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Acrylamide Neurotoxicity
Role of Oxidative Metabolism

Medical Coll. of Georgia, Augusta

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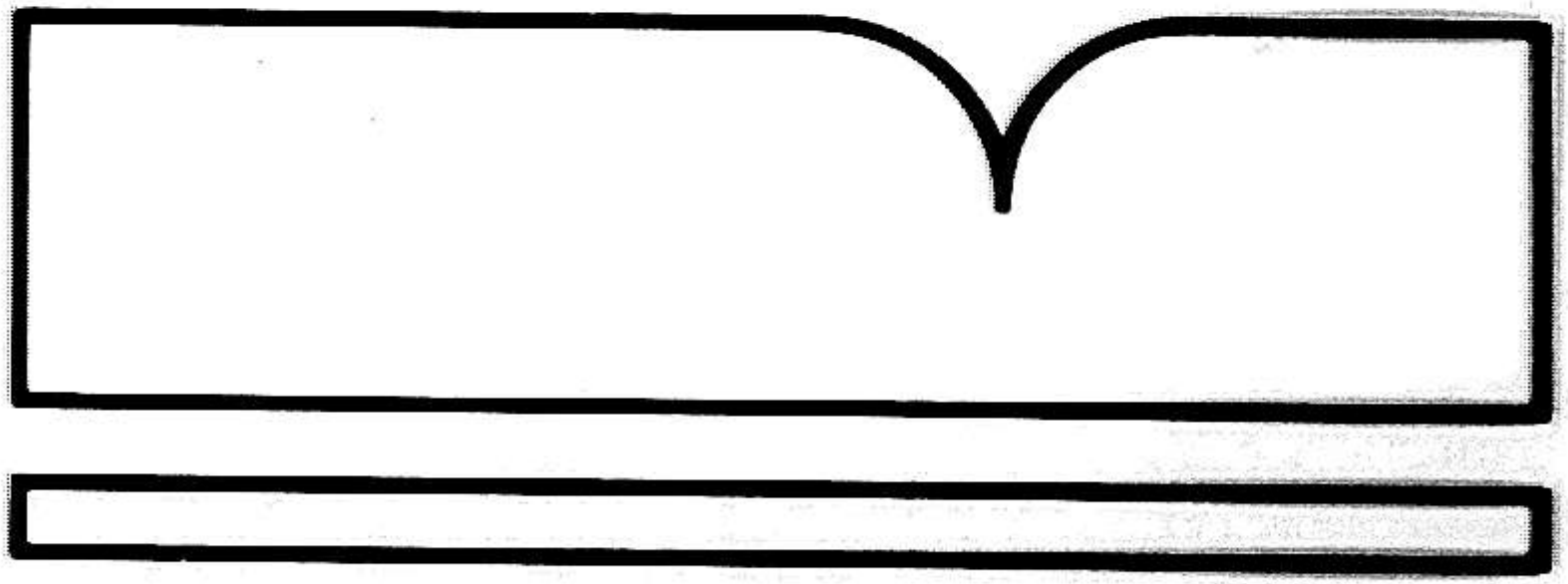

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15. Supplementary Notes

16. Abstract (Limit: 200 words): The possible selective inhibition of oxidative enzymes in neural tissues by acrylamide (79061) (ACR) given intraperitoneally at dose levels of 50mg/kg/day for 5 or 10 days was investigated. A 17 to 20 percent reduction in NADH-tetrazolium-reductase (NADH-TR) activity in one motoneuron type was demonstrated along with a progressive decrease in retrograde transport of horseradish-peroxidase from the muscle to the motoneuron. Following an acute exposure, activity returned to control levels in 72 hours, indicating that ACR did not irreversibly damage the neuron oxidative metabolism. The nonneurotoxic analogue, methylene-bis-acrylamide was less effective in enzyme inhibition. A study of the effects of ACR and a nonneurotoxic analogue on the NADH-TR activity of neural and nonneural tissues indicated that ACR specifically inhibited neural specific NADH-TR activity. The effect of ACR on the specific enzymes lipoamide-dehydrogenase (LdPH) and cytochrome-c-reductase (CCR) in neural and nonneural tissues was examined. The results agreed with the NADH-TR histochemical data, except for the kidney studies. In general the neural tissue enzymes were more significantly affected than nonneural. In studies of axoplasmic transport and nerve high energy phosphates the depletion of high energy phosphate was determined not to be the causative factor for deficits in axoplasmic transport caused by any of the neurotoxicants under study.

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms: NIOSH-Publication, NIOSH-Grant, Grant-Number-R01-OH-02020, End-Date-01-31-1987, Neurotoxic-effects, Laboratory-animals, Enzyme-activity, Metabolic-study

c. COSATI Field/Group

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Acrylamide Neurotoxicity: Role of Oxidative Metabolism

FINAL PERFORMANCE REPORT NIOSH #0H02020 August 1, 1984 to June 1, 1987

I. Professional Personnel

Dale W Sickles, Ph D	Prin Invest	8/84-6/87	40%
Robert W Wrenn, Ph D	Collaborator	8/84-6/87	5%
Barry D Goldstein, PhD	Collaborator	8/84-6/87	5%
Martha Coles	Res Assist I	8/84-12/84	100%
Carol Keldahl		12/84-8/86	
Angela Testino		8/86-6/87	

II. Specific Aims of Previous Grant

1. To determine whether or not acrylamide selectively inhibits the activity of oxidative enzymes in neural tissues.

A variety of quantitative histochemical and biochemical assays for oxidative enzymes in neural and non-neural tissues after exposure to acrylamide and its non-neurotoxic analoque methylene bis acrylamide were proposed to determine the specific inhibition by the neurotoxicant.

2. To determine whether or not inhibition of glycolytic (previously reported) and/or oxidative enzymes by acrylamide produced a decrease in high energy phosphates in peripheral nerves.

Bioluminescence assay for ATP and creatine phosphate (CP) in the peripheral nerves of animals exposed to acrylamide were proposed to detect such changes. In addition, the ability of ATP production by isolated mitochondria in the presence of exogenous pyruvate was included to differentiate an effect upon oxidative as compared to glycolytic metabolism.

3. To determine whether or not a correlation between the reduction in axoplasmic transport was paralleled by a decrease in high energy phosphates.

Fast anterograde transport rates and capacities of nerves exposed to increasing concentrations of acrylamide were compared to ATP plus CP levels in the nerve of the opposite limb.

III. REPORT

1. Effects of acrylamide upon oxidative enzymes.

Intraperitoneal acrylamide injections of 50mg/kg/day were given for either 5 or 10 days, controls received saline vehicle only. Quantitative histochemical assays for NADH-tetrazolium reductase (NADH-TR) activity (composite of lipoamide dehydrogenase activity of pyruvate and alpha-ketoglutarate dehydrogenase complexes and NADH dehydrogenase of the electron transport system) in two motoneuron types demonstrated a 17-20% decrease in NADH-TR activity in one motoneuron type. A progressive decrease in retrograde transport of horseradish peroxidase from the muscle to the motoneuron occured in the same motoneurons. This was the first report of inhibition of an enzyme of oxidative metabolism by acrylamide. (Sickles and Goldstein. Second Internat Conf Selected Neurotox Chem. Chicago, 1983; Sickles and Goldstein, Toxicol Lett 26:111-118,1985)

2. Time-course and dose-dependent inhibition of NADH-TR activity

The acute effects of ACR upon NADH-TR activity and the recovery time were assessed by measuring this enzyme activity in motoneurons following a single 50 mg/kg ip injection of ACR. The NADH-TR activity decreased rapidly, a significant change occured in 1/2h, the maximum decrease of 35% was at 1h. A direct enzyme inhibition by acrylamide (not a metabolite) was concluded. Following an acute injection, the activity slowly recovered to control levels in 72 hours indicating ACR did not irreversibly damage the neuron oxidative metabolism. In vitro incubation of spinal cord sections in buffer with varying doses of acrylamide showed a dose-dependent inhibition of NADH-TR activity in motoneurons from 1-10mM ACR; the maximum was 54% at 10mM (Sickles and Goldstein. Neurotoxicol 7:187-196, 1986).

3. Chemical specificity

The effect of a non-neurotoxic analogue, methylene bis acrylamide (MbACR), on NADH-TR activity in vivo and in vitro demonstrated the specificity of action of the neurotoxicant upon this enzyme. MbACR injection at equimolar doses to ACR produced only 6.7% inhibition of NADH-TR activity in motoneurons compared to 33.4% by ACR. In vitro incubation of tissue sections with MbACR produced a maximum inhibition of 14% of NADH-TR activity at 3mM. This data supports the importance of NADH-TR activity inhibitions as a key site of action since the non-neurotoxic analogue is less effective in enzyme inhibition. (Sickles, Goldstein, Pearson. Toxicologist 5:199,1985; Sickles and Goldstein. Neurotoxicol 7:187-196,1986.)

4. Neural specificity of ACR and MbACR action (Figure 1)

The effects of ACR and a non-neurotoxic analogue upon the NADH-TR activity of neural and non-neural tissues were determined. A single dose of 50mg/kg ACR or equimolar MbACR (108.4mg/kg) or saline control was given 1h prior to sacrifice. In 15 animals (5 in each group), the sciatic nerve in the distal thigh had been ligated three days prior to injection. The nerve swelling on the proximal side of the ligation served as a concentrated pool of axonal enzyme activity. At sacrifice several neural and non-neural tissues were processed for quantitative histochemical assays of NADH-TR activity. ACR produced a significant decrease in enzyme activity in axons (9.5%). Purkinje cerebellar neurons (17.3%) and in DRG neurons (17.8%). No significant effect of ACR on kidney cortical tubule cells, stomach parietal cells or hepatocytes or MbACR upon any of the tissues (except cerebellar Purkinje neurons) was observed. We concluded that ACR specifically inhibited neural-specific NADH-TR activity. The tissue specificity of ACR enzyme inhibition may be explained by glutathione buffering capacity; i.e. the tissue concentration of glutathione is inversely related to the enzyme inhibition (figure 1 & table 2).

Although these data supported the energy hypothesis, two disturbing points were raised. First, the degree of neural enzyme inhibitions was lower than previously reported in the motoneurons; raising the issue as to the physiological or pathological significance of the findings. Second, the inhibition of cerebellar Purkinje neuron NADH-TR activity by the non-neurotoxic analogue should result in a significant

effect upon these neurons. To our knowledge, such an observation has not been reported.

A similar study has been conducted using single doses of 4mM 2,5HD and 0.25mM DMHD. Neither of these chemicals produced significant changes in neural or non-neural tissues, except DMHD in dorsal root ganglion neurons (table 1)(Pearson and Sickles. Toxicologist 7:131, 1987; two manuscripts submitted).

5. Lipoamide dehydrogenase (LpDH) and cytochrome c reductase (CCR) activity

The NADH-TR activity previously discussed is a composite of activity from LpDH and CCR enzymes. The effect of ACR upon these specific enzymes in neural and non-neural tissues was examined several ways.

a. in vivo assays in neural and non-neural tissues (figures 2 & 3).

Animals were given single 50 mg/kg doses of acrylamide or saline vehicle only. The brain, spinal cord, liver, kidney and stomach were removed under pentobarbital anesthesia one hour later. Homogenates of brain and spinal cord showed a 16 and 16.7% decrease of LpDH activity from control and 8.7 and 14.9% decrease in CCR activity, respectively. LpDH of kidney and stomach was decreased 21.7 and 12%, respectively. No other decreases were significant.

b. in vitro dose response of CCR to ACR (figure 4)

Tissue homogenates were incubated at 37 C for 30 minutes with varying doses of acrylamide. No preincubation or cold preincubation of toxicant with tissue resulted in the absence of an effect. Unfortunately, the preincubation caused total loss of LpDH activity even in the presence of several protease inhibitors; data on the effect of the toxicant was not obtained. Inhibition of brain/spinal cord, DRG and stomach CCR activity was significant. Inhibition of 29.6, 11, and 16.3% by 1 mM ACR was observed for these tissues, respectively. Liver and kidney were unaffected.

c. in vitro assays of neuron-enriched brain fractions (figures 5 & 6)

Neuron-enriched fractions of rat cerebral cortex were obtained by disruption of tissue by forcing through progressively finer screens and gradient centrifugation in sucrose/ficoll gradients and verified under light microscopy. The fractions were exposed to 1mM ACR for 30 minutes at 37 C (figure 5). Assays showed a highly significant inhibition of both LpDH (-44.8%) and CCR (-16.9) activity (fig 6).

d. in vivo assays of neuron and glial fractions (fig 7)

Adult Sprague-Dawley rats were given 50 mg/kg ACR in a single ip injection (or saline vehicle for controls) and sacrificed one hour later; their brains were processed as illustrated in figure 5 for neuron and glial fractions and assayed for CCR and LpDH activities. CCR activity of neurons and glial fractions were reduced 25 and 27%, respectively, following ACR exposure. Glial LpDH activity was reduced 29%; neuronal LpDH activity was inhibited by 11%.

The biochemical data on LpDH and CCR activity in general agrees with the NADH-TR histochemical data with the exception of the kidney. This may be due to sampling of only cortical tubules in the histochemical methods

while biochemical methods incorporated the entire organ. In general, neural tissue enzymes were more significantly affected than non-neural. However, kidney and stomach LpDH activity and stomach CCR activity were decreased also. Inhibitions of these enzymes in glial and non-neural tissues which equal or exceed those in neural tissues is evidence against these enzymes as being the critical site of neurotoxicity. With the exception of the 29.6% inhibition of central nervous sytem enyzmes by 1mM acrylamide, the percent change in enzyme activities appear insufficient to cause significant depletion of high energy phosphates. (Sickles. Toxicologist 7:132, 1987; manuscript submitted)

6. Mitochondrial ATP production

Rat brain mitochondria were isolated (figure 8) and preincubated with 0.73 mM acrylamide (equivalent to 50mg/kg single dose) or in phosphate buffer only at 37 C for 30 minutes. ATP production by the mitochondria in the presence of exogenous substrate is measured with a luciferin-luciferase bioluminescence assay in a scintillation counter (illustrated in figures 9, 10, & 11). Absence of pyruvate (substrate) or addition of 1mM dinitrophenol (uncoupler of oxidative phosphorylation) completely inhibited the production of ATP. Acrylamide did not significantly affect the rate of production of ATP (table 3) nor the endogenous concentration of ATP (table 7). Brain mitochondria from rats receiving 50mg/kg/day for 5 days showed an 11% reduction in ATP production rate.

This project was expanded to evaluate the effects of the other neurofilamentous axonopathy-producing neurotoxicants on the same functions. DMHD (0.25mM) preincubations had no effect upon mitochondrial ATP production or content (tables 5 & 7). IDPN (0.1%) reduced the rate of ATP production 16.3% (table 6) but did not change the endogenous ATP content of the fraction (table 7). However, 4mM HD significantly reduced both the rate of production (22.2%) (table 4) and endogenous ATP content (22%)(table 7). Mitochondrial ATP production does not appear to be the common site of action of these toxicants. HD may cause a general toxicity through inhibited mitochondrial function or changes specific to 2,5-HD toxicity may be caused by this differential effect upon mitochondria. The 11% reduction in ATP generation by chronically-exposed ACR would be insufficient to produce a neuropathy.

(Sickles, Fowler, Testino. Toxicologist 7:132, 1987; manuscript submitted

7. Axoplasmic Transport and Nerve High Energy Phosphates (figure 12, 13, 14, table 8,9).

The effect of 50-100 mg/kg single doses of acrylamide upon fast anterograde axoplasmic transport rate and capacity as well as ATP and CP content of the nerves have been determined. Figure 12 shows the typical downflow of radioactivity in the sciatic nerve following DRG injection with 3H-leucine. ACR produced a dose-dependent decrease in rate (9.2-20.7%) and capacity (42.9-51.3%) of transport by 50 and 100 mg/kg ACR, respectively (figures 13, 14 & table 8,9). Matched data for ATP plus CP levels and axoplasmic transport in the same animal (opposite limbs) is available for 50 and 100 mg/kg ACR-exposed animals as shown in figure 15. By comparing the data in figure 12 with figure 15, it is obvious that the rate and capacity for transport are decreased while the high energy phosphates are not significantly affected. In addition, we have assayed for ATP and CP in nerves

of animals exposed to a single 50mg/kg dose of ACR for 1,2, or 3 hours to determine if transient changes in high energy phosphates occur (fig 16). While slight decreases in ATP were found 1 hour post injection and slight decreases in CP at 1-2 hours, none of the changes were significant (figure 16). We conclude that ACR does block axoplasmic transport but the block is not due to depletion of high energy phosphates. (Sickles and Pearson. Toxicologist 7:132,1987)

We are currently conducting similar studies with the neurotoxic hexacarbons. Briefly, the rate and capacity of the fast anterograde transport are decreased by gamma-diketones (see preliminary data). We have found that single doses of 4mmoles/kg 2,5-HD and 0.25 mmoles/kg DMHD do not significantly change the concentration of ATP or CP in the sciatic nerve one hour after injection. Therefore, it appears that high energy phosphate depletion is not the causative factor for deficits in axoplasmic transport caused by any of the neurotoxicants under study.

IV. SUMMARY of Progress Toward Original Specific Aims

The above work was generated during the last 2 1/2 years of the grant. Note that the start date was delayed by one month and the budget was cut 15% the first fiscal year and 10% the second. All of the specific aims have been completed and manuscripts have either been submitted or are in preparation. We have not determined the effects of acrylamide upon all of the enzymes mentioned in the specific aims. This was done in consultation with officials at NIOSH. It appeared inappropriate to spend money and effort testing the effects of acrylamide upon oxidative enzymes when the mitochondrial ATP production was not inhibited. The budget cuts were thus absorbed by not performing all of these assays.

VI. PUBLICATIONS:

Abstracts pertinent to grant:

Sickles DW and Goldstein BD Further investigations into the inhibition of oxidative enzymes by acrylamide (ACR). Neuroscience Abstract 10:1195 1984.

Sickles DW, Goldstein BD and Pearson JK. Time course and specificity of acrylamide inhibition of oxidative enzymes. Toxicologist 5:199, 1985.

Pearson JK and Sickles DW. Effects of ACR, HD and DMHD on neural and non-neural NADH-TR activity in the rat. Toxicologist 7: 131, 1987.

Sickles DW. Neuron-specific inhibition of lipoamide dehydrogenase and cytochrome c reductase by acrylamide. Toxicologist 7: 132,1987.

Sickles DW and Pearson JK. ATP, CP and axoplasmic transport in sciatic nerves of ACR, 2,5HD and DMHD exposed rats. Toxicologist 7: 132, 1987.

Sickles DW, Fowler SR and Testino AR. ATP production by brain mitochondria exposed to neurofilamentous axonopathy-producing neurotoxins. Toxicologist 7: 132, 1987.

Sickles DW, Tischfield JA, Welter DA, Testino AR, Pearson JK and Oblak TG. Toxic action of ACR, 2,5HD and DMHD upon the microtubule: mitosis and tubulin crosslinking. Toxicologist 7: 131, 1987.

Sickles DW, Tischfield JA and Oblak TG. Effects of ACR, 2,5HD, DMHD and IDPN upon microtubule cytoskeletal networks. Toxicologist 7: 131, 1987.

Abstracts supported by time commitment:

Pearson JK and Sickles DW. Effects of synergist tenotomy on rat soleus muscle and motoneuron metabolism. Neuroscience Abstracts 11:1102, 1985.

Sickles DW, Oblak TG and Scholer J. Effects of hyperthyroidism upon slow oxidative and fast glycolytic motoneuron metabolism. Neuroscience Abstracts 11:1102, 1985.

Sohal GS and Sickles DW. Embryonic differentiation of muscle fiber types. Anat Rec 214:126A, 1986.

Papers pertinent to grant:

Sickles DW and Goldstein BD. Acrylamide alters oxidative enzyme activity of rat motoneurons. Toxicology Letters 26: 111-118, 1985.

Sickles DW and Goldstein BD. Acrylamide produces a direct, dose-dependent and specific inhibition of oxidative metabolism in motoneurons. Neurotoxicology 7: 187-196,1986.

Sickles DW. Neural specificity of acrylamide action upon enzymes of oxidative energy-producing pathways. I. Histochemical analysis of NADH-TR activity (in press).

Sickles DW. Neural specificity of acrylamide action upon enzymes of oxidative energy-producing pathways. II. Biochemical analysis of lipoamide dehydrogenase and cytochrome c reductase (submitted).

Pearson JK and Sickles DW. The effects of 2,5 hexanedione and 3,4-dimethyl 2,5-hexanedione on NADH-TR activity of neural and non-neural tissues. (submitted)

Sickles DW, Fowler S and Testino AR. Effects of neurofilamentous axonopathy-producing neurotoxins upon ATP production by brain mitochondria. (submitted)

Sickles DW and Pearson JK. High energy phosphates and axoplasmic transport in acrylamide neurotoxicity. (submitted)

Sickles DW. Changes in fast axoplasmic transport following single injections of gamma-diketone hexacarbons. (in preparation)

Sickles DW, Goldstein BD and Tischfield JA. Acrylamide and gamma-diketones alter the structural and functional properties of microtubules in cultured cells. (in preparation)

Sickles DW. The microtubule as the critical site of action of neurofilamentous axonopathy-producing neurotoxins, an hypothesis. (in preparation).

Papers supported by time commitment:

Sohal GS and Sickles DW. Embryonic differentiation of fiber types in the normal, paralyzed and aneural superior oblique muscle. J Embryol Exp Morph 96: 79-97, 1986.

Sickles DW, Oblak TG and Scholer J. Hyperthyroidism causes a selective increase in oxidative metabolism of slow oxidative motoneurons. Exp Neurol 97: 90-105, 1987

Faith SD, Kirby ML and Sickles DW. Carbohydrate, lipid and oxidative metabolism in the sympathetically aneural chick heart. J Mol Cell Cardiol 19: 349-356, 1987

Pearson JK and Sickles DW. Effects of synergist ablation upon the oxidative metabolism of soleus motoneurons and muscle fibers. J Appl Physiol (in press)

Sickles DW and Oblak TG. Factors responsible for variations in horseradish peroxidase labeling of specific motoneuron types. Exp Neurol (in press)

Sickles DW. Metabolic variation among alpha-motoneurons innervating different muscle fiber types. II. Hexokinase and Phosphorylase activities. (submitted).

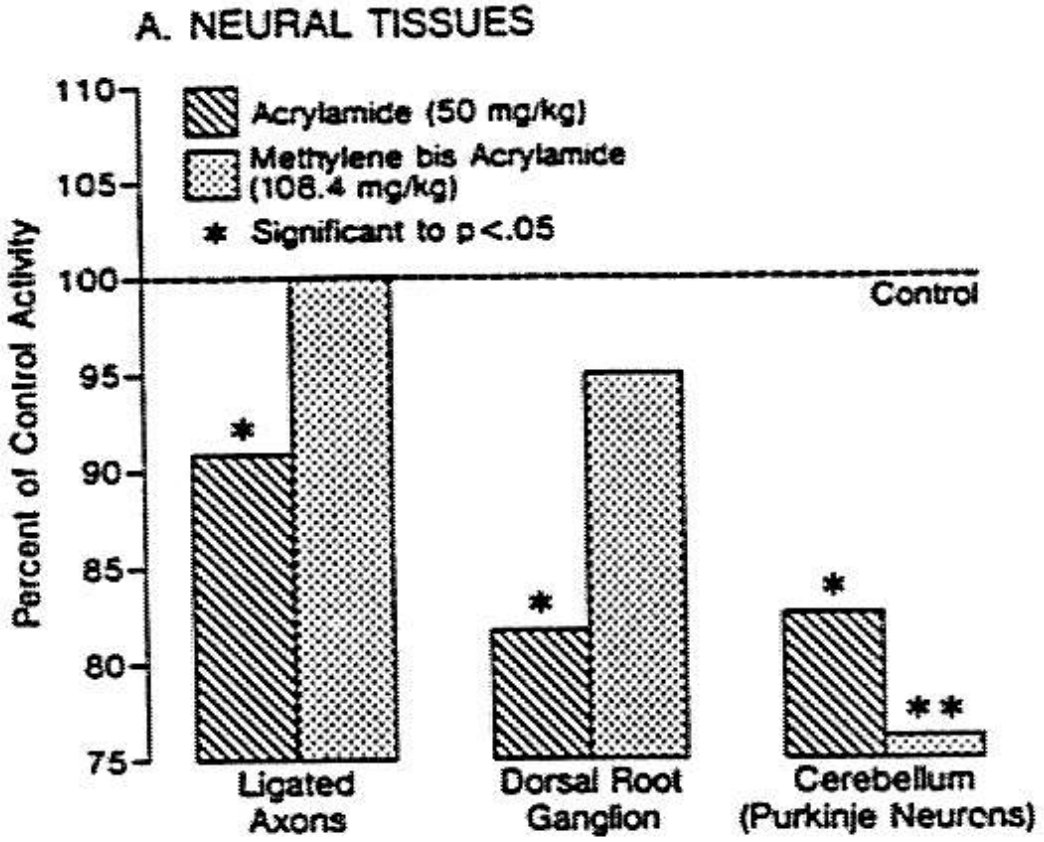
EQUIPMENT INVENTORY

Funds from this grant were used to acquire an update version of the Zeiss microdensitometry system. The total purchase price of the update was $9150; $4000 was from this grant. The total price of the equipment is approximately $65-70,000. The percentage of grant funds contributing to this instrument is 5.7%; the balance was from state funds. No serial numbers on the update are available since most of this acquisition is software and small unlabeled components. This instrument was used for all of the quantitative histochemical enzyme assays.

FINAL INVENTION STATEMENT

No inventions were conceived under this assistance award.

Figure 1


A. NEURAL TISSUES
Acrylamide (50 mg/kg)
Methylene bis Acrylamide (108.4 mg/kg)
* Significant to p<.05
Percent of Control Activity
110
105
100
95
90
85
80
75
Control
*
*
*
**
Ligated Axons
Dorsal Root Ganglion
Cerebellum (Purkinje Neurons)

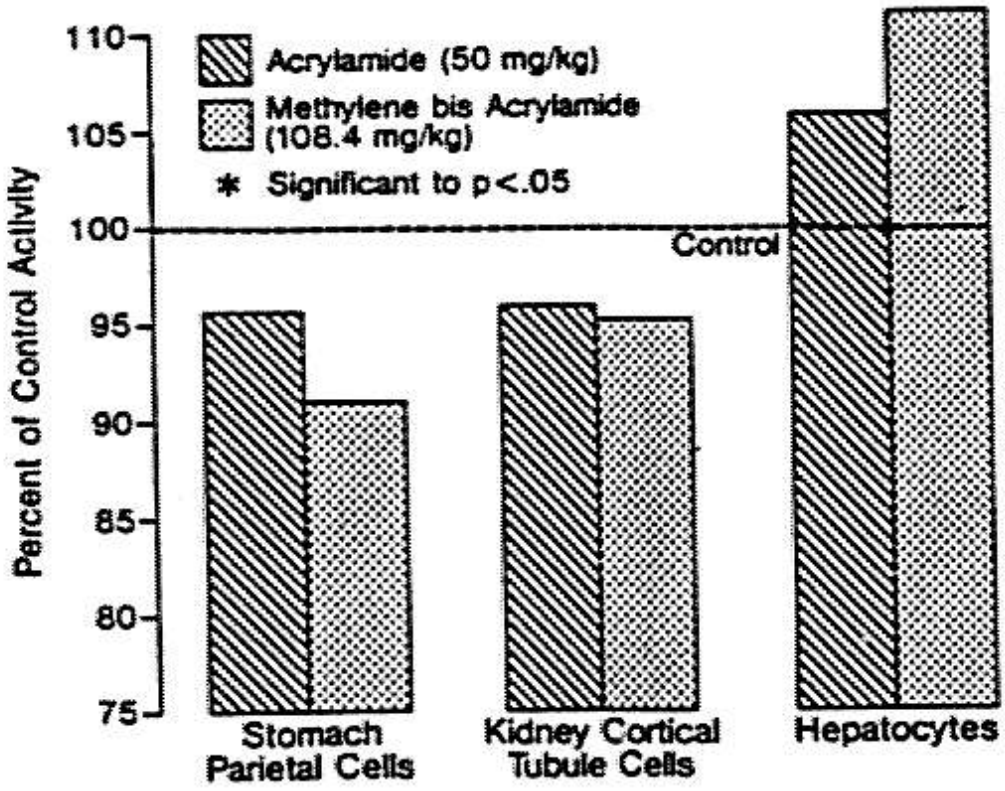

B. NON-NEURAL TISSUES
Acrylamide (50 mg/kg)
Methylene bis Acrylamide (108.4 mg/kg)
* Significant to p<.05
Percent of Control Activity
110
105
100
95
90
85
80
75
Control
Stomach Parietal Cells
Kidney Cortical Tubule Cells
Hepatocytes

Table 1

NADH-TR ACTIVITY
(O.D. Units)

	Purkinje	DRG	Hepatocytes	Kidney
Control	.289 ± .010	.343 ± .010	.380 ± .014	.529 ± .034
HD (4mM 1 hr)	.268 ± .020 (-7.2%)	.302 ± .009 (-12.0%)	.388 ± .016 (+2.1%)	.551 ± .027 (+4.2%)
DMHD (.25mM 1 hr)	.257 ± .024 (-11.1%)	.287 ± .012* (-16.3%)	.399 ± .026 (+5.0%)	.492 ± .020 (+7.0%)

* significant to p <.05

Table 2

GLUTATHIONE CONCENTRATION
(μmoles/g tissue)

LIVER	6.95[1]
STOMACH	2.7[2]
BRAIN	1.7[1]
SPINAL CORD	1.22[1]
CEREBELLUM	1.4-1.53[3,4]
PERIPHERAL NERVE	1.94[5]

1. Hashimoto & Aldridge, 1970
2. Robert et al., 1984
3. Hiramatsu & Mori, 1980
4. Pasha & Sadasivudu, 1984
5. Stein & Wenreich, 1984 (30μmole/mg protein) and 65μg protein/g nerve determined in our laboratory

Figure 2

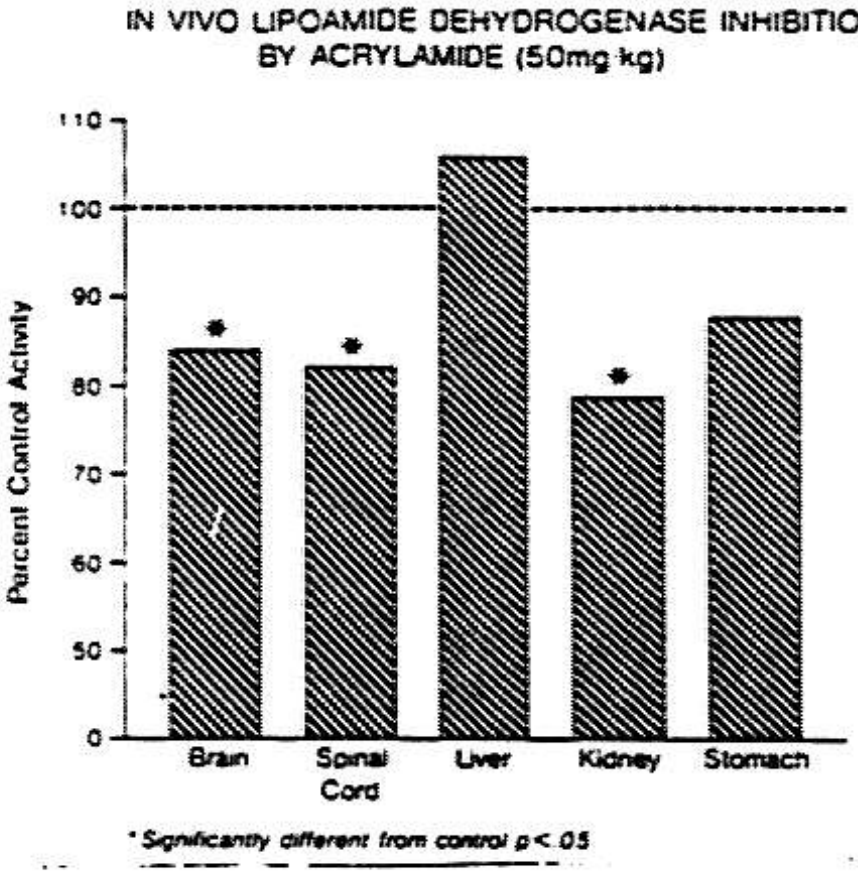


Figure 3

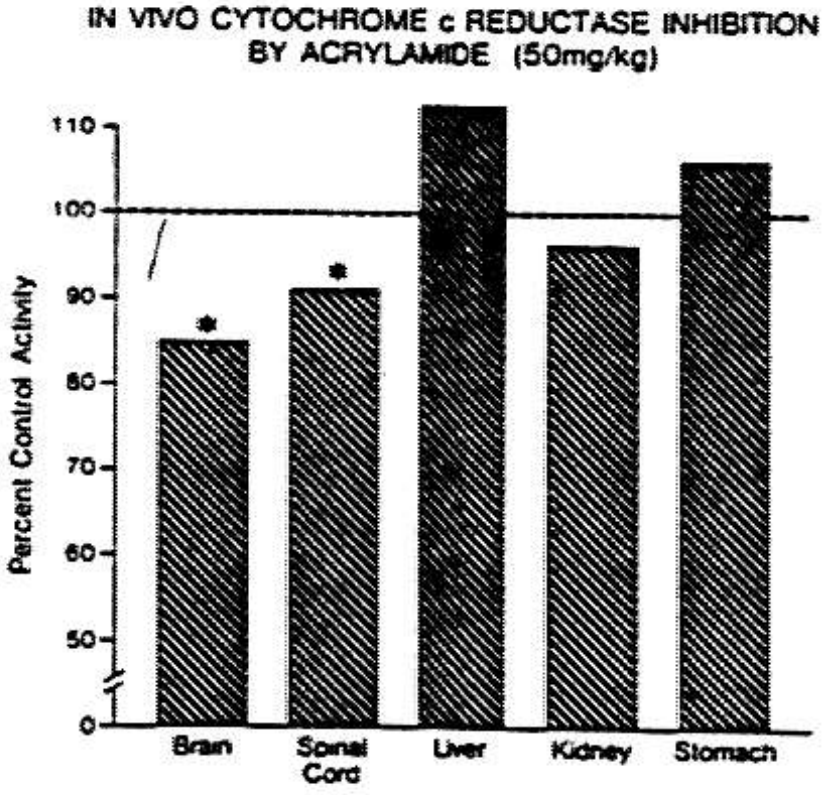


Figure 4

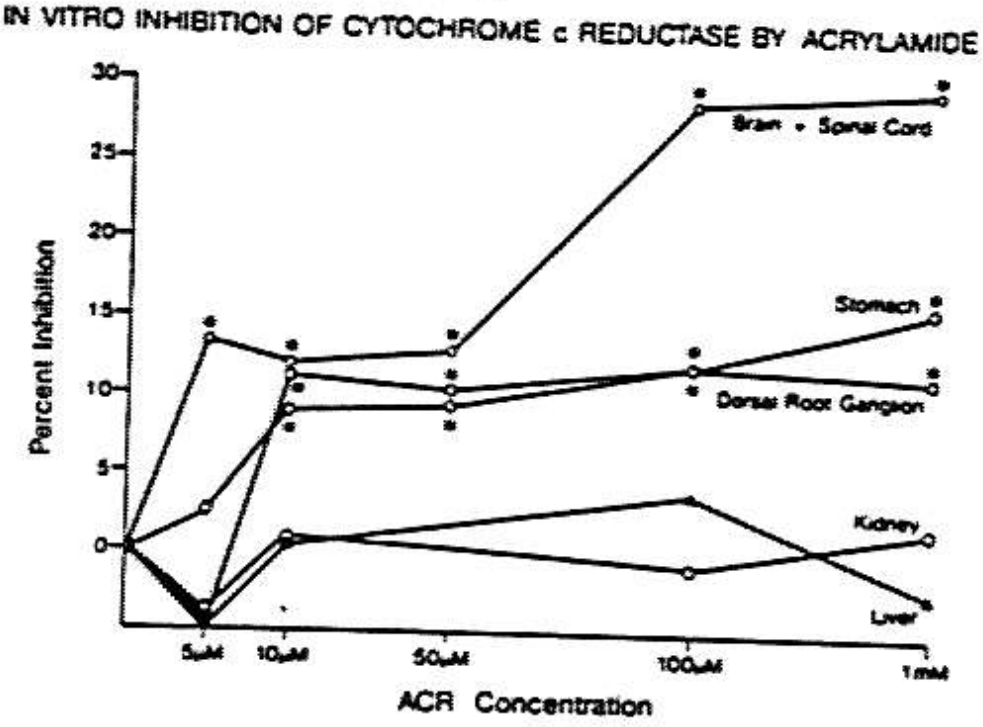


Figure 5

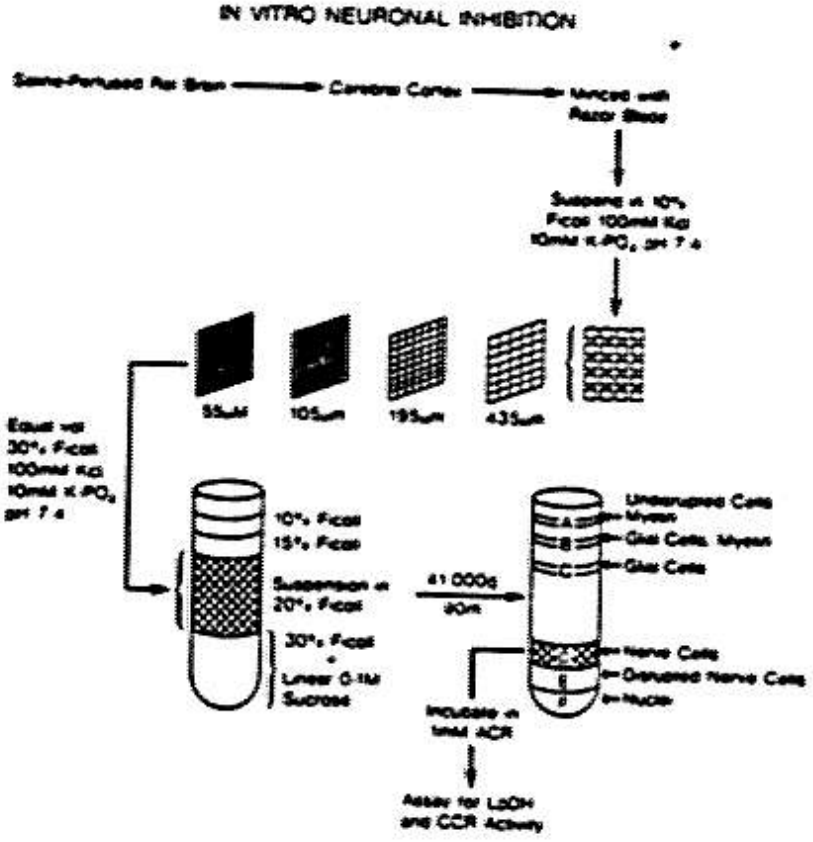
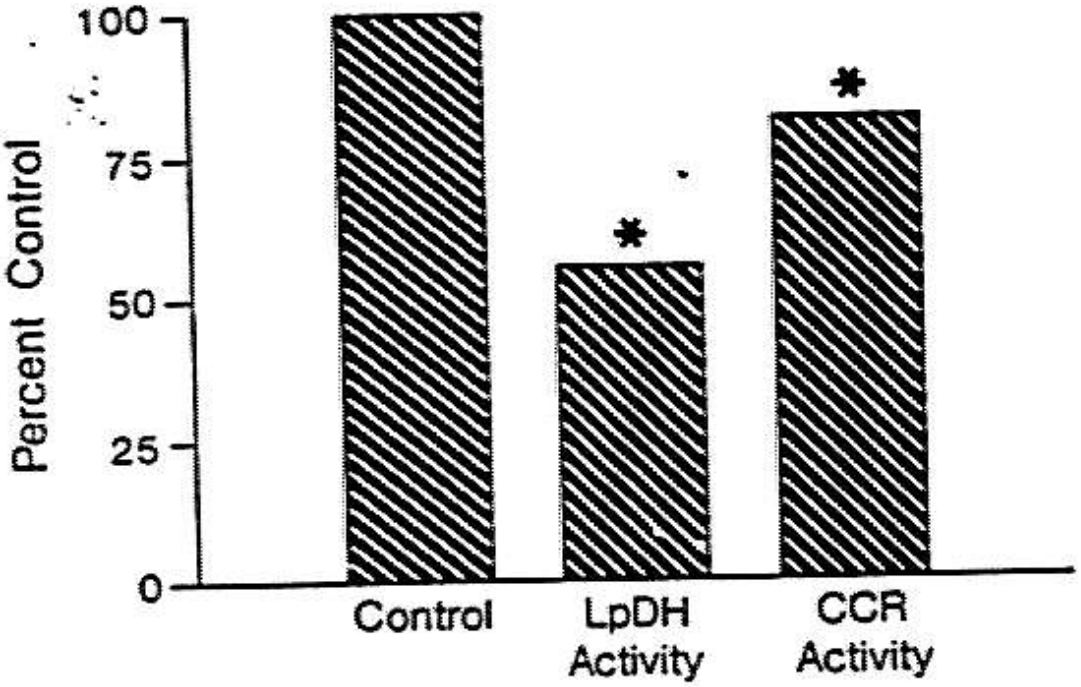


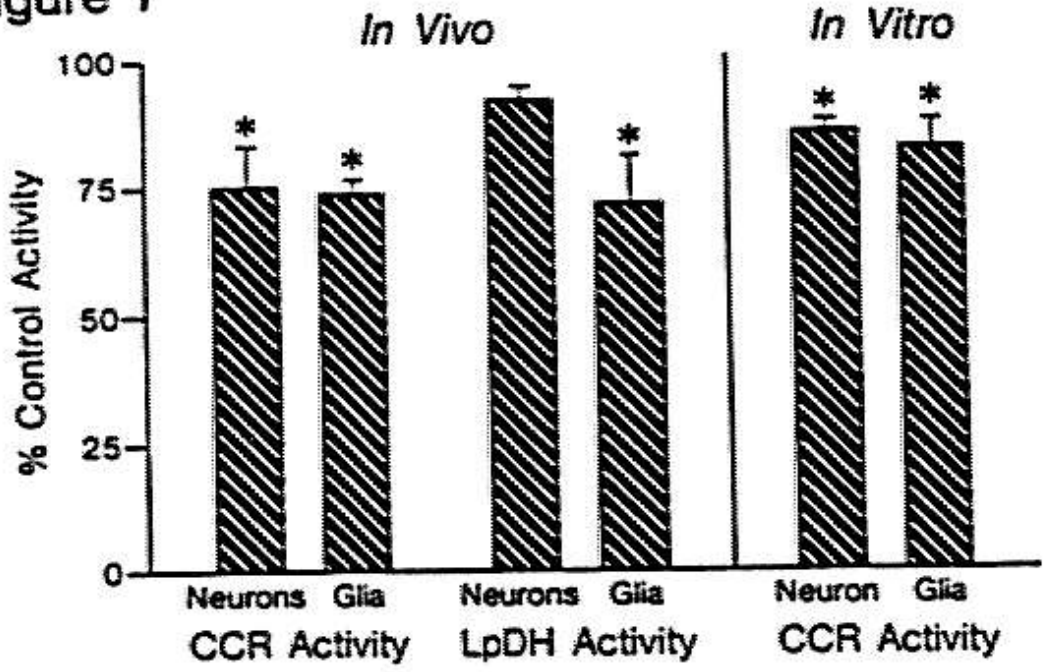
Figure 6

IN VITRO INHIBITION OF NEURONAL LIPOAMIDE DEHYDROGENASE AND CYTOCHROME c REDUCTASE BY ACRYLAMIDE (1mM)



*Significantly different from control $p<.05$

Figure 7



*Significantly different from control $p<.05$

Figure 8

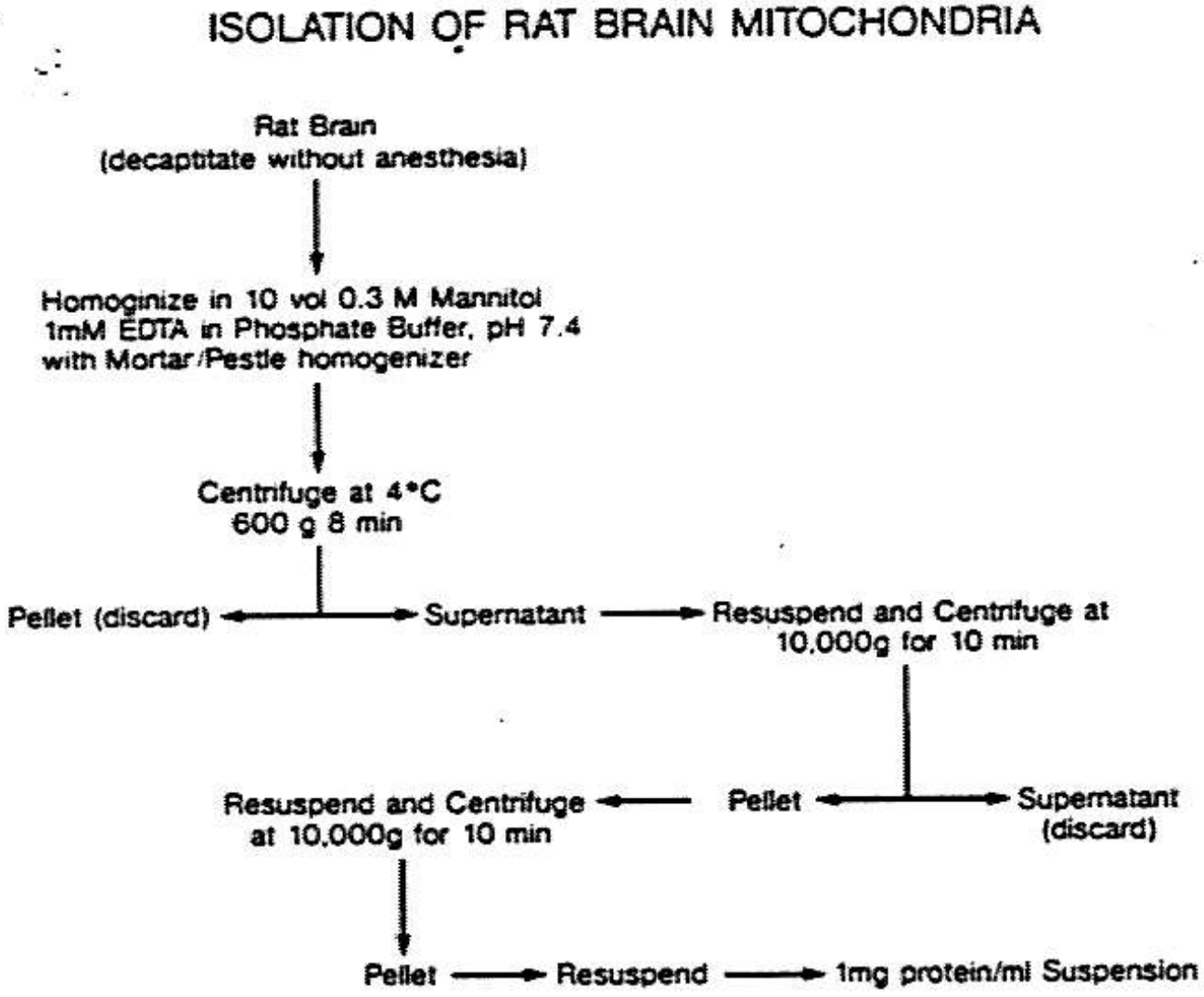
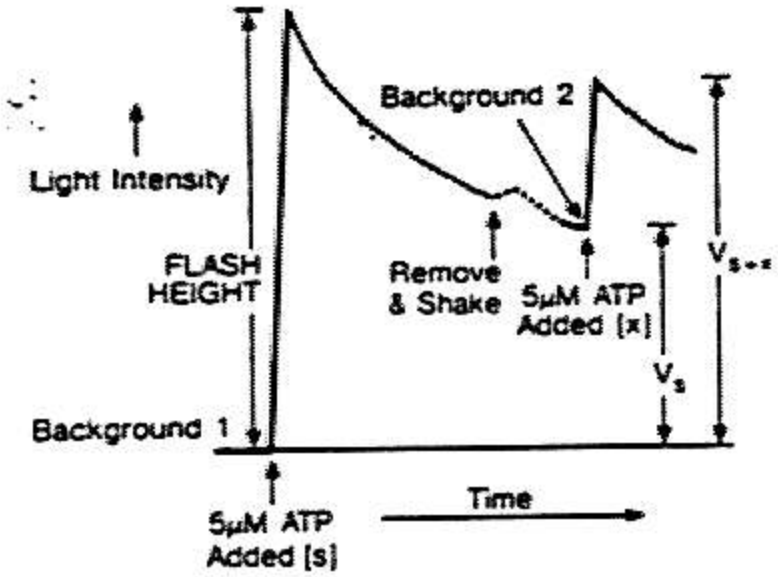

ISOLATION OF RAT BRAIN MITOCHONDRIA
Rat Brain
(decapitate without anesthesia)
Homoginize in 10 vol 0.3 M Mannitol
1mM EDTA in Phosphate Buffer, pH 7.4
with Mortar/Pestle homogenizer
Centrifuge at 4°C
600 g 8 min
Pellet (discard)
Supernatant
Resuspend and Centrifuge at
10,000g for 10 min
Pellet
Supernatant
(discard)
Resuspend and Centrifuge
at 10,000g for 10 min
Pellet
Resuspend
1mg protein/ml Suspension

Figure 9

CALCULATION OF MICHAELIS-MENTON CONSTANT



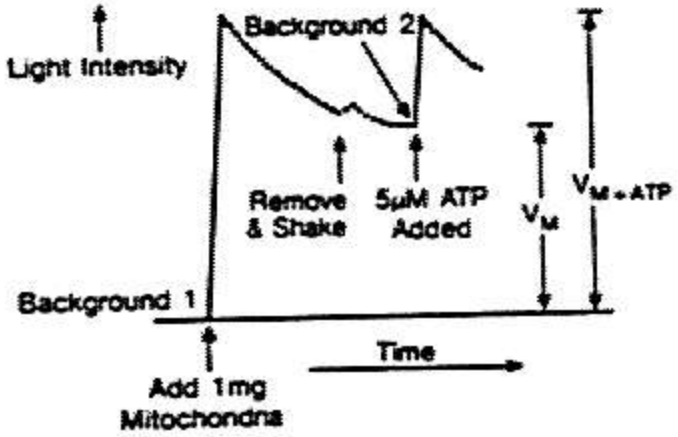
$$K_s = \frac{[s]\,(1+V_s/V_{s+x})}{V_s/V_{s+x} - ([s]+[x])/([s]+[x])}$$

V_s = Background 2 - Background 1

V_{s+x} = Peak 2 - Background 1

Figure 10

CALCULATION OF ENDOGENOUS ATP



$$[ATP_M] = \frac{5\mu M\,(V_M/V_{M+ATP})}{(V_s/V_{s+x})}$$

or

$$[s] = \frac{-(K_s+[x])}{2} + \sqrt{\frac{V_s/V_{s+x}\,(K_s-[x])^2 - (K_s+[x])^2}{4\,(V_s/V_{s+x}-1)}}$$

Figure 11

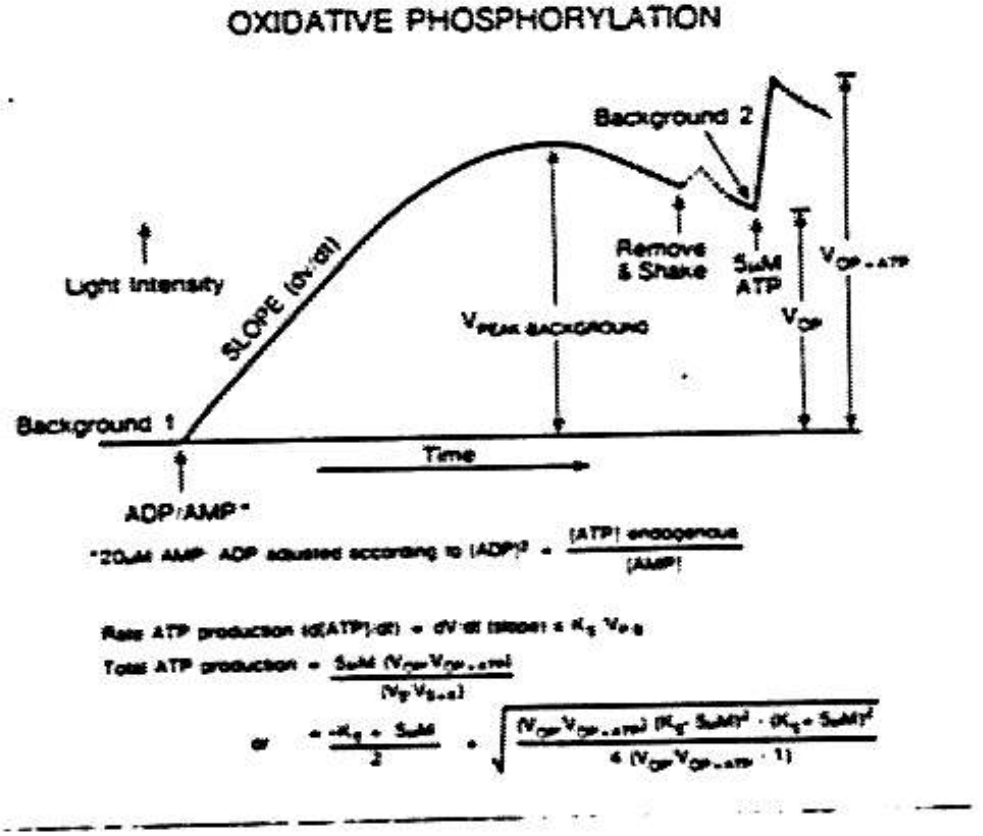

OXIDATIVE PHOSPHORYLATION
Background 2
Light Intensity
SLOPE (dV/dt)
Remove & Shake
5uM ATP
V_{OP-ATP}
$V_{PEAK\ BACKGROUND}$
V_{OP}
Background 1
Time
ADP/AMP*
*20uM AMP ADP adjusted according to (ADP)² = [ATP] endogenous / [AMP]
Rate ATP production d[ATP]/dt = dV/dt (slope) x K_q V_{P-Q}
Total ATP production = $\frac{5uM\ (V_{OP}V_{OP-ATP})}{(V_P V_{P-Q})}$
or $= \frac{-K_q + 5uM}{2} + \sqrt{\frac{(V_{OP}V_{OP-ATP})(K_q - 5uM)^2 \cdot (K_q + 5uM)^2}{4\ (V_{OP}V_{OP-ATP} \cdot 1)}}$

Table 3

ACR

	K_s	Endo ATP (nmoles/mg prot)	Prod ATP (nmoles/mg prot)	d[ATP]/dt (nmoles/mg prot/m)
Control	15.87	1.55	6.00	5.45
	13.06	1.76	5.96	3.60
	14.60	1.80	5.76	4.56
$\bar{x} \pm$ SEM	14.51 ± .81	1.70 ± .08	5.91 ± .07	4.54 ± .53
ACR	10.68	1.50	5.79	5.16
	12.14	1.68	5.45	3.35
	14.70	1.80	5.38	5.39
$\bar{x} \pm$ SEM	12.51 ± 1 17	1.66 ± .09*	5.54 ± .13*	4.63 ± .65
% Control	86.2	97.6	93.7	101.9

Significantly different from control $p < .05$

Table 4

2.5 HD

	K_s	Endo ATP (nmoles/mg prot)	Prod ATP (nmoles/mg prot)	d[ATP]/dt (nmoles/mg prot/m)
Control	11.10	4.40	5.68	3.78
	11.76	4.36	6.39	3.36
	13.33	1.50	5.81	4.59
	14.26	1.00	7.86	4.65
$\bar{x} \pm$ SEM	12.61 ± .72	2.81 ± .91	6.43 ± .53	4.09 ± .32
2.5 HD	13.06	1.70	6.92	3.19
	12.35	1.54	7.36	2.46
	14.62	1.25	6.29	3.22
	15.67	0.71	8.25	3.85
$\bar{x} \pm$ SEM	13.97 ± .79*	1.30 ± .22*	7.20 ± .41*	3.18 ± .28*
% Control	107.8	53.7	119.8	77.8

Significantly different from control $p < .05$

Table 5

DMHD

	K_s	Endo ATP (nmoles/mg prot)	Prod ATP (nmoles/mg prot)	d[ATP]/dt (nmoles/mg prot/m)
Control	11.10	4.40	5.68	3.20
	11.41	0.42	5.54	2.28
	10.81	1.43	6.06	3.26
$\bar{x}$ ± SEM	11.11 ± .17	2.08 ± 1.19	5.76 ± .16	2.91 ± .32
DMHD	14.26	3.30	5.88	3.87
	11.58	0.56	5.22	1.73
	10.81	1.43	5.81	3.04
$\bar{x}$ ± SEM	12.22 ± 1.05	1.76 ± .81	5.64 ± .21	2.88 ± .62
% Control	109.9	84.6	97.9	99.0

*Significantly different from control $p < .05$

Table 6

IDPN

	K_s	Endo ATP (nmoles/mg prot)	Prod ATP (nmoles/mg prot)	d[ATP]/dt (nmoles/mg prot/m)
Control	10.43	1.32	7.33	3.11
	13.33	0.88	8.75	4.47
	13.93	2.70	6.16	6.46
$\bar{x}$ ± SEM	12.56 ± 1.08	1.63 ± .55	7.41 ± .75	4.68 ± .97
IDPN	10.68	1.05	6.53	2.85
	12.35	0.75	7.78	3.71
	14.62	1.85	5.58	5.15
$\bar{x}$ ± SEM	12.55 ± 1.14	1.22 ± .33*	6.63 ± .64*	3.90 ± .67*
% Control	100	74.8	89.5	83.3

*Significantly different from control $p < .05$

Table 7

ENDOGENOUS MITOCHONDRIAL ATP
(nmoles ATP/mg mitochondrial protein)

	Control	ACR (0.7mM)	2.5-HD (4mM)	DMHD (0.25mM)	IDPN (0.1%)
	1.21	1.21	0.84	1.29	1.25
	1.04	1.09	0.87	1.00	1.38
	1.46	1.58	1.20	1.36	1.50
$\bar{x} \pm$ SEM	1.24 ± 0.12	1.29 ± 0.15	0.97 ± 0.12*	1.22 ± 0.11	1.38 ± 0.07
% Control		105	78	100	111

*Significantly different from control $p < .05$

Figure 12

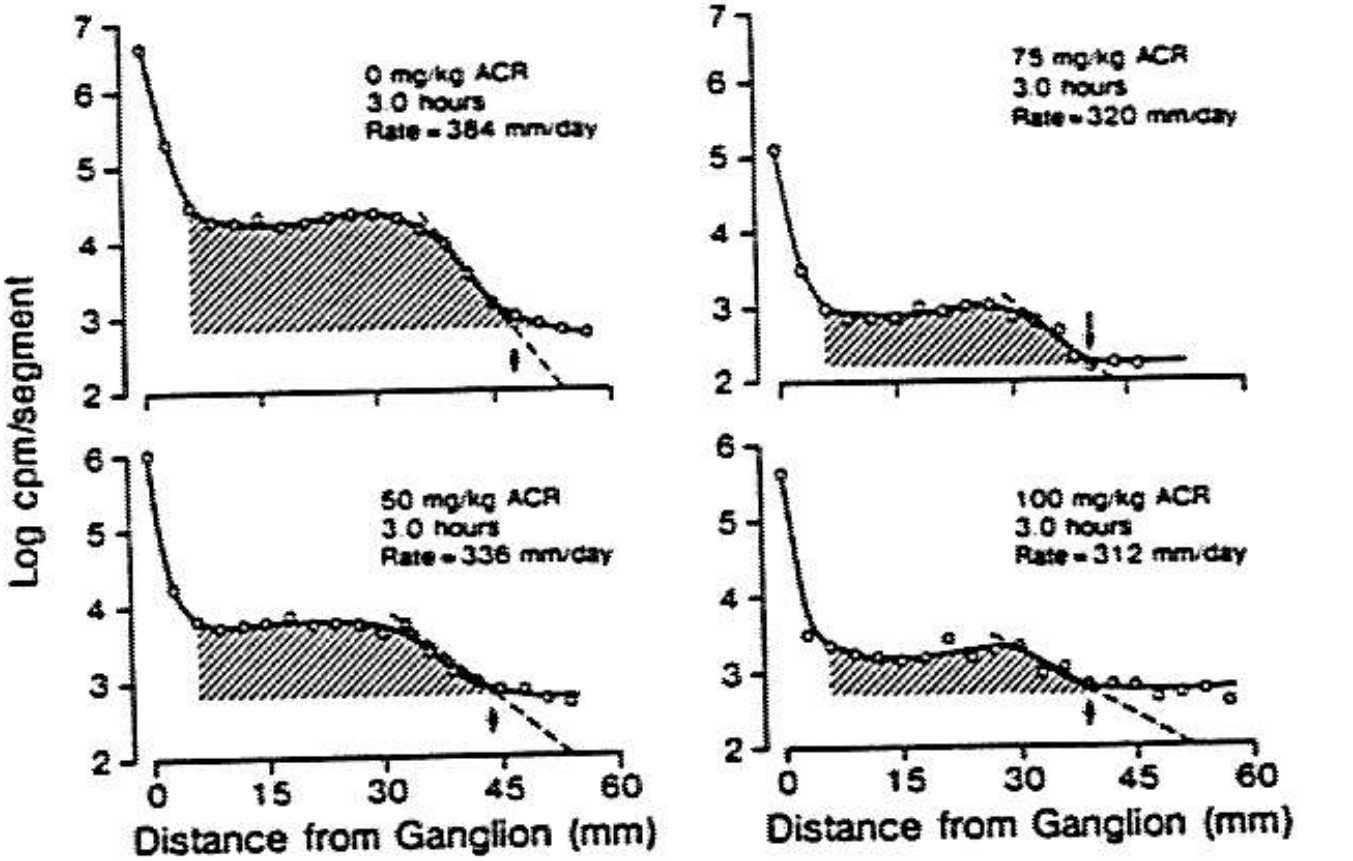
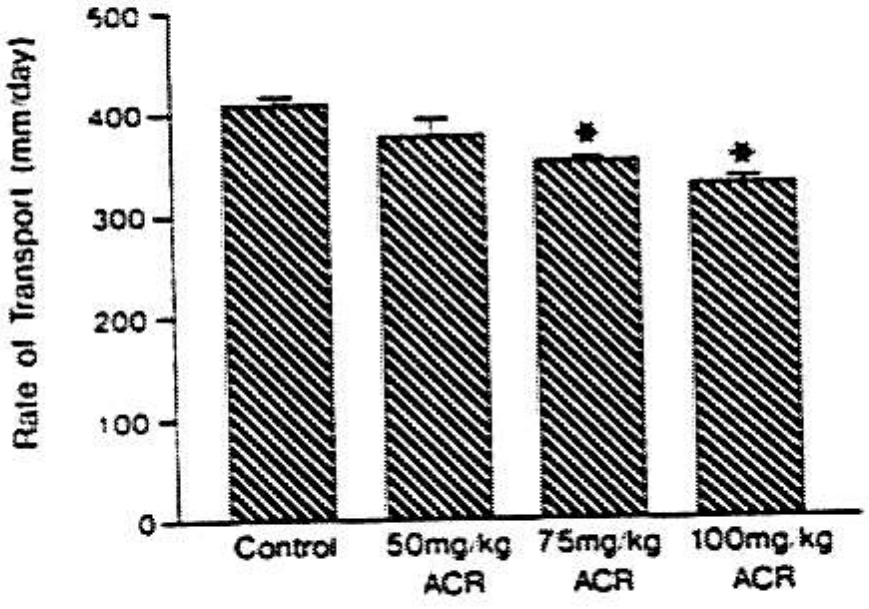

0 mg/kg ACR
3.0 hours
Rate = 384 mm/day
75 mg/kg ACR
3.0 hours
Rate = 320 mm/day
50 mg/kg ACR
3.0 hours
Rate = 336 mm/day
100 mg/kg ACR
3.0 hours
Rate = 312 mm/day
Log cpm/segment
Distance from Ganglion (mm)
Distance from Ganglion (mm)

Figure 13

ACRYLAMIDE EFFECT UPON FAST ANTEROGRADE AXOPLASMIC TRANSPORT RATE



*Significantly different from control $p<.05$

Figure 14

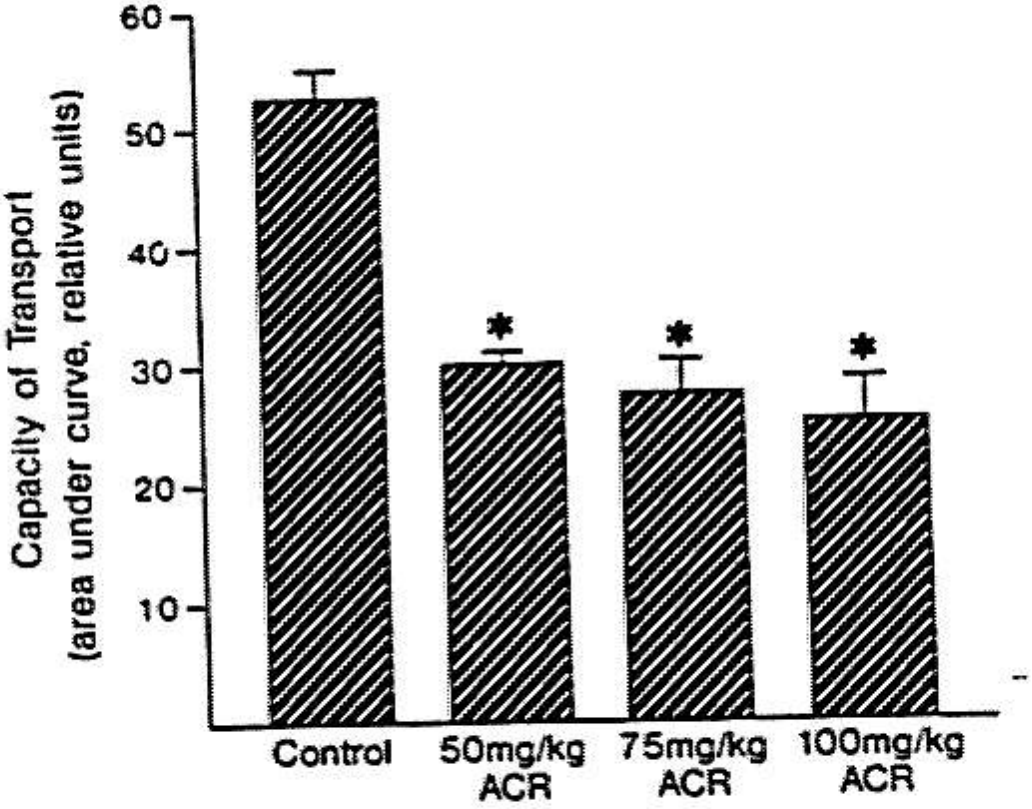


Table 8

RATE OF TRANSPORT (mm/day)

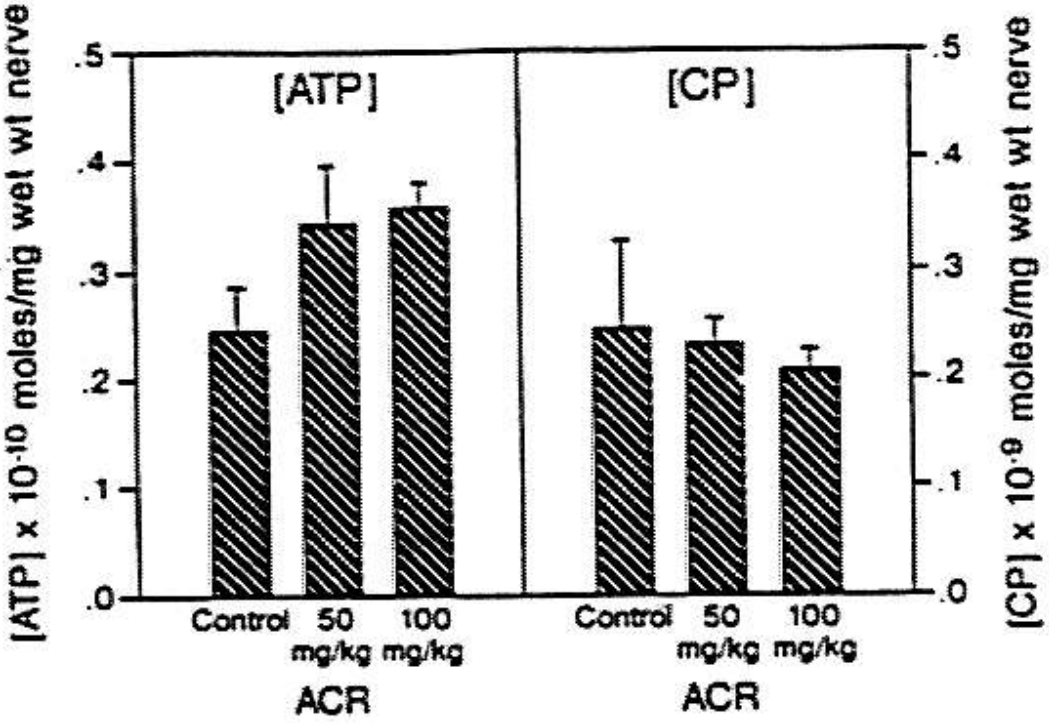
Control	1	424
	2	408
	3	384
	4	432
	5	396
$\bar{x} \pm$ SEM		409 ± 8.8
ACR (50mg/kg)	1	416
	2	367
	3	360
	4	344
$\bar{x} \pm$ SEM		371 ± 15.5
ACR (75mg/kg)	1	344
	2	352
	3	360
	4	340
$\bar{x} \pm$ SEM		349 ± 4.4
ACR (100mg/kg)	1	336
	2	312
	3	312
	4	336
$\bar{x} \pm$ SEM		324 ± 6.9

Table 9

CAPACITY OF TRANSPORT
(Area Under Curve; Relative Units)

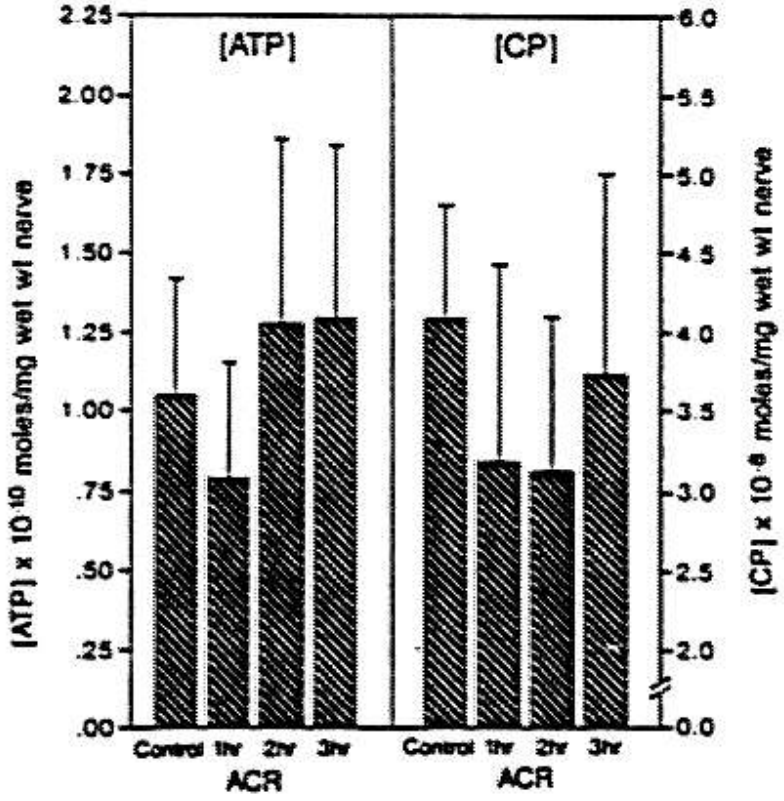
Control	1	52,130
	2	61,270
	3	51,490
	4	45,010
	5	53,490
$\bar{x} \pm$ SEM		52,678 ± 2597
ACR (50mg/kg)	1	31,310
	2	30,790
	3	27,230
	4	32,070
$\bar{x} \pm$ SEM		30,050 ± 1072
ACR (75mg/kg)	1	23,070
	2	34,580
	3	24,010
	4	29,730
$\bar{x} \pm$ SEM		27,847 ± 2683
ACR (100mg/kg)	1	27,740
	2	34,260
	3	23,290
	4	17,260
$\bar{x} \pm$ SEM		25,637 ± 3587

Figure 15



*Significantly different from control $p<.05$

Figure 16



*Significantly different from control $p<.05$