,−	−,−
Day of 100% failure	−,− (440 mg/kg)	+,+ (520 mg/kg)	+,+ (600 mg/kg)
Day of functional recovery	++,+	++,++	++,++

[a] Figures in parentheses = cumulative dose of acrylamide received.

DISCUSSION

We have found that treatment with cortisol and deficiencies in pyridoxine or thiamine had no measurable effect on the cumulative dose of acrylamide required to produce neurologic deficit. Although cortisone was therapeutic in TOCP poisoning (Glees, 1961), the lack of any beneficial effect of cortisol in acrylamide poisoning was not surprising in light of recent findings that the "dying back" neuropathies produced by TOCP and acrylamide seemed to involve two distinct pathophysiological processes. Pleasure *et al.* (1969) reported an interruption in the slow axoplasmic flow of proteins migrating along dorsal and ventral spinal nerve roots in acrylamide-treated cats; whereas, in both control and TOCP-poisoned cats proteins migrated along nerve roots at the rate of 1–2 mm/day. The ultrastructural changes produced in peripheral nerves and the spinal cord in cats poisoned by acrylamide were distinctly different from those observed in TOCP-poisoned cats (Prineas, 1969).

The increase in susceptibility to acrylamide after bilateral adrenalectomy was nonspecific and probably resulted from the generalized weakness and debility seen in these animals. Neither pyridoxine or thiamine deficiency increased the susceptibility of rats to acrylamide treatment. The ability of the pyridoxine-deficient rats to recover neurologic function after acrylamide was discontinued suggests that either this vitamin was not required for the recovery process or that the deficiency state induced was marginal. Rats have been shown to be resistant to the peripheral nerve degeneration produced by chronic thiamine deficiency (Collins et al., 1964); however, as the untreated thiamine-deficient control rats in our experiment developed nervous signs and died, we must assume that a frank deficiency state existed but that it did not influence the onset of functional impairment in acrylamide-treated rats.

In young rats and adult rats pretreated with DDT or phenobarbital the onset of neurologic deficit and functional recovery from acrylamide poisoning were both delayed. The increased disappearance of acrylamide when incubated with 9000 *g* supernatants from phenobarbital-pretreated rat livers when compared to controls suggested a possible basis for the delay in onset of neurologic impairment. The severity of most responses to toxic agents is dependent upon the concentration of the active compound present at its specific receptor. If acrylamide is detoxified by a hepatic microsomal enzyme system, then young rats with increased microsomal enzyme activity (Kato *et al.*, 1964) and adult rats pretreated with microsomal enzyme inducers might be better able to

metabolize each daily dose of acrylamide than unpretreated adult rats, and more prolonged administration would be required to produce functional impairment. The greater degree of peripheral nerve injury observed in young rats and adult rats pretreated with phenobarbital could have resulted from the higher total cumulative doses of acrylamide received by these rats as compared to the adult unpretreated rats. This observation, however, fails to clarify the reason for the delay in onset of impairment that was seen in these animals.

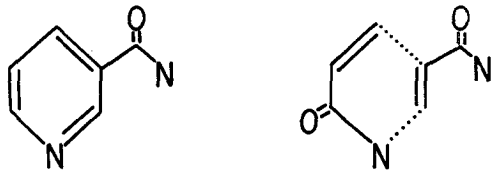

FIG. 8. Structural similarity between nicotinamide (left) and two acrylamide (right) molecules.

Since the structure of acrylamide is contained within the nicotinamide molecule it is tempting to speculate that acrylamide is a nicotinamide antagonist and could compete with nicotinamide for sites necessary in the production of the coenzymes NAD and NADP as has been reported for 3-acetylpyridine (Barnes, 1969) and 6-aminonicotinamide (Dietrich *et al.*, 1958). The similarity between nicotinamide and two acrylamide molecules can be seen in Fig. 8. The greater sensitivity of cats to acrylamide (Kuperman, 1958; McCollister *et al.*, 1964; Leswing and Ribelin, 1969) as compared to rats (Fullerton and Barnes, 1966), baboons (Hopkins, 1970) and monkeys (McCollister *et al.*, 1964; Leswing and Ribelin, 1969), might then be explained by their inability to convert tryptophan to nicotinamide (Leklem *et al.*, 1969), and unlike the primate or rat, cats would be less able to cope with the resulting interference in pyridine nucleotide coenzyme metabolism.

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