Endocrinological Responses to the Administration of Nicotine: Interactions with Drug Initiation, Conditioned Effects, and Conditions of Stress

by

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Dissertation

submitted to the Faculty of the Department of Medical Psychology

Graduate Program of the Uniformed Services University

of the Health Sciences in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

1984

Volume II

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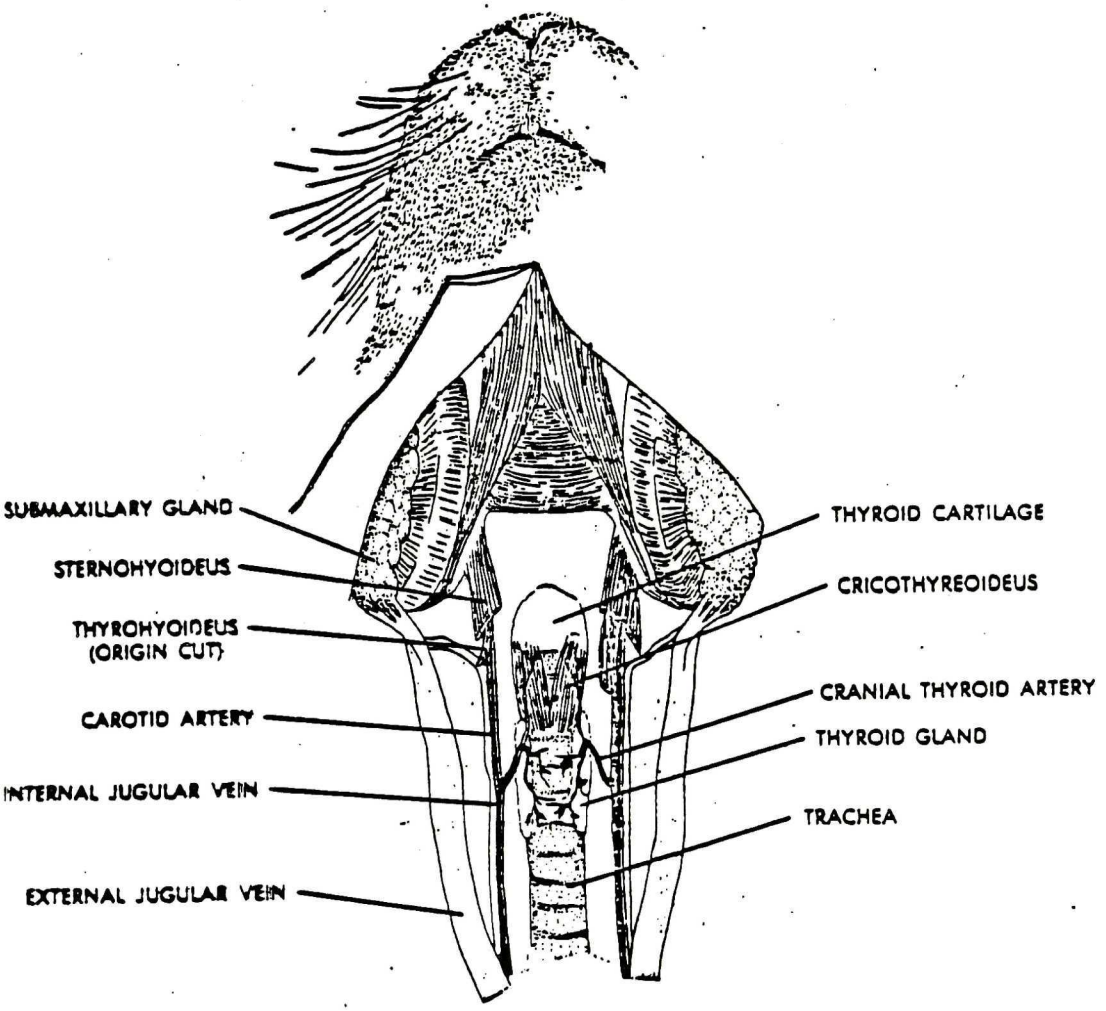
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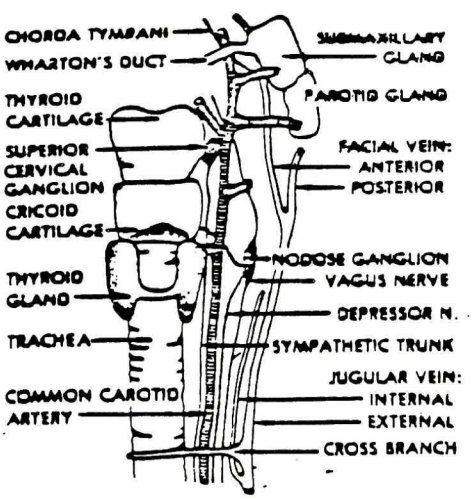
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Appendix A

TIME LINE OF EXPERIMENTAL EVENTS

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
GENTAMICIN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BICILLIN	X			X														
CATHETER IMPLANTATION				X														
POST-OPERATIVE RECOVERY					X													
DRUG ADMINISTRATION						X 1	X 2	X 3	X 4	X 5	X 6	X 7	X 8	X 9	X 10	X 11	X 12	X 13
OVERNIGHT FAST PERIOD			X		X		X				X		X		X		X	
COLLECT PLASMA SAMPLES						X		X				X		X		X		X
TEST OF CONDITIONING														X				
STRESS W/WO NICOTINE																X		X

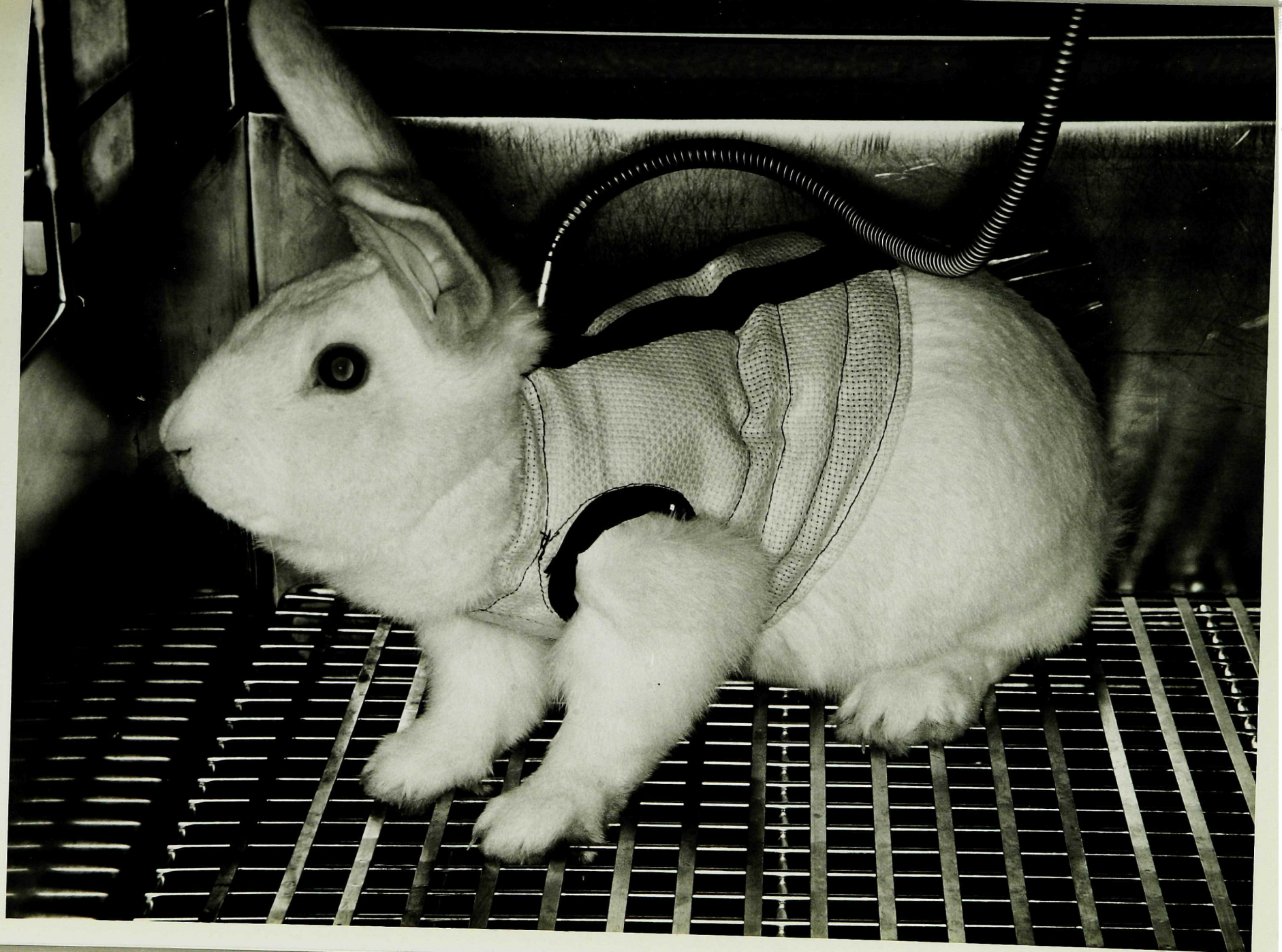


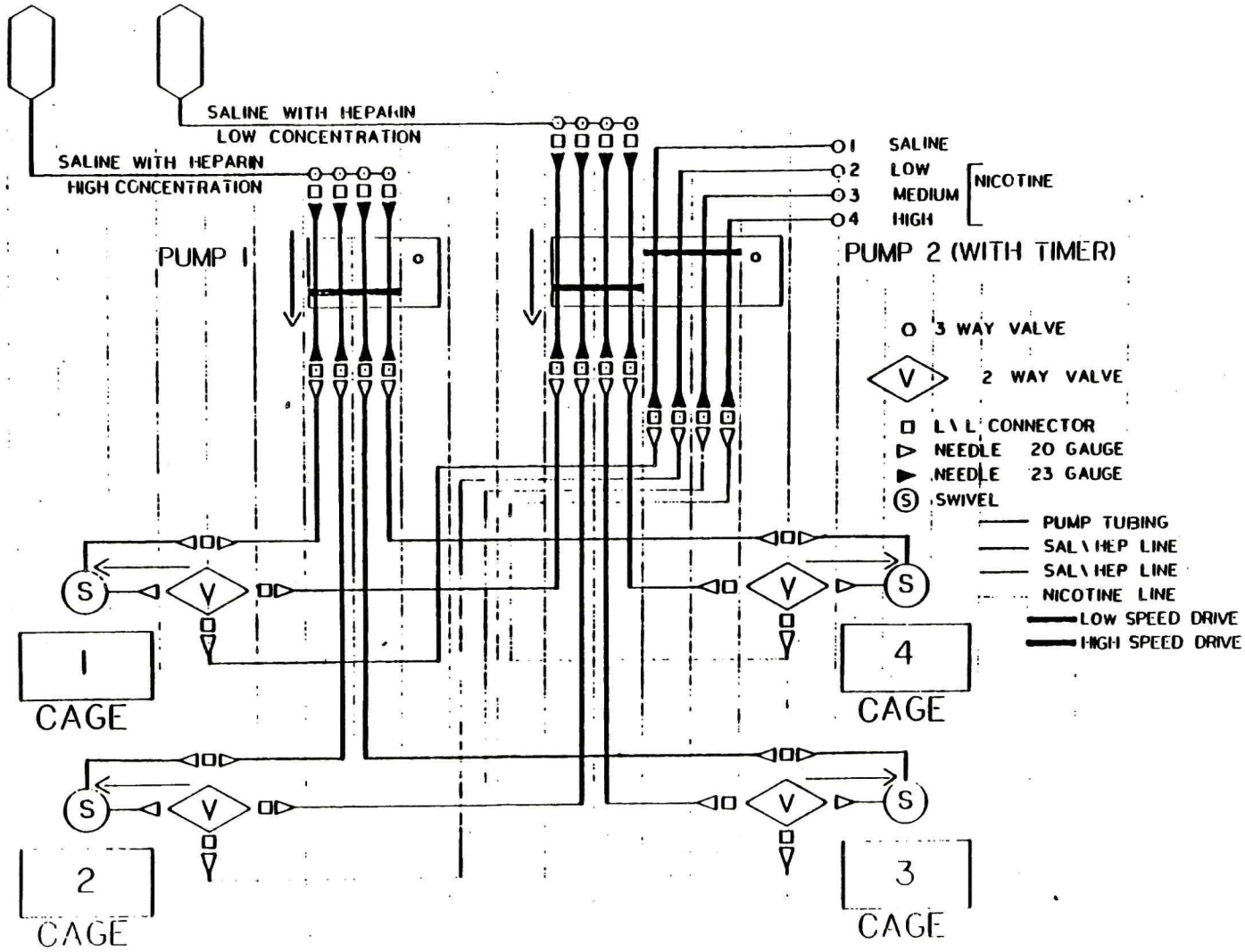


Figs. 1a and b; Neck Anatomy.

Major structures seen in ventral dissection of the neck. (Superficial structures have been removed.)

Figure 2: General Equipment System
(Photographic Plate)




SALINE WITH HEPARIN
LOW CONCENTRATION
SALINE WITH HEPARIN
HIGH CONCENTRATION
PUMP 1
PUMP 2 (WITH TIMER)
1 SALINE
2 LOW
3 MEDIUM
4 HIGH
NICOTINE
3 WAY VALVE
2 WAY VALVE
L \ L CONNECTOR
NEEDLE 20 GAUGE
NEEDLE 23 GAUGE
SWIVEL
PUMP TUBING
SAL \ HEP LINE
SAL \ HEP LINE
NICOTINE LINE
LOW SPEED DRIVE
HIGH SPEED DRIVE
1
CAGE
2
CAGE
3
CAGE
4
CAGE

Appendix B

COMT Assay for Catecholamines

Source: Modified procedure of Durrett, L.R. and Ziegler, M.G. A sensitive radioenzymatic assay for catechol drugs. J. of Neuroscience Research, 1980, 5, 587-598.

I. Reagents

A. Stock Standards:

D,L-Norepinephrine HCl MW = 205.7 $\frac{205.7}{169.2} = 1.216$

(N) Norepinephrine (MW = 169.2)

(-)-Norepinephrine Bitartrate (hydrate)
MW = 337.3 $\frac{337.3}{169.2} = 1.993$

L-Arterenol Bitartrate (hydrate - 1.5 H_2O/mole)
MW = 319.3 $\frac{319.3}{169.2} = 1.887$

(E) Epinephrine (MW = 183.2)

L-Epinephrine Bitartrate MW = 333.3
$\frac{333.3}{183.2} = 1.819$

(D) Dopamine (MW = 153.2)

3-Hydroxytyramine HCl MW = 189.7
$\frac{187.7}{153.2} = 1.238$

1. 1 mg/ml in 0.2 N HAC
   - Prepare 10 ml of each (N, E, & D) separately and store in refrigerator
2. 100 ug/ml NED combination for "NED-A" Aliquots
   - Combine 0.5 ml of each std (N+E+D, 1 mg/ml in 0.2 N HAC)
   - Add 3.5 ml 0.2 N HAC
   - Aliquot 100 l into a series of Sartstedt tubes labeled "NED-A" and store in freezer (10 ug of NED/100 ul).

3. Dil. A - 100 ul aliquot NED - A + 9.9 ml 0.01 N HCl (prepared fresh) (1 ug/1 ml.: 1 ng/ul).

4. Further dilutions are made from dilution A.

B. TEM pH 8.3

- 0.09 M $MgCl_2$ ($MgCl_2$-6 H_2O, 4.57 g/250 ml) MW = 203.31
- 20 mM EGTA (ehtylene glycol-bis-(B-amino-ethyl ether) N,N'-tetracetic acid, 1.9 g/250 ml) MW = 380.4
- 0.2 M TRIS (6.1 g/250 ml) MW = 121.14
- Adjust pH with HCl
- Will go into solution once TRIS is added

C. .75 M Borate buffer with 25 mg/ml EDTA, pH 10

- 23.18 g boric acid crystal + 12.5 g EDTA, add 350 ml H_2O, then add NaOH until pH 10.
- Adjust total volume to 500 ml

D. Cold Carriers
- 1 mg/ml of each in 0.01 N HCl

(NME) Normetanephrine . HCl MW = 219.7

Normetanephrine (MW = 183.2) $\frac{\text{NME . HCl}}{\text{NME}} = \frac{219.7}{183.2} = 1.20$ (NME . HCl 60 mg/50 ml)

(ME) Metanephrine . HCl MW = 233.7

Metanephrine (MW = 197.2) $\frac{\text{ME . HCl}}{\text{ME}} = \frac{233.7}{197.2} = 1.19$ (ME . HCl 59.3 mg/50 ml)

(3MT) 3-Methoxytyramine . HCl MW = 203.7

3-Methoxytyramine (MW = 167.2) (3-Methoxy-4-hydroxyphenylethylamine)
$\frac{\text{3MT . HCl}}{\text{3MT}} = \frac{203.7}{167.2} = 1.22$ (3MT . HCl 60 mg/50 ml)

E. 3:2 Toluene:Isoamyl alcohol (2400 ml toluene:1600 ml isoamyl alcohol)

F. 0.1 N HAC (Acetic acid 99.5%, 5.7 ml/L)

G. Cold Carrier + Ethanol/HCl

- 5 ml H_2O + 100 ml ethanol + 10 ul 1 N HCl + 32 mg NME . HCl + 31 mg ME . HCl + 32 mg 3 MT . HCl

H. Ethylamine Solvent - prepared fresh for each developing jar

- 80 ml Chloroform
- 15 ml Ethanol
- 10 ml Ethylamine
- (Add in this order and solvent will remain clear)

I. 2 N NH_4OH

- 135 ml/1 of 28% solution

J. 4% $NaIO_4$

- Freshly prepared (0.4 g/10 ml)

K. 10% Glycerol

- Refrigerate (10 ml glycerol + 90 ml H_2O)

L. 10 N Acetic Acid

- 288 ml/500 ml of 99.5% (glacial)

M. Phosphor Only

- 50 ml PPO-POPOP in 1000 ml toluene

N. "TIAL": in 1 gal bottle

- 2100 ml toluene
- 900 ml isoamyl alcohol
- 150 ml fluor (RPI, Liquifluor (PPO-POPOP), Spectrofluor)

II. Procedure

A. DAY 1 - Incubation

1. Make sure lyophilizer is running.

2. Pipette 100 ul aliquot for each replicate of each sample into 538, 14 ml round bottom, polypropylene tubes.

   Plasma, CSF - take note of any hemolysis on protocol. Centrifuge, if necessary, to remove fibrin clots.

   Urine - make 1:100 dilution of 100 ul aliquot w/GDW.

3. Plasma Blanks - 100 ul plasma in one tube, 200 ul COMT mix in second tube, after incubation add borate and cold carrier to plasma tube, then transfer 100 ul of incubated COMT mix to plasma tube, vortex.

   Plasma Controls - 100 ul plasma.

   Plasma Standards - Various concentrations of N, Epi and DA added to 100 ml plasma.

4. Keep samples in ice water bath.

5. Add 10 ul 0.01 N HCl (1 ml 1 N HCl + 99 ml GDW) to each sample that doesn't have 10 ul std added to it.

6. Add 100 ul of COMT incubation mix to each tube.

   The mix consists of: 1 ul of benzylhydroxylamine
   84 ul TEM pH 8.3
   0.6 mg/ml glutathione (reduced)
   5 ul H^3-SAM
   10 ul COMT - put in a 14 ml tube for easy pipetting.

   Incubate for 90 min at 37^{o}C.

7. Return tubes to ice water bath. Add 200 ul .75 M borate buffer with 25 mg/ml EDTA (pH 10) to each tube.
8. Add 50 ul cold carrier to each tube, vortex.
9. Add 50 ul 1% TPB, vortex.
10. Add 7 ml 3:2 toluene:isoamyl alcohol to each tube, cap and shake for 10 min. Centrifuge at 3000 rpm for 5 min. Uncap.
11. Place tubes in a dry ice/ethanol bath to freeze aqueous layer. Decant organic phase into 14 ml (538) polypropylene tubes containing 250 ul of 0.1 N acetic acid.
12. Cap and shake for 10 min. Centrifuge at 3000 rpm for 5 min. Uncap.
13. Aspirate organic phase. Wash remaining aqueous layer with 3 ml 3:2 toluene: isoamyl alcohol, recap tubes, shake 10 min. and centrifuge at 3000 rpm for 5 min. Uncap.
14. Aspirate organic phase. Freeze samples -70^{o}C freezer. Turn on shelf refrigeration on lyophilizer.
15. Put samples in shelf chamber. Turn off shelf refrigeration if doing overnight work. If more than one night, leave refrigeration on. Lyophilize remaining aqueous layer in all tubes.

B. <u>DAY 2 - TLC Separation of methylated products</u>

1. Take out samples. Turn system back on.

2. Add 50 ul cold carrier + HCl/ethanol solution and centrifuge for 30 sec. at 3000 rpm.
3. Spot solution onto prescored silica gel TLC plates with fluorescent indicator and preadsorbent area (usually requires 2-3 separate applications). (Whatman LKSDF TLC plates) Dry plates with a hair dryer, if necessary.
4. Add 50 ul cold carrier + HCl/ethanol solution again and spot as above (no need to centrifuge second time).
5. Develop plates in TLC jars containing ethylamine solvent system (line jars with chromatography paper to equalize solvent vapor, if necessary).
6. Visualize spots on plate using U.V. light and mark with soft pencil. The bands are in the order: Origin, normetanephrine, metanephrine, 3-methoxytyramine, solvent front.
7. 3-methoxytyramine, normetanephrine, and metanephrine spots are scraped and placed into separate liquid scintillation vials.

C. Preparation for Counting

1. Dopamine (non-beta-hydroxylated products)
   a. Add 1 ml 0.05 N NH_4OH to each sample and shake (slow) for 15 min.
   b. Add 5 ml TIAL, cap, shake vigorously, and count 5 min/vial.

2. Epinephrine and Norepinephrine (beta-hydroxylated products)

   a. Add 1 ml 2 N NH_4OH to each counting vial and shake for 15 min.

   b. Add 50 ul freshly prepared 4% $NaIO_4$ to each vial.

   c. After 5 min., stop the reaction by adding 50 ul 10% glycerol to each vial.

   d. Add 200 ul 10 N acetic acid to each vial.

   e. Add 5 ml Phosphor-Only, cap, shake vigorously, and count 5 min/vial.

D. Calculation of Sample Concentration

Formula:

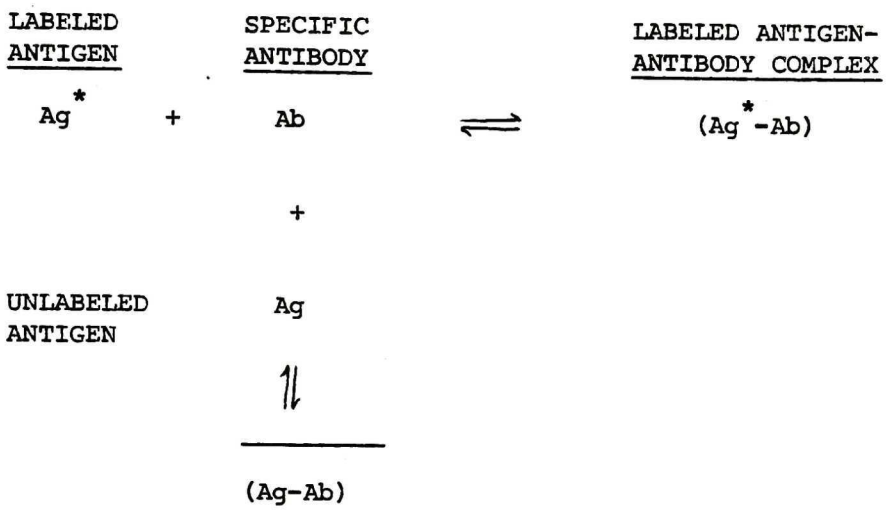
$$\frac{\bar{x}\ \text{CTS Sample} - \bar{x}\ \text{CTS blank}}{\bar{x}\ \text{CTS}_{5000 + p} - \bar{x}\ \text{CTS}_{p}} \text{ (5000 pg/ml) (dilution factor)}$$

$\bar{x}\ \text{CTS}_{p}$ = mean counts for endogenous catecholamines

$\bar{x}\ \text{CTS}_{5000 + p}$ = endogenous catecholamines + 5000 pg/ml

Concepts of Radioimmunoassay

The reactions involved in any competitive radioimmunoassay procedure can be illustrated by the reactions below.



The unlabeled antigen (Ag) competes with the labeled antigen (Ag^*) for a limited number of antibody (Ab) binding sites and decreases the amount of labeled antigen bound to the antibody (Ag^*-Ab).

If the amount of the antibody and labeled antigen are the same for all samples, the only variable is the amount of unlabeled antigen. Therefore, unknown samples can be quantitated by comparing the decrease in antibody binding of labeled antigen in unknowns and in known standard solutions.

The unbound antigen may be separated from the antibody-bound antigen, and the amount of binding of the radioactive antigen can be determined by counting the radioactivity of either fraction.

Procedure for the Radioimmunoassay (H^3) of Plasma Corticosterone

Source: Radioassay Systems Laboratories, Inc., Carson, CA 90746.

I. Kit Contents

11 ml Corticosterone antibody

12 ml Corticosterone - H^3, 4,000-6,000 cpm/0.1 ml

6 x 5 ml Corticosterone Standards, Range: 0.025-1.0 ng/0.5 nl

22 ml Charcoal Dextran Solution

112 ml Assay Buffer

II. Sample Preparation

Dilute plasma sample 1:250 with diluent buffer in glass test tubes (suggested; 0.01 ml plasma diluted with 2.5 ml of buffer).

III. Assay Procedure

A. Using 10 x 75 mm glass test tubes, add 0.6 ml of diluent buffer to tubes number 1 and 2 (Blank or Non-Specific Binding Tubes).

B. Add 0.5 ml of buffer to tubes 3 and 4 (100% binding tube or Zero Standard tube).

C. Add 0.5 ml (in duplicate) of each corticosterone standard (0.025-1.0 ng/0.5 ml) to tubes number 5 through 16 (Standard Curve).

D. Add 0.5 ml (in duplicate) of 1:250 dilutes sample to tube numbers 17 to end of assay.

E. Incubate all tubes at 98°C for ten minutes. (This step is necessary to denature the corticosterone binding proteins in plasma.)

F. Allow samples to cool to room temperature following incubation (approximately thirty minutes).

G. With the exception of tubes number 1 and 2, add 0.1 ml of antiserum to the assay tubes.

H. Add 0.1 ml of corticosterone - H^3 to all the assay tubes.

I. Mix and incubate tubes for a minimum of one hour or a maximum of twenty-four hours in a 4°C water bath.

J. After incubation, add 0.2 ml of cold (4°C) charcoal-dextran solution (swirling the charcoal solution constantly). Mix for twenty seconds by shaking the whole test tube rack, then let sit for twenty minutes at 4°C.

K. Centrifuge at 2500 rpm for fifteen minutes.

L. Decant supernatant solution into scintillation vial and add 5 ml of counting cocktail (Scint-A, Packard Instrument Co. Inc., Downers Grove, IL).

M. Count each tube for a minimum of two minutes in a scintillation counter set for H^3.

IV. Calculations

A. Percent Bound

1. Average the counts of tubes 1 and 2 (Blank) and subtract the value from all other duplicate assay tubes.

2. Divide the value obtained from tubes 3 and 4 into the values obtained for duplicate sample tubes. Multiply value by 100 to determine percent bound.

   Formula:

$$\frac{\text{CTS (sample - CTS (Blank)}}{\text{CTS (0 Standard) - CTS (Blank)}} \text{ X 100 - \% Bound}$$

   CTS = average counts of duplicate tubes

   Sample = standard, control or unknown

   Blank = non-specific binding tube

   0 Standard = 100% binding tube, 0 Corticosterone

B. Final Results

1. Plot percent bound against the corticosterone standards ranging from 0.025-1.0 ng/0.5 ml on semi-logarithmic paper (use 100% as the starting point).

2. Locate % bound of sample on the standard curve, then read ng off axis; multiply by 500 to determine ng Corticosterone/ml of sample.

Procedure for the Radioimmunoassay (I^{125}) of Plasma Insulin

Source: Amersham Corporation, Arlington Heights, IL 60005. Code IM.78, March 1977.

PRINCIPLES OF THE PROCEDURE

Samples of the unknown and standard insulin solutions are incubated for 45 minutes with suspended insulin binding reagent. Then, Insulin I^{125} is added and the samples incubated for an additional 2 hours and 15 minutes.

The insulin binding reagent is an insoluble complex of guinea pig antibody to insulin which has been reacted with a second rabbit antibody to the guinea pig antibody. This provides both an antibody site for reaction with insulin and the basis for separation of antibody-bound insulin and the unbound insulin.

Insoluble antigen-antibody complex is separated from the soluble free insulin by centrifugation.

By counting the radioactivity in the precipitate for both standards and unknowns, a standard curve can be constructed and unknown values can be interpolated.

REAGENTS

1. Insulin Binding Reagent

Description

The insulin binding reagent is a freeze-dried preparation of anti-insulin serum (guinea pig) precipitated by anti-guinea pig serum (rabbit), which contains phosphate buffer, preservative, EDTA and bovine serum albumin as a stabilizer.

Reconstitution

Add 8.0 ml of distilled water to the vial containing the insulin binding reagent.

Storage of Reconstituted Insulin Binding Reagent

Use within one month after reconstitution, storing at -20°C, thawing only once.

2. Insulin I^{125}

Description

This component is prepared from purified bovine insulin, which has a potency of 24 international units/mg. Each vial contains 0.04 ug iodinated insulin dissolved in 2 ml of stabilized phosphate buffer. The vial contains not more than 10 uCi of I^{125}.

Dilution

Prepare an insulin I^{125} working solution by pipetting into a glass vial 1.0 ml of the reagent insulin I^{125} into 7.0 ml of Buffer.

Storage of Diluted Insulin I^{125}

Store at 2-4°C for not more than one week or at -20°C for several weeks, but do not refreeze after thawing.

3. Human Insulin Standard

Description

This component is a freeze-dried preparation of human insulin. After reconstitution according to directions, the solution will contain 1000 microunits of insulin per ml.

Reconstitution of Insulin Standard Stock Solution

The number of microunits of insulin contained in the vials is

stated on the label. Reconstitute with sufficient buffer to prepare a stock solution containing 1000 microunits/ml. For example, if the vial is labeled 2300 microunits add 2.30 ml Buffer. Reconstitution is best accomplished according to the following procedure.

Give the bottle several sharp taps to dislodge any solid particles on the cap. Carefully remove the cap and add the appropriate amount of Buffer using a micropipette; replace the cap. Allow to stand about one minute, vortex mix gently and then invert bottle several times until all solid is dissolved.

Dispense two 400 ul aliquots of the insulin standard stock solution into separate glass vials. If the Insulin RIA Kit is to be used on two separate occasions, store one aliquot as described below. The remaining aliquot should be diluted according to the method described under "Details of Procedure," and used immediately. The dilution is used to prepare the assay standards.

Storage of Insulin Standard Stock Solution

Store at -20°C. Use within one month of reconstitution, thawing only once. Do not use the stored aliquot after the expiry date stated on the vial label.

4. Buffer Component

Description

This component contains freeze-dried stabilized phosphate buffer.

Reconstitution

Dissolve the contents of one container of buffer using about 30 ml freshly distilled water. Transfer the solution to a measuring

cylinder. Rinse out the container with more freshly distilled water and adjust the final volume to 100 ml. Mix carefully.

Storage of Reconstituted Buffer

This reconstituted buffer may be stored at 2-4°C for up to four weeks.

STORAGE

The Insulin RIA Kit should be stored at 2-4°C prior to reconstitution of reagents. The expiration date is stamped on the package and will normally be 6-8 weeks from date of shipment.

SPECIMEN COLLECTION AND PREPARATION

The Amersham Insulin RIA Kit may be used to determine insulin levels in serum or heparinized plasma samples. Sufficient blood should be drawn to provide 1 ml of plasma. The plasma should be separated as soon as possible. These samples may be stored for several weeks at -20°C. Thawed plasma samples should be mixed, then centrifuged to remove fibrin or other solids.

In using combined insulin/glucose testing, the patient should be prepared for testing according to the laboratory's standard procedures for the glucose tolerance test. This may include a controlled diet for several days prior to testing and/or an overnight fast prior to testing.

In addition, baseline (fasting) samples are often collected prior to the administration of the glucose dose.

Samples for glucose levels should be collected according to standard laboratory procedures and, in some cases, the sample may be split for both insulin and glucose levels.

In combined procedures, strict adherence to the established testing protocol and patient preparation procedures are required to allow valid comparisons with the laboratory's established norms.

Hemolyzed samples will show decreased insulin levels due to insulin degradation and should not be used. Patient samples containing radioactivity should not be used.

PROCEDURE

Materials Provided

- Insulin I^{125} solution - one vial
- Insulin Binding Reagent - two vials
- Human Insulin Standard - one vial
- Buffer Component - two containers

Materials Required But Not Provided

- Vortex mixer
- Gamma scintillation counter
- Refrigerator or ice bath (2-4^{o}C)
- Reaction and counting tubes - 4 ml or larger round bottomed polystyrene tubes are suitable (e.g. 12 x 75 mm)
- Pipettes - 100 ul, 200 ul, 500 ul
- Repeating sampler or pipette to measure 700 ul
- Distilled water
- Centrifuge - preferably refrigerated, capable of at least 1500g with load
- Clean glass vials (10 ml size)
- Stoppered glass measuring cylinder (100 ml)

- Tube racks
- Absorbent paper

Details of Procedure

1. Reconstitute all reagents and prepare the insulin stock solution as described under Reagents. All reagents should be at room temperature prior to pipetting.

2. Prepare an insulin working standard solution of 160 microunits/ml by adding a 400 ul aliquot of the stock standard to 2.1 ml Buffer in a clean glass vial. Mix thoroughly.

(This volume of working standard is sufficient for the preparation of one set of assay standards. The small amount of standard left after preparation of the assay standards should be discarded and not stored for later use.)

3. Prepare insulin assay standards of 80, 40, 20, 10 and 0 microunits/ml by adding the 160 microunits/ml working standard to the appropriate amount of Buffer according to the scheme in Table 1. Add Buffer to assay tubes before adding the working solution aliquot. Use clean glass vials for these solutions. Mix each thoroughly.

The preparation of working standards by a series of doubling dilutions is not recommended.

4. Arrange polystyrene assay tubes according to the scheme shown in Table 2. Standards should be assayed in triplicate and unknowns in duplicate (see Note 1).

5. Pipette 100 ul aliquots of Buffer, insulin standard, or unknown sera according to the scheme shown in Table 2 (see Note 2). Blank

tubes require 200 ul of buffer. Pipette solutions directly to the bottom of the assay tubes in all steps.

6. Pipette 100 ul aliquots of insulin binding reagent into all tubes except the total counts and blank tubes. Vortex mix the tubes and incubate at 2-4°C for 45 minutes.

7. Pipette 100 ul aliquots of insulin I^{125} solution into each tube. The total counts tubes should be set aside at this stage awaiting counting at step 13 of the protocol. Vortex mix the tubes and incubate at 2-4°C for not less than two hours and fifteen minutes.

8. Add 700 ul cold Buffer (2-4°C) to all tubes except the total counts. Deliver the Buffer so as to wash down the tube walls. A repeating sampler is sufficiently accurate for this step. Again vortex mix.

9. Centrifuge tubes for 25 minutes ($\pm$ 2 minutes) applying a force of at least 1500g in a refrigerated (2-4°C) or room temperature centrifuge. Refrigeration and/or greater force will assist the separation. If a refrigerated centrifuge is not available, tubes should not be subjected to long periods of centrifugation in a warm centrifuge.

10. At the end of the centrifugation allow the rotor to slow without application of the brake. Immediately remove the tubes with the minimum of agitation and place into racks. Invert each tube gently in one continuous movement, so that the precipitate will remain undisturbed at the bottom of the tube. Do not decant the total counts tubes. Avoid tapping or shaking the tubes during decantation. It

should be noted that the supernatant fluid which is poured off will be slightly radioactive and should be treated accordingly."

11. Keeping the tubes inverted, place over tissue paper to drain for at least 15 minutes. Any remaining liquid in the necks of the tubes may be removed by touching with tissue. Note that the precipitate may not be visible.

12. After draining, the bottoms and rims of each tube should be wiped clean using dampened tissue paper.

13. Count the tubes in a gamma counter (see Note 3).

Notes

1. Special precautions

The buffer used in the Insulin RIA Kit is specially formulated to reduce absorption of insulin. Nevertheless, certain precautions are necessary to minimize absorption of insulin:

a. Scrupulously cleaned glass vials should be used for the preparation and storage of insulin stock standards, insulin working standard and insulin I^{125} solutions.

b. Pipette tips or microsyringes should be wetted thoroughly by drawing up and discharging pure buffer solution several times before pipetting solutions of insulin.

2. Pipetting

As with other tests of this type, precision pipetting is an essential feature. The use of precision microsyringes or micropipettes with disposable tips is strongly recommended as providing the desired convenience and precision (better than 1% standard deviation).

3. Determination of radioactivity

The time of counting will depend on the efficiency of the instrument and the age of the kit. One minute counts will usually be found sufficient for all tubes, accumulating 10,000 or more counts for the zero standard.

The use of glass tubes for counting or for secondary containment of the counting tubes should be avoided since the attenuation of the low energy I^{125} radiation by glass is relatively high. Not only is

the count rate markedly reduced, but serious errors may arise due to variations in the wall thickness of the glass container.

Increased Precision Assay

Slightly greater precision may be obtained by delaying the addition of the insulin I^{125} until the other reagents have been incubated at 2-4°C for 6 hours. After the addition of the insulin I^{125} the contents of the tubes are mixed, then returned to the refrigerator (2-4°C) for an additional 18 hours incubation prior to separation.

RESULTS

Calculation of Results

1. Express the count-rate for each tube in counts per unit time. Compute the mean count-rates for each series of replicates, rejecting any count-rates which are grossly aberrant.

2. Plot on linear gragh paper the counts/time of insulin I^{125} in the precipitate at each concentration of insulin in the standards and for the zero standard. The concentration of insulin in each of the unknowns, expressed in terms of the insulin used as a standard, can be read directly from the graph. A typical graph is shown in Figure 1.

Procedure for Plasma Glucose Determination

Source: Fales, F. W. Standard Methods in Clinical Chemistry 4:101, 1963. Kingsley, G. R., & Gatchel, G. Clinical Chemistry 6:466, 1966

Glucose Oxidase Method

Glucose is oxidized by the enzyme glucose oxidase in the presence of air to glucuronic acid with the formation of hydrogen peroxide. In the presence of the added enzyme, peroxidase, the hydrogen peroxide will oxidize the chromogen o-dianisidine to a compound that gives a red color in strongly acid solution. The reaction is very specific for glucose and thus gives true glucose values. High concentrations of reducing substances, particularly ascorbic acid, will interfere by competing with the chromogen for the liberated oxygen and thus cause low results. Hemoglobulin will also interfere by causing premature decomposition of the hydrogen peroxide and also give low results. If hemoglobin is absent, the reaction can be run directly on serum or plasma. For hemolyzed samples or whole blood a Somogyi filtrate must be used.

REAGENTS

1. Phosphate buffer, 0.1M, pH 7.0. Dissolve 8.7 g Na_2HPO_4 and 5.3 g KH_2PO_4 in about 950 ml water. Check the pH and adjust to 7.0 if necessary with small amounts of 1N acid or base; then dilute to 1L.

2. Buffered peroxidase solution. Mix 125 ml phosphate buffer, 175 ml water, and 200 ml glycerin. Dissolve 10 mg peroxidase (Sigma type II*) in this solution, then add 100 mg o-dianisidine dissolved in

10 ml methanol, and mix well. Because of the added glycerin, this solution may be stored in the freezer compartment for added stability.

3. Glucose oxidase solution. Dissolve 500 mg glucose oxidase (Sigma type II) in 50 ml 40% glycerin. This may also be stored in the freezer.

4. Sulfuric acid, 30%. Carefully add 300 ml concentrated sulfuric acid to 700 ml water. Mix well and cool to room temperature.

5. Procedure for direct method: Pipet 4.5 ml buffered peroxidase to a test tube. Warm to 37°C and add 0.02 ml serum or plasma. Add 0.5 ml glucose oxidase solution and incubate at 37°C for 30 minutes. After incubation, add 3 ml 30% sulfuric acid and mix. Run standards and blank similarly. Read standards and samples against blank at 530 nm.

Calculation: Since the standards and samples are treated similarly:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Conc. of standard} = \text{Conc. of sample}$$

Appendix C

Table 1

Part I

Experiment Phase I: Norepinephrine

Analysis of Variance Summary Table

Response Grand Mean = 367.49 pg/ml

Source	df	Sum of Squares	Mean Square	F Value	p<
Between Subjects Factors					
Drug	3	748484.79	249494.93	0.71	0.5722
s/Drug	8	2806951.79	350868.97		
Within Subject Factors					
Day	2	596300.47	296800.23	2.97	0.0800
Drug x Day	6	475865.99	79310.99	0.79	0.5908
Day x s/Drug	16	1606581.27	100411.32		
Sample	5	942116.38	188423.27	11.50	0.0001
Drug x Sample	15	397449.82	26496.65	1.62	0.1124
Sample x s/Drug	40	655305.55	16382.63		
Day x Sample	10	137520.74	13752.07	0.78	0.6457
Drug x Day x Sample	30	398201.71	13273.39	0.75	0.8045
Day x Sample x s/Drug	80	1406665.02	17583.31		

Table 1
Part II
Norepinephrine

Tukey's Studentized Range Test
Alpha = 0.05

A) Drug Condition:
Mean Square Error = 350868.97, df = 8
Critical Value of Studentized Range = 4.53
Minimum Significant Difference = 365.15

Drug Condition	Mean Response (pg/ml)	N
Saline (control)	284.97	54
0.025 mg Nic/kg	398.53	54
0.050 mg Nic/kg	344.45	54
0.100 mg Nic/kg	442.04	54

B) Test Day:
Mean Square Error = 100411.32, df = 16
Critical Value of Studentized Range = 3.65
Minimum Significant Difference = 136.30

Test Day	Mean Response	N
1	441.64	72
3	334.70	72
7	326.16	72

C) Sample (0-5):
Mean Square Error = 16382.63, df = 40
Critical Value of Studentized Range = 4.23
Minimum Significant Difference = 90.23

Sample	Mean Response	N
0	247.55	36
-- Nicotine infusion		
1	336.58	36
2	361.56	36
-- Nicotine infusion		
3	382.72	36
4	454.70	36
5	421.87	36

Table 1
Part II (Continued)
Norepinephrine

Tukey's Studentized Range Test

Drug x Sample Interaction

Alpha = 0.05, N = 9 for each mean
Mean Square Error = 16382.63, df = 40
Critical Value of the Studentized Range = 4.23
Minimum Significant Difference = 180.47

	Sample					
	0	1	2	3	4	5
Saline (Control)	270.75	286.02	287.98	261.94	302.09	301.02
0.025 mg Nic/kg	258.92	422.59	405.57	376.09	451.02	476.97
0.050 mg Nic/kg	195.70	268.36	328.75	379.89	486.23	407.78
0.100 mg Nic/kg	264.84	369.33	423.95	512.95	579.46	501.69

All values are in ng/ml of plasma

Table 1
Part III
Norepinephrine

Group Mean Response for Saline Control and
All Nicotine Doses by Samples (0-5) on Day 7

(Values are in pg Norepinephrine/ml of plasma)

	Sample					
	0	1	2	3	4	5
Saline (Control)	262.82	273.91	243.65	264.75	292.50	303.72
Standard Error	39.65	72.46	55.52	52.44	50.89	37.66
▵ from baseline		11.09	-19.17	1.93	29.68	40.90
All Nicotine Doses	194.15	317.18	336.83	334.49	463.60	463.00
Standard Error	21.80	45.00	44.74	63.11	92.42	100.23
▵ from baseline		123.03	142.68	140.34	269.45	268.85
Student's t Statistic: Control vs. All Nicotine Doses (df = 9)						
t	1.60	-0.50	-1.14	-0.63	-1.08	-0.93
p <	0.1435	0.6266	0.2826	0.5434	0.3087	0.3747

Table 1
Part IV
Norepinephrine

A. Group Mean Responses by Drug Condition and Day

(Values are in pg/ml of plasma)

Drug Condition	Day 1	3	7
Saline Control	259.41	321.94	273.56
0.025 mg Nic/mg	536.25	322.17	330.14
0.050 mg Nic/mg	400.21	284.60	285.97
0.100 mg Nic/mg	431.34	381.25	416.67

B. Group Mean Responses for All Drug Conditions by Sample

(Values are in pg/ml of plasma)

		Sample				
Drug Condition	0	1	2	3	4	5
Saline Control	270.75	286.03	287.98	261.94	302.10	301.02
Δ from baseline		15.28	17.23	-8.81	31.35	30.27
0.025 mg Nic/kg	274.01	422.60	405.57	376.09	451.03	476.97
Δ from baseline		148.59	131.56	102.08	177.02	202.96
0.050 mg Nic/kg	189.12	255.89	333.20	361.36	485.09	399.72
Δ from baseline		66.77	144.08	172.24	295.97	210.60
0.100 mg Nic/kg	249.63	341.11	402.15	461.74	539.11	461.00
Δ from baseline		91.48	152.52	212.11	289.48	211.37

Table 2

Part I

Experiment Phase I: Epinephrine

Analysis of Variance Summary Table

Response Grand Mean = 76.59 pg/ml

Source	df	Sum of Squares	Mean Square	F Value	p
Between Subjects Factors					
Drug	3	310971.97	103657.32	1.77	0.2300
s/Drug	8	467861.01	58482.62		
Within Subject Factors					
Day	2	271456.59	135728.29	4.29	0.0323
Drug x Day	6	237050.51	39508.41	1.25	0.3342
Day x s/Drug	16	506424.55	31651.53		
Sample	5	320230.29	64046.05	1.67	0.1651
Drug x Sample	15	609705.47	40647.03	1.06	0.4227
Sample x s/Drug	40	1537514.13	38437.85		
Day x Sample	10	187204.08	18720.41	0.99	0.4561
Drug x Day x Sample	30	611952.76	20398.42	1.08	0.3789
Day x Sample x s/Drug	80	1507350.23	18841.88		

Table 2
Part II
Epinephrine

Tukey's Studentized Range Test
Alpha = 0.05

A) Drug Condition:
Mean Square Error = 58482.62, df = 8
Critical Value of Studentized Range = 4.53
Minimum Significant Difference = 149.07

Drug Condition	Mean Response (pg/ml)	N
Saline (control)	40.89	54
0.025 mg Nic/kg	70.93	54
0.050 mg Nic/kg	54.86	54
0.100 mg Nic/kg	139.67	54

B) Test Day:
Mean Square Error = 31651.53, df = 16
Critical Value of Studentized Range = 3.65
Minimum Significant Difference = 76.52

Test Day	Mean Response	N
1	123.99	72
3	67.04	72
7	38.74	72

C) Sample (0-5):
Mean Square Error = 38437.85, df = 40
Critical Value of Studentized Range = 4.23
Minimum Significant Difference = 138.21

Sample	Mean Response	N
0	43.03	36
-- Nicotine infusion		
1	52.80	36
2	61.94	36
-- Nicotine infusion		
3	159.77	36
4	72.72	36
5	69.26	36

Table 2
Part III
Epinephrine

Group Mean Response for Saline Control and
All Nicotine Doses by Samples (0-5) on Day 7

(Values are in pg Epinephrine/ml of plasma)

	Sample					
	0	1	2	3	4	5
Saline (Control)	34.14	36.29	29.97	35.69	42.41	38.53
Standard Error	4.60	7.16	1.78	1.41	4.08	7.29
Δ from baseline		2.15	-4.17	1.55	8.27	4.39
All Nicotine Doses	28.24	32.33	33.94	59.51	36.95	37.98
Standard Error	3.52	3.57	8.10	20.39	4.36	6.06
Δ from baseline		4.09	5.70	31.27	8.71	9.74
Student's t Statistic: Control vs. All Nicotine Doses (df = 9)						
t	0.91	0.55	-0.29	-0.69	0.71	0.05
p<	0.3853	0.5968	0.7784	0.5067	0.4963	0.9613

Table 2
Part IV
Epinephrine

A. Group Mean Responses by Drug Condition and Day

(Values are in pg/ml of plasma)

Drug Condition	Day 1	3	7
Saline Control	46.11	40.38	36.17
0.025 mg Nic/kg	128.22	54.48	26.23
0.050 mg Nic/kg	66.18	41.58	52.37
0.100 mg Nic/kg	195.42	111.98	40.63

B. Group Mean Responses for All Drug Conditions by Sample

(Values are in pg/ml of plasma)

	Sample					
Drug Condition	0	1	2	3	4	5
Saline Control	42.09	42.25	37.42	38.47	42.02	43.07
▲ from baseline		0.16	-4.67	-3.62	-0.07	0.98
0.025 mg Nic/kg	43.37	57.22	77.15	69.69	84.32	95.53
▲ from baseline		13.85	33.78	26.32	40.95	52.16
0.050 mg Nic/kg	35.91	34.52	49.28	97.93	66.86	43.80
▲ from baseline		-1.39	13.37	62.02	30.95	7.89
0.100 mg Nic/kg	49.56	69.46	77.80	352.57	99.82	87.96
▲ from baseline		19.90	28.24	303.01	50.26	38.40

Table 3

Part I

Experiment Phase I: Dopamine

Analysis of Variance Summary Table

Response Grand Mean = 474.25 pg/ml

Source	df	Sum of Squares	Mean Square	F Value	p<
Between Subjects Factors					
Drug	3	568056.98	189352.32	1.45	0.2985
s/Drug	8	1043090.41	130386.30		
Within Subject Factors					
Day	2	899501.51	449750.75	2.53	0.1107
Drug x Day	6	1452880.82	242146.80	1.36	0.2874
Day x s/Drug	16	2840293.62	177518.35		
Sample	5	89471.66	17894.33	3.50	0.0102
Drug x Sample	15	105918.62	7061.24	1.38	0.2038
Sample x s/Drug	40	204576.65	5114.41		
Day x Sample	10	96215.45	9621.54	1.48	0.1604
Drug x Day x Sample	30	137226.93	4574.23	0.71	0.8570
Drug x Sample x s/Drug	80	518387.51	6479.84		

Table 3
Part II
Dopamine

Tukey's Studentized Range Test
Alpha = 0.05

A) Drug Condition:
Mean Square Error = 130386.30, df = 8
Critical Value of Studentized Range = 4.53
Minimum Significant Difference = 222.59

Drug Condition	Mean Response (pg/ml)	N
Saline (control)	454.98	54
0.025 mg Nic/kg	401.69	54
0.050 mg Nic/kg	538.44	54
0.100 mg Nic/kg	501.89	54

B) Test Day:
Mean Square Error = 177518.35, df = 16
Critical Value of Studentized Range = 3.65
Minimum Significant Difference = 181.23

Test Day	Mean Response	N
1	562.77	72
3	410.77	72
7	449.20	72

C) Sample (0-5):
Mean Square Error = 5114.41, df = 40
Critical Value of Studentized Range = 4.23
Minimum Significant Difference = 50.41

Sample	Mean Response	N
0	434.44	36
-- Nicotine infusion		
1	472.33	36
2	474.17	36
-- Nicotine infusion		
3	483.37	36
4	478.78	36
5	502.41	36

Table 3
Part III
Dopamine

Group Mean Response for Saline Control and
All Nicotine Doses by Samples (0-5) on Day 7

(Values are in pg Dopamine/ml of plasma)

	Sample 0	1	2	3	4	5
Saline (Control)	389.38	427.68	439.73	455.62	353.88	439.17
Standard Error	102.16	98.26	90.19	109.96	76.27	126.99
Δ from baseline		38.30	50.35	66.24	-35.50	49.79
All Nicotine Doses	384.44	422.28	424.35	460.17	426.75	464.17
Standard Error	66.12	71.47	66.91	71.21	59.06	103.20
Δ from baseline		37.84	39.91	75.73	42.31	79.73
Student's t Statistic: Control vs. All Nicotine Doses (df = 9)						
t	0.04	0.04	0.12	-0.03	-0.67	-0.12
p<	0.9694	0.9683	0.9036	0.9738	0.5179	0.9037

Table 3
Part IV
Dopamine

A. Group Mean Responses by Drug Condition and Day

(Values are in pg/ml of plasma)

Drug Condition	Day 1	3	7
Saline Control	448.70	495.31	420.91
0.025 mg Nic/kg	519.05	368.52	320.70
0.050 mg Nic/kg	489.04	368.85	666.13
0.100 mg Nic/kg	618.56	459.64	391.03

B. Group Mean Responses for All Drug Conditions by Sample

(Values are in pg/ml of plasma)

	Sample					
Drug Condition	0	1	2	3	4	5
Saline Control	458.82	468.29	462.72	456.52	413.98	469.52
Δ from baseline		9.47	3.90	-2.30	-44.84	10.70
0.025 mg Nic/kg	383.45	392.14	413.45	405.41	416.32	420.85
Δ from baseline		8.69	30.00	21.96	32.87	37.40
0.050 mg Nic/kg	421.44	444.29	470.59	498.57	518.99	511.29
Δ from baseline		22.85	49.15	77.13	97.55	89.85
0.100 mg Nic/kg	466.91	506.17	501.22	514.18	491.71	512.12
Δ from baseline		39.26	34.31	47.27	24.80	45.21

Table 4

Part I

Experiment Phase I: Corticosterone

Analysis of Variance Summary Table

Response Grand Mean = 36.98 ng/ml

Source	df	Sum of Squares	Mean Square	F Value	p <
Between Subjects Factors					
Drug	3	4819.21	1606.40	2.08	0.1818
s/Drug	8	6189.55	773.69		
Within Subject Factors					
Day	2	63.15	31.57	0.02	0.9758
Drug x Day	6	2868.48	478.08	0.37	0.8863
Day x s/Drug	16	20581.73	1286.35		
Sample	5	5028.46	1005.69	4.90	0.0014
Drug x Sample	15	4361.34	290.75	1.42	0.1865
Sample x s/Drug	40	8208.20	205.20		
Day x Sample	10	768.36	76.84	0.95	0.4933
Drug x Day x Sample	30	4226.65	140.88	1.74	0.0263
Day x Sample x s/Drug	80	6471.78	80.89		

Table 4
Part II
Corticosterone

Tukey's Studentized Range Test
Alpha = 0.05

A) Drug Condition:
Mean Square Error = 773.69, df = 8
Critical Value of Studentized Range = 4.53
Minimum Significant Difference = 17.14

Drug Condition	Mean Response (ng/ml)	N
Saline (control)	31.07	54
0.025 mg Nic/kg	39.89	54
0.050 mg Nic/kg	43.03	54
0.100 mg Nic/kg	33.93	54

B) Test Day:
Mean Square Error = 1286.35, df = 16
Critical Value of Studentized Range = 3.65
Minimum Significant Difference = 15.42

Test Day	Mean Response	N
1	36.22	72
3	37.45	72
7	37.26	72

C) Sample (0-5):
Mean Square Error = 205.20, df = 40
Critical Value of Studentized Range = 4.23
Minimum Significant Difference = 10.09

Sample	Mean Response	N
0	29.89	36
-- Nicotine infusion		
1	41.26	36
2	33.42	36
-- Nicotine infusion		
3	43.95	36
4	38.89	36
5	34.47	36

Table 4
Part II (Continued)
Corticosterone

Tukey's Studentized Range Test

Drug x Sample Interaction

Alpha = 0.05, N = 9 for each mean
Mean Square Error = 205.20, df = 40
Critical Value of the Studentized Range = 4.23
Minimum Significant Difference = 20.19

	Sample					
	0	1	2	3	4	5
Saline (Control)	28.28	31.39	31.89	31.89	33.94	29.05
0.025 mg Nic/kg	34.55	38.78	42.72	42.83	36.22	44.22
0.050 mg Nic/kg	31.19	52.19	34.67	54.18	49.17	36.76
0.100 mg Nic/kg	25.56	42.67	24.39	46.89	36.22	27.83

Drug x Day x Sample Interaction

Alpha = 0.05, N = 3 for each mean
Mean Square Error = 80.89, df = 80
Critical Value of the Studentized Range = 5.15
Minimum Significant Difference = 26.74

	Day	Sample					
		0	1	2	3	4	5
Saline (Control)	1	17.50	22.50	22.33	20.67	26.33	21.00
	2	32.67	37.83	41.50	36.33	37.17	30.83
	3	34.67	33.83	31.83	38.67	38.33	35.33
0.025 mg Nic/kg	1	40.33	46.00	48.17	39.83	39.67	39.83
	2	29.83	32.00	32.33	47.00	37.17	55.83
	3	33.50	38.33	47.67	41.67	31.83	37.00
0.050 mg Nic/kg	1	35.00	46.50	46.83	64.17	52.00	33.33
	2	28.33	59.33	33.33	46.50	45.67	26.67
	3	30.25	50.24	23.83	51.81	49.84	50.29
0.100 mg Nic/kg	1	26.33	54.17	24.17	43.67	36.50	22.50
	2	26.00	40.17	27.17	51.17	34.00	29.50
	3	24.33	33.67	21.83	45.83	38.17	31.50

Table 4
Part III
Corticosterone

Group Mean Response for Saline Control and
All Nicotine Doses by Samples (0-5) on Day 7

(Values are in ng Corticosterone/ml of plasma)

	Sample					
	0	1	2	3	4	5
Saline (Control)	34.67	33.83	31.83	38.66	38.33	35.33
Standard Error	7.33	7.58	8.07	7.51	7.43	9.47
Δ from baseline		-0.84	-2.84	3.99	3.66	0.66
All Nicotine Doses	25.50	39.50	32.06	45.75	38.75	38.37
Standard Error	1.70	3.17	6.32	5.68	3.55	6.33
Δ from baseline		14.00	6.56	20.25	13.25	12.87
Student's t Statistic: Control vs. All Nicotine Doses (df = 9)						
t	1.09	-0.83	-0.02	-0.68	-0.06	-0.26
p<	0.3037	0.4263	0.9846	0.5151	0.9556	0.8042

Table 4
Part IV
Corticosterone

A. Group Mean Responses by Drug Condition and Day

(Values are in ng/ml of plasma)

Drug Condition	Day 1	3	7
Saline Control	21.72	36.06	35.44
0.025 mg Nic/kg	40.84	39.03	38.33
0.050 mg Nic/kg	43.10	35.33	42.79
0.100 mg Nic/kg	33.92	33.98	32.56

B. Group Mean Responses for All Drug Conditions by Sample

(Values are in ng/ml of plasma)

		Sample				
Drug Condition	0	1	2	3	4	5
Saline Control	28.28	31.39	31.89	31.89	33.94	29.06
Δ from baseline		3.11	3.61	3.61	5.66	0.78
0.025 mg Nic/kg	32.55	38.78	42.72	42.83	36.22	44.22
Δ from baseline		6.23	10.17	10.28	3.67	11.67
0.050 mg Nic/kg	28.77	46.41	34.95	49.64	46.18	35.36
Δ from baseline		17.64	6.18	20.87	17.41	6.59
0.100 mg Nic/kg	23.91	38.18	28.00	44.95	36.91	28.45
Δ from baseline		14.27	4.09	21.04	13.00	4.54

Table 5

Part I

Experiment Phase I: Glucose

Analysis of Variance Summary Table

Response Grand Mean = 103.45 mg/dl

Source	df	Sum of Squares	Mean Square	F Value	p <
Between Subjects Factors					
Drug	3	2214.09	738.03	5.58	0.0231
s/Drug	8	1057.96	132.24		
Within Subject Factors					
Day	2	1846.95	923.47	2.88	0.0857
Drug x Day	6	2008.12	334.68	1.04	0.4350
Day x s/Drug	16	5138.48	321.15		
Sample	5	1768.86	353.77	7.10	0.0001
Drug x Sample	15	1115.38	74.35	1.49	0.1544
Sample x s/Drug	40	1992.48	49.81		
Day x Sample	10	217.71	21.77	0.50	0.8843
Drug x Day x Sample	30	1077.65	35.92	0.83	0.7150
Day x Sample x s/Drug	80	3473.74	43.42		

Table 5
Part II
Glucose

Tukey's Studentized Range Test
Alpha = 0.05

A) Drug Condition:
Mean Square Error = 132.24, df = 8
Critical Value of Studentized Range = 4.53
Minimum Significant Difference = 7.08

Drug Condition	Mean Response (mg%)	N
Saline (control)	101.09	54
0.025 mg Nic/kg	103.11	54
0.050 mg Nic/kg	108.78	54
0.100 mg Nic/kg	100.81	54

B) Test Day:
Mean Square Error = 321.15, df = 16
Critical Value of Studentized Range = 3.65
Minimum Significant Difference = 7.70

Test Day	Mean Response	N
1	106.92	72
3	103.67	72
7	99.76	72

C) Sample (0-5):
Mean Square Error = 49.81, df = 40
Critical Value of Studentized Range = 4.23
Minimum Significant Difference = 4.97

Sample	Mean Response	N
0	98.75	36
-- Nicotine infusion		
1	101.92	36
2	101.83	36
-- Nicotine infusion		
3	106.22	36
4	106.86	36
5	105.11	36

Table 5
Part II (Continued)
Glucose

Tukey's Studentized Range Test

Drug x Sample Interaction

Alpha = 0.05, N = 9 for each mean
Mean Square Error = 49.81, df = 40
Critical Value of the Studentized Range = 4.23
Minimum Significant Difference = 9.95

	Sample					
	0	1	2	3	4	5
Saline (Control)	99.11	99.22	102.00	101.55	104.00	100.67
0.025 mg Nic/kg	100.67	101.55	103.33	105.56	102.00	105.56
0.050 mg Nic/kg	102.67	109.00	106.56	111.00	112.22	111.22
0.100 mg Nic/kg	92.56	97.89	95.44	106.78	109.22	103.00

All values are in mg% (i.e., mg/dl)

Table 5
Part III
Glucose

Group Mean Response for Saline Control and
All Nicotine Doses by Samples (0-5) on Day 7

(Values are in mg Glucose/dl of plasma, i.e. mg%)

	Sample					
	0	1	2	3	4	5
Saline (Control)	92.00	94.00	94.67	98.67	102.67	95.67
Standard Error	1.00	1.53	4.26	4.33	1.45	1.20
Δ from baseline		2.00	2.67	6.67	10.67	3.67
All Nicotine Doses	93.37	99.37	100.62	103.37	104.12	102.87
Standard Error	2.51	2.78	2.99	3.39	3.59	3.26
Δ from baseline		6.00	7.25	10.00	10.75	9.50
Student's t Statistic: Control vs. All Nicotine Doses (df = 9)						
t	-0.32	-1.13	-1.07	-0.76	-0.24	-1.30
p<	0.7554	0.2885	0.3123	0.4670	0.8168	0.2258

Table 5
Part IV
Glucose

A. Group Mean Responses by Drug Condition and Day

(Values are in mg%, i.e., mg/dl of plasma)

Drug Condition	Day 1	3	7
Saline Control	101.89	105.11	96.28
0.025 mg Nic/kg	103.05	102.22	104.11
0.050 mg Nic/kg	108.87	108.20	102.75
0.100 mg Nic/kg	107.83	99.50	95.72

B. Group Mean Responses for All Drug Conditions by Sample

(Values are in mg%, i.e., mg/dl of plasma)

	Sample					
Drug Condition	0	1	2	3	4	5
Saline Control	99.11	99.22	102.00	101.56	104.00	100.67
Δ from baseline		0.11	2.89	2.45	4.89	1.56
0.025 mg Nic/kg	101.00	101.56	103.33	105.56	102.00	105.56
Δ from baseline		0.56	2.33	4.56	1.00	4.56
0.050 mg Nic/kg	102.90	107.64	105.91	109.82	109.82	109.00
Δ from baseline		4.74	3.01	6.92	6.92	6.10
0.100 mg Nic/kg	94.09	98.36	97.18	106.55	108.64	104.18
Δ from baseline		4.27	3.09	12.46	14.55	10.09

Table 6

Part I

Experiment Phase I: Insulin

Analysis of Variance Summary Table

Response Grand Mean = 3.60 micro units/ml

Source	df	Sum of Squares	Mean Square	F Value	p<
Between Subjects Factors					
Drug	3	116.64	38.88	0.54	0.6676
s/Drug	8	574.95	71.86		
Within Subject Factors					
Day	2	13.16	6.58	0.44	0.6503
Drug x Day	6	67.61	11.27	0.76	0.6134
Day x s/Drug	16	238.10	14.88		
Sample	5	8.23	1.65	0.29	0.9158
Drug x Sample	15	175.02	11.66	2.06	0.0352
Sample x s/Drug	40	227.09	5.67		
Day x Sample	10	77.41	7.74	1.91	0.0556
Drug x Day x Sample	30	194.46	6.48	1.60	0.0505
Day x Sample x s/Drug	80	324.17	4.05		

Table 6
Part II
Insulin

Tukey's Studentized Range Test
Alpha = 0.05

A) Drug Condition:
Mean Square Error = 71.86, df = 8
Critical Value of Studentized Range = 4.53
Minimum Significant Difference = 5.22

Drug Condition	Mean Response (u units/ml)	N
Saline (control)	3.65	54
0.025 mg Nic/kg	2.39	54
0.050 mg Nic/kg	4.27	54
0.100 mg Nic/kg	4.10	54

B) Test Day:
Mean Square Error = 14.88, df = 16
Critical Value of Studentized Range = 3.65
Minimum Significant Difference = 1.65

Test Day	Mean Response	N
1	3.93	72
3	3.34	72
7	3.53	72

C) Sample (0-5):
Mean Square Error = 5.67, df = 40
Critical Value of Studentized Range = 4.23
Minimum Significant Difference = 1.67

Sample	Mean Response	N
0	3.90	36
-- Nicotine infusion		
1	3.56	36
2	3.27	36
-- Nicotine infusion		
3	3.58	36
4	3.77	36
5	3.52	36

Table 6
Part II (Continued)
Insulin

Tukey's Studentized Range Test

Drug x Sample Interaction

Alpha = 0.05, N = 9 for each mean
Mean Square Error = 5.67, df = 40
Critical Value of the Studentized Range = 4.23
Minimum Significant Difference = 3.35

	Sample					
	0	1	2	3	4	5
Saline (Control)	6.65	3.85	3.01	4.07	2.18	2.13
0.025 mg Nic/kg	2.19	2.59	2.30	2.36	2.19	2.68
0.050 mg Nic/kg	3.18	4.10	4.13	4.55	5.02	4.61
0.100 mg Nic/kg	3.56	3.69	3.65	3.33	5.67	4.65

All values are in micro units/ml of plasma

Table 6
Part III
Insulin

Group Mean Response for Saline Control and
All Nicotine Doses by Samples (0-5) on Day 7

(Values are in u units Insulin/ml of plasma)

	Sample					
	0	1	2	3	4	5
Saline (Control)	5.62	5.79	2.73	4.56	1.39	1.85
Standard Error	2.46	2.43	1.03	1.54	0.27	0.71
▲ from baseline		0.17	-2.89	-1.06	-4.23	-3.77
All Nicotine Doses	3.61	3.59	3.49	2.76	2.57	3.72
Standard Error	1.12	1.16	1.13	0.57	0.53	0.73
▲ from baseline		-0.02	-0.12	-0.85	-1.04	0.11
Student's t Statistic: Control vs. All Nicotine Doses (df = 9)						
t	0.86	0.93	-0.38	1.40	-1.31	-1.45
p <	0.4096	0.3778	0.7111	0.1956	0.2220	0.1801

Table 6
Part IV
Insulin

A. Group Mean Responses by Drug Condition and Day

(Values are in u units/ml of plasma)

Drug Condition	Day 1	3	7
Saline Control	4.83	2.46	3.66
0.025 mg Nic/kg	2.35	2.60	2.16
0.050 mg Nic/kg	2.91	3.54	4.85
0.100 mg Nic/kg	3.89	3.62	3.39

B. Group Mean Responses for All Drug Conditions by Sample

(Values are in u units/ml of plasma)

		Sample				
Drug Condition	0	1	2	3	4	5
Saline Control	6.65	3.85	3.01	4.07	2.18	2.13
Δ from baseline		-2.80	-3.64	-2.58	-4.47	-4.52
0.025 mg Nic/kg	2.10	2.60	2.31	2.36	2.20	2.69
Δ from baseline		0.50	0.21	0.26	0.10	0.59
0.050 mg Nic/kg	2.82	3.29	3.29	3.72	4.14	3.71
Δ from baseline		0.47	0.47	0.90	1.32	0.89
0.100 mg Nic/kg	3.25	3.27	3.36	3.06	4.89	4.12
Δ from baseline		0.02	0.11	-0.19	1.64	0.87

Table 7

Part I

Experiment Phase II: Norepinephrine

Analysis of Variance Summary Table

Response Grand Mean = 297.65 pg/ml

Source	df	Sum of Squares	Mean Square	F Value	p<
Between Subjects Factors					
Drug	3	279864.33	93288.11	0.70	0.5781
s/Drug	8	1066619.03	133327.37		
Within Subject Factors					
Day	1	117030.13	117030.13	6.24	0.0371
Drug x Day	3	104059.48	34686.49	1.85	0.2167
Day x s/Drug	8	150132.65	18766.58		
Sample	5	369585.64	73917.12	8.46	0.0001
Drug x Sample	15	158408.81	10560.58	1.21	0.3050
Sample x s/Drug	40	349415.41	8735.38		
Day x Sample	5	57360.77	11472.15	1.65	0.1694
Drug x Day x Sample	15	197838.60	13189.24	1.90	0.0540
Day x Sample x s/Drug	40	278276.37	6956.91		

Table 7
Part II
Norepinephrine

Tukey's Studentized Range Test
Alpha = 0.05

A) Drug Condition:
Mean Square Error = 133327.37, df = 8
Critical Value of Studentized Range = 4.53
Minimum Significant Difference = 275.68

Drug Condition	Mean Response (pg/ml)	N
Saline (control)	290.45	36
0.025 mg Nic/kg	280.30	36
0.050 mg Nic/kg	250.26	36
0.100 mg Nic/kg	369.59	36

B) Test Day:
Mean Square Error = 18766.58, df = 8
Critical Value of Studentized Range = 4.04
Minimum Significant Difference = 65.22

Test Day	Mean Response	N
7	326.16	72
9 Test of Conditioning	269.14	72

C) Sample (0-5):
Mean Square Error = 8735.38, df = 40
Critical Value of Studentized Range = 4.23
Minimum Significant Difference = 80.70

Sample	Mean Response	N
0	214.23	24
-- Infusion (Nicotine or Saline)		
1	282.63	24
2	281.37	24
-- Infusion (Nicotine or Saline)		
3	287.27	24
4	357.25	24
5	363.15	24

Table 7
Part III
Norepinephrine

Group Mean Response for Saline Control and
All Nicotine Doses by Sample (0-5) and Day (7 or 9)

(Values are in pg Norepinephrine/ml of plasma)

	Sample 0	1	2	3	4	5
Saline (Control) combined data for Days 7 & 9	283.27	282.00	293.05	282.85	298.49	303.00
Δ from baseline		-1.27	9.78	-.042	15.22	19.73
Day 7	194.15	317.18	336.83	334.49	463.60	463.00
All Nicotine Doses						
Standard Error	21.80	45.00	44.74	63.11	92.42	100.23
Δ from baseline		123.03	142.68	140.34	269.45	268.85
Student's t Statistic:						
Control Day 7 vs. All Nicotine Doses						
(df = 9)						
t	1.60	-0.50	-1.14	-0.63	-1.08	-0.93
p <	0.1435	0.6266	0.2826	0.5434	0.3087	0.3747
Day 9	193.15	259.85	219.94	241.46	303.48	320.56
Test of Conditioning						
All Nicotine Doses						
Standard Error	26.53	38.08	29.64	33.32	50.41	48.83
Δ from baseline		66.70	26.79	48.31	110.33	127.41
Student's t Statistic:						
Control Day 9 vs. All Nicotine Doses						
(df = 10)						
t	2.20	0.41	2.29	0.93	0.01	-0.21
p <	0.0523	0.6890	0.0449	0.3742	0.9917	0.8410

Table 8

Part I

Experiment Phase II: Epinephrine

Analysis of Variance Summary Table

Response Grand Mean = 31.35 pg/ml

Source	df	Sum of Squares	Mean Square	F Value	p <
Between Subjects Factors					
Drug	3	2650.33	883.44	1.08	0.4111
s/Drug	8	6545.86	818.23		
Within Subject Factors					
Day	1	7870.35	7870.35	13.49	0.0063
Drug x Day	3	4397.63	1465.87	2.51	0.1323
Day x s/Drug	8	4668.07	583.50		
Sample	5	2568.52	513.70	3.23	0.0153
Drug x Sample	15	4242.26	282.81	1.78	0.0738
Sample x s/Drug	40	6361.52	159.03		
Day x Sample	5	3041.60	608.32	3.69	0.0077
Drug x Day x Sample	15	4756.66	317.11	1.93	0.0499
Day x Sample x s/Drug	40	6588.52	164.71		

Table 8
Part II
Epinephrine

Tukey's Studentized Range Test
Alpha = 0.05

A) Drug Condition:
Mean Square Error = 818.23, df = 8
Critical Value of Studentized Range = 4.53
Minimum Significant Difference = 21.59

Drug Condition	Mean Response (pg/ml)	N
Saline (control)	32.37	36
0.025 mg Nic/kg	24.19	36
0.050 mg Nic/kg	35.57	36
0.100 mg Nic/kg	33.25	36

B) Test Day:
Mean Square Error = 583.50, df = 8
Critical Value of Studentized Range = 4.04
Minimum Significant Difference = 15.42

Test Day	Mean Response	N
7	38.74	72
9 Test of Conditioning	23.95	72

C) Sample (0-5):
Mean Square Error = 159.03, df = 40
Critical Value of Studentized Range = 4.23
Minimum Significant Difference = 10.88

Sample	Mean Response	N
0	27.11	24
-- Infusion (Nicotine or Saline)		
1	28.64	24
2	28.41	24
-- Infusion (Nicotine or Saline)		
3	39.84	24
4	31.81	24
5	32.26	24

Table 8
Part III
Epinephrine

Group Mean Response for Saline Control and
All Nicotine Doses by Sample (0-5) and Day (7 or 9)

(Values are in pg Epinephrine/ml of plasma)

	Sample					
	0	1	2	3	4	5
Saline (Control) combined data for Days 7 & 9	32.69	32.27	27.03	30.84	35.71	35.67
Δ from baseline		-0.42	-5.66	-1.85	3.02	2.98
Day 7	28.24	32.33	33.94	59.51	36.95	37.98
All Nicotine Doses						
Standard Error	3.52	3.57	8.10	20.39	4.36	6.06
Δ from baseline		4.09	5.70	31.27	8.71	9.74
Student's t Statistic:						
Control Day 7 vs. All Nicotine Doses (df = 9)						
t	0.91	0.55	-0.29	-0.69	-0.71	0.05
p <	0.3853	0.5968	0.7784	0.5067	0.4963	0.9613
Day 9	21.79	21.71	22.30	21.38	24.29	23.00
Test of Conditioning						
All Nicotine Doses						
Standard Error	1.82	1.84	2.45	3.03	2.53	0.91
Δ from baseline		-0.08	0.51	-0.41	2.50	1.21
Student's t Statistic:						
Control Day 9 vs. All Nicotine Doses (df = 10)						
t	1.79	1.72	0.39	0.74	0.98	2.67
p <	0.1039	0.1155	0.7051	0.4791	0.3513	0.0236

Table 9

Part I

Experiment Phase II: Dopamine

Analysis of Variance Summary Table

Response Grand Mean = 419.65 pg/ml

Source	df	Sum of Squares	Mean Square	F Value	p <
Between Subjects Factors					
Drug	3	597487.38	199162.46	4.54	0.0387
s/Drug	8	351223.71	43902.96		
Within Subject Factors					
Day	1	125736.43	125736.43	0.88	0.3750
Drug x Day	3	716820.57	238940.19	1.68	0.2482
Day x s/Drug	8	1139644.89	142455.61		
Sample	5	57150.72	11430.14	2.08	0.0882
Drug x Sample	15	104511.63	6967.44	1.27	0.2670
Sample x s/Drug	40	219948.07	5498.70		
Day x Sample	5	42980.27	8596.05	2.30	0.0628
Drug x Day x Sample	15	67371.89	4491.45	1.20	0.3097
Day x Sample x s/Drug	40	149450.79	3736.26		

Table 9
Part II
Dopamine

Tukey's Studentized Range Test
Alpha = 0.05

A) Drug Condition:
Mean Square Error = 43902.96, df = 8
Critical Value of Studentized Range = 4.53
Minimum Significant Difference = 158.19

Drug Condition	Mean Response (pg/ml)	N
Saline (control)	421.68	36
0.025 mg Nic/kg	331.25	36
0.050 mg Nic/kg	513.08	36
0.100 mg Nic/kg	412.59	36

B) Test Day:
Mean Square Error = 142455.61, df = 8
Critical Value of Studentized Range = 4.04
Minimum Significant Difference = 179.70

Test Day	Mean Response	N
7	449.20	72
9 Test of Conditioning	390.10	72

C) Sample (0-5):
Mean Square Error = 5498.70, df = 40
Critical Value of Studentized Range = 4.23
Minimum Significant Difference = 64.02

Sample	Mean Response	N
0	384.66	24
-- Infusion (Nicotine or Saline)		
1	412.74	24
2	413.98	24
-- Infusion (Nicotine or Saline)		
3	433.05	24
4	424.21	24
5	449.26	24

Table 9
Part III
Dopamine

Group Mean Response for Saline Control and
All Nicotine Doses by Sample (0-5) and Day (7 or 9)

(Values are in pg Dopamine/ml of plasma)

	Sample					
	0	1	2	3	4	5
Saline (Control) combined data for Days 7 & 9	391.90	419.09	425.37	435.46	404.75	453.50
Δ from baseline		27.19	33.47	43.56	12.85	61.60
Day 7	384.44	422.28	424.35	460.17	426.75	464.17
All Nicotine Doses						
Standard Error	66.12	71.47	66.91	71.21	59.06	103.20
Δ from baseline		37.84	39.91	75.73	42.31	79.73
Student's t Statistic:						
Control Day 7 vs. All Nicotine Doses (df = 9)						
t	0.04	0.04	0.12	-0.03	-0.67	-0.12
p <	0.9694	0.9683	0.9036	0.9738	0.5179	0.9037
Day 9	361.30	371.88	372.21	380.29	411.81	378.40
Test of Conditioning						
All Nicotine Doses						
Standard Error	29.91	25.18	35.05	38.91	34.21	22.93
Δ from baseline		10.58	10.91	18.99	50.51	17.10
Student's t Statistic:						
Control Day 9 vs. All Nicotine Doses (df = 10)						
t	0.47	0.59	0.54	0.37	0.52	1.09
p <	0.6493	0.5701	0.6003	0.7162	0.6134	0.2999

Table 10

Part I

Experiment Phase II: Corticosterone

Analysis of Variance Summary Table

Response Grand Mean = 32.85 ng/ml

Source	df	Sum of Squares	Mean Square	F Value	p <
Between Subjects Factors					
Drug	3	1826.22	608.74	0.96	0.4574
s/Drug	8	5076.62	634.57		
Within Subject Factors					
Day	1	2804.58	2804.58	5.61	0.0454
Drug x Day	3	1101.35	367.11	0.73	0.5604
Day x s/Drug	8	4001.62	500.20		
Sample	5	1005.71	201.14	3.80	0.0065
Drug x Sample	15	1162.73	77.51	1.47	0.1653
Sample x s/Drug	40	2115.61	52.89		
Day x Sample	5	771.72	154.34	1.56	0.1925
Drug x Day x Sample	15	1761.21	117.41	1.19	0.3187
Day x Sample x s/Drug	40	3948.90	98.72		

Table 10
Part II
Corticosterone

Tukey's Studentized Range Test
Alpha = 0.05

A) Drug Condition:
Mean Square Error = 634.57, df = 8
Critical Value of Studentized Range = 4.53
Minimum Significant Difference = 19.01

Drug Condition	Mean Response (ng/ml)	N
Saline (control)	34.73	36
0.025 mg Nic/kg	35.26	36
0.050 mg Nic/kg	34.70	36
0.100 mg Nic/kg	26.69	36

B) Test Day:
Mean Square Error = 500.20, df = 8
Critical Value of Studentized Range = 4.04
Minimum Significant Difference = 10.64

Test Day	Mean Response	N
7	37.26	72
9 Test of Conditioning	28.43	72

C) Sample (0-5):
Mean Square Error = 52.89, df = 40
Critical Value of Studentized Range = 4.23
Minimum Significant Difference = 6.27

Sample	Mean Response	N
0	29.53	24
-- Infusion (Nicotine or Saline)		
1	34.42	24
2	29.73	24
-- Infusion (Nicotine or Saline)		
3	37.07	24
4	33.75	24
5	32.59	24

Table 10
Part III
Corticosterone

Group Mean Response for Saline Control and
All Nicotine Doses by Sample (0-5) and Day (7 or 9)

(Values are in ng Corticosterone/ml of plasma)

	Sample 0	1	2	3	4	5
Saline (Control) combined data for Days 7 & 9	33.00	33.58	32.75	38.83	36.16	34.08
Δ from baseline		0.58	-0.25	5.83	3.16	1.08
Day 7	25.50	39.50	32.06	45.75	38.75	38.37
All Nicotine Doses						
Standard Error	1.70	3.17	6.32	5.68	3.55	6.33
Δ from baseline		14.00	6.56	20.25	13.25	12.87
Student's t Statistic:						
Control Day 7 vs. All Nicotine Doses (df = 9)						
t	1.09	-0.83	-0.02	-0.68	-0.06	-0.26
p <	0.3037	0.4263	0.9846	0.5151	0.9556	0.8042
Day 9	27.33	28.61	26.33	26.50	25.94	24.61
Test of Conditioning						
All Nicotine Doses						
Standard Error	3.51	3.19	3.38	5.85	5.19	3.82
Δ from baseline		1.21	-1.00	-0.83	-1.39	-2.72
Student's t Statistic:						
Control Day 9 vs. All Nicotine Doses (df = 10)						
t	0.56	0.68	1.09	1.14	0.78	1.19
p <	0.5854	0.5122	0.2981	0.2821	0.4549	0.2606

Table 11

Part I

Experiment Phase II: Glucose

Analysis of Variance Summary Table

Response Grand Mean = 103.74 mg/dl

Source	df	Sum of Squares	Mean Square	F Value	p <
Between Subjects Factors					
Drug	3	2122.47	707.49	4.27	0.0447
s/Drug	8	1326.00	165.75		
Within Subject Factors					
Day	1	2272.11	2272.11	13.82	0.0059
Drug x Day	3	162.72	54.24	0.33	0.8042
Day x s/Drug	8	1315.33	164.41		
Sample	5	1083.31	216.66	13.55	0.0001
Drug x Sample	15	354.53	23.63	1.48	0.1603
Sample x s/Drug	40	639.67	15.99		
Day x Sample	5	192.39	38.47	4.17	0.0038
Drug x Day x Sample	15	170.44	11.36	1.23	0.2896
Day x Sample x s/Drug	40	369.00	9.22		

Table 11
Part II
Glucose

Tukey's Studentized Range Test
Alpha = 0.05

A) Drug Condition:
Mean Square Error = 165.75, df = 8
Critical Value of Studentized Range = 4.53
Minimum Significant Difference = 9.72

Drug Condition	Mean Response (mg%)	N
Saline (control)	99.06	36
0.025 mg Nic/kg	107.17	36
0.050 mg Nic/kg	107.86	36
0.100 mg Nic/kg	100.86	36

B) Test Day:
Mean Square Error = 164.41, df = 8
Critical Value of Studentized Range = 4.04
Minimum Significant Difference = 6.10

Test Day	Mean Response	N
7	99.76	72
9 Test of Conditioning	107.71	72

C) Sample (0-5):
Mean Square Error = 15.99, df = 40
Critical Value of Studentized Range = 4.23
Minimum Significant Difference = 3.45

Sample	Mean Response	N
0	99.33	24
-- Infusion (Nicotine or Saline)		
1	101.29	24
2	103.46	24
-- Infusion (Nicotine or Saline)		
3	105.79	24
4	107.42	24
5	105.13	24

Table 11
Part III
Glucose

Group Mean Response for Saline Control and
All Nicotine Doses by Sample (0-5) and Day (7 or 9)

(Values are in mg Glucose/dl of plasma, i.e., mg%)

	Sample					
	0	1	2	3	4	5
Saline (Control) combined data for Days 7 & 9	95.16	95.00	97.16	101.00	105.00	101.00
Δ from baseline		-0.16	2.00	5.84	9.84	5.84
Day 7	93.37	99.37	100.62	103.37	104.12	102.87
All Nicotine Doses						
Standard Error	2.51	2.78	2.99	3.39	3.59	3.26
Δ from baseline		6.00	7.25	10.00	10.75	9.50
Student's t Statistic:						
Control Day 7 vs. All Nicotine Doses (df = 9)						
t	-0.32	-1.13	-1.07	-0.76	-0.24	-1.30
p <	0.7554	0.2885	0.3123	0.4670	0.8168	0.2258
Day 9	108.11	106.77	110.44	111.22	111.66	109.77
Test of Conditioning						
All Nicotine Doses						
Standard Error	1.78	1.61	1.28	1.71	1.61	1.49
Δ from baseline		-1.34	2.33	3.11	3.55	1.66
Student's t Statistic:						
Control Day 9 vs. All Nicotine Doses (df = 10)						
t	-2.99	-3.71	-2.89	-2.15	-1.18	-1.07
p <	0.0134	0.0040	0.0158	0.0575	0.2647	0.3088

Table 12

Part I

Experiment Phase II: Insulin

Analysis of Variance Summary Table

Response Grand Mean = 3.46 micro units/ml

Source	df	Sum of Squares	Mean Square	F Value	p <
Between Subjects Factors					
Drug	3	148.43	49.47	0.49	0.7003
s/Drug	8	811.71	101.46		
Within Subject Factors					
Day	1	0.63	0.63	0.02	0.8848
Drug x Day	3	50.48	16.82	0.60	0.6319
Day x s/Drug	8	223.73	27.96		
Sample	5	40.44	8.08	2.10	0.0853
Drug x Sample	15	53.76	3.58	0.93	0.5398
Sample x s/Drug	40	154.02	3.85		
Day x Sample	5	38.26	7.65	2.05	0.0920
Drug x Day x Sample	15	101.12	6.74	1.81	0.0684
Day x Sample x s/Drug	40	149.25	3.73		

Table 12
Part II
Insulin

Tukey's Studentized Range Test
Alpha = 0.05

A) Drug Condition:
Mean Square Error = 101.46, df = 8
Critical Value of Studentized Range = 4.53
Minimum Significant Difference = 7.60

Drug Condition	Mean Response (u units/ml)	N
Saline (control)	4.45	36
0.025 mg Nic/kg	2.29	36
0.050 mg Nic/kg	4.49	36
0.100 mg Nic/kg	2.61	36

B) Test Day:
Mean Square Error = 27.96, df = 8
Critical Value of Studentized Range = 4.04
Minimum Significant Difference = 2.51

Test Day	Mean Response	N
7	3.53	72
9 Test of Conditioning	3.39	72

C) Sample (0-5):
Mean Square Error = 3.85, df = 40
Critical Value of Studentized Range = 4.23
Minimum Significant Difference = 1.69

Sample	Mean Response	N
0	4.50	24
-- Infusion (Nicotine or Saline)		
1	3.77	24
2	3.28	24
-- Infusion (Nicotine or Saline)		
3	3.05	24
4	3.24	24
5	2.95	24

Table 12
Part III
Insulin

Group Mean Response for Saline Control and
All Nicotine Doses by Samples (0-5) by Day (7 or 9)

(Values are in u units Insulin/ml of plasma)

	Sample					
	0	1	2	3	4	5
Saline (Control) combined data for Days 7 & 9	5.34	6.46	3.91	5.04	3.70	3.14
Δ from baseline		1.12	-1.43	-0.30	-1.64	-2.20
Day 7	3.61	3.59	3.49	2.76	2.57	3.72
All Nicotine Doses						
Standard Error	1.12	1.16	1.13	0.57	0.53	0.73
Δ from baseline		-0.02	-0.12	-0.85	-1.04	0.11
Student's t Statistic:						
Control Day 7 vs. All Nicotine Doses (df = 9)						
t	0.86	0.93	-0.38	1.40	-1.31	-1.45
p <	0.4096	0.3778	0.7111	0.1956	0.2220	0.1801
Day 9	4.78	2.52	2.37	1.88	3.44	1.67
Test of Conditioning						
All Nicotine Doses						
Standard Error	2.19	0.83	0.73	0.29	1.06	0.26
Δ from baseline		-2.26	-2.41	-2.90	-1.34	-3.11
Student's t Statistic:						
Control Day 9 vs. All Nicotine Doses (df = 10)						
t	0.06	1.06	1.11	1.44	0.85	1.50
p <	0.9543	0.3125	0.2939	0.1817	0.4160	0.1634

Table 13
Part I
Phase III: Norepinephrine

Student's t Statistic for Related Samples
Paired Comparisons: Baseline vs. Samples 1-5 by Day and Drug Condition

(Values are in pg/ml of plasma)

	Sample	1	2	3	4	5
Saline Control, No Stress	Mean Diff	11.11	3.76	-38.72	9.99	-1.06
	S.E.	18.10	31.61	23.87	34.96	15.62
	t	0.61	0.12	-1.62	0.29	-0.07
	p <	0.6018	0.9161	0.2463	0.8019	0.9519
Saline Control, With Stress	Mean Diff	10.29	515.59	319.94	342.62	294.78
	S.E.	18.83	126.99	106.07	97.71	64.92
	t	0.55	4.06	3.02	3.51	4.54
	p <	0.6397	0.0556	0.0946	0.0726	0.0452
Stress with Nicotine						
0.025 mg Nic/kg,	Mean Diff	17.10	187.95	26.19	117.74	117.21
	S.E.	25.35	28.19	15.75	41.21	43.83
	t	0.67	6.67	1.66	2.86	2.67
	p <	0.5695	0.0218	0.2383	0.1038	0.1160
0.050 mg Nic/kg,	Mean Diff	26.44	425.14	303.73	442.70	302.26
	S.E.	30.69	194.05	95.70	215.51	77.08
	t	0.86	2.19	3.17	2.05	3.92
	p <	0.4798	0.1598	0.0866	0.1763	0.0593
0.100 mg Nic/kg,	Mean Diff	228.71	709.03	407.97	374.81	453.63
	S.E.	97.72	313.23	173.29	162.49	173.79
	t	2.34	2.26	2.35	2.31	2.61
	p <	0.1441	0.1519	0.1428	0.1475	0.1208
Stress without Nicotine						
0.025 mg Nic/kg,	Mean Diff	22.21	74.21	11.60	76.38	66.59
	S.E.	21.06	53.61	18.29	30.99	37.76
	t	1.05	1.38	0.63	2.46	1.76
	p <	0.4021	0.3005	0.5907	0.1327	0.2199
0.050 mg Nic/kg,	Mean Diff	-18.73	279.65	166.45	168.28	269.56
	S.E.	31.28	267.64	117.49	87.39	142.81
	t	-0.60	1.04	1.42	1.93	1.89
	p <	0.6101	0.4058	0.2923	0.1940	0.1997
0.100 mg Nic/kg,	Mean Diff	93.56	278.15	161.94	262.88	324.27
	S.E.	67.62	116.02	51.14	59.35	40.49
	t	1.38	2.40	3.17	4.43	8.01
	p <	0.3007	0.1387	0.0869	0.0474	0.0152

Table 13
Part II
Phase III: Norepinephrine

Student's t Statistic for Related Samples
Paired Comparison of Day 11 (stress with Nicotine) vs. Day 13 (stress without Nioctine) by Drug Condition and Sample

(Values are in pg/ml of plasma)

		Saline Control	0.025 mg Nicotine/kg	0.050 mg Nicotine/kg	0.100 mg Nicotine/kg
Sample 0 (baseline)					
	Mean Diff	3.22	64.06	52.75	-31.75
	S.E.	70.99	26.80	8.75	35.21
	t	0.05	2.39	6.03	-0.90
	p <	0.9680	0.1394	0.0264	0.4624
Sample 1	Mean Diff	2.39	69.17	7.58	-166.89
	S.E.	83.88	64.76	29.75	200.47
	t	0.03	1.07	0.25	-0.83
	p <	0.9799	0.3973	0.8227	0.4927
Sample 2 (Physical Restraint Stress Initiated)					
	Mean Diff	515.04	-49.68	-92.74	-462.63
	S.E.	143.11	39.25	67.67	445.59
	t	3.60	-1.27	-1.37	-1.04
	p <	0.0693	0.3331	0.3041	0.4082
Sample 3	Mean Diff	361.88	49.47	-84.53	-277.78
	S.E.	94.36	51.89	40.43	228.15
	t	3.84	0.95	-2.09	-1.22
	p <	0.0618	0.4411	0.1717	0.3476
Sample 4	Mean Diff	335.84	22.69	-221.68	-143.67
	S.E.	115.85	66.31	138.85	127.97
	t	2.90	0.34	-1.60	-1.12
	p <	0.1012	0.7648	0.2514	0.3782
Sample 5	Mean Diff	299.06	13.43	20.05	-161.11
	S.E.	92.39	30.46	58.79	195.99
	t	3.24	0.44	0.34	-0.82
	p <	0.0836	0.7023	0.7656	0.4975

Table 13
Part III

Phase III: Norepinephrine

Student's t Statistic: Control vs. All Nicotine Doses by Test Day and Sample

(Values are in pg/ml of plasma)

	Saline Control		Test Day: Stress with Nicotine 0.025 mg		0.050 mg		0.100 mg		Test Day: Stress without Nicotine 0.025 mg		0.050 mg		0.100 mg	
Sample 0 (baseline)	No Stress Test	Stress Test												
$\bar{x}$	207.58	210.79	186.71		152.86		267.25		250.77		205.61		235.50	
S.E.	35.90	39.18	54.69		39.08		75.06		59.11		34.80		44.49	
No Stress Control vs. df, t			4	0.32	4	1.03	4	-0.72	4	-0.62	4	0.04	4	-0.49
p <				0.7658		0.3607		0.5129		0.5661		0.9705		0.6508
Stress Control vs. df, t			4	0.36	4	1.05	4	-0.67	4	-0.56	4	0.10	4	-0.42
p <				0.7385		0.3542		0.5414		0.6030		0.9260		0.6982
Sample 1														
$\bar{x}$	218.69	221.08	203.82		179.30		495.96		272.99		186.88		329.07	
S.E.	40.91	49.34	77.96		19.72		168.85		70.70		18.43		47.20	
No Stress Control vs. df, t			4	0.17	4	0.87	4	-1.60	4	-0.66	4	0.71	4	-1.77
p <				0.8740		0.4347		0.1857		0.5426		0.5175		0.1520
Stress Control vs. df, t			4	0.19	4	0.79	4	-1.56	4	-0.60	4	0.65	4	-1.58
p <				0.8607		0.4757		0.1932		0.5796		0.5516		0.1889
Sample 2 (Physical Restraint Stress Initiated)														
$\bar{x}$	211.34	726.38	374.67		578.00		976.28		324.98		485.26		513.65	
S.E.	36.81	158.67	77.36		232.88		375.89		82.86		300.29		80.47	
No Stress Control vs. df, t			4	-1.91	4	-1.56	4	-2.03	4	-1.25	4	-0.91	4	-3.42
p <				0.1293		0.1949		0.1128		0.2783		0.4164		0.0269
Stress Control vs. df, t			4	1.99	4	0.53	4	-0.61	4	2.24	4	0.71	4	1.20
p <				0.1171		0.6264		0.5733		0.0884		0.5169		0.2978
Sample 3														
$\bar{x}$	168.85	530.73	212.91		456.59		675.22		262.37		372.06		397.44	
S.E.	24.18	113.96	39.74		130.90		229.73		74.74		149.86		50.67	
No Stress Control vs. df, t			4	-0.95	4	-2.16	4	-2.19	4	-1.19	4	-1.34	4	-4.07
p <				0.3972		0.0967		0.0935		0.2996		0.2517		0.0152
Stress Control vs. df, t			4	2.63	4	0.43	4	-0.56	4	1.97	4	0.84	4	1.07
p <				0.0580		0.6912		0.6032		0.1203		0.4468		0.3454

Table 13, Part III continued

	Saline Control		Test Day: Stress with Nicotine			Test Day: Stress without Nicotine		
			0.025 mg	0.050 mg	0.100 mg	0.025 mg	0.050 mg	0.100 mg
Sample 4	No Stress Test	Stress Test						
$\bar{x}$	217.57	553.41	304.46	595.56	642.06	327.15	373.89	498.38
S.E.	52.56	127.23	95.89	253.48	230.41	84.14	115.71	103.72
No Stress Control vs.		df, t	4 -0.79	4 -1.46	4 -1.80	4 -1.10	4 -1.23	4 -2.42
		p <	0.4713	0.2180	0.1469	0.3313	0.2861	0.0731
Stress Control vs.		df, t	4 1.56	4 -0.15	4 -0.34	4 1.48	4 1.04	4 0.34
		p <	0.1932	0.8890	0.7532	0.2121	0.3555	0.7543
Sample 5								
x	206.51	505.58	303.93	455.12	720.88	317.36	475.17	559.77
S.E.	23.93	91.46	81.33	113.74	229.92	90.88	169.39	68.01
No Stress Control vs.		df, t	4 -1.15	4 -2.14	4 -2.23	4 -1.18	4 -1.57	4 -4.90
		p <	0.3146	0.0992	0.0901	0.3036	0.1914	0.0080
Stress Control vs.		df, t	4 1.65	4 0.35	4 -0.87	4 1.46	4 0.16	4 -0.48
		p <	0.1748	0.7470	0.4333	0.2181	0.8822	0.6592

Table 13
Part IV
Norepinephrine

Percent of Baseline Response: Mean Values for All Drug Conditions by Sample and Test Day (Stress with/without Nicotine)

(x - Indicates initiation of Physical Restraint Stress)

	Sample					
	0 (baseline)	1	x	2	3	4	5
Saline Control No Stress or Nicotine	100	105.79		104.34	83.29	105.06	101.64
Saline Control Stress, No Nicotine	100	103.66		308.40	195.52	231.11	211.81
Stress with Nicotine							
0.025 mg Nic/kg	100	104.63		210.16	121.64	161.04	165.38
0.050 mg Nic/kg	100	126.96		353.49	298.43	362.38	300.73
0.100 mg Nic/kg	100	177.74		365.27	260.55	237.41	280.65
Stress without Nicotine							
0.025 mg Nic/kg	100	107.03		135.43	100.99	128.40	124.39
0.050 mg Nic/kg	100	95.00		206.17	169.53	177.34	224.45
0.100 mg Nic/kg	100	149.26		240.09	176.41	210.27	244.85

Table 14
Part I
Phase III: Epinephrine

Student's t Statistic for Related Samples
Paired Comparisons: Baseline vs. Samples 1-5 by Day and Drug Condition

(Values are in pg/ml of plasma)

	Sample	1	2	3	4	5
Saline Control, No Stress	Mean Diff	-2.74	-6.35	-4.55	-5.13	-1.56
	S.E.	5.04	3.99	3.83	3.22	5.67
	t	-0.54	-1.59	-1.19	-1.59	-0.28
	p <	0.6410	0.2520	0.3574	0.2523	0.8091
Saline Control, With Stress	Mean Diff	-2.92	71.85	544.18	704.51	570.51
	S.E.	2.62	29.02	526.89	671.76	537.73
	t	-1.12	2.48	1.03	1.05	1.06
	p <	0.3803	0.1317	0.4102	0.4043	0.3999
Stress with Nicotine						
0.025 mg Nic/kg,	Mean Diff	-0.24	34.27	12.15	9.88	1.88
	S.E.	2.19	10.69	5.17	4.62	1.43
	t	-0.11	3.20	2.35	2.14	1.32
	p <	0.9231	0.0852	0.1433	0.1661	0.3174
0.050 mg Nic/kg,	Mean Diff	15.65	42.74	75.61	37.80	17.49
	S.E.	16.86	14.86	38.60	18.15	11.04
	t	0.93	2.88	1.96	2.08	1.58
	p <	0.4513	0.1026	0.1893	0.1727	0.2541
0.100 mg Nic/kg,	Mean Diff	1.91	194.14	183.48	69.59	120.45
	S.E.	2.04	51.25	19.79	18.98	81.59
	t	0.94	3.79	9.27	3.67	1.48
	p <	0.4478	0.0632	0.0114	0.0670	0.2779
Stress without Nicotine						
0.025 mg Nic/kg,	Mean Diff	0.79	15.33	16.72	25.25	2.75
	S.E.	4.67	5.74	6.08	14.45	7.69
	t	0.17	2.67	2.75	1.75	0.36
	p <	0.8807	0.1164	0.1108	0.2228	0.7550
0.050 mg Nic/kg,	Mean Diff	-0.31	51.98	28.48	31.41	35.04
	S.E.	2.25	33.11	13.62	16.52	17.19
	t	-0.14	1.57	2.09	1.90	2.04
	p <	0.9020	0.2570	0.1717	0.1977	0.1785
0.100 mg Nic/kg,	Mean Diff	3.29	51.41	25.98	67.37	96.84
	S.E.	6.23	12.20	9.88	57.69	79.97
	t	0.53	4.21	2.63	1.17	1.21
	p <	0.6505	0.0520	0.1193	0.3633	0.3496

Table 14
Part II
Phase III: Epinephrine

Student's t Statistic for Related Samples
Paired Comparison of Day 11 (stress with Nicotine) vs. Day 13 (stress without Nicotine) by Drug Condition and Sample

(Values are in pg/ml of plasma)

		Saline Control	0.025 mg Nicotine/kg	0.050 mg Nicotine/kg	0.100 mg Nicotine/kg
Sample 0 (baseline)					
	Mean Diff	0.43	5.87	-2.06	1.72
	S.E.	0.63	1.47	4.55	3.28
	t	0.69	4.00	-0.45	0.53
	p <	0.5638	0.0572	0.6949	0.6519
Sample 1	Mean Diff	0.25	6.90	-18.02	3.10
	S.E.	1.87	7.31	20.59	5.31
	t	0.13	0.94	-0.88	0.58
	p <	0.9056	0.4445	0.4736	0.6179
Sample 2 (Physical Restraint Stress Initiated)					
	Mean Diff	78.64	-13.07	7.19	-141.01
	S.E.	31.85	16.13	26.06	51.89
	t	2.47	-0.81	0.28	-2.72
	p <	0.1323	0.5030	0.8086	0.1129
Sample 3	Mean Diff	549.16	10.44	-49.19	-155.78
	S.E.	528.05	12.61	30.71	24.15
	t	1.04	0.83	-1.60	-6.45
	p <	0.4076	0.4948	0.2504	0.0232
Sample 4	Mean Diff	710.07	21.24	-8.45	-0.49
	S.E.	669.73	11.27	8.87	60.16
	t	1.06	1.88	-0.95	-0.01
	p <	0.4002	0.2001	0.4414	0.9942
Sample 5	Mean Diff	572.50	6.74	15.49	-21.89
	S.E.	534.64	6.77	4.03	5.59
	t	1.07	1.00	3.84	-3.91
	p <	0.3963	0.4245	0.0616	0.0596

Table 14
Part III
Phase III: Epinephrine
Student's t Statistic: Control vs. All Nicotine Doses by Test Day and Sample
(Values are in pg/ml of plasma)

	Saline Control		Test Day: Stress with Nicotine						Test Day: Stress without Nicotine					
			0.025 mg		0.050 mg		0.100 mg		0.025 mg		0.050 mg		0.100 mg	
Sample 0 (baseline)	No Stress Test	Stress Test												
x	28.58	29.01	27.75		27.92		39.02		33.62		25.86		40.75	
S.E.	6.16	5.60	2.92		2.52		11.67		3.78		6.50		14.93	
No Stress Control vs.			df, t	4	0.12	4	0.10	4	-0.79	4	-0.70	4	0.30	4	-0.75
			p <		0.9087		0.9255		0.4730		0.5245		0.7763		0.4933
Stress Control vs.			df, t	4	0.20	4	0.18	4	-0.77	4	-0.68	4	0.37	4	-0.74
			p <		0.8509		0.8669		0.4824		0.5330		0.7316		0.5027
Sample 1														
x	25.84	26.09	27.51		43.57		40.93		34.41		25.54		44.04	
S.E.	1.26	2.98	0.80		14.58		11.97		7.51		6.39		15.92	
No Stress Control vs.			df, t	4	-1.11	4	-1.21	4	-1.25	4	-1.13	4	0.05	4	-1.14
			p <		0.3281		0.2926		0.2781		0.3233		0.9658		0.3182
Stress Control vs.			df, t	4	-0.46	4	-1.17	4	-1.20	4	-1.03	4	0.08	4	-1.11
			p <		0.6700		0.3055		0.2952		0.3612		0.9419		0.3301
Sample 2 (Physical Restraint Stress Initiated)														
x	22.23	100.87	62.02		70.66		233.17		48.95		77.84		92.16	
S.E.	4.55	13.59	13.59		15.44		62.54		3.09		26.64		13.80	
No Stress Control vs.			df, t	4	-2.78	4	-3.01	4	-3.36	4	-4.86	4	-2.06	4	-4.81
			p <		0.0500		0.0396		0.0282		0.0083		0.1087		0.0086
Stress Control vs.			df, t	4	1.05	4	0.80	4	-1.85	4	1.50	4	0.53	4	0.23
			p <		0.3548		0.4696		0.1378		0.2091		0.6257		0.8266
Sample 3														
x	24.04	573.19	39.90		103.53		222.51		50.34		54.34		66.73	
S.E.	3.34	525.48	5.24		37.17		26.25		8.35		7.12		23.67	
No Stress Control vs.			df, t	4	-2.56	4	-2.13	4	-7.50	4	-2.92	4	-3.85	4	-1.78
			p <		0.0629		0.1002		0.0017		0.0431		0.0183		0.1487
Stress Control vs.			df, t	4	1.01	4	0.89	4	0.67	4	0.99	4	0.99	4	0.96
			p <		0.3676		0.4230		0.5415		0.3761		0.3794		0.3901

Table 14, Part III continued

	Saline Control		Test Day: Stress with Nicotine			Test Day: Stress without Nicotine		
			0.025 mg	0.050 mg	0.100 mg	0.025 mg	0.050 mg	0.100 mg
Sample 4	No Stress Test	Stress Test						
$\bar{x}$	23.46	733.52	37.63	65.72	108.61	58.87	57.27	108.12
S.E.	3.87	670.42	5.90	17.39	23.73	16.76	13.61	72.58
No Stress Control vs. df, t			4 -2.01	4 -2.37	4 -3.54	4 -2.06	4 -2.39	4 -1.16
p <			0.1151	0.0766	0.0240	0.1086	0.0752	0.3088
Stress Control vs. df, t			4 1.04	4 0.99	4 0.93	4 1.01	4 1.01	4 0.93
p <			0.3579	0.3757	0.4043	0.3713	0.3703	0.4062
Sample 5								
$\bar{x}$	27.02	599.53	29.63	45.41	159.48	36.37	60.90	137.59
S.E.	2.28	536.40	2.16	12.19	93.18	6.65	14.51	94.57
No Stress Control vs. df, t			4 -0.83	4 -1.48	4 -1.42	4 -1.33	4 -2.31	4 -1.17
p <			0.4528	0.2125	0.2283	0.2545	0.0823	0.3074
Stress Control vs. df, t			4 1.06	4 1.03	4 0.81	4 1.05	4 1.00	4 0.85
p <			0.3479	0.3601	0.4643	0.3530	0.3723	0.4442

Table 14
Part IV
Epinephrine

Percent of Baseline Response: Mean Value for All Drug Conditions by Sample and Test Day (Stress with/without Nicotine)

(x - Indicates initiation of Physical Restraint Stress)

	Sample					
	0 (baseline)	1	x	2	3	4	5
Saline Control No Stress or Nicotine	100	96.59		80.31	87.91	83.96	101.10
Saline Control Stress, No Nicotine	100	92.56		327.77	162.38	200.46	203.24
Stress with Nicotine							
0.025 mg Nic/kg	100	101.00		217.46	145.98	135.87	107.86
0.050 mg Nic/kg	100	168.05		255.01	449.20	243.22	161.48
0.100 mg Nic/kg	100	105.04		613.47	634.15	308.35	347.01
Stress without Nicotine							
0.025 mg Nic/kg	100	100.79		150.63	149.75	172.60	111.67
0.050 mg Nic/kg	100	100.76		439.51	272.78	271.37	287.24
0.100 mg Nic/kg	100	107.87		275.41	169.05	208.96	270.55

Table 15
Part I
Phase III: Dopamine

Student's t Statistic for Related Samples
Paired Comparisons: Baseline vs. Samples 1-5 by Day and Drug Condition

(Values are in pg/ml of plasma)

	Sample	1	2	3	4	5
Saline Control, No Stress	Mean Diff	-28.94	-37.59	-72.79	-62.69	-44.74
	S.E.	8.52	62.89	20.62	25.13	47.93
	t	-3.40	-0.60	-3.53	-2.49	-0.93
	p <	0.0768	0.6108	0.0717	0.1300	0.4492
Saline Control, With Stress	Mean Diff	-0.45	43.03	21.92	37.32	7.74
	S.E.	9.15	54.88	62.52	67.28	55.28
	t	-0.05	0.78	0.35	0.55	0.14
	p <	0.9655	0.5151	0.7593	0.6348	0.9015
Stress with Nicotine						
0.025 mg Nic/kg,	Mean Diff	30.96	-41.56	-59.12	-60.09	-23.63
	S.E.	9.64	44.22	36.09	25.77	17.52
	t	3.21	-0.94	-1.64	-2.33	-1.35
	p <	0.0849	0.4465	0.2430	0.1450	0.3100
0.050 mg Nic/kg,	Mean Diff	-4.91	-29.78	6.03	-27.78	-83.02
	S.E.	38.39	69.38	51.32	48.02	5.92
	t	-0.13	-0.43	0.12	-0.58	-14.04
	p <	0.9099	0.7096	0.9172	0.6214	0.0050
0.100 mg Nic/kg,	Mean Diff	60.04	51.76	51.89	33.39	54.26
	S.E.	51.35	72.62	72.33	61.93	79.54
	t	1.17	0.71	0.72	0.54	0.68
	p <	0.3628	0.5499	0.5476	0.6438	0.5655
Stress without Nicotine						
0.025 mg Nic/kg,	Mean Diff	33.22	-20.64	17.37	64.86	18.72
	S.E.	0.75	29.38	27.28	12.34	31.64
	t	44.42	-0.70	0.64	5.26	0.59
	p <	0.0005	0.5551	0.5894	0.0343	0.6141
0.050 mg Nic/kg,	Mean Diff	-3.14	6.36	15.64	29.51	28.07
	S.E.	27.79	7.56	32.98	44.06	28.59
	t	-0.11	0.84	0.47	0.67	0.98
	p <	0.9203	0.4887	0.6820	0.5720	0.4298
0.100 mg Nic/kg,	Mean Diff	17.43	7.40	-40.19	-48.44	-40.54
	S.E.	22.70	19.82	14.61	58.48	40.54
	t	0.77	0.37	-2.75	-0.83	-1.00
	p <	0.5230	0.7446	0.1107	0.4946	0.4227

Table 15
Part II
Phase III: Dopamine

Student's t Statistic for Related Samples
Paired Comparison of Day 11 (stress with Nicotine) vs. Day 13 (stress without Nicotine) by Drug Condition and Sample

(Values are in pg/ml of plasma)

		Saline Control	0.025 mg Nicotine/kg	0.050 mg Nicotine/kg	0.100 mg Nicotine/kg
Sample 0 (baseline)					
	Mean Diff	44.29	27.77	-33.98	32.00
	S.E.	44.87	29.52	71.56	43.44
	t	0.99	0.94	-0.47	0.74
	p <	0.4276	0.4462	0.6817	0.5380
Sample 1	Mean Diff	72.79	30.02	-32.22	-10.61
	S.E.	49.92	22.34	65.24	57.39
	t	1.46	1.34	-0.49	-0.18
	p <	0.2822	0.3112	0.6703	0.8704
Sample 2	(Physical Restraint Stress Initiated)				
	Mean Diff	124.92	48.69	2.15	-12.36
	S.E.	11.72	46.16	33.89	81.40
	t	10.66	1.05	0.06	-0.15
	p <	0.0087	0.4021	0.9552	0.8933
Sample 3	Mean Diff	139.01	104.26	-24.37	-60.08
	S.E.	67.23	35.83	64.36	94.38
	t	2.07	2.91	-0.38	-0.64
	p <	0.1746	0.1006	0.7414	0.5896
Sample 4	Mean Diff	144.31	152.71	23.31	-49.83
	S.E.	63.96	56.51	14.75	121.07
	t	2.26	2.70	1.58	-0.41
	p <	0.1527	0.1140	0.2549	0.7206
Sample 5	Mean Diff	96.77	70.11	77.11	-62.79
	S.E.	31.20	74.49	60.31	117.30
	t	3.10	0.94	1.28	-0.54
	p <	0.0901	0.4459	0.3294	0.6460

Table 15
Part III
Phase III: Dopamine
Student's t Statistic: Control vs. All Nicotine Doses by Test Day and Sample
(Values are in pg/ml of plasma)

	Saline Control		Test Day: Stress with Nicotine			Test Day: Stress without Nicotine		
			0.025 mg	0.050 mg	0.100 mg	0.025 mg	0.050 mg	0.100 mg
Sample 0 (baseline)	No Stress Test	Stress Test						
$\bar{x}$	370.45	414.74	375.49	426.20	418.72	403.25	392.21	450.73
S.E.	48.85	49.35	21.15	82.09	132.90	17.31	28.63	91.08
No Stress Control vs. df, t			4 -0.09	4 -0.58	4 -0.34	4 -0.63	4 -0.38	4 -0.78
p <			0.9291	0.5908	0.7503	0.5611	0.7202	0.4807
Stress Control vs. df, t			4 0.73	4 -0.12	4 -0.03	4 0.22	4 0.39	4 -0.35
p <			0.5052	0.9106	0.9709	0.8369	0.7131	0.7458
Sample 1								
$\bar{x}$	341.50	414.30	406.45	421.29	478.76	436.47	389.07	468.16
S.E.	40.33	57.97	11.51	57.85	102.92	17.71	31.08	69.66
No Stress Control vs. df, t			4 -1.55	4 -1.13	4 -1.24	4 -2.16	4 -0.93	4 -1.57
p <			0.1964	0.3211	0.2822	0.0973	0.4031	0.1907
Stress Control vs. df, t			4 0.13	4 -0.09	4 -0.55	4 -0.37	4 0.38	4 -0.59
p <			0.9008	0.9361	0.6142	0.7330	0.7209	0.5843
Sample 2 (Physical Restraint Stress Initiated)								
$\bar{x}$	332.86	457.78	333.92	396.42	470.49	382.61	398.57	458.13
S.E.	23.37	29.47	39.14	21.24	108.22	22.17	26.12	76.82
No Stress Control vs. df, t			4 -0.02	4 -2.01	4 -1.24	4 -1.54	4 -1.88	4 -1.56
p <			0.9825	0.1144	0.2817	0.1974	0.1341	0.1938
Stress Control vs. df, t			4 2.53	4 1.69	4 -0.11	4 2.04	4 1.50	4 -0.004
p <			0.0648	0.1664	0.9152	0.1111	0.2071	0.9967
Sample 3								
$\bar{x}$	297.66	436.67	316.36	432.22	470.62	420.63	407.85	410.54
S.E.	38.03	39.94	46.67	48.24	147.38	10.86	58.17	76.58
No Stress Control vs. df, t			4 -0.31	4 -2.19	4 -1.14	4 -3.11	4 -1.59	4 -1.32
p <			0.7716	0.0936	0.3193	0.0359	0.1880	0.2573
Stress Control vs. df, t			4 1.96	4 0.07	4 -0.22	4 0.39	4 0.41	4 0.30
p <			0.1218	0.9468	0.8349	0.7181	0.7040	0.7774

Table 15, Part III continued

	Saline Control		Test Day: Stress with Nicotine 0.025 mg	0.050 mg	0.100 mg	Test Day: Stress without Nicotine 0.025 mg	0.050 mg	0.100 mg
Sample 4	No Stress Test	Stress Test						
$\bar{x}$	307.75	452.07	315.40	398.42	452.12	468.11	421.73	402.29
S.E.	26.61	44.15	36.20	34.07	157.40	20.31	38.37	36.72
No Stress Control vs. df, t			4 -0.17	4 -2.10	4 -0.90	4 -4.79	4 -2.44	4 -2.08
p <			0.8731	0.1040	0.4169	0.0087	0.0711	0.1055
Stress Control vs. df, t			4 2.39	4 0.96	4 -0.00	4 -0.33	4 0.52	4 0.87
p <			0.0749	0.3905	0.9998	0.7579	0.6313	0.4349
Sample 5								
$\bar{x}$	325.71	422.48	351.86	343.18	472.99	421.97	420.28	410.19
S.E.	27.85	22.07	37.55	78.95	156.38	37.20	18.90	50.74
No Stress Control vs. df, t			4 -0.56	4 -0.21	4 -0.93	4 -2.07	4 -2.81	4 -1.46
p <			0.6058	0.8449	0.4063	0.1071	0.0483	0.2182
Stress Control vs. df, t			4 1.62	4 0.97	4 -0.32	4 0.01	4 0.08	4 0.22
p <			0.1803	0.3881	0.7651	0.9912	0.9433	0.8351

Table 15
Part IV
Dopamine

Percent of Baseline Response: Mean Value for All Drug Conditions by Sample and Test Day (Stress with/without Nicotine)

(x - Indicates initiation of Physical Restraint Stress)

	Sample					
	0 (baseline)	1	x	2	3	4	5
Saline Control No Stress or Nicotine	100	92.57		94.47	80.82	84.40	90.92
Saline Control Stress, No Nicotine	100	99.42		102.51	94.76	97.37	95.84
Stress with Nicotine							
0.025 mg Nic/kg	100	108.60		89.46	83.79	83.75	93.17
0.050 mg Nic/kg	100	101.09		98.09	105.09	96.94	79.84
0.100 mg Nic/kg	100	121.41		118.93	112.54	105.11	111.95
Stress without Nicotine							
0.025 mg Nic/kg	100	108.26		95.29	104.92	116.13	104.59
0.050 mg Nic/kg	100	99.64		101.77	102.98	108.51	108.14
0.100 mg Nic/kg	100	105.98		103.12	91.59	92.99	93.62

Table 16
Part I
Phase III: Corticosterone

Student's t Statistic for Related Samples
Paired Comparisons: Baseline vs. Samples 1-5 by Day and Drug Condition

(Values are in ng/ml of plasma)

	Sample	1	2	3	4	5
Saline Control, No Stress	Mean Diff	-1.67	-5.67	-6.83	-6.83	0.33
	S.E.	3.11	0.88	3.28	1.88	1.59
	t	-0.54	-6.43	-2.08	-3.64	0.21
	p <	0.6460	0.0234	0.1729	0.0679	0.8534
Saline Control, With Stress	Mean Diff	1.83	35.83	119.67	149.00	106.83
	S.E.	4.59	13.85	17.05	30.50	41.37
	t	0.40	2.59	7.02	4.89	2.58
	p <	0.7280	0.1226	0.0197	0.0394	0.1229
Stress with Nicotine						
0.025 mg Nic/kg,	Mean Diff	7.67	42.50	163.50	241.00	187.67
	S.E.	5.93	9.66	16.31	29.02	27.45
	t	1.29	4.40	10.02	8.30	6.84
	p <	0.3250	0.0479	0.0098	0.0142	0.0207
0.050 mg Nic/kg,	Mean Diff	-0.50	66.83	161.50	230.50	158.00
	S.E.	6.17	51.59	84.77	84.75	88.67
	t	-0.08	1.30	1.91	2.72	1.78
	p <	0.9428	0.3245	0.1970	0.1128	0.2167
0.100 mg Nic/kg,	Mean Diff	-0.33	31.50	157.17	180.67	111.67
	S.E.	3.84	9.99	15.49	55.49	54.70
	t	-0.09	3.15	10.14	3.26	2.04
	p <	0.9388	0.0875	0.0096	0.0828	0.1780
Stress without Nicotine						
0.025 mg Nic/kg,	Mean Diff	8.50	54.00	111.17	119.00	50.83
	S.E.	0.76	20.48	7.69	22.57	24.99
	t	11.13	2.64	14.45	5.27	2.03
	p <	0.0080	0.1187	0.0048	0.0341	0.1789
0.050 mg Nic/kg,	Mean Diff	-3.67	72.50	150.33	129.00	67.67
	S.E.	7.05	7.55	26.67	25.66	16.60
	t	-0.52	9.60	5.64	5.03	4.08
	p <	0.6548	0.0107	0.0301	0.0374	0.0553
0.100 mg Nic/kg,	Mean Diff	-9.50	26.17	114.50	108.50	60.67
	S.E.	1.61	9.58	28.94	13.25	25.07
	t	-5.91	2.73	3.96	8.19	2.42
	p <	0.0275	0.1119	0.0584	0.0146	0.1366

Table 16
Part II
Phase III: Corticosterone

Student's t Statistic for Related Samples
Paired Comparison of Day 11 (stress with Nicotine) vs. Day 13 (stress without Nicotine) by Drug Condition and Sample

(Values are in ng/ml of plasma)

		Saline Control	0.025 mg Nicotine/kg	0.050 mg Nicotine/kg	0.100 mg Nicotine/kg
Sample 0 (baseline)					
	Mean Diff	3.50	-16.83	-11.33	-13.17
	S.E.	4.92	18.22	16.37	6.56
	t	0.71	-0.92	-0.69	-2.01
	p <	0.5509	0.4532	0.5602	0.1825
Sample 1	Mean Diff	7.00	-16.00	-14.50	-22.33
	S.E.	11.36	22.14	12.22	8.74
	t	0.62	-0.72	-1.19	-2.55
	p <	0.6005	0.5449	0.3572	0.1251
Sample 2 (Physical Restraint Stress Initiated)					
	Mean Diff	45.00	-5.33	-5.67	-18.50
	S.E.	17.89	46.58	65.49	7.29
	t	2.51	-0.11	-0.09	-2.54
	p <	0.1284	0.9193	0.9389	0.1264
Sample 3	Mean Diff	130.00	-69.17	-22.50	-55.83
	S.E.	22.64	30.43	73.87	38.99
	t	5.74	-2.27	-0.30	-1.43
	p <	0.0290	0.1509	0.7894	0.2885
Sample 4	Mean Diff	159.33	-138.83	-112.83	-85.33
	S.E.	36.84	24.88	76.81	52.41
	t	4.33	-5.58	-1.47	-1.63
	p <	0.0495	0.0306	0.2796	0.2450
Sample 5	Mean Diff	110.00	-153.67	-101.67	-64.17
	S.E.	43.75	8.35	95.69	52.28
	t	2.51	-18.41	-1.06	-1.23
	p <	0.1284	0.0029	0.3994	0.3446

Table 16
Part III

Phase III: Corticosterone

Student's t Statistic: Control vs. All Nicotine Doses by Test Day and Sample

(Values are in ng/ml of plasma)

	Saline Control		Test Day: Stress with Nicotine			Test Day: Stress without Nicotine		
			0.025 mg	0.050 mg	0.100 mg	0.025 mg	0.050 mg	0.100 mg
Sample 0 (baseline)	No Stress Test	Stress Test						
$\bar{x}$	35.83	39.33	52.33	54.83	53.00	35.50	43.50	39.83
S.E.	0.60	5.45	20.74	22.66	10.79	3.05	6.60	4.83
No Stress Control vs. df, t			4 -0.79	4 -0.84	4 -1.59	4 -0.11	4 -1.16	4 -0.82
p <			0.4710	0.4491	0.1872	0.9199	0.3118	0.4576
Stress Control vs. df, t			4 -0.61	4 -0.67	4 -1.13	4 0.61	4 -0.49	4 -0.07
p <			0.5771	0.5424	0.3214	0.5730	0.6521	0.9486
Sample 1								
$\bar{x}$	34.17	41.17	60.00	54.33	52.67	44.00	39.83	30.33
S.E.	3.09	10.00	25.66	16.91	13.87	3.77	7.26	6.36
No Stress Control vs. df, t			4 -0.99	4 -1.17	4 -1.30	4 -2.02	4 -0.72	4 0.54
p <			0.3741	0.3059	0.2629	0.1139	0.5122	0.6164
Stress Control vs. df, t			4 -0.68	4 -0.67	4 -0.67	4 -0.27	4 0.11	4 0.91
p <			0.5316	0.5395	0.5381	0.8041	0.9193	0.4124
Sample 2 (Physical Restraint Stress Initiated)								
$\bar{x}$	30.17	75.17	94.83	121.67	84.50	89.50	116.00	66.00
S.E.	1.42	19.30	30.28	74.20	20.76	17.42	12.47	13.58
No Stress Control vs. df, t			4 -2.13	4 -1.23	4 -2.61	4 -3.39	4 -6.84	4 -2.62
p <			0.0998	0.2851	0.0594	0.0274	0.0024	0.0585
Stress Control vs. df, t			4 -0.55	4 -0.61	4 -0.33	4 -0.55	4 -1.78	4 0.39
p <			0.6130	0.5769	0.7585	0.6108	0.1502	0.7175
Sample 3								
$\bar{x}$	29.00	159.00	215.83	216.33	210.17	146.67	193.83	154.33
S.E.	3.01	21.55	25.83	107.10	24.92	4.64	32.25	28.21
No Stress Control vs. df, t			4 -7.18	4 -1.75	4 -7.22	4 -21.27	4 -4.94	4 -4.42
p <			0.0020	0.1553	0.0020	0.0001	0.0078	0.0115
Stress Control vs. df, t			4 -1.69	4 -0.52	4 -1.55	4 0.56	4 -0.88	4 0.13
p <			0.1664	0.6274	0.1953	0.6056	0.4290	0.9018

Table 16, Part III continued

	Saline Control		Test Day: Stress with Nicotine 0.025 mg		0.050 mg		0.100 mg		Test Day: Stress without Nicotine 0.025 mg		0.050 mg		0.100 mg	
Sample 4	No Stress Test	Stress Test												
$\bar{x}$	29.00	188.33	293.33		285.33		233.67		154.50		172.50		148.33	
S.E.	1.32	35.86	15.90		107.36		66.23		20.21		32.24		17.81	
No Stress Control vs.	df, t		4	-16.57	4	-2.39	4	-3.09	4	-6.20	4	-4.45	4	-6.68
	p <			0.0001		0.0754		0.0366		0.0035		0.0113		0.0026
Stress Control vs.	df, t		4	-2.68	4	-0.86	4	-0.60	4	0.82	4	0.33	4	0.99
	p <			0.0554		0.4398		0.5797		0.4573		0.7591		0.3744
Sample 5														
$\bar{x}$	36.17	146.17	240.00		212.83		164.67		86.33		111.17		100.50	
S.E.	2.17	45.89	20.82		110.32		64.16		22.69		22.88		29.88	
No Stress Control vs.	df, t		4	-9.74	4	-1.60	4	-2.00	4	-2.20	4	-3.26	4	-2.15
	p <			0.0006		0.1846		0.1159		0.0925		0.0310		0.0982
Stress Control vs.	df, t		4	-1.86	4	-0.56	4	-0.23	4	1.17	4	0.68	4	0.83
	p <			0.1361		0.6066		0.8261		0.3074		0.5324		0.4512

Table 16
Part IV
Corticosterone

Percent of Baseline Response: Mean Value for All Drug Conditions by Sample and Test Day (Stress with/without Nicotine)

(x - Indicates initiation of Physical Restraint Stress)

	Sample					
	0 (baseline)	1	x	2	3	4	5
Saline Control No Stress or Nicotine	100	95.38		84.10	81.07	119.18	100.78
Saline Control Stress, No Nicotine	100	100.26		182.02	400.99	465.54	357.08
Stress with Nicotine							
0.025 mg Nic/kg	100	114.56		198.61	567.35	802.90	637.14
0.050 mg Nic/kg	100	106.79		188.36	374.75	537.45	354.32
0.100 mg Nic/kg	100	96.62		156.49	413.81	426.97	286.93
Stress without Nicotine							
0.025 mg Nic/kg	100	123.94		223.63	421.07	447.87	254.51
0.050 mg Nic/kg	100	93.56		270.73	443.31	392.54	251.85
0.100 mg Nic/kg	100	74.66		163.46	398.96	372.89	242.50

Table 17
Part I
Phase III: Glucose

Student's t Statistic for Related Samples
Paired Comparisons: Baseline vs. Samples 1-5 by Day and Drug Condition

(Values are in mg%, i.e., mg/dl)

	Sample	1	2	3	4	5
Saline Control, No Stress	Mean Diff	1.67	6.33	3.67	9.00	0.67
	S.E.	1.76	3.48	3.84	3.00	4.91
	t	0.94	1.82	0.95	3.00	0.14
	p <	0.4444	0.2104	0.4408	0.0955	0.9044
Saline Control, With Stress	Mean Diff	-3.67	23.00	28.67	34.00	48.67
	S.E.	5.36	6.11	3.48	10.15	13.04
	t	-0.68	3.76	8.24	3.35	3.73
	p <	0.5648	0.0639	0.0144	0.0787	0.0649
Stress with Nicotine						
0.025 mg Nic/kg,	Mean Diff	4.00	22.00	18.33	21.00	21.67
	S.E.	0.58	5.69	3.67	9.02	10.65
	t	6.93	3.87	5.00	2.33	2.03
	p <	0.0202	0.0608	0.0377	0.1453	0.1789
0.050 mg Nic/kg,	Mean Diff	1.67	31.00	27.67	23.00	14.33
	S.E.	1.76	7.57	5.46	5.51	3.38
	t	0.94	4.09	5.07	4.18	4.24
	p <	0.4444	0.0548	0.0368	0.0528	0.0514
0.100 mg Nic/kg,	Mean Diff	2.00	36.67	38.67	40.67	40.33
	S.E.	0.58	4.33	4.91	13.22	15.89
	t	3.46	8.46	7.87	3.08	2.54
	p <	0.0742	0.0137	0.0157	0.0914	0.1266
Stress without Nicotine						
0.025 mg Nic/kg,	Mean Diff	2.33	14.00	20.00	19.00	17.00
	S.E.	2.67	4.93	4.51	3.06	9.07
	t	0.88	2.84	4.44	6.22	1.87
	p <	0.4738	0.1050	0.0473	0.0249	0.2019
0.050 mg Nic/kg,	Mean Diff	2.33	26.00	29.67	33.00	30.33
	S.E.	2.60	16.04	10.68	12.53	10.04
	t	0.90	1.62	2.78	2.63	3.02
	p <	0.4647	0.2465	0.1089	0.1190	0.0943
0.100 mg Nic/kg,	Mean Diff	-1.33	26.67	21.67	30.67	40.00
	S.E.	1.67	3.71	2.91	9.94	21.17
	t	-0.80	7.18	7.46	3.09	1.89
	p <	0.5076	0.0188	0.0175	0.0909	0.1994

Table 17
Part II
Phase III: Glucose

Student's t Statistic for Related Samples
Paired Comparison of Day 11 (stress with Nicotine) vs. Day 13 (stress without Nicotine) by Drug Condition and Sample

(Values are in mg%, i.e., mg/dl)

		Saline Control	0.025 mg Nicotine/kg	0.050 mg Nicotine/kg	0.100 mg Nicotine/kg
Sample 0 (baseline)					
	Mean Diff	7.67	0.0	-0.33	10.67
	S.E.	3.93	1.15	2.40	1.67
	t	1.95	0.0	-0.14	6.40
	p <	0.1904	1.00	0.9024	0.0236
Sample 1	Mean Diff	2.33	-1.67	0.33	7.33
	S.E.	6.36	2.73	1.20	1.76
	t	0.37	-0.61	0.28	4.16
	p <	0.7489	0.6035	0.8075	0.0533
Sample 2 (Physical Restraint Stress Initiated)					
	Mean Diff	24.33	-8.00	-5.33	0.67
	S.E.	6.49	9.50	12.13	7.26
	t	3.75	-0.84	-0.44	0.09
	p <	0.0643	0.4886	0.7031	0.9352
Sample 3	Mean Diff	32.67	1.67	1.67	-6.33
	S.E.	3.84	3.33	9.13	6.69
	t	8.50	0.50	0.18	-0.95
	p <	0.0136	0.6667	0.8720	0.4438
Sample 4	Mean Diff	32.67	-2.00	9.67	0.67
	S.E.	7.36	7.02	10.48	17.37
	t	4.44	-0.28	0.92	0.04
	p <	0.0472	0.8026	0.4536	0.9729
Sample 5	Mean Diff	55.67	-4.67	15.67	10.33
	S.E.	9.39	5.36	9.24	17.68
	t	5.93	-0.87	1.69	0.58
	p <	0.0273	0.4761	0.2322	0.6180

Table 17
Part III
Phase III: Glucose
Student's t Statistic: Control vs. All Nicotine Doses by Test Day and Sample
(Values are in mg%, i.e., mg/dl)

	Saline Control		Test Day: Stress with Nicotine			Test Day: Stress without Nicotine		
			0.025 mg	0.050 mg	0.100 mg	0.025 mg	0.050 mg	0.100 mg
Sample 0 (baseline)	No Stress Test	Stress Test						
$\bar{x}$	101.67	109.33	111.33	103.33	95.67	111.33	103.00	106.33
S.E.	2.73	1.20	4.06	3.48	1.86	2.91	1.53	3.18
No Stress Control vs. df, t			4 -1.98	4 -0.38	4 1.82	4 -2.42	4 -0.43	4 -1.11
p <			0.1191	0.7254	0.1432	0.0724	0.6918	0.3278
Stress Control vs. df, t			4 -0.47	4 1.63	4 6.18	4 -0.64	4 3.26	4 0.88
p <			0.6610	0.1785	0.0035	0.5593	0.0311	0.4273
Sample 1								
$\bar{x}$	103.33	105.67	115.33	105.00	97.67	113.67	105.33	105.00
S.E.	3.28	4.84	3.48	4.51	1.33	3.71	3.84	2.00
No Stress Control vs. df, t			4 -2.51	4 -0.30	4 1.60	4 -2.09	4 -0.40	4 -0.43
p <			0.0662	0.7800	0.1850	0.1054	0.7126	0.6870
Stress Control vs. df, t			4 -1.62	4 0.10	4 1.59	4 -1.31	4 0.05	4 0.13
p <			0.1803	0.9246	0.1864	0.2600	0.9596	0.9049
Sample 2 (Physical Restraint Stress Initiated)								
$\bar{x}$	108.00	132.33	133.33	134.33	132.33	125.33	129.00	133.00
S.E.	1.00	5.55	8.01	5.78	5.93	6.84	14.57	1.53
No Stress Control vs. df, t			4 -3.14	4 -4.49	4 -4.05	4 -2.51	4 -1.44	4 -13.69
p <			0.0349	0.0109	0.0155	0.0662	0.2239	0.0002
Stress Control vs. df, t			4 -0.10	4 -0.25	4 0.00	4 0.79	4 0.21	4 -0.12
p <			0.9239	0.8152	1.00	0.4712	0.8412	0.9133
Sample 3								
$\bar{x}$	105.33	138.00	129.67	131.00	134.33	131.33	132.67	128.00
S.E.	2.03	2.31	5.24	2.65	6.49	7.13	9.17	0.58
No Stress Control vs. df, t			4 -4.33	4 -7.70	4 -4.27	4 -3.51	4 -2.91	4 -10.75
p <			0.0123	0.0015	0.0130	0.0247	0.0437	0.0004
Stress Control vs. df, t			4 1.46	4 1.99	4 0.53	4 0.89	4 0.56	4 4.20
p <			0.2192	0.1170	0.6227	0.4238	0.6029	0.0137

Table 17, Part III continued

	Saline Control		Test Day: Stress with Nicotine 0.025 mg	0.050 mg	0.100 mg	Test Day: Stress without Nicotine 0.025 mg	0.050 mg	0.100 mg
Sample 4	No Stress Test	Stress Test						
$\bar{x}$	110.67	143.33	132.33	126.33	136.33	130.33	136.00	137.00
S.E.	2.33	9.68	12.67	4.81	15.07	5.81	11.02	11.02
No Stress Control vs. df, t			4 -1.68	4 -2.93	4 -1.68	4 -3.14	4 -2.25	4 -2.34
p <			0.1678	0.0427	0.1676	0.0348	0.0877	0.0795
Stress Control vs. df, t			4 0.69	4 1.57	4 0.39	4 1.15	4 0.50	4 0.43
p <			0.5282	0.1910	0.7159	0.3138	0.6433	0.6881
Sample 5								
$\bar{x}$	102.33	158.00	133.00	117.67	136.00	128.33	133.33	146.33
S.E.	5.17	13.28	14.57	2.03	17.67	11.35	8.65	23.48
No Stress Control vs. df, t			4 -1.98	4 -2.76	4 -1.83	4 -2.08	4 -3.08	4 -1.83
p <			0.1184	0.0509	0.1415	0.1055	0.0371	0.1413
Stress Control vs. df, t			4 1.27	4 3.00	4 0.99	4 1.70	4 1.56	4 0.43
p <			0.2735	0.0398	0.3760	0.1647	0.1946	0.6877

Table 17
Part IV
Glucose

Percent of Baseline Response: Mean Value for All Drug Conditions by Sample and Test Day (Stress with/without Nicotine)

(x - Indicates initiation of Physical Restraint Stress)

	Sample					
	0 (baseline)	1	x	2	3	4	5
Saline Control No Stress or Nicotine	100	101.63		106.41	103.78	112.30	100.70
Saline Control Stress, No Nicotine	100	96.69		121.10	126.29	131.19	144.48
Stress with Nicotine							
0.025 mg Nic/kg	100	103.64		119.67	116.54	118.41	118.84
0.050 mg Nic/kg	100	101.56		130.42	127.16	122.50	114.07
0.100 mg Nic/kg	100	102.11		138.21	140.28	141.99	141.56
Stress without Nicotine							
0.025 mg Nic/kg	100	102.11		112.48	117.81	116.96	114.98
0.050 mg Nic/kg	100	102.21		125.70	129.12	132.41	129.73
0.100 mg Nic/kg	100	98.82		125.31	120.57	128.79	136.88

Table 18
Part I
Phase III: Insulin

Student's t Statistic for Related Samples
Paired Comparisons: Baseline vs. Samples 1-5 by Day and Drug Condition

(Values are in u units/ml of plasma)

	Sample	1	2	3	4	5
Saline Control, No Stress	Mean Diff	-0.64	0.19	2.73	1.19	0.29
	S.E.	0.36	0.27	2.91	0.52	0.06
	t	-1.79	0.73	0.94	2.27	4.79
	p <	0.2162	0.5435	0.4474	0.1516	0.0409
Saline Control, With Stress	Mean Diff	-0.53	1.23	-0.24	1.38	-0.27
	S.E.	0.29	1.04	0.45	1.35	0.36
	t	-1.80	1.18	-0.53	1.02	-0.75
	p <	0.2139	0.3585	0.6516	0.4133	0.5305
Stress with Nicotine						
0.025 mg Nic/kg,	Mean Diff	2.05	3.52	0.09	0.58	-0.07
	S.E.	1.89	2.78	0.28	0.20	0.19
	t	1.08	1.27	0.33	2.90	-0.35
	p <	0.3934	0.3331	0.7751	0.1013	0.7578
0.050 mg Nic/kg,	Mean Diff	-0.24	2.95	3.04	0.71	0.56
	S.E.	0.39	1.49	1.69	0.58	0.81
	t	-0.61	1.98	1.79	1.24	0.69
	p <	0.6031	0.1863	0.2149	0.3413	0.5610
0.100 mg Nic/kg,	Mean Diff	-0.26	-0.23	1.09	0.81	1.41
	S.E.	0.21	0.45	1.27	0.80	1.11
	t	-1.23	-0.50	0.86	1.01	1.26
	p <	0.3445	0.6650	0.4804	0.4193	0.3333
Stress without Nicotine						
0.025 mg Nic/kg,	Mean Diff	-0.26	-0.31	-0.48	0.16	-0.22
	S.E.	0.28	0.08	0.35	0.09	0.24
	t	-0.95	-4.10	-1.37	1.62	-0.90
	p <	0.4437	0.0548	0.3042	0.2464	0.4651
0.050 mg Nic/kg,	Mean Diff	-0.05	0.98	1.70	0.67	0.74
	S.E.	0.62	0.66	1.84	0.57	1.02
	t	-0.08	1.48	0.92	1.18	0.72
	p <	0.9465	0.2762	0.4533	0.3602	0.5446
0.100 mg Nic/kg,	Mean Diff	1.68	6.33	1.24	2.69	1.76
	S.E.	2.09	5.73	1.21	2.50	0.99
	t	0.81	1.11	1.02	1.07	1.78
	p <	0.5049	0.3842	0.4153	0.3950	0.2164

Table 18
Part II
Phase III: Insulin

Student's t Statistic for Related Samples
Paired Comparison of Day 11 (stress with Nicotine) vs. Day 13 (stress without Nicotine) by Drug Condition and Sample

(Values are in u units/ml of plasma)

		Saline Control	0.025 mg Nicotine/kg	0.050 mg Nicotine/kg	0.100 mg Nicotine/kg
Sample 0 (baseline)					
	Mean Diff	-0.08	0.31	-0.39	-1.21
	S.E.	0.42	0.25	0.36	1.24
	t	-0.20	1.25	-1.09	-0.98
	p <	0.8621	0.3392	0.3899	0.4314
Sample 1	Mean Diff	0.03	-2.00	-0.19	0.73
	S.E.	0.74	1.75	0.58	1.11
	t	0.04	-1.15	-0.34	0.66
	p <	0.9703	0.3704	0.7639	0.5781
Sample 2 (Physical Restraint Stress Initiated)					
	Mean Diff	0.95	-3.52	-2.36	5.35
	S.E.	1.29	2.98	1.28	4.75
	t	0.74	-1.18	-1.85	1.12
	p <	0.5369	0.3584	0.2056	0.3777
Sample 3	Mean Diff	-3.05	-0.26	-1.72	-1.06
	S.E.	2.89	0.41	3.30	1.38
	t	-1.05	-0.63	-0.52	-0.77
	p <	0.4022	0.5929	0.6540	0.5200
Sample 4	Mean Diff	0.12	-0.11	-0.43	0.67
	S.E.	1.42	0.23	0.35	1.09
	t	0.08	-0.49	-1.23	0.62
	p <	0.9427	0.6747	0.3448	0.6004
Sample 5	Mean Diff	-0.64	0.16	-0.21	-0.85
	S.E.	0.24	0.33	0.49	1.25
	t	-2.66	0.49	-0.43	-0.69
	p <	0.1168	0.6726	0.7087	0.5635

Table 18
Part III
Phase III: Insulin
Student's t Statistic: Control vs. All Nicotine Doses by Test Day and Sample
(Values are in u units/ml of plasma)

	Saline Control		Test Day: Stress with Nicotine						Test Day: Stress without Nicotine					
			0.025 mg		0.050 mg		0.100 mg		0.025 mg		0.050 mg		0.100 mg	
Sample 0 (baseline)	No Stress Test	Stress Test												
$\bar{x}$	2.40	2.32	1.74		2.29		3.41		2.05		1.90		2.20	
S.E.	0.40	0.78	0.07		0.30		1.82		0.30		0.52		0.59	
No Stress Control vs.			df, t	4	1.63	4	0.23	4	-0.54	4	0.70	4	0.76	4	0.28
			p <		0.1783		0.8327		0.6174		0.5226		0.4888		0.7907
Stress Control vs.			df, t	4	0.74	4	0.04	4	-0.55	4	0.32	4	0.45	4	0.12
			p <		0.5009		0.9730		0.6115		0.7641		0.6788		0.9081
Sample 1														
$\bar{x}$	1.76	1.79	3.79		2.05		3.15		1.79		1.85		3.88	
S.E.	0.28	0.64	1.87		0.52		1.63		0.12		0.36		2.68	
No Stress Control vs.			df, t	4	-1.08	4	-0.50	4	-0.84	4	-0.10	4	-0.21	4	-0.79
			p <		0.3427		0.6446		0.4466		0.9267		0.8441		0.4744
Stress Control vs.			df, t	4	-1.01	4	-0.32	4	-0.78	4	0.001	4	-0.09	4	-0.76
			p <		0.3681		0.7650		0.4795		0.9988		0.9338		0.4895
Sample 2 (Physical Restraint Stress Initiated)														
$\bar{x}$	2.60	3.55	5.27		5.24		3.18		1.74		2.88		8.53	
S.E.	0.67	1.80	2.74		1.19		1.56		0.28		0.70		6.32	
No Stress Control vs.			df, t	4	-0.94	4	-1.93	4	-0.34	4	1.19	4	-0.29	4	-0.93
			p <		0.3982		0.1257		0.7490		0.3006		0.7869		0.4034
Stress Control vs.			df, t	4	-0.52	4	-0.78	4	0.16	4	0.99	4	0.35	4	-0.76
			p <		0.6291		0.4788		0.8834		0.3759		0.7442		0.4909
Sample 3														
$\bar{x}$	5.13	2.08	1.83		5.33		4.50		1.57		3.60		3.44	
S.E.	3.21	0.33	0.35		1.99		3.07		0.10		1.88		1.79	
No Stress Control vs.			df, t	4	1.02	4	-0.05	4	0.14	4	1.11	4	0.41	4	0.46
			p <		0.3654		0.9609		0.8948		0.3309		0.7031		0.6699
Stress Control vs.			df, t	4	0.52	4	-1.61	4	-0.78	4	1.46	4	-0.80	4	-0.74
			p <		0.6297		0.1834		0.4779		0.2173		0.4706		0.5000

Table 18, Part III continued

	Saline Control		Test Day: Stress with Nicotine 0.025 mg		0.050 mg		0.100 mg		Test Day: Stress without Nicotine 0.025 mg		0.050 mg		0.100 mg	
Sample 4	No Stress Test	Stress Test												
$\bar{x}$	3.59	3.70		2.32		3.00		4.22		2.21		2.58		4.89
S.E.	0.91	0.57		0.26		0.36		2.05		0.29		0.08		3.09
No Stress Control vs.		df, t	4	1.34	4	0.60	4	-0.28	4	1.45	4	1.11	4	-0.40
		p <		0.2513		0.5817		0.7923		0.2216		0.3280		0.7066
Stress Control vs.		df, t	4	2.19	4	1.03	4	-0.24	4	2.33	4	1.96	4	-0.38
		p <		0.0930		0.3598		0.8205		0.0805		0.1219		0.7248
Sample 5														
$\bar{x}$	2.69	2.05		1.67		2.85		4.82		1.83		2.64		3.96
S.E.	0.40	0.45		0.27		0.51		2.69		0.17		0.50		1.52
No Stress Control vs.		df, t	4	2.34	4	-0.26	4	-0.78	4	2.25	4	0.08	4	-0.82
		p <		0.0797		0.8066		0.4762		0.0873		0.9384		0.4585
Stress Control vs.		df, t	4	0.71	4	-1.17	4	-1.02	4	0.44	4	-0.88	4	-1.21
		p <		0.5174		0.3075		0.3674		0.6805		0.4309		0.2934

Table 18
Part IV
Insulin

Percent of Baseline Response: Mean Value for All Drug Conditions by Sample and Test Day (Stress with/without Nicotine)

(x - Indicates initiation of Physical Restraint Stress)

	Sample					
	0 (baseline)	1	x	2	3	4	5
Saline Control No Stress or Nicotine	100	75.50		104.15	187.76	144.10	113.74
Saline Control Stress, No Nicotine	100	80.86		133.33	106.33	237.49	100.79
Stress with Nicotine							
0.025 mg Nic/kg	100	222.19		310.85	104.06	132.93	95.04
0.050 mg Nic/kg	100	99.66		305.45	257.91	170.56	160.72
0.100 mg Nic/kg	100	95.94		105.50	110.83	150.72	155.30
Stress without Nicotine							
0.025 mg Nic/kg	100	90.33		84.90	80.71	108.86	91.69
0.050 mg Nic/kg	100	110.32		161.45	192.02	159.97	179.98
0.100 mg Nic/kg	100	141.04		300.39	137.57	184.02	172.29

Appendix D

Comments Regarding the Graphic Presentation of the Experimental Data

The graphs presented on the following pages were created using the SAS:Graphics data presentation package (SAS Users Guide:Graphics, 1982 Edition, SAS Institute Inc., Cary, NC 27511). Contained in the title of each graph is information regarding the phase (i.e., Phases I, II and III) of the study from which the data originated, the dependent measure (i.e., norepinephrine, epinephrine, dopamine, corticosterone, glucose, insulin) and the experimental drug condition which is presented (i.e., saline control, 0.025, 0.050 or 0.100 mg Nic/kg body weight). Individual graphs are presented for each of the drug conditions within each phase of the experiment.

The graphs (except graphs 5, 10, 15, 20, 25 and 30) present the individual response values using different emblems to indicate the Test Day of their origin (the Legend for individual graphs indicates the symbols used and the Test Day to which each is related). Samples (0-5) are presented on the abscissa which indicates the time (in minutes) separating each from the baseline sample. Drug infusions occurred during the time periods 0-20 and 120-140 minutes. Response values are presented on the ordinate axis (in an attempt to optimize the presentation of the data the ordinate axis of each graph has been individually determined according to the values being presented).

A cubic regression function was used for drawing the curves presented in each graph. The regression curves are presented only as an aid to the reader in the interpretation of the experimental

results, and should not be construed as necessarily being the "best" fit function.

Figures 5, 10, 15, 20, 25 and 30 present the regression curves for each of the six dependent variables measured in Phase I of the experiment according to the experimental Test Day. Test Day is presented on the abscissa of each graph. The legends for the graphs are as follows:

Drug Condition 1 = Saline Control

Drug Condition 2 = 0.025 mg Nic/kg

Drug Condition 3 = 0.050 mg Nic/kg

Drug Condition 4 = 0.100 mg Nic/kg

The legend for graphs of Phase III results is as follows:

Saline Control Day 11 = No Stress Condition

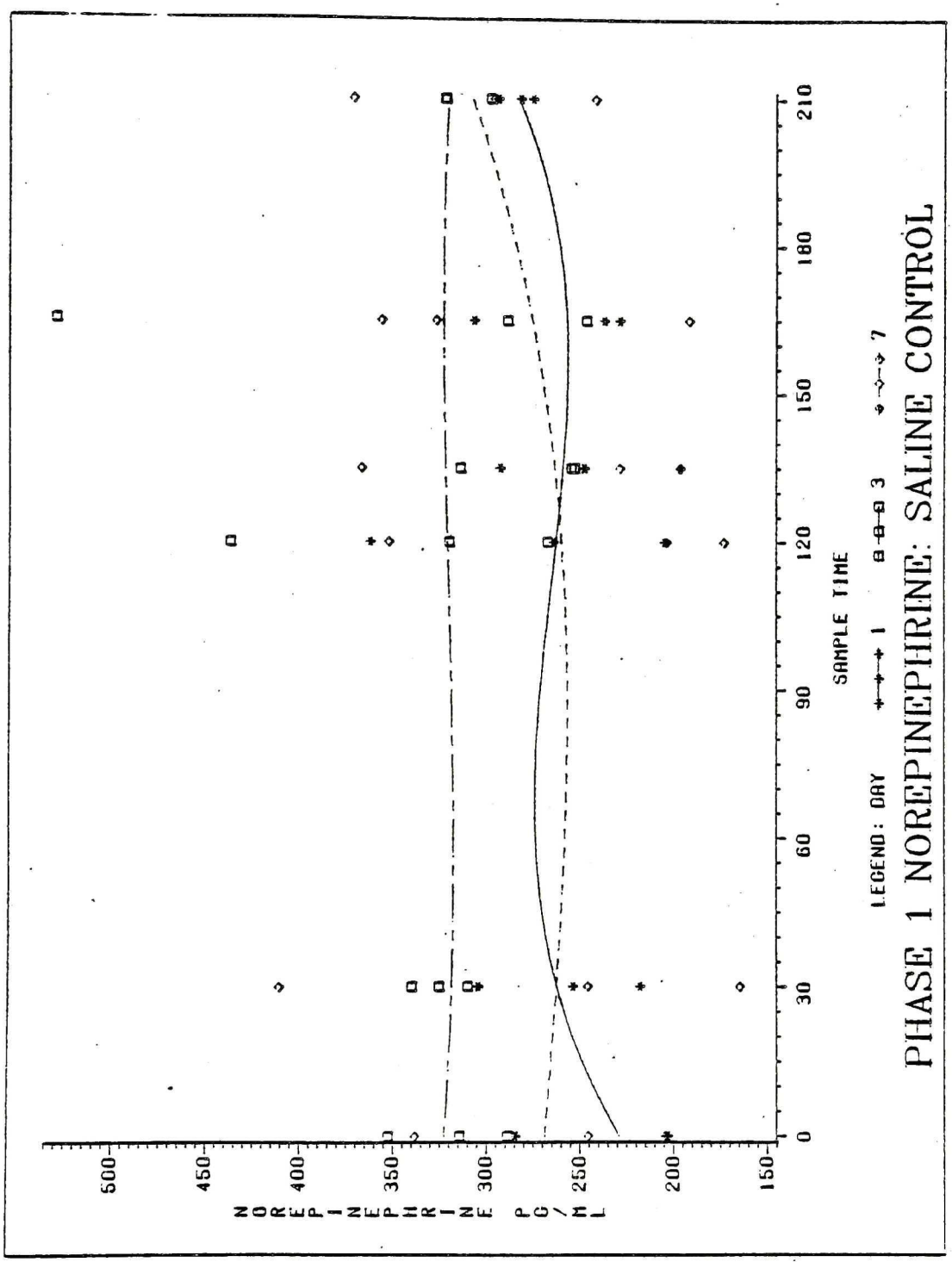
Saline Control Day 13 = Stress Condition

All Nicotine Doses Day 11 = Stress with Nicotine

All Nicotine Doses Day 13 = Stress without Nicotine

FIGURE 1

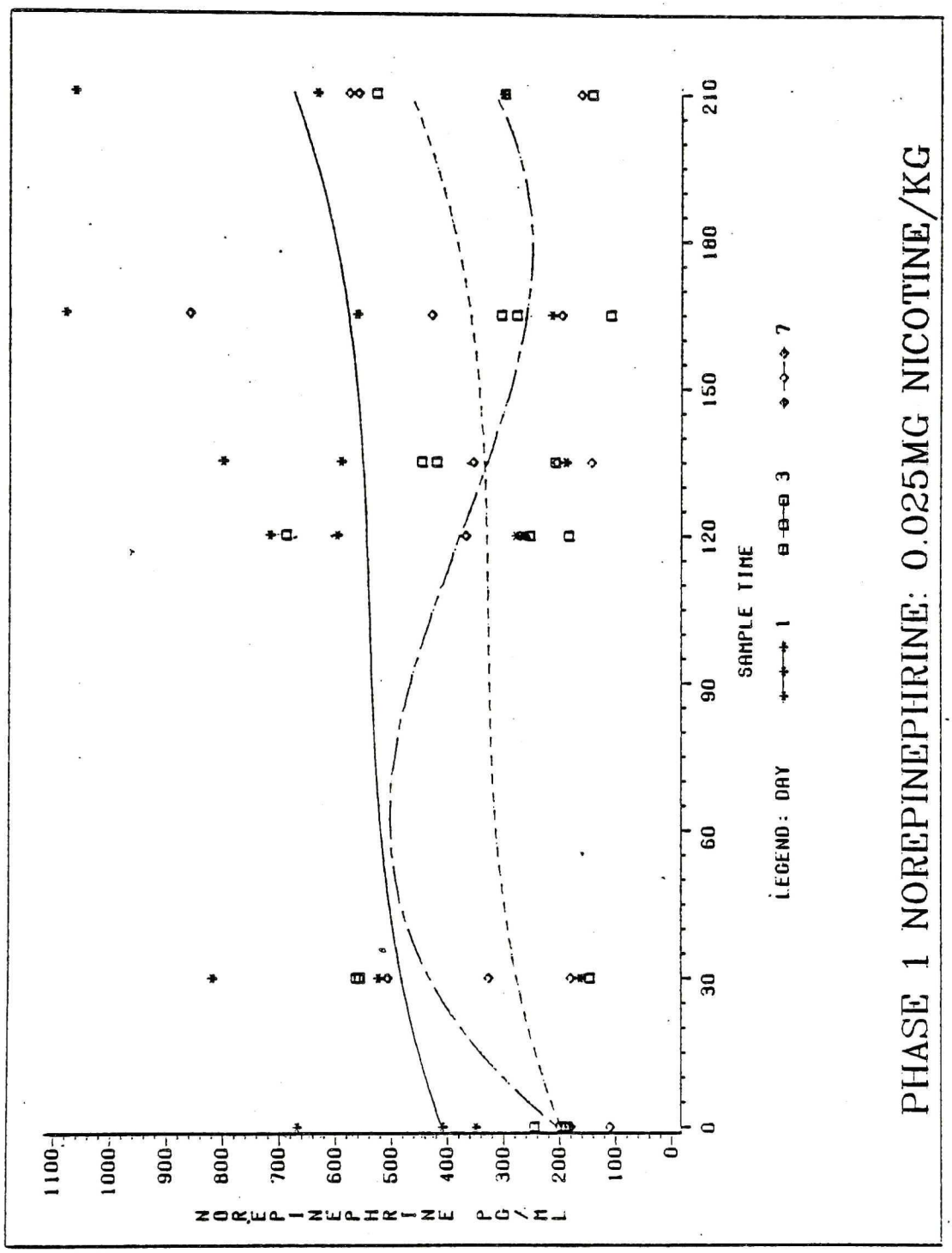
PHASE I: NOREPINEPHRINE RESPONSES FOR SALINE CONTROL CONDITION



PHASE 1 NOREPINEPHRINE: SALINE CONTROL

FIGURE 2

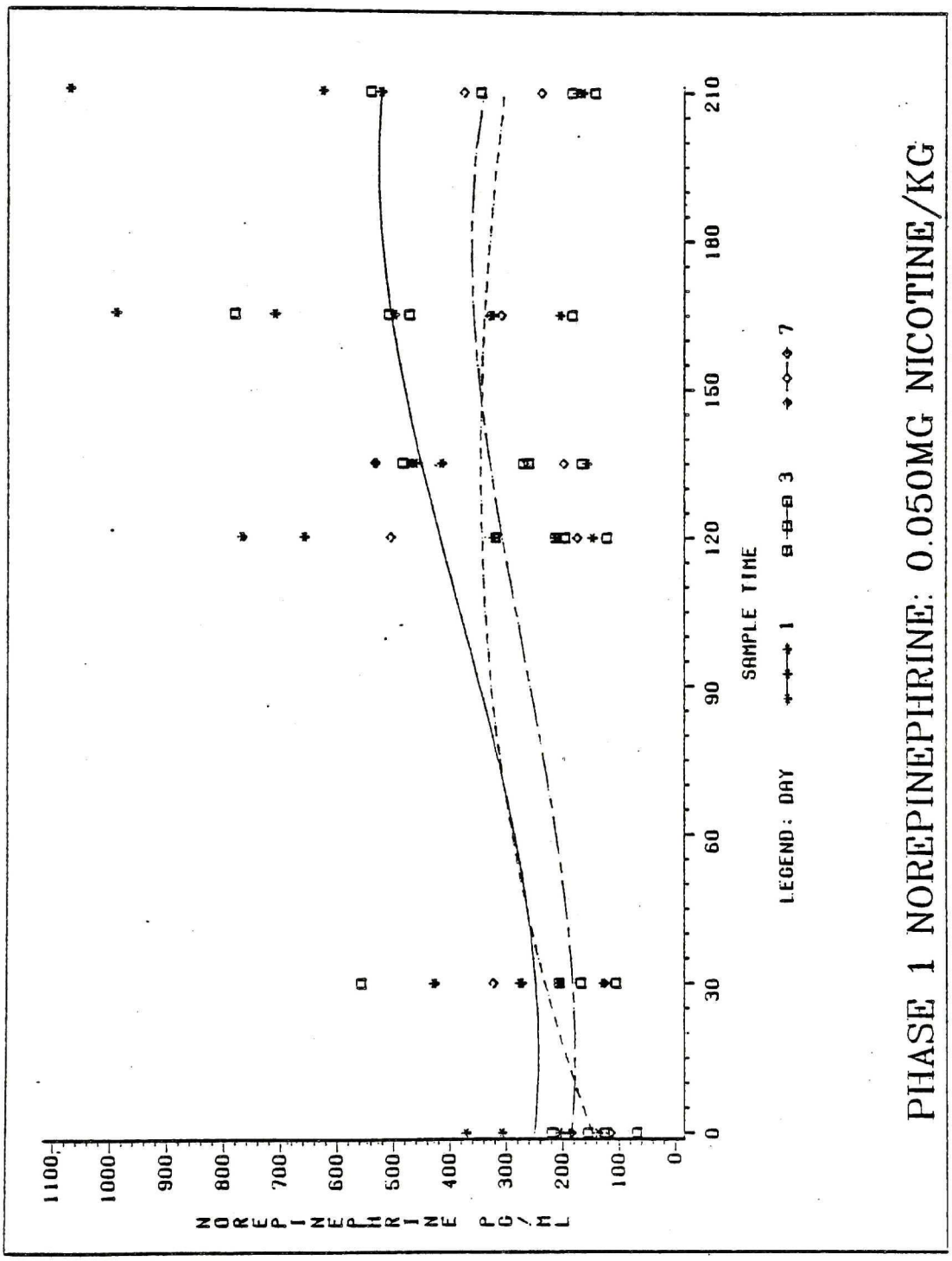
PHASE I: NOREPINEPHRINE RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION



PHASE 1 NOREPINEPHRINE: 0.025MG NICOTINE/KG

FIGURE 3

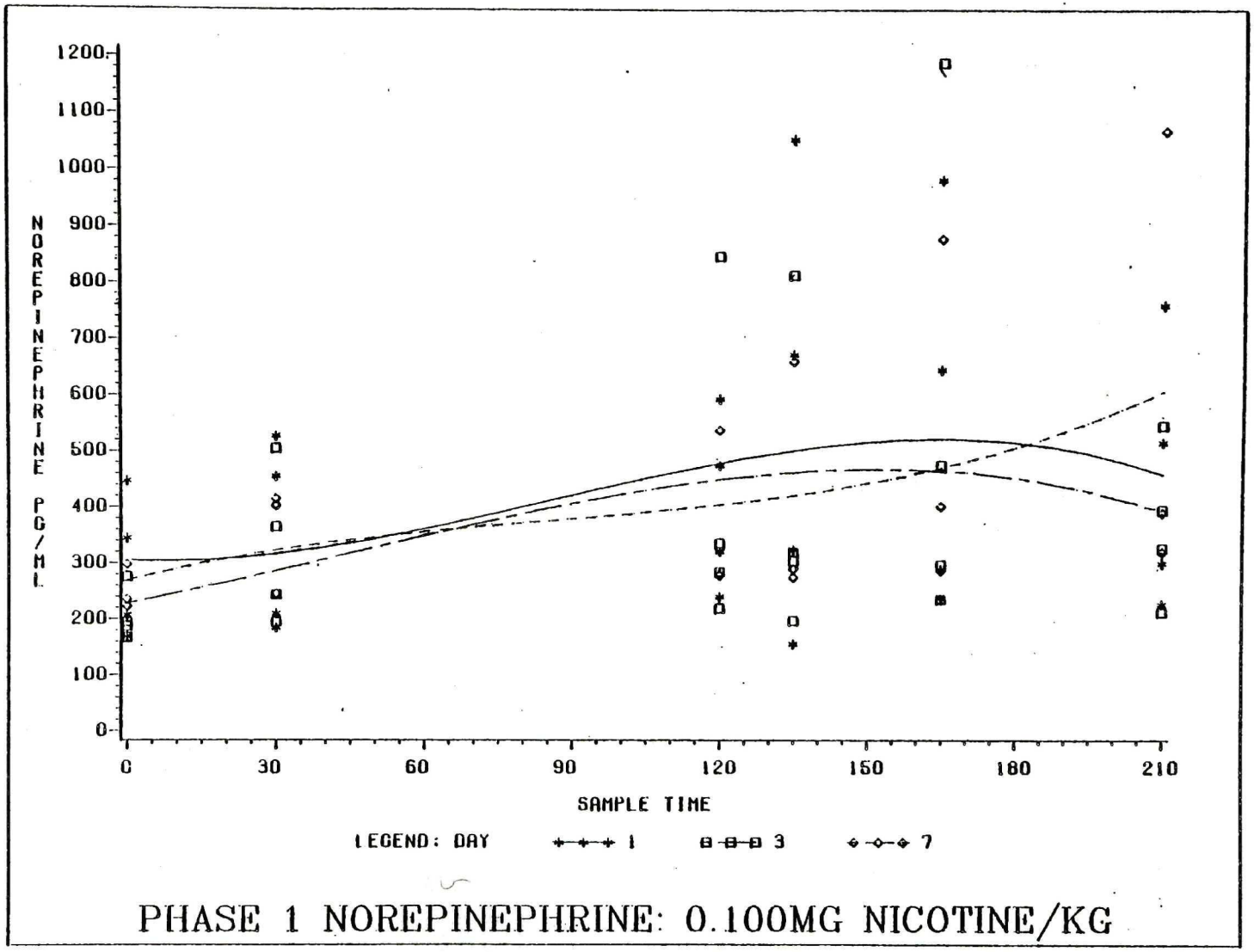
PHASE I: NOREPINEPHRINE RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION



PHASE 1 NOREPINEPHRINE: 0.050MG NICOTINE/KG

FIGURE 4

PHASE I: NOREPINEPHRINE RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION



PHASE 1 NOREPINEPHRINE: 0.100MG NICOTINE/KG

FIGURE 5

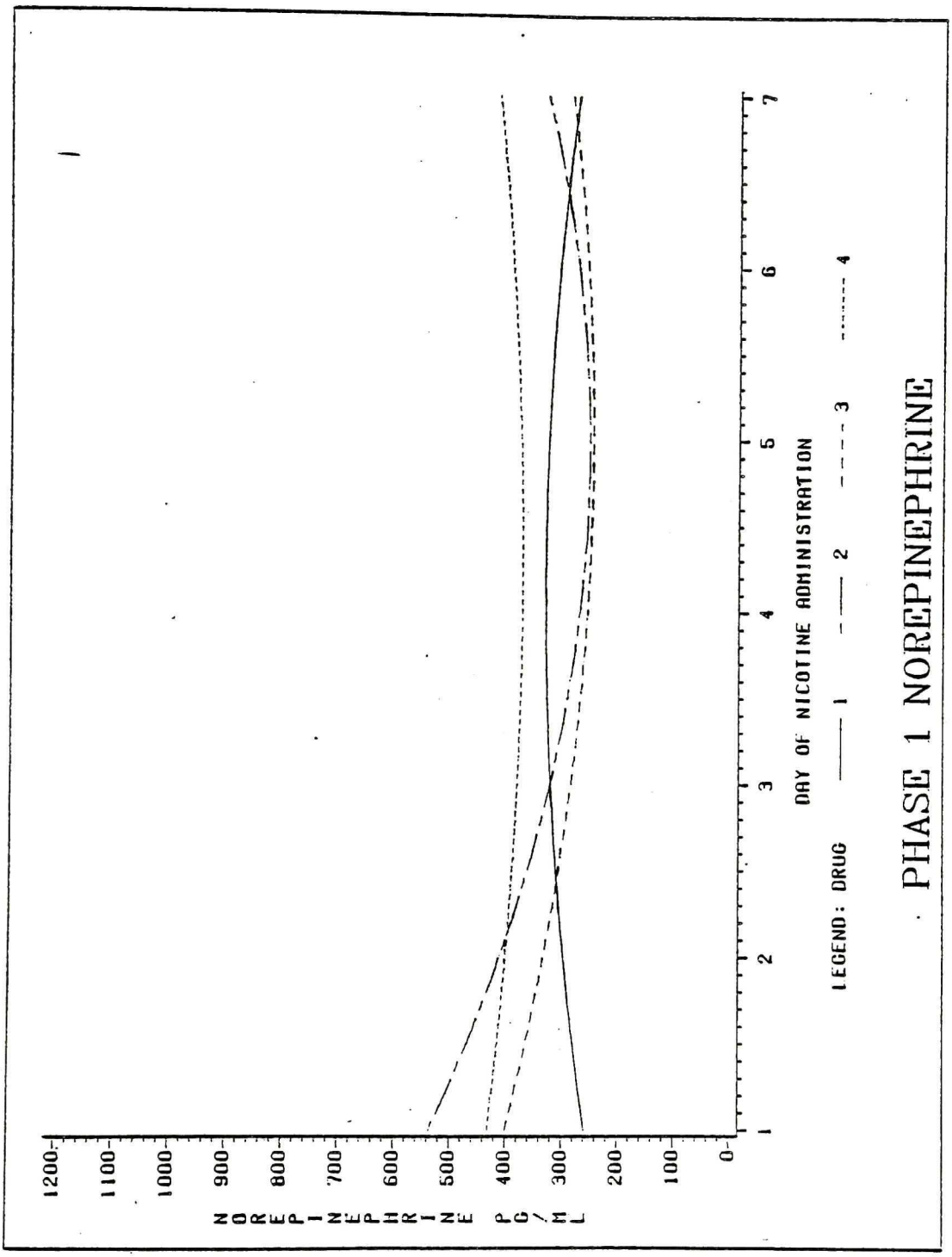
LEGEND

DRUG CONDITION 1 = SALINE CONTROL

DRUG CONDITION 2 = 0.025 mg NICOTINE/kg

DRUG CONDITION 3 = 0.050 mg NICOTINE/kg

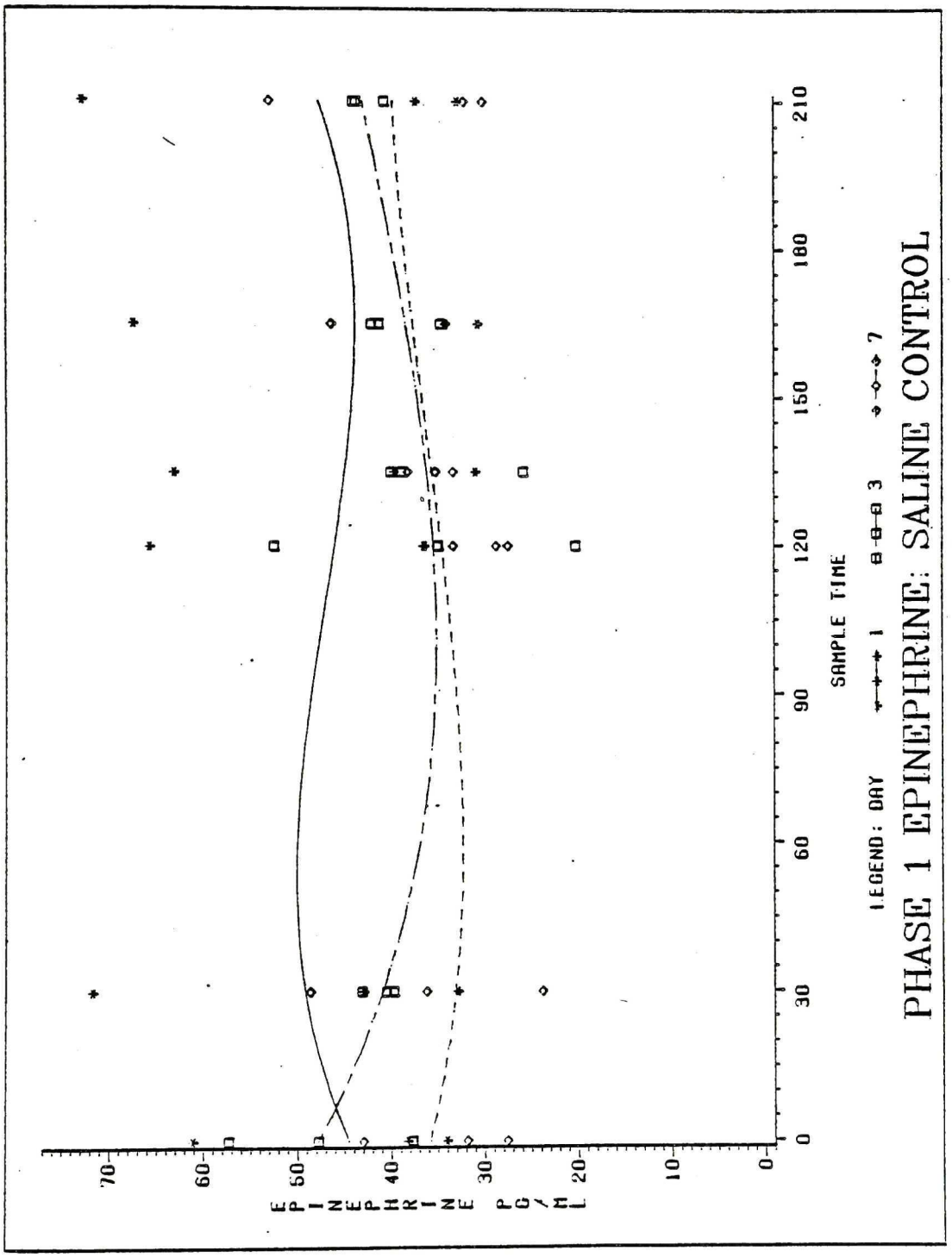
DRUG CONDITION 4 = 0.100 mg NICOTINE/kg



PHASE 1 NOREPINEPHRINE

FIGURE 6

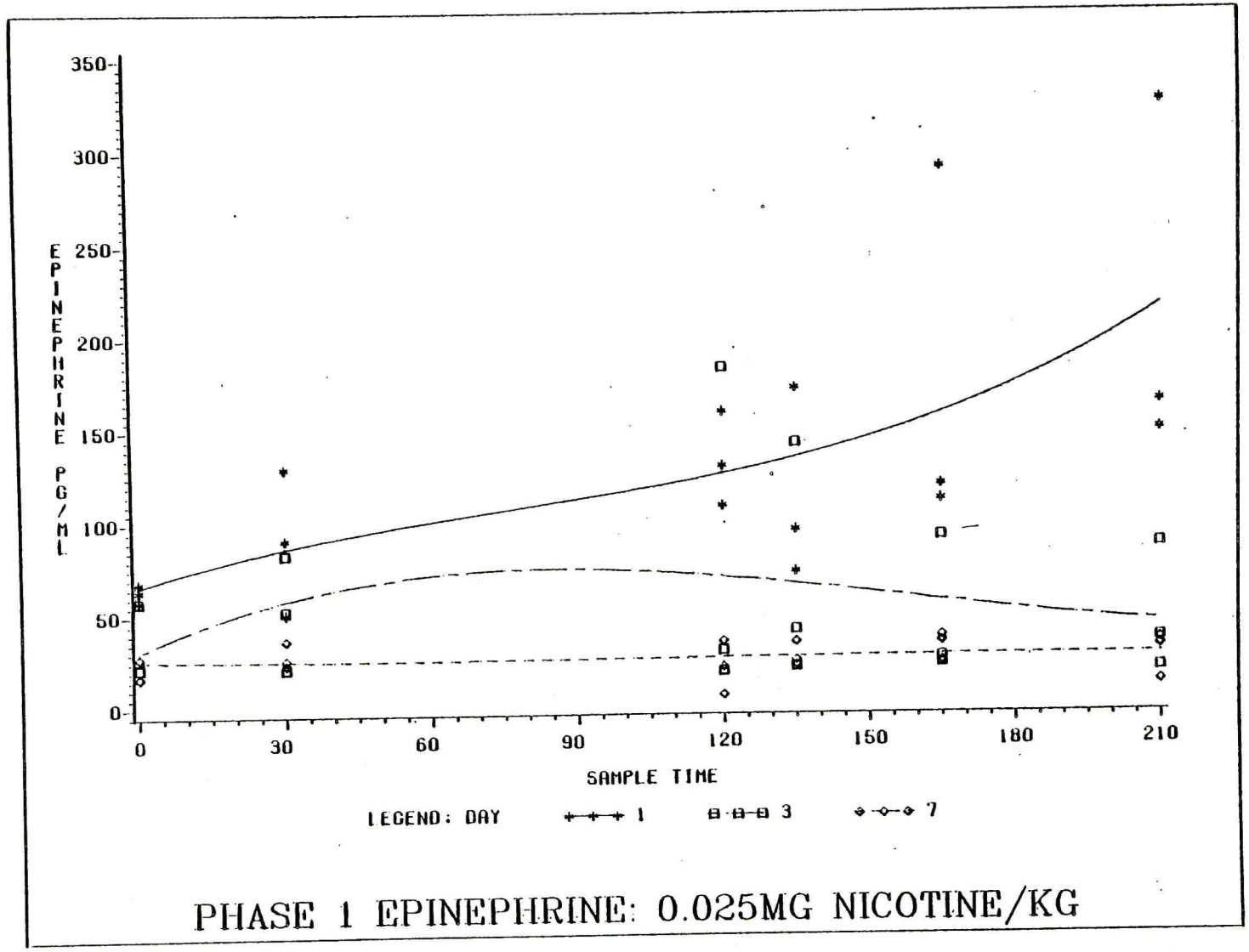
PHASE I: EPINEPHRINE RESPONSES FOR SALINE CONTROL CONDITION



PHASE 1 EPINEPHRINE: SALINE CONTROL

FIGURE 7

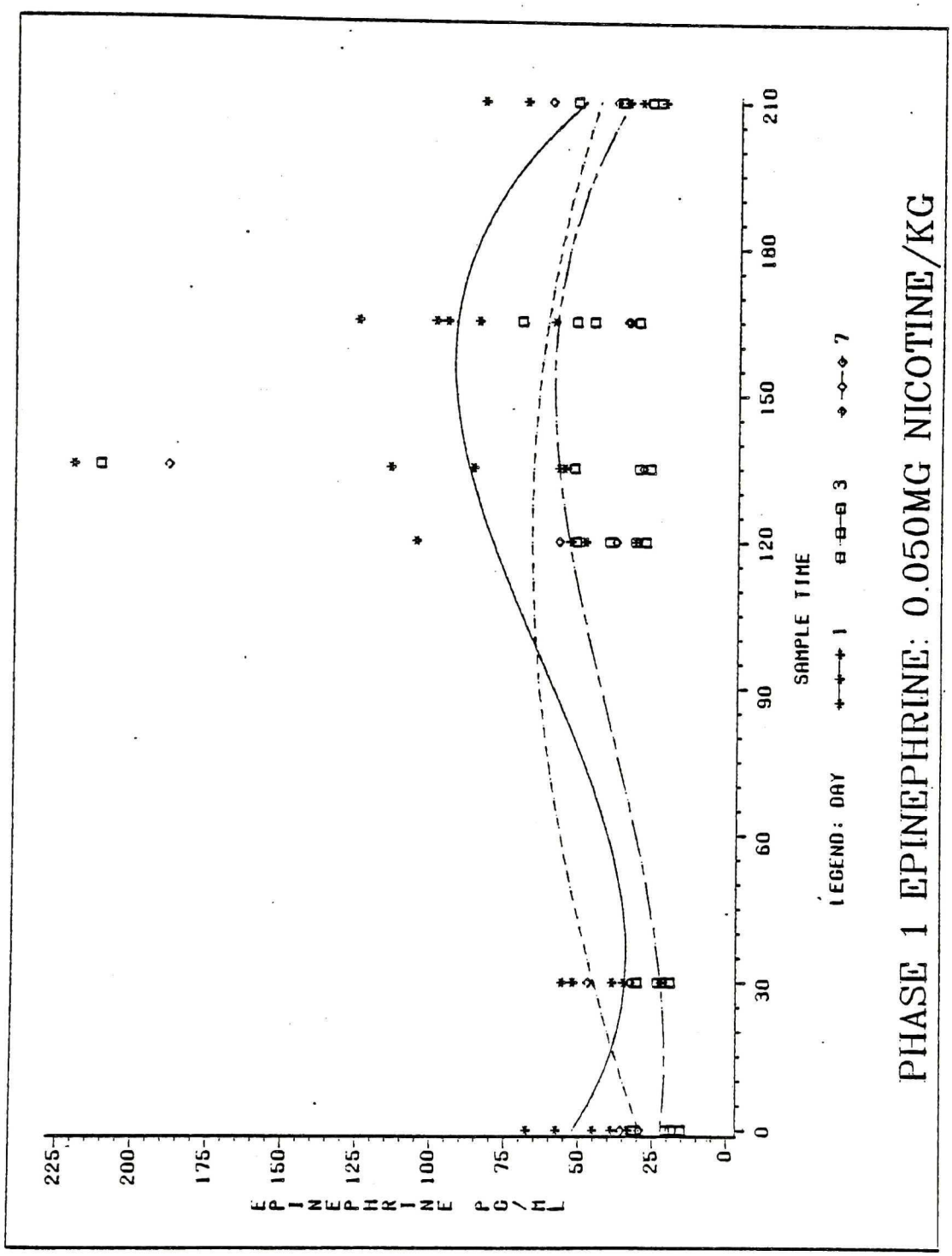
PHASE I: EPINEPHRINE RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION



PHASE 1 EPINEPHRINE: 0.025MG NICOTINE/KG

FIGURE 8

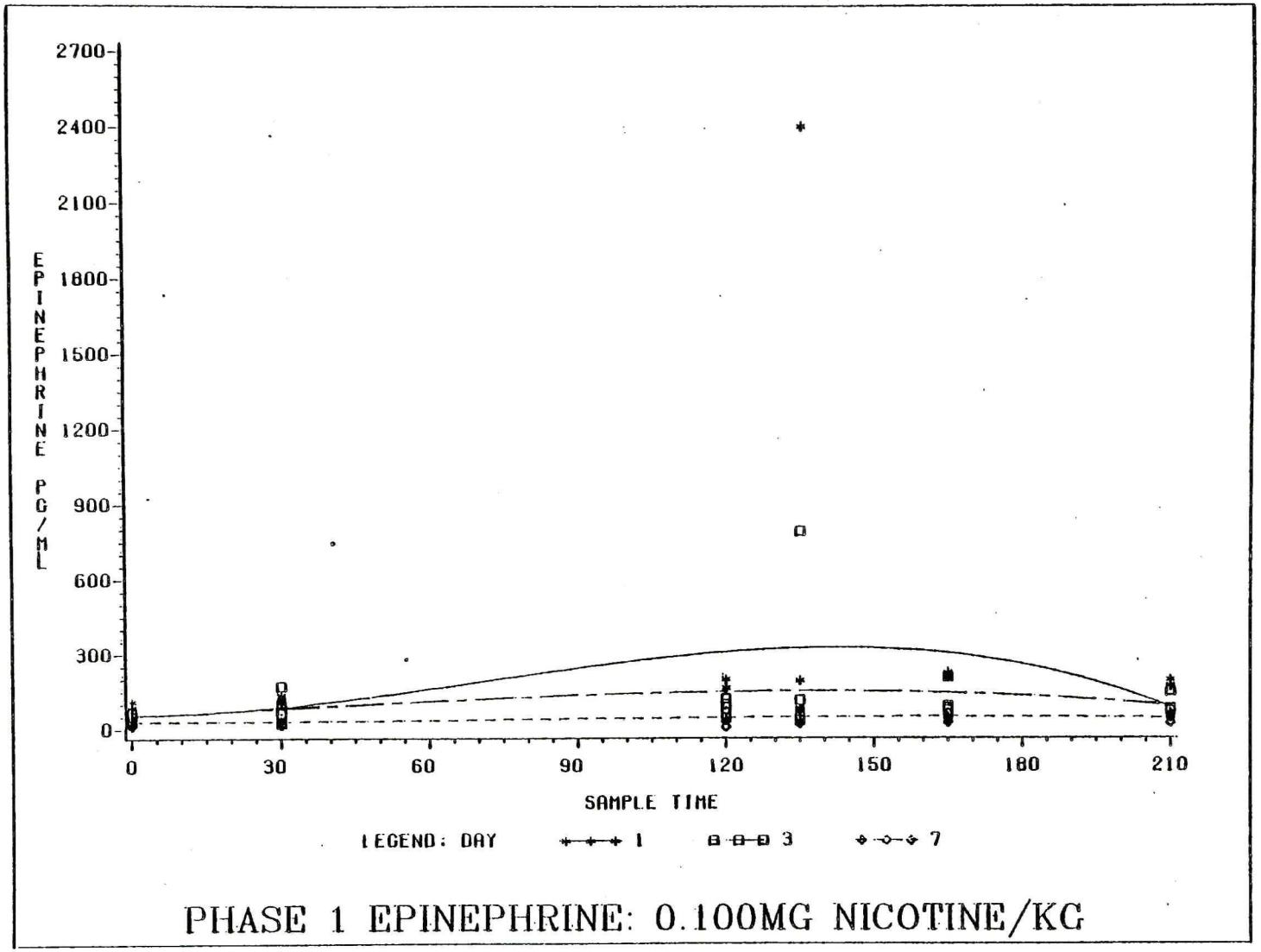
PHASE I: EPINEPHRINE RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION



PHASE 1 EPINEPHRINE: 0.050MG NICOTINE/KG

FIGURE 9

PHASE I: EPINEPHRINE RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION



PHASE 1 EPINEPHRINE: 0.100MG NICOTINE/KG

FIGURE 10

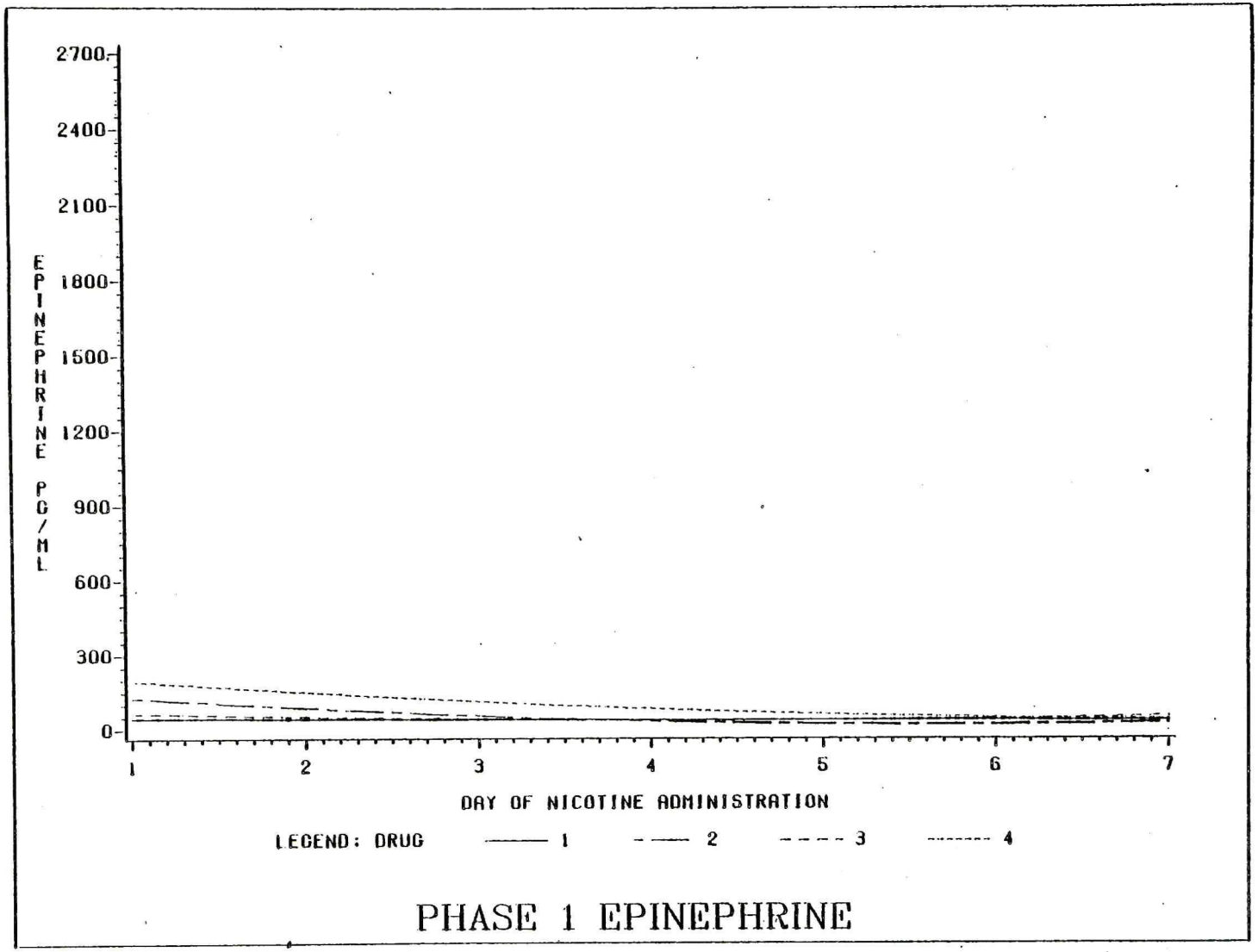
LEGEND

DRUG CONDITION 1 = SALINE CONTROL

DRUG CONDITION 2 = 0.025 mg NICOTINE/kg

DRUG CONDITION 3 = 0.050 mg NICOTINE/kg

DRUG CONDITION 4 = 0.100 mg NICOTINE/kg



PHASE 1 EPINEPHRINE

FIGURE 11

PHASE I: DOPAMINE RESPONSES FOR SALINE CONTROL CONDITION

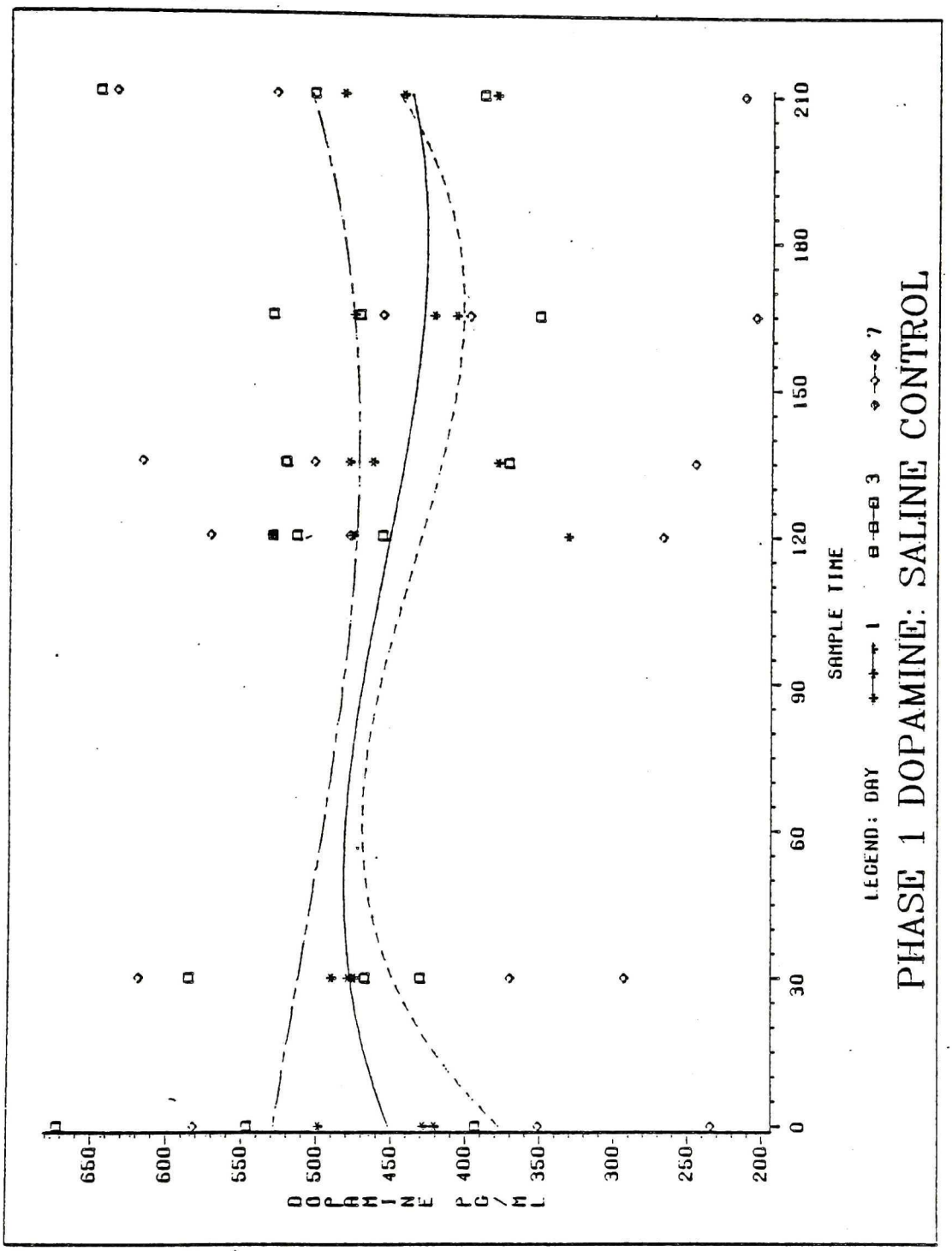
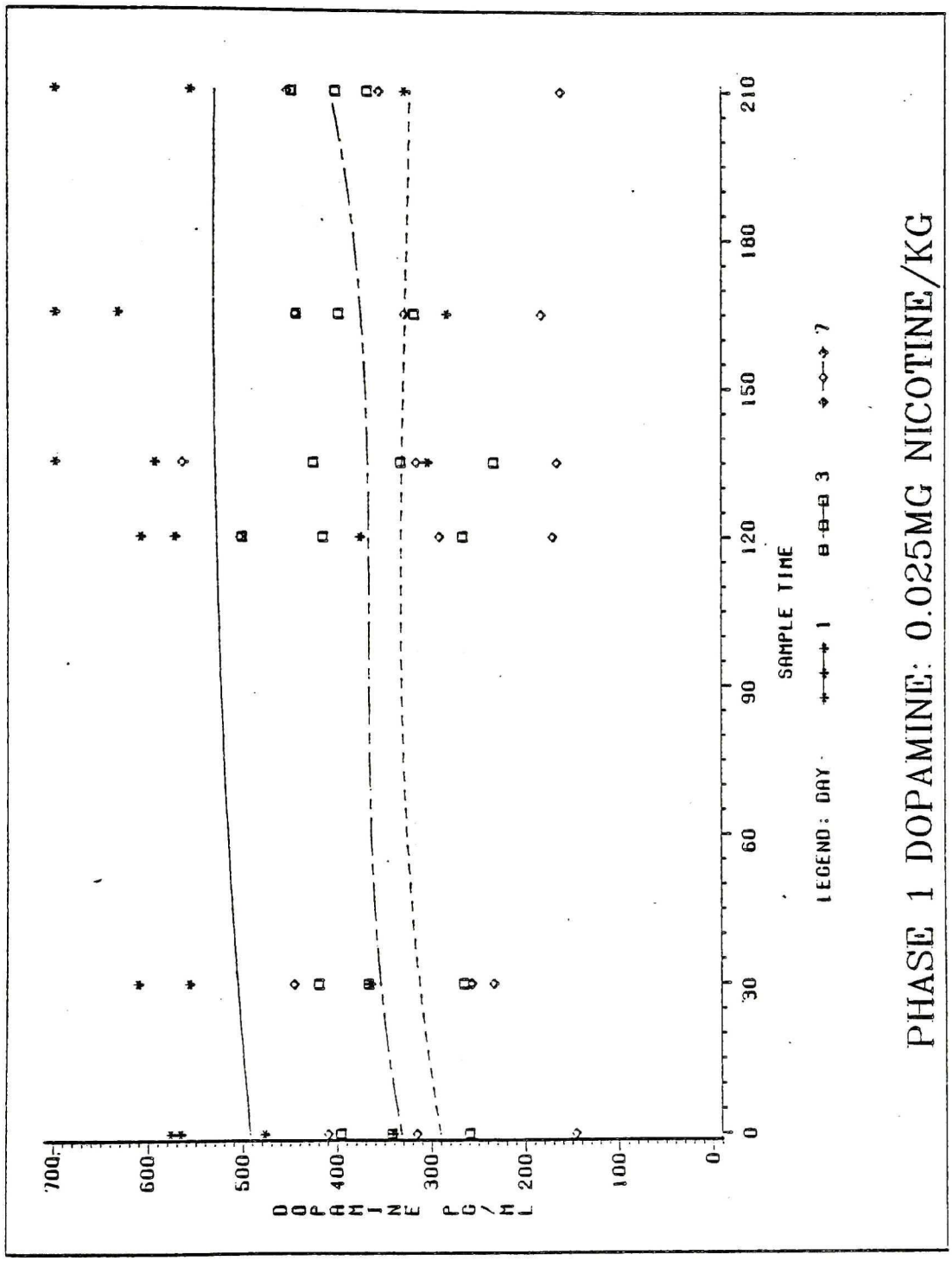

PHASE 1 DOPAMINE: SALINE CONTROL
LEGEND: DAY 1 3 7
SAMPLE TIME
0
30
60
90
120
150
180
210
DOPAMINE PG/ML
650
600
550
500
450
400
350
300
250
200

FIGURE 12

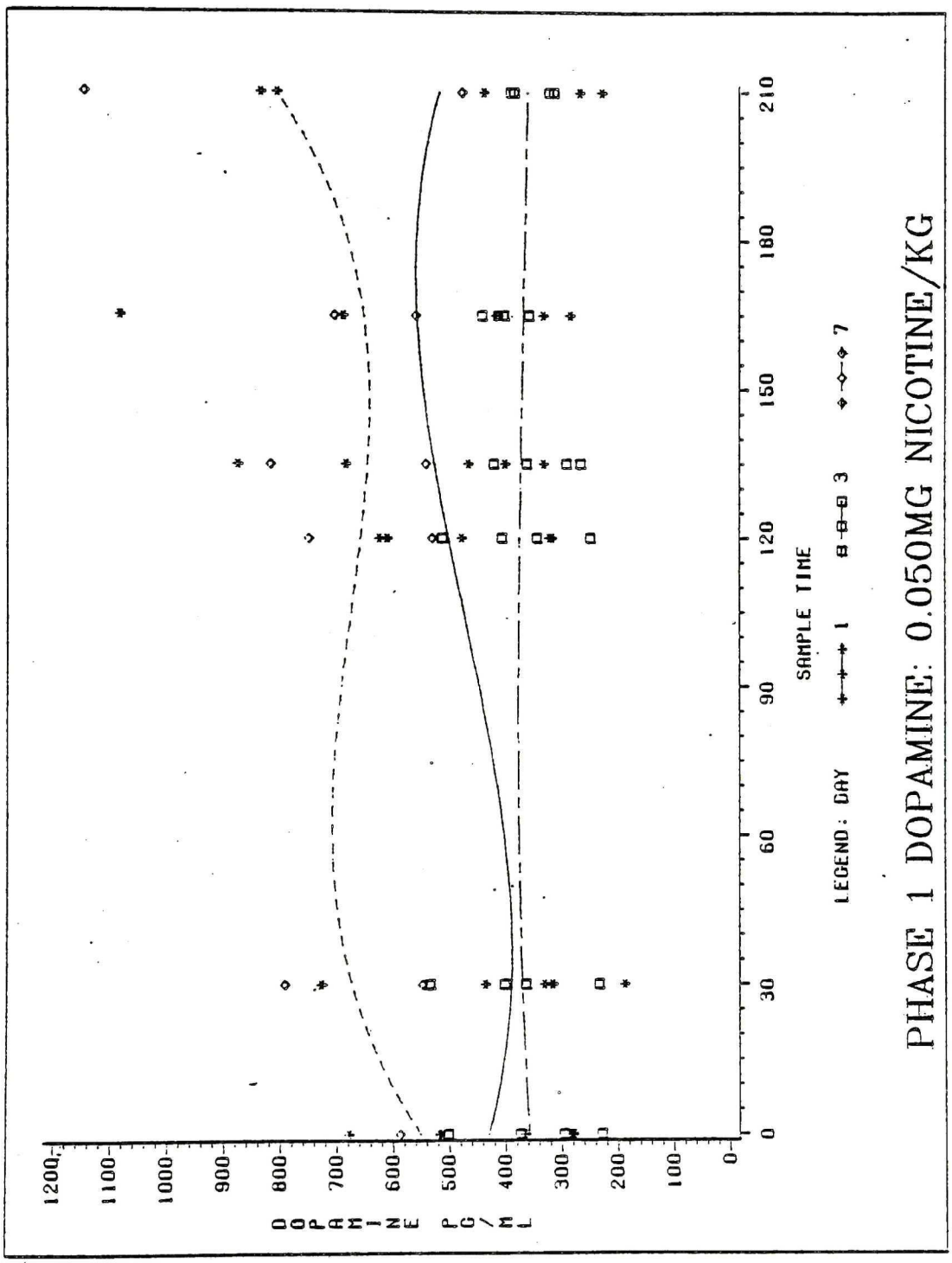
PHASE I: DOPAMINE RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION



PHASE 1 DOPAMINE: 0.025MG NICOTINE/KG

FIGURE 13

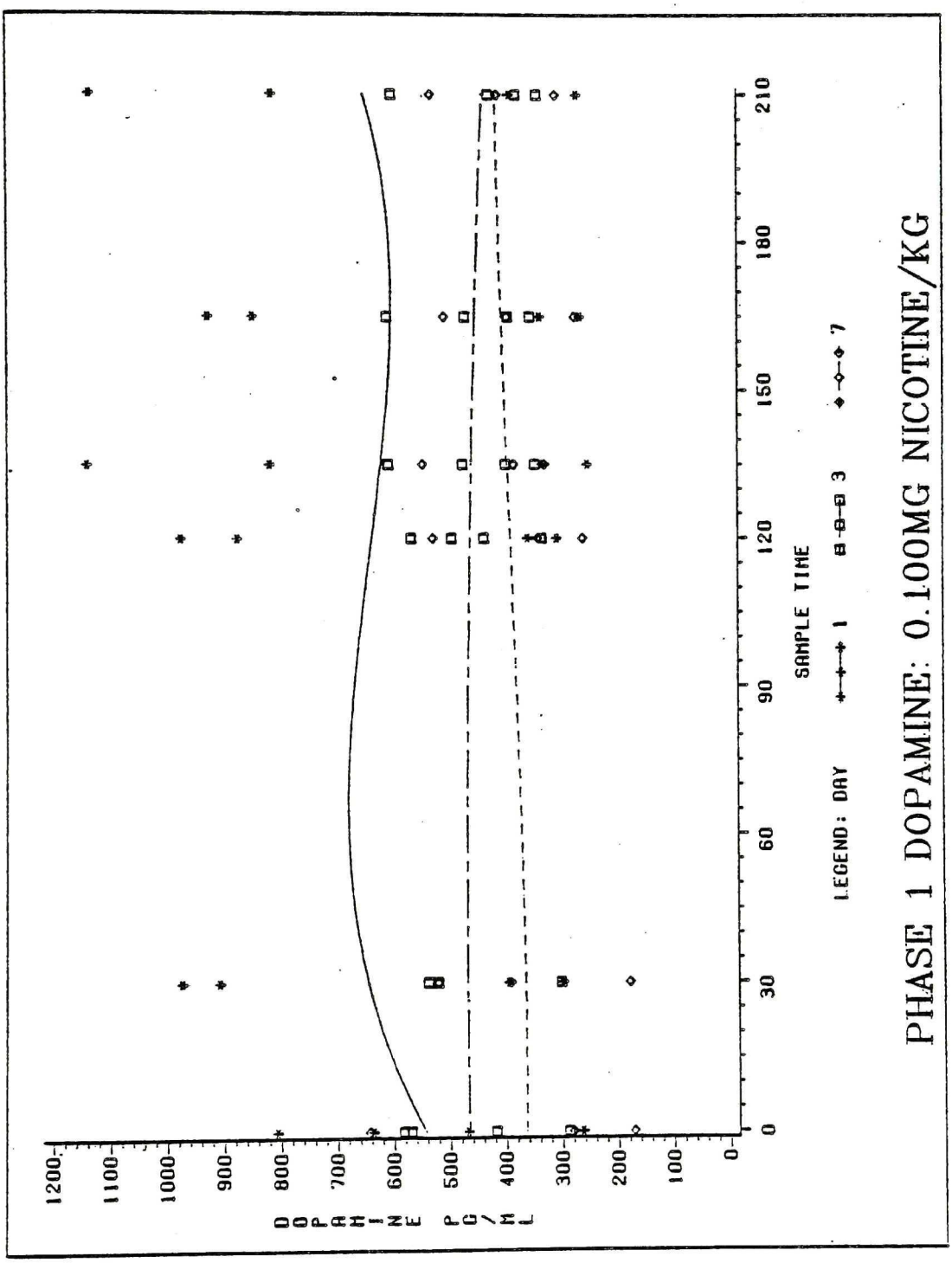
PHASE I: DOPAMINE RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION



PHASE 1 DOPAMINE: 0.050MG NICOTINE/KG

FIGURE 14

PHASE I: DOPAMINE RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION



PHASE 1 DOPAMINE: 0.100MG NICOTINE/KG

FIGURE 15

LEGEND

DRUG CONDITION 1 = SALINE CONTROL

DRUG CONDITION 2 = 0.025 mg NICOTINE/kg

DRUG CONDITION 3 = 0.050 mg NICOTINE/kg

DRUG CONDITION 4 = 0.100 mg NICOTINE/kg

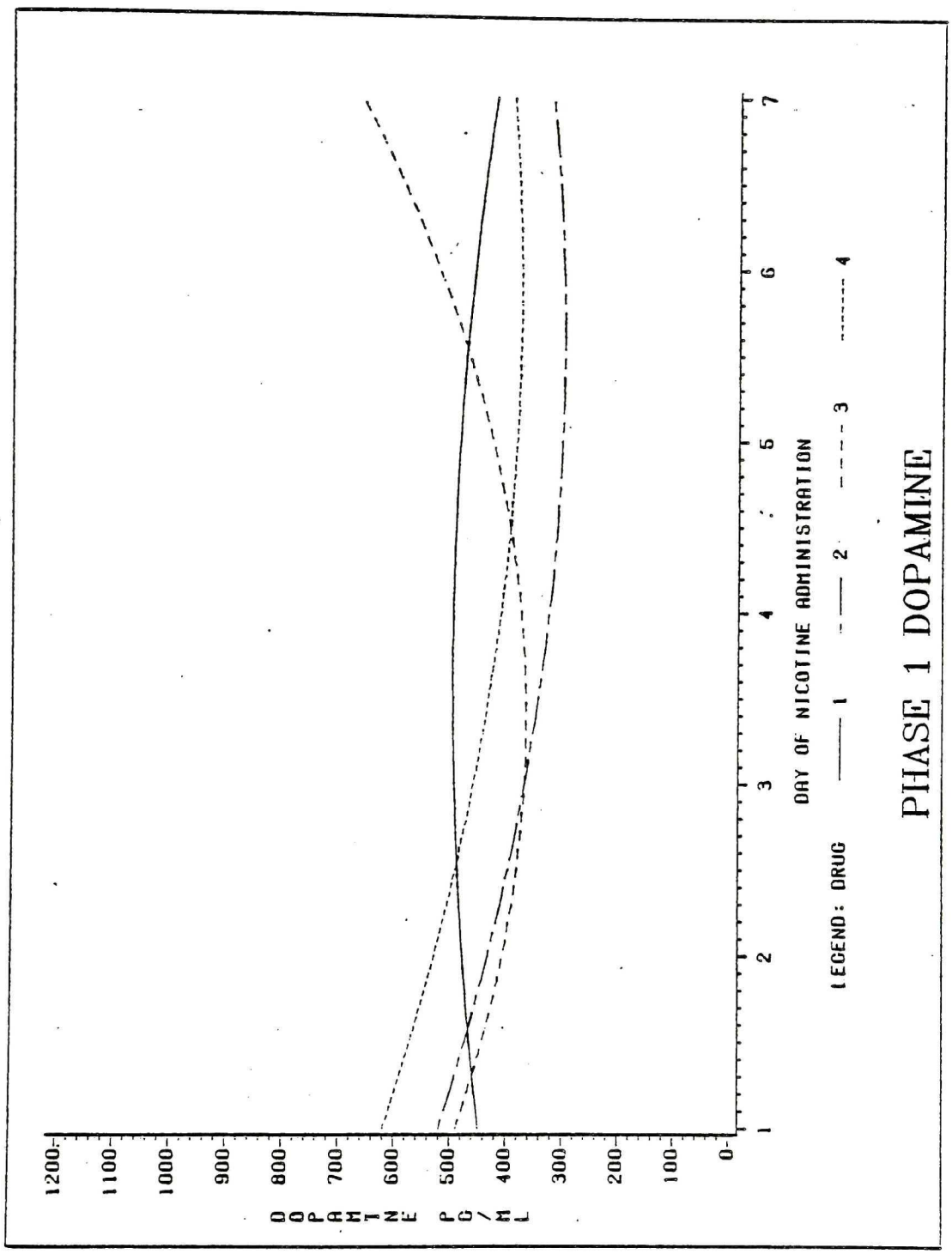
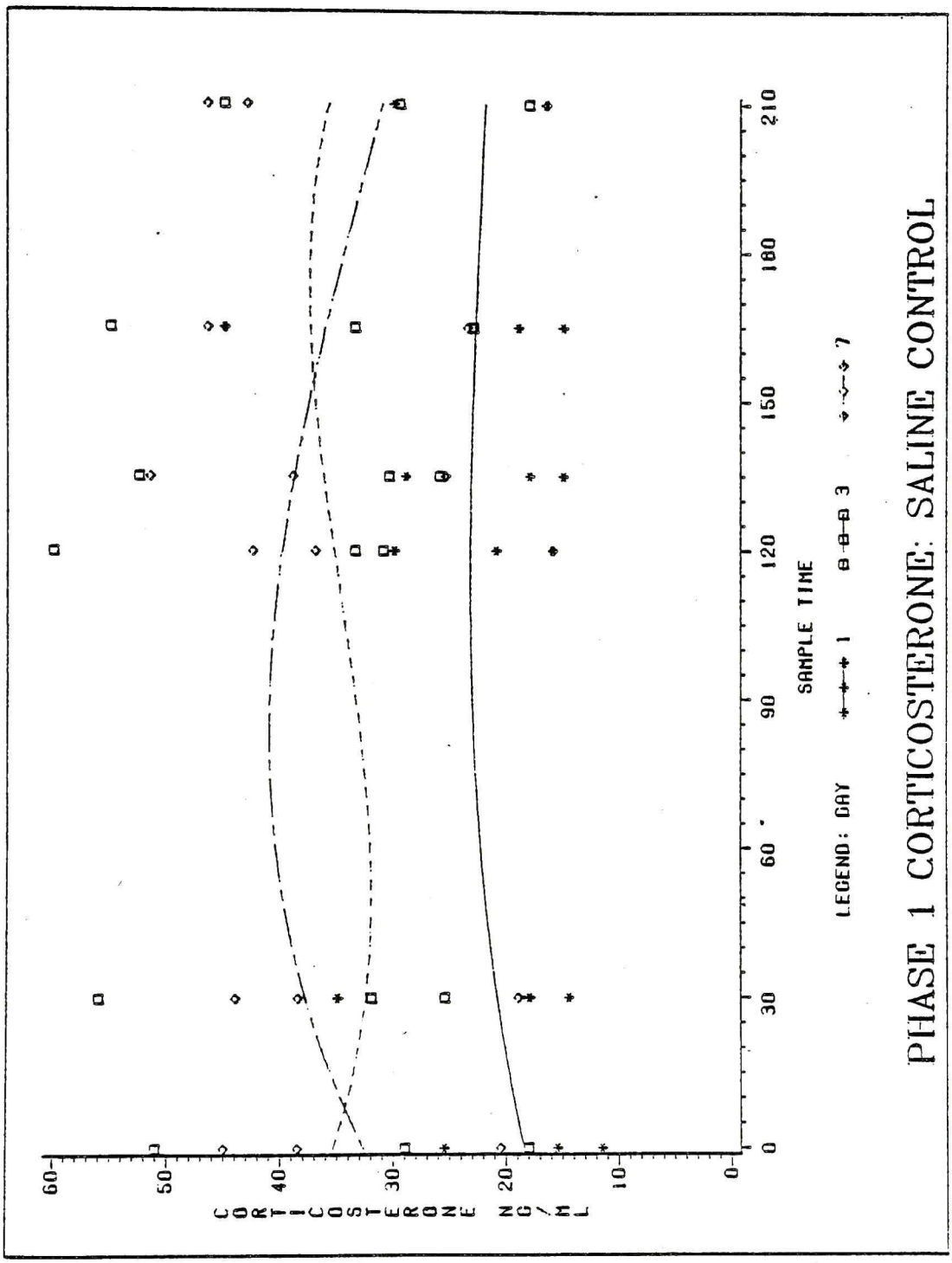

PHASE 1 DOPAMINE
DOPAMINE PG/ML
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
DAY OF NICOTINE ADMINISTRATION
LEGEND: DRUG 1 2 3 4

FIGURE 16

PHASE I: CORTICOSTERONE RESPONSES FOR SALINE CONTROL CONDITION



PHASE 1 CORTICOSTERONE: SALINE CONTROL

FIGURE 17

PHASE I: CORTICOSTERONE RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION

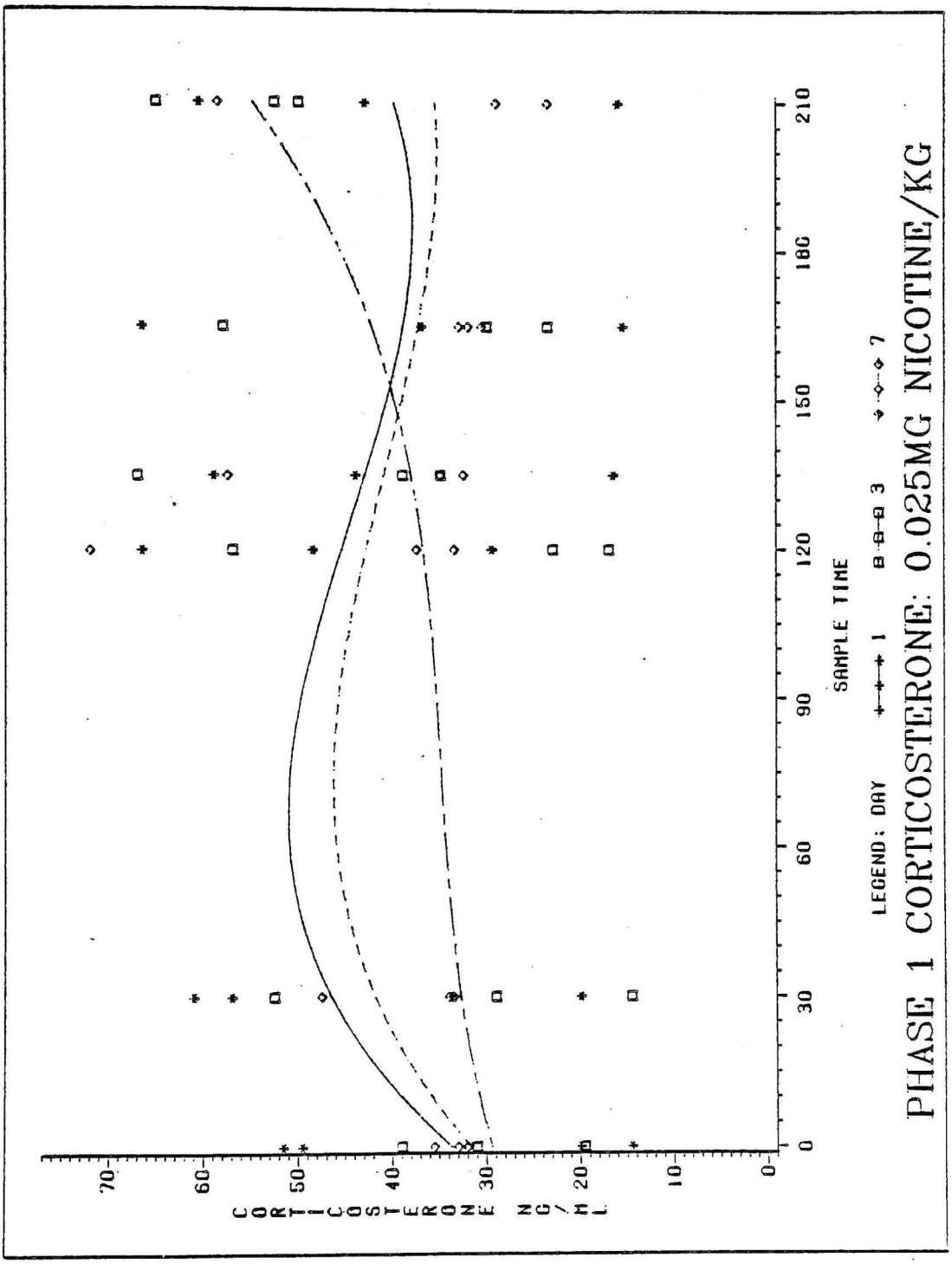
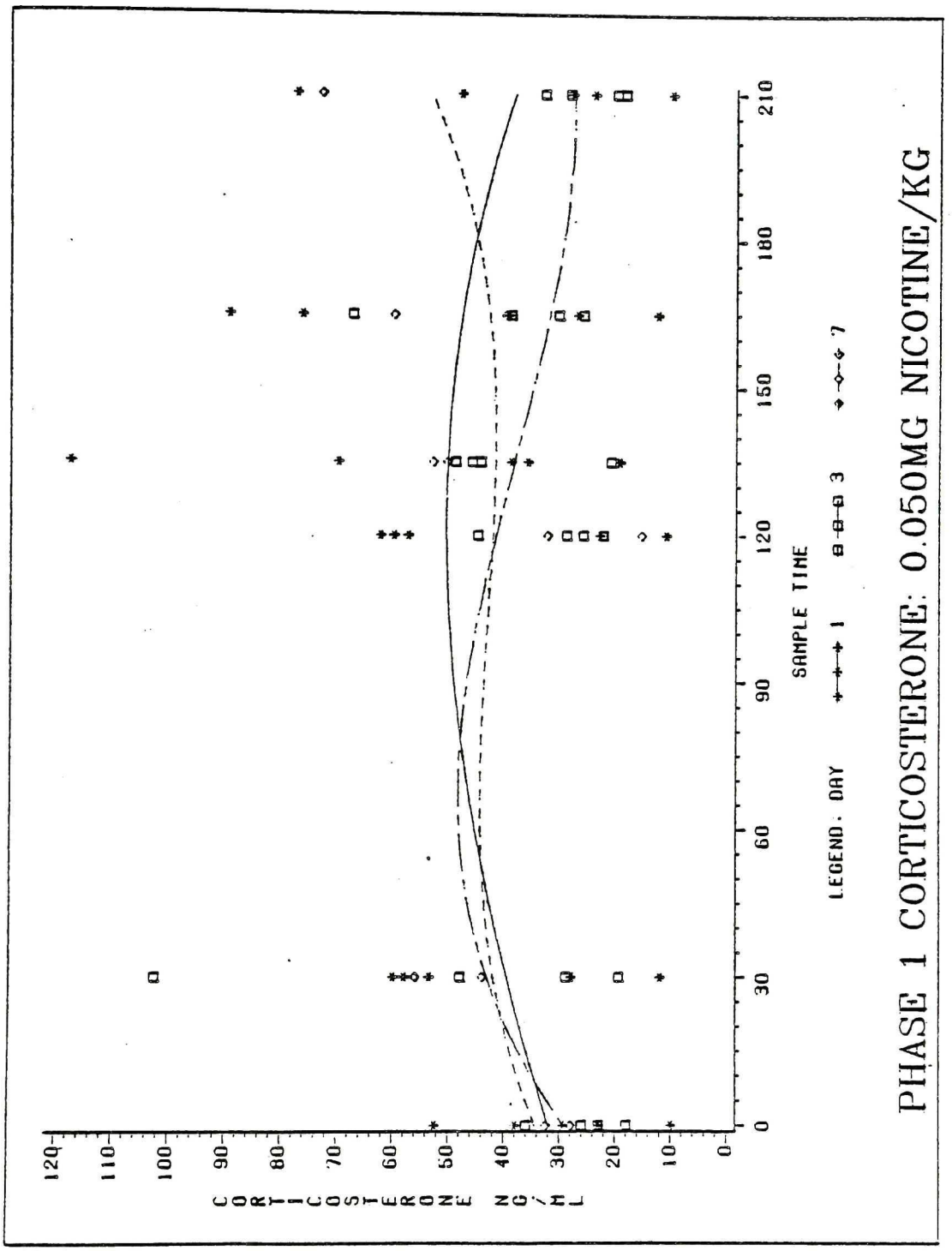

PHASE 1 CORTICOSTERONE: 0.025MG NICOTINE/KG
CORTICOSTERONE NG/ML
0
10
20
30
40
50
60
70
0
30
60
90
120
150
180
210
SAMPLE TIME
LEGEND: DAY 1 3 7

FIGURE 18

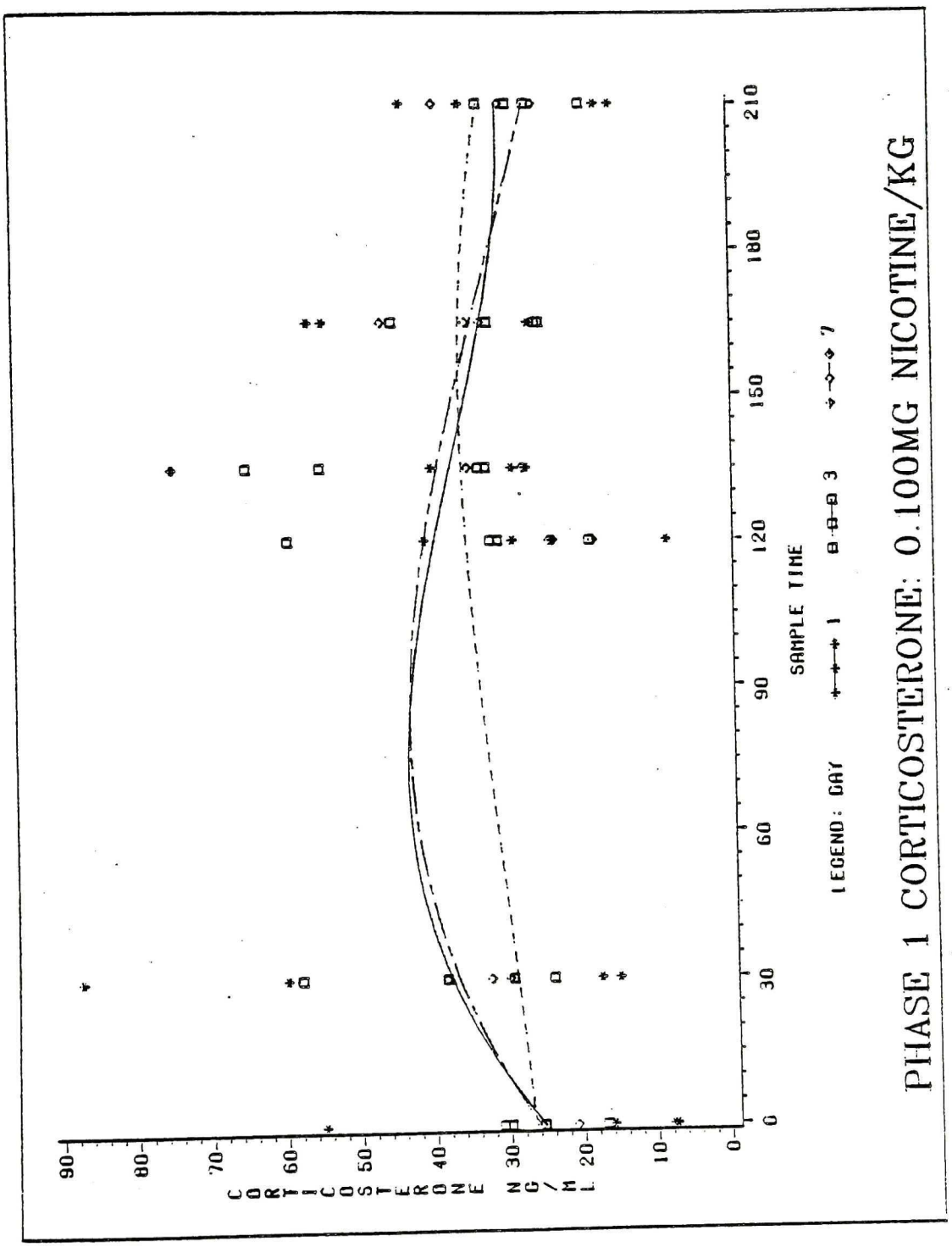
PHASE I: CORTICOSTERONE RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION



PHASE 1 CORTICOSTERONE: 0.050MG NICOTINE/KG

FIGURE 19

PHASE I: CORTICOSTERONE RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION



PHASE 1 CORTICOSTERONE: 0.100MG NICOTINE/KG

FIGURE 20

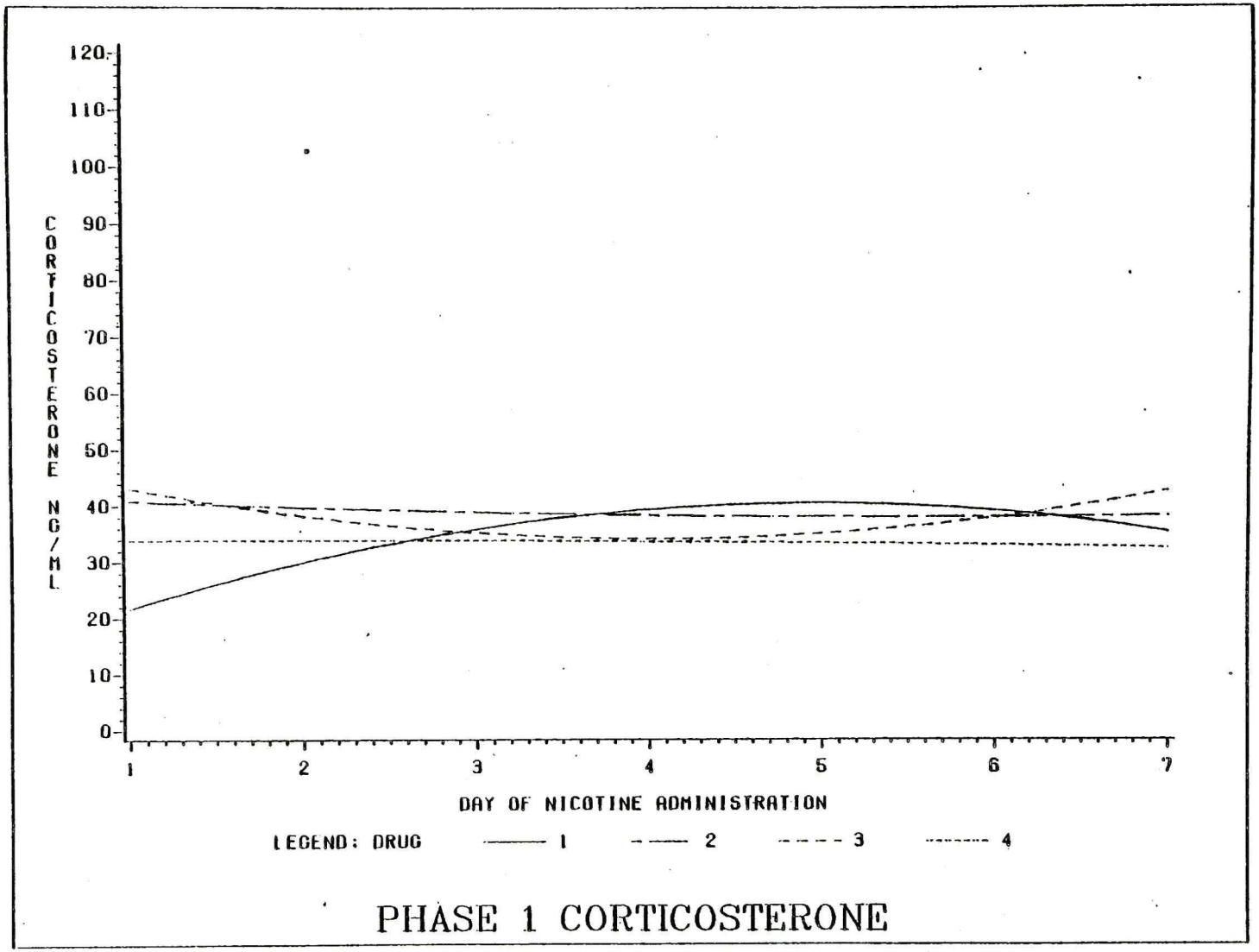
LEGEND

DRUG CONDITION 1 = SALINE CONTROL

DRUG CONDITION 2 = 0.025 mg NICOTINE/kg

DRUG CONDITION 3 = 0.050 mg NICOTINE/kg

DRUG CONDITION 4 = 0.100 mg NICOTINE/kg



PHASE 1 CORTICOSTERONE

D150

FIGURE 21

PHASE I: GLUCOSE RESPONSES FOR SALINE CONTROL CONDITION

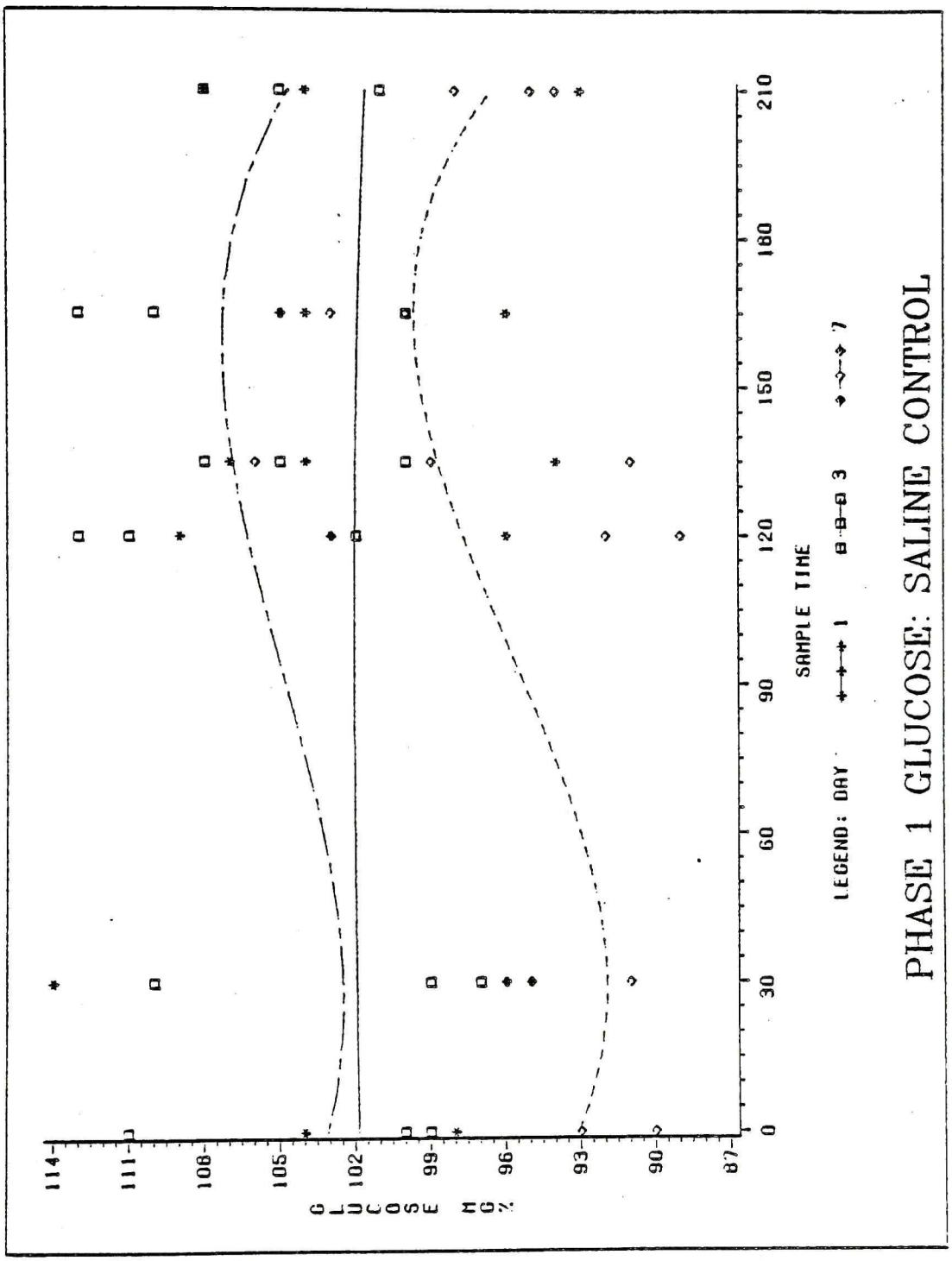

PHASE 1 GLUCOSE: SALINE CONTROL
LEGEND: DAY 1 3 7
SAMPLE TIME
0
30
60
90
120
150
180
210
GLUCOSE MG%
114
111
108
105
102
99
96
93
90
87

FIGURE 22

PHASE I: GLUCOSE RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION

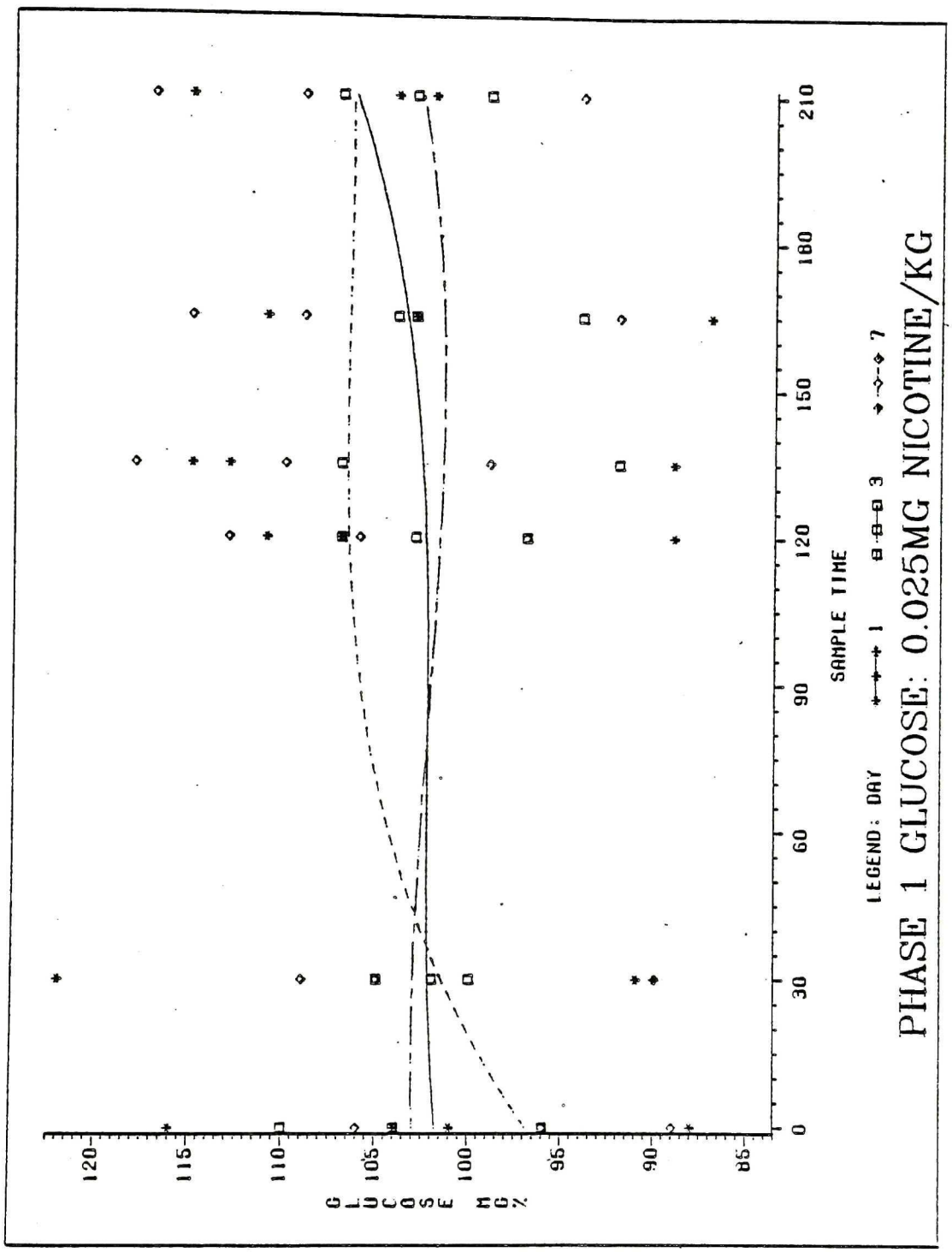
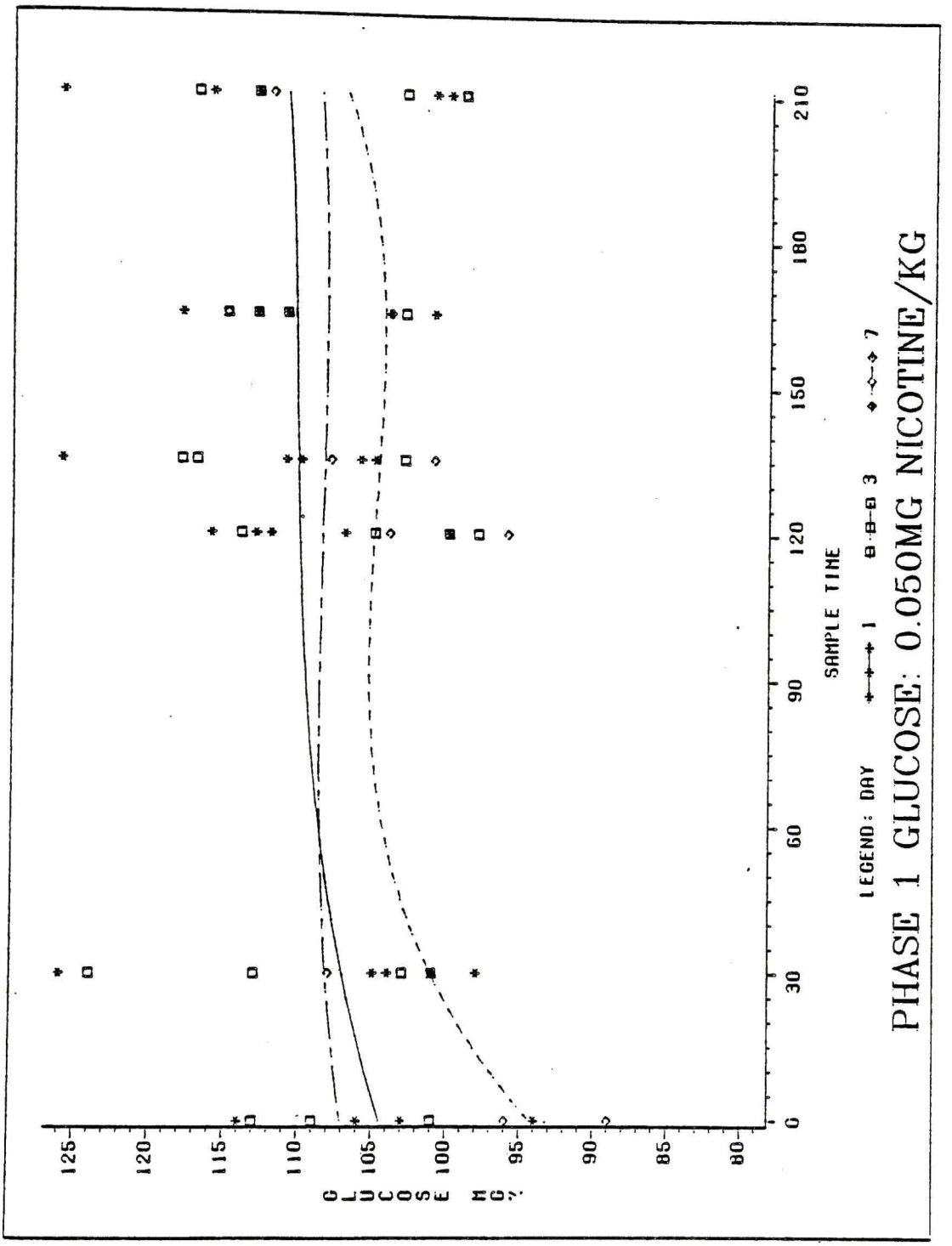

PHASE 1 GLUCOSE: 0.025MG NICOTINE/KG
LEGEND: DAY 1 3 7
SAMPLE TIME
0
30
60
90
120
150
180
210
GLUCOSE MG%
120
115
110
105
100
95
90
85

FIGURE 23

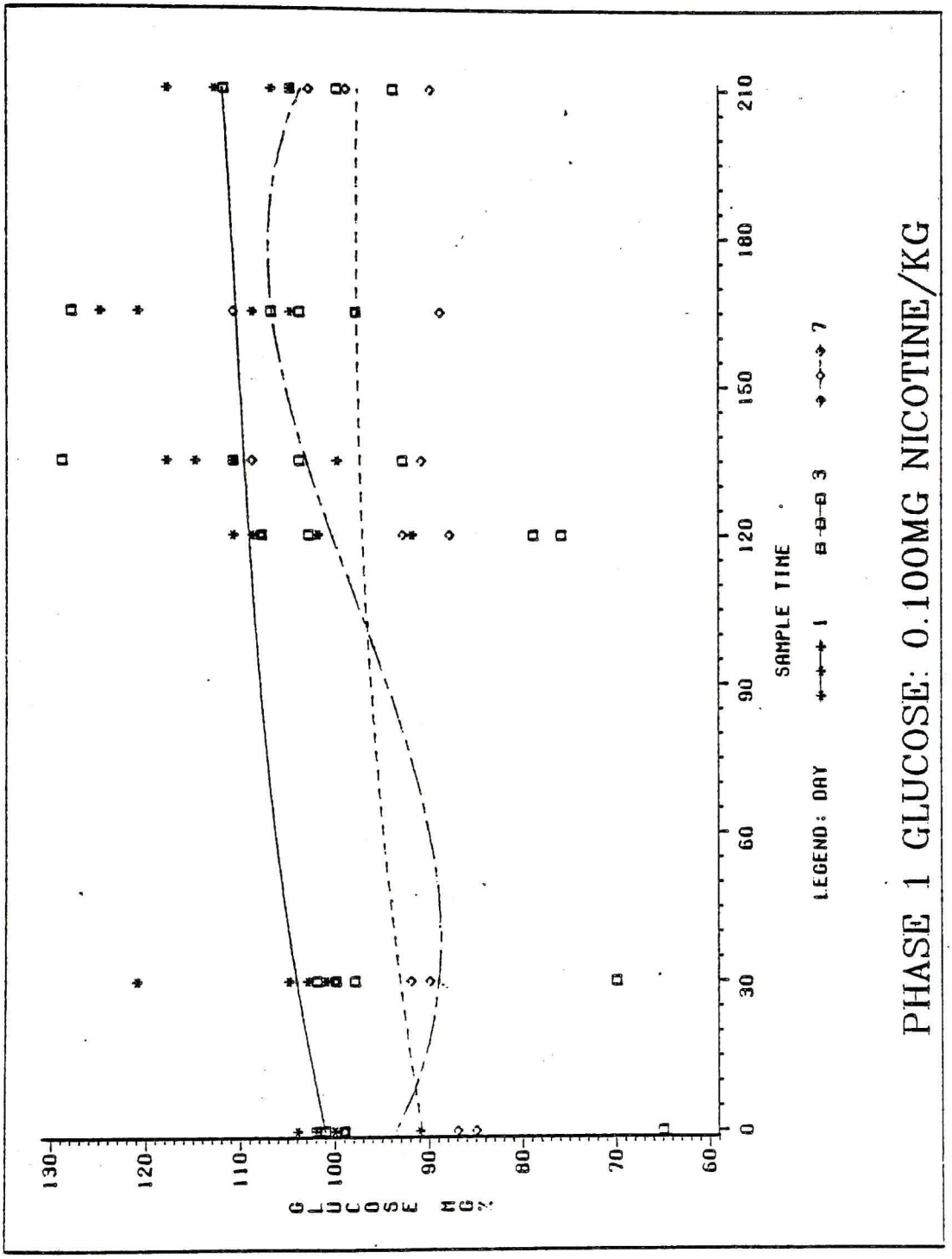
PHASE I: GLUCOSE RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION



PHASE 1 GLUCOSE: 0.050MG NICOTINE/KG

FIGURE 24

PHASE I: GLUCOSE RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION



PHASE 1 GLUCOSE: 0.100MG NICOTINE/KG

FIGURE 25

LEGEND

DRUG CONDITION 1 = SALINE CONTROL

DRUG CONDITION 2 = 0.025 mg NICOTINE/kg

DRUG CONDITION 3 = 0.050 mg NICOTINE/kg

DRUG CONDITION 4 = 0.100 mg NICOTINE/kg

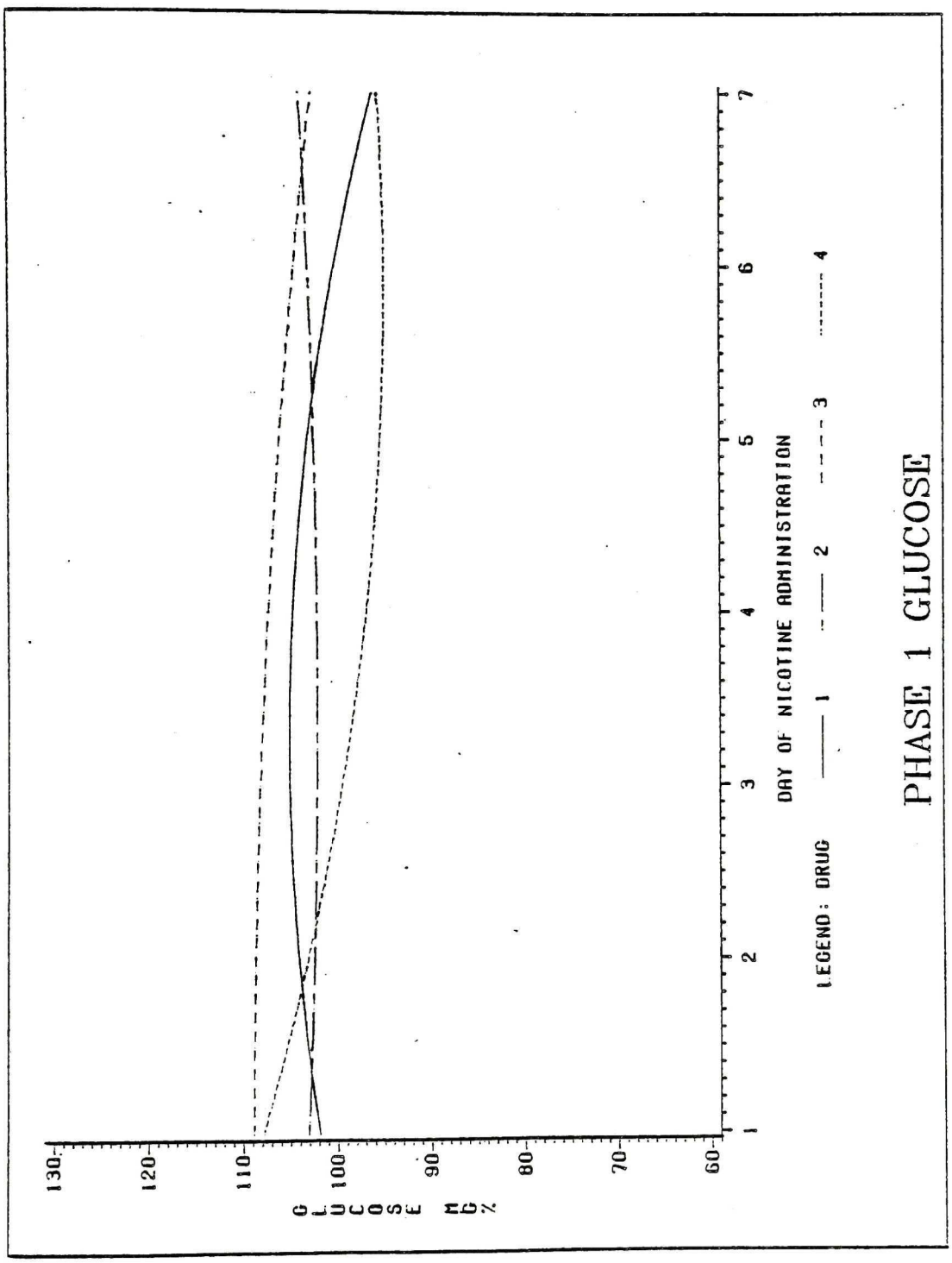
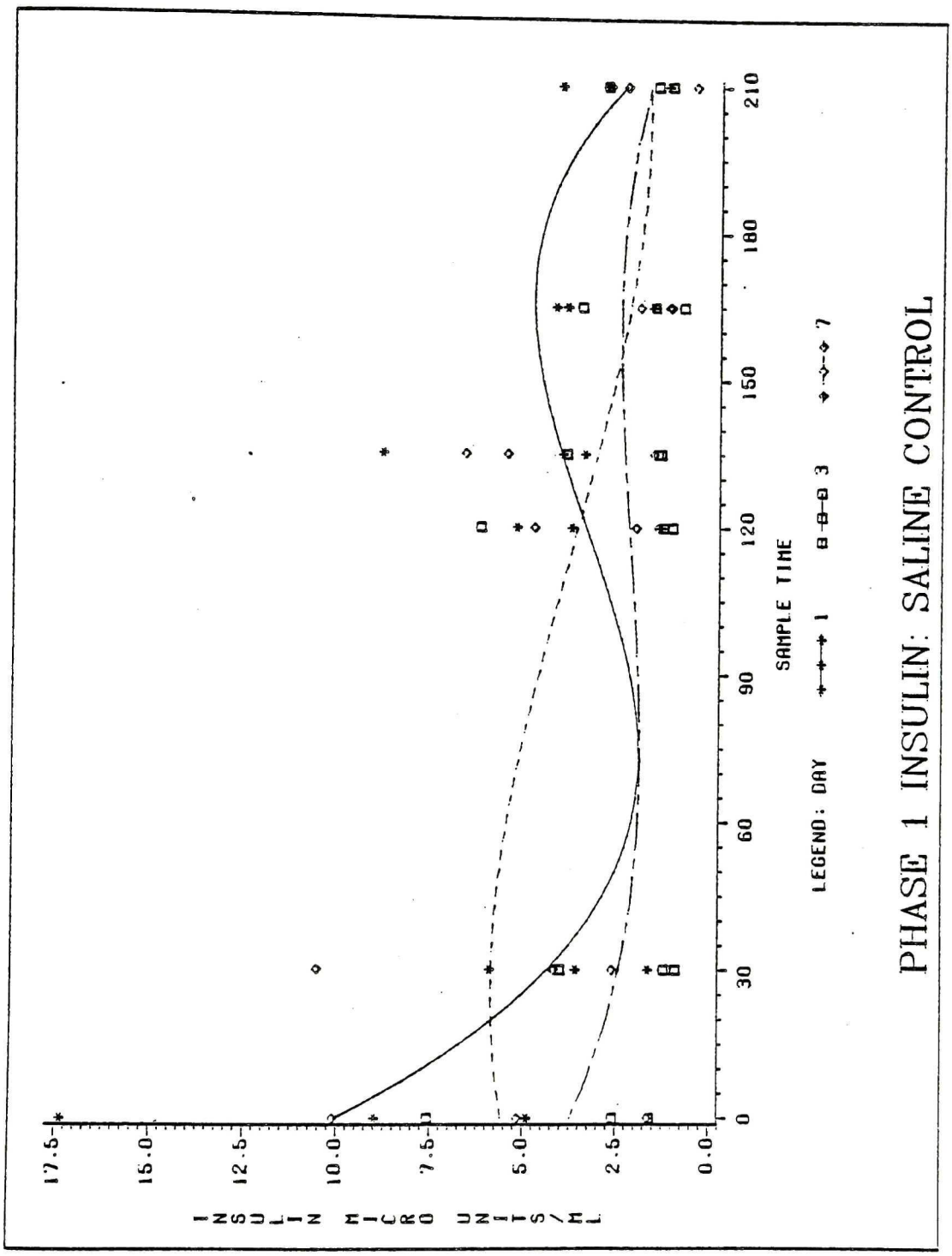

PHASE 1 GLUCOSE
GLUCOSE MG%
130
120
110
100
90
80
70
60
1
2
3
4
5
6
7
DAY OF NICOTINE ADMINISTRATION
LEGEND: DRUG
1
2
3
4

FIGURE 26

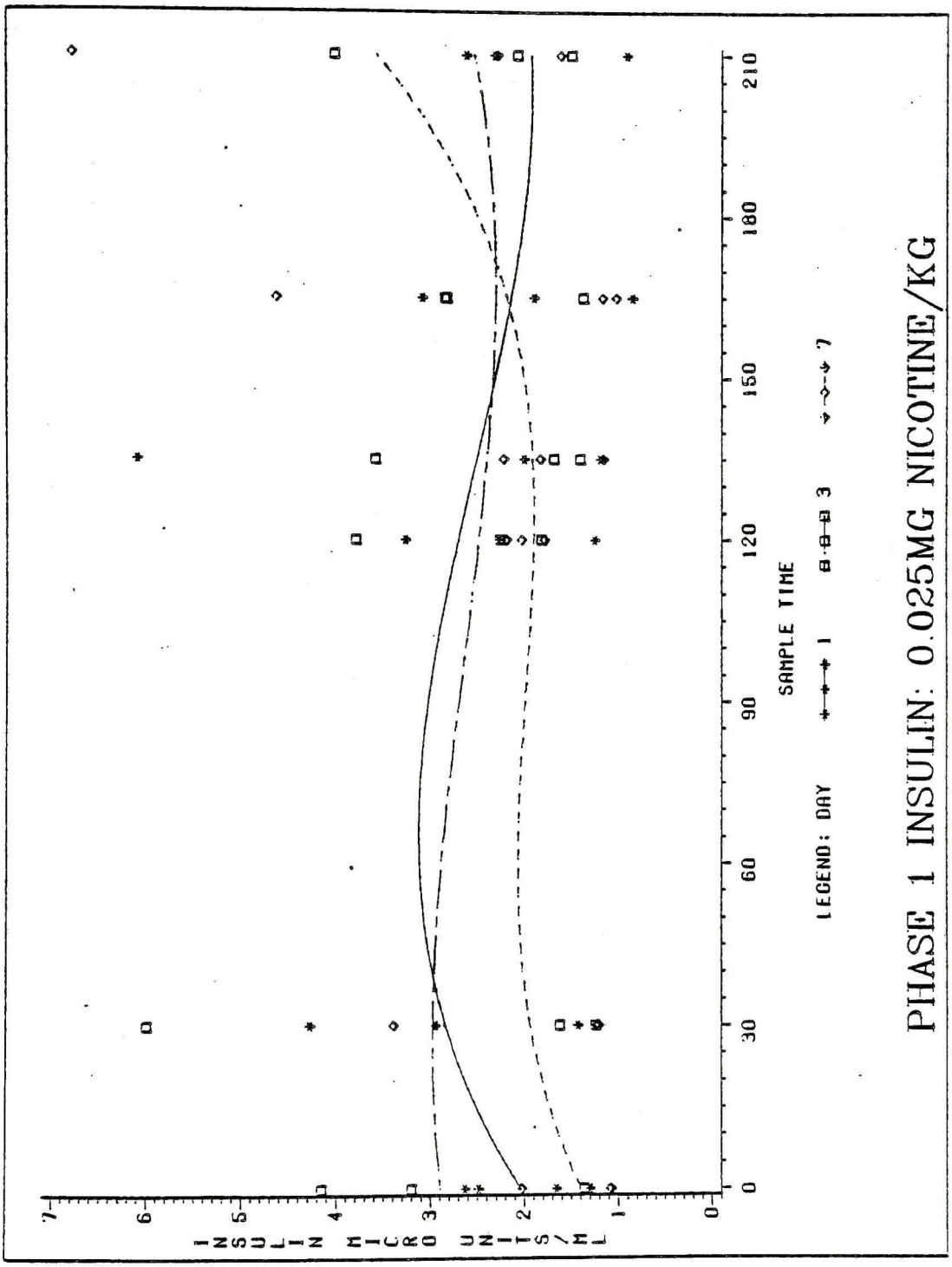
PHASE I: INSULIN RESPONSES FOR SALINE CONTROL CONDITION



PHASE 1 INSULIN: SALINE CONTROL

FIGURE 27

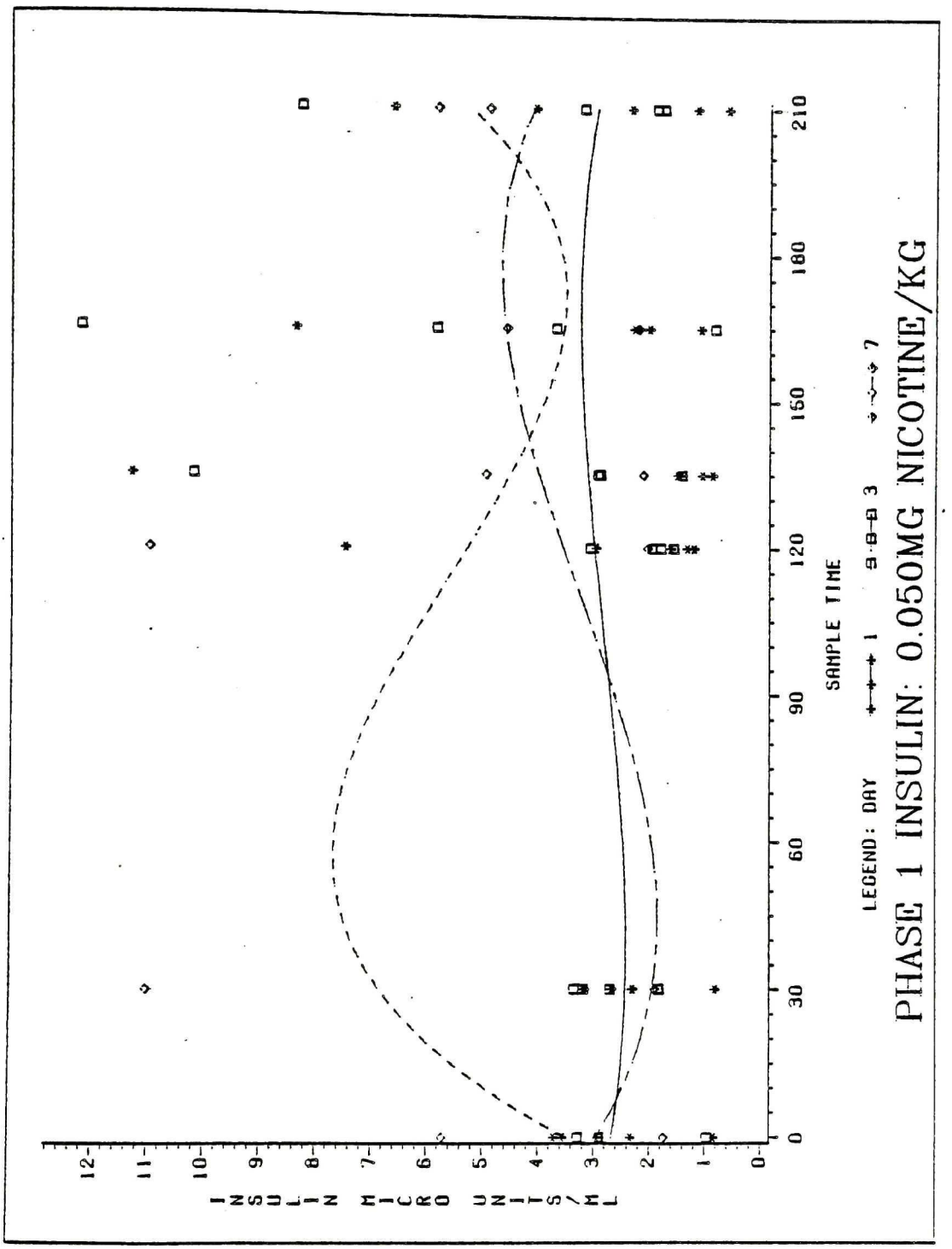
PHASE I: INSULIN RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION



PHASE 1 INSULIN: 0.025MG NICOTINE/KG

FIGURE 28

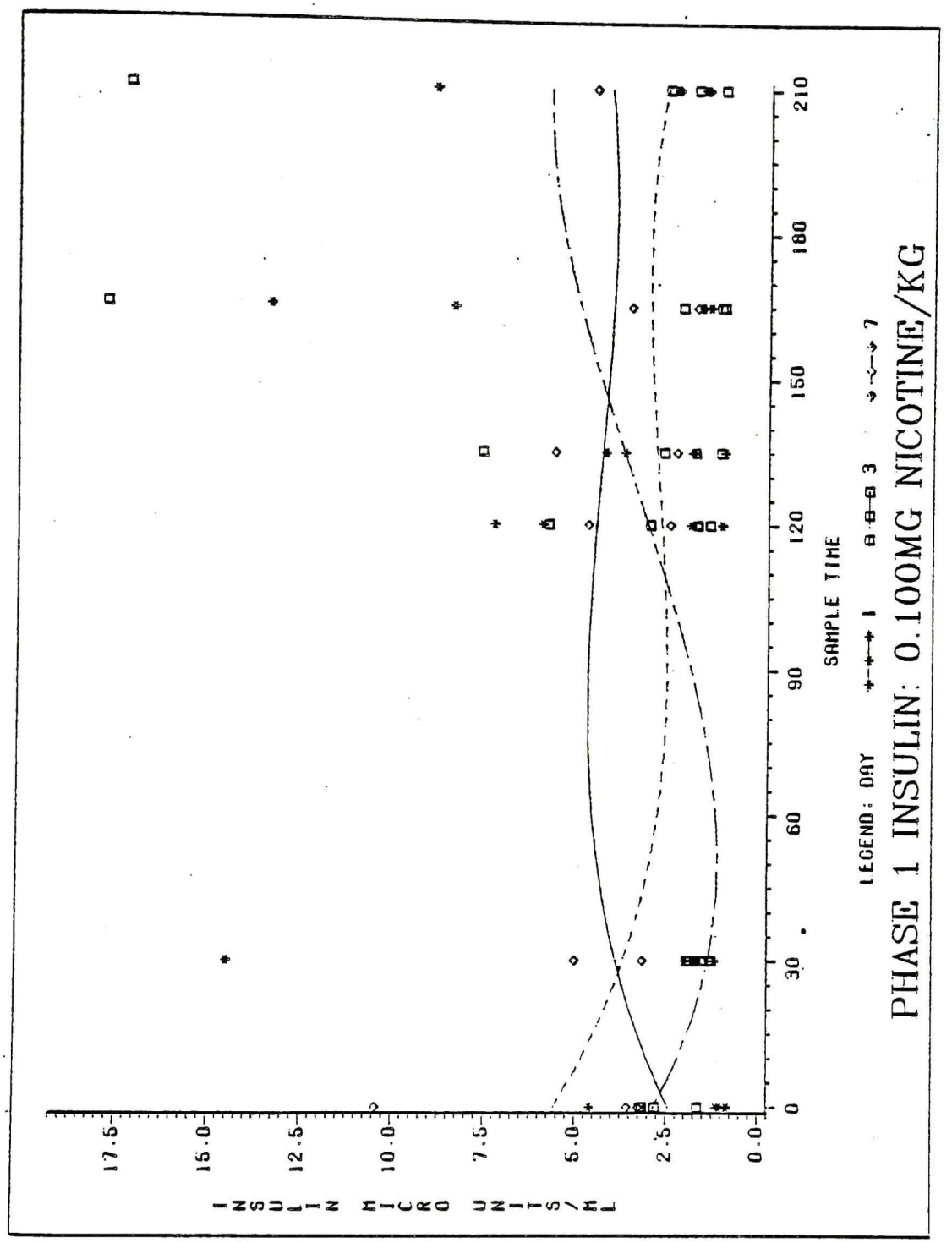
PHASE I: INSULIN RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION



PHASE 1 INSULIN: 0.050MG NICOTINE/KG

FIGURE 29

PHASE I: INSULIN RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION


PHASE 1 INSULIN: 0.100MG NICOTINE/KG
INSULIN MICRO UNITS/ML
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
0
30
60
90
120
150
180
210
SAMPLE TIME
LEGEND: DAY 1 3 7

LIBRARY Use

FIGURE 30

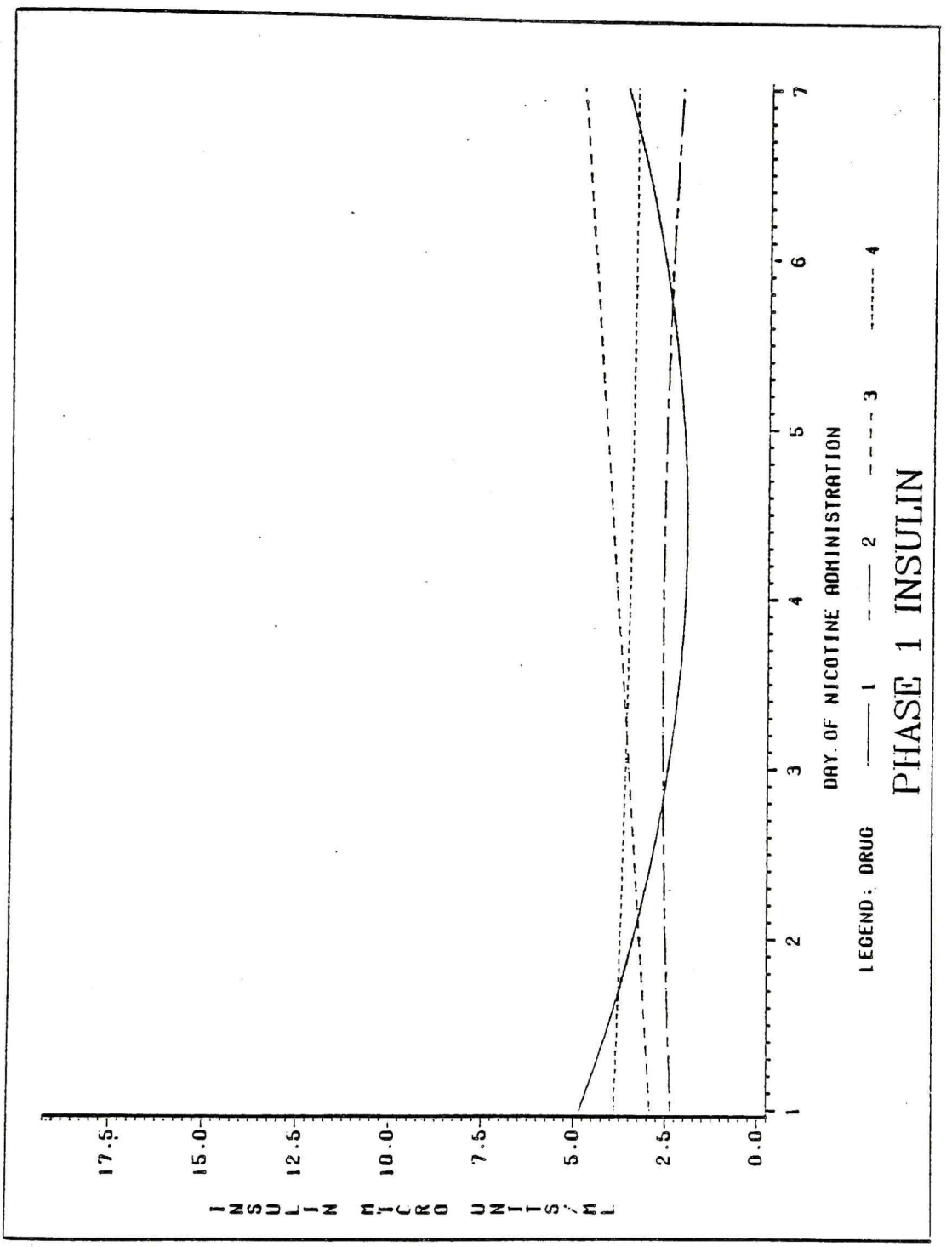
LEGEND

DRUG CONDITION 1 = SALINE CONTROL

DRUG CONDITION 2 = 0.025 mg NICOTINE/kg

DRUG CONDITION 3 = 0.050 mg NICOTINE/kg

DRUG CONDITION 4 = 0.100 mg NICOTINE/kg


PHASE 1 INSULIN
INSULIN MICRO UNITS/ML
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
1
2
3
4
5
6
7
DAY OF NICOTINE ADMINISTRATION
LEGEND: DRUG
1
2
3
4

LIBRARY

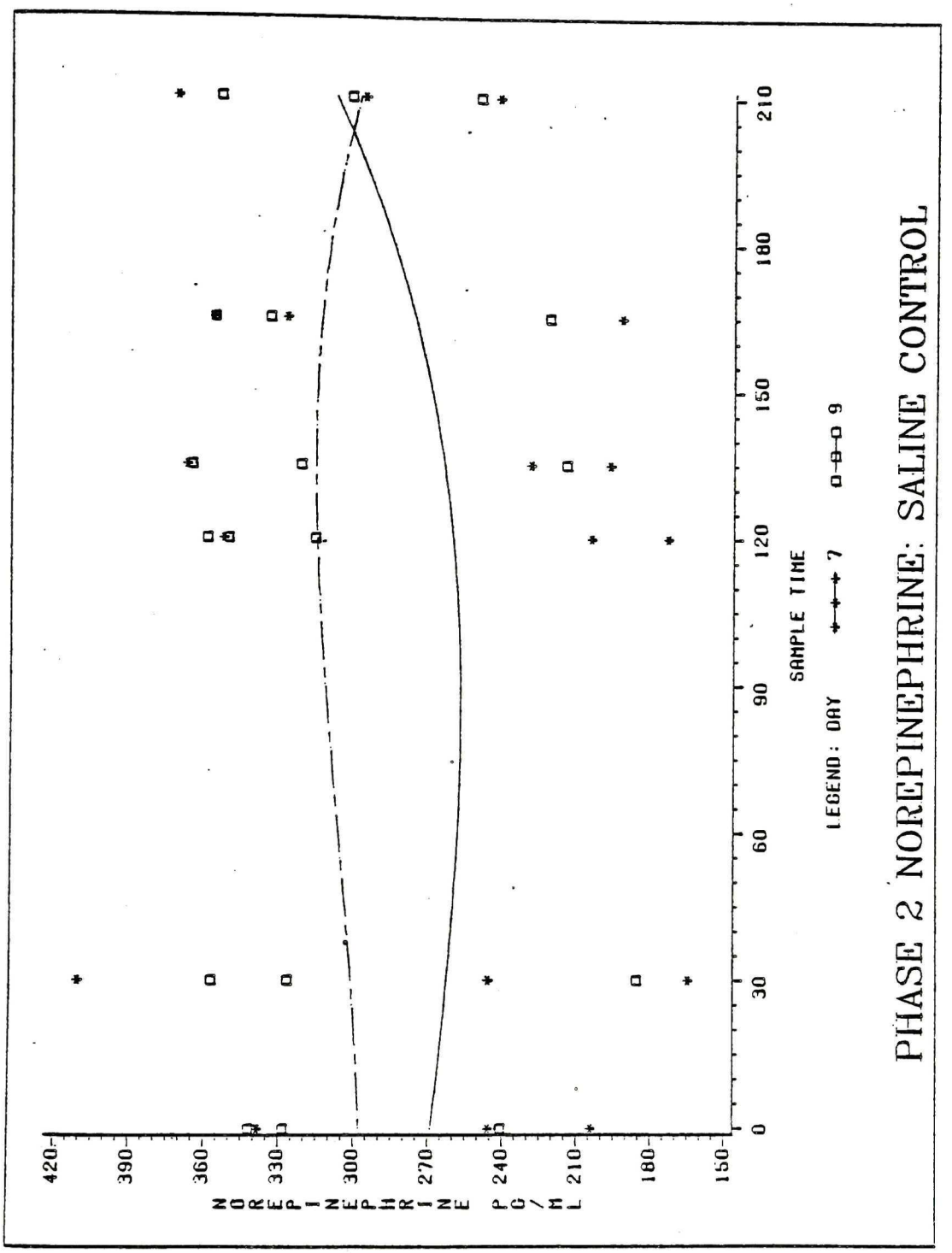
FIGURE 31

PHASE II: NOREPINEPHRINE RESPONSES FOR SALINE CONTROL CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 NOREPINEPHRINE: SALINE CONTROL

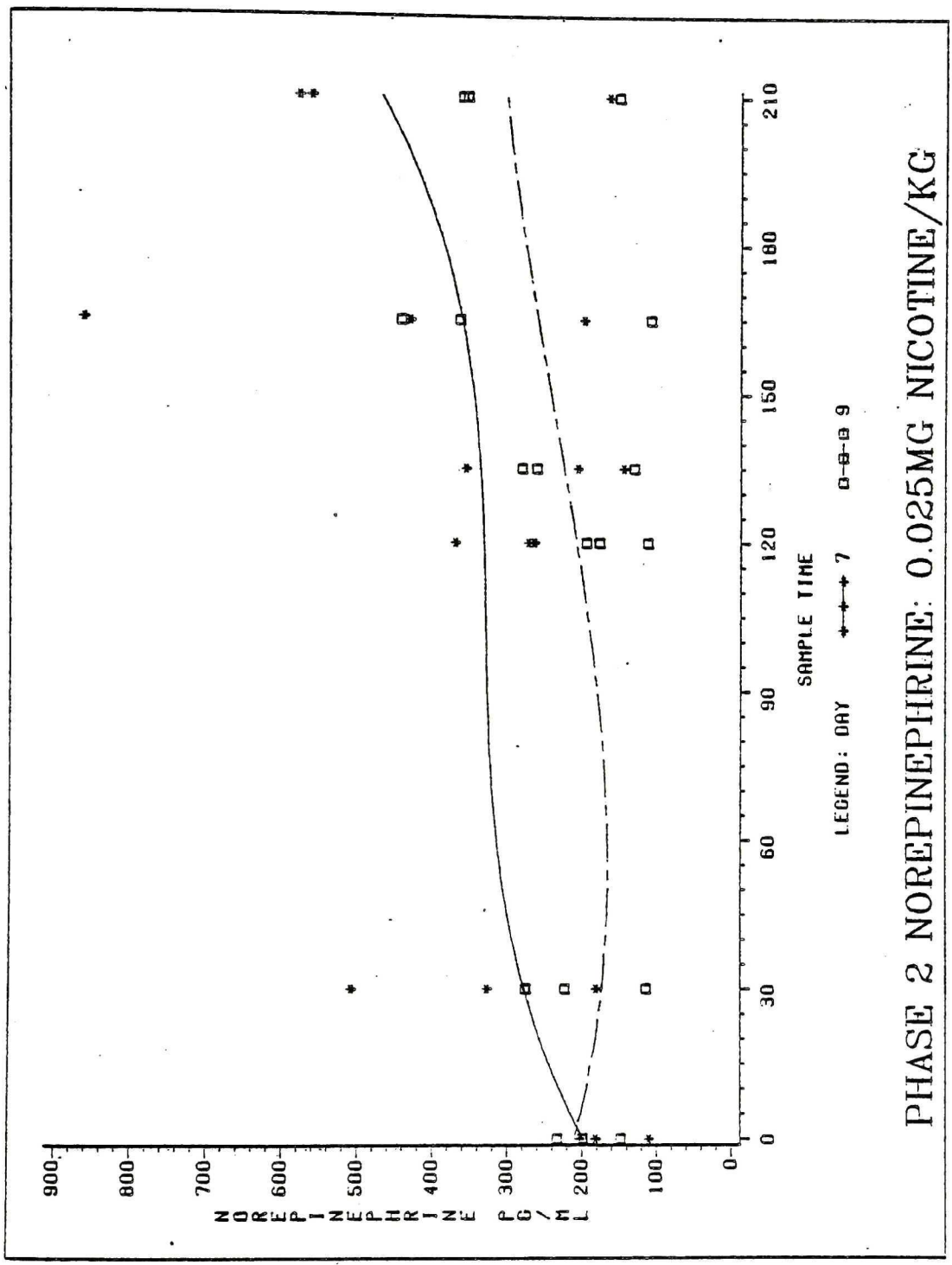
FIGURE 32

PHASE II: NOREPINEPHRINE RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES


PHASE 2 NOREPINEPHRINE: 0.025MG NICOTINE/KG
NOREPINEPHRINE PG/ML
900
800
700
600
500
400
300
200
100
0
0
30
60
90
120
150
180
210
SAMPLE TIME
LEGEND: DAY 7 9

LIBRARY USE

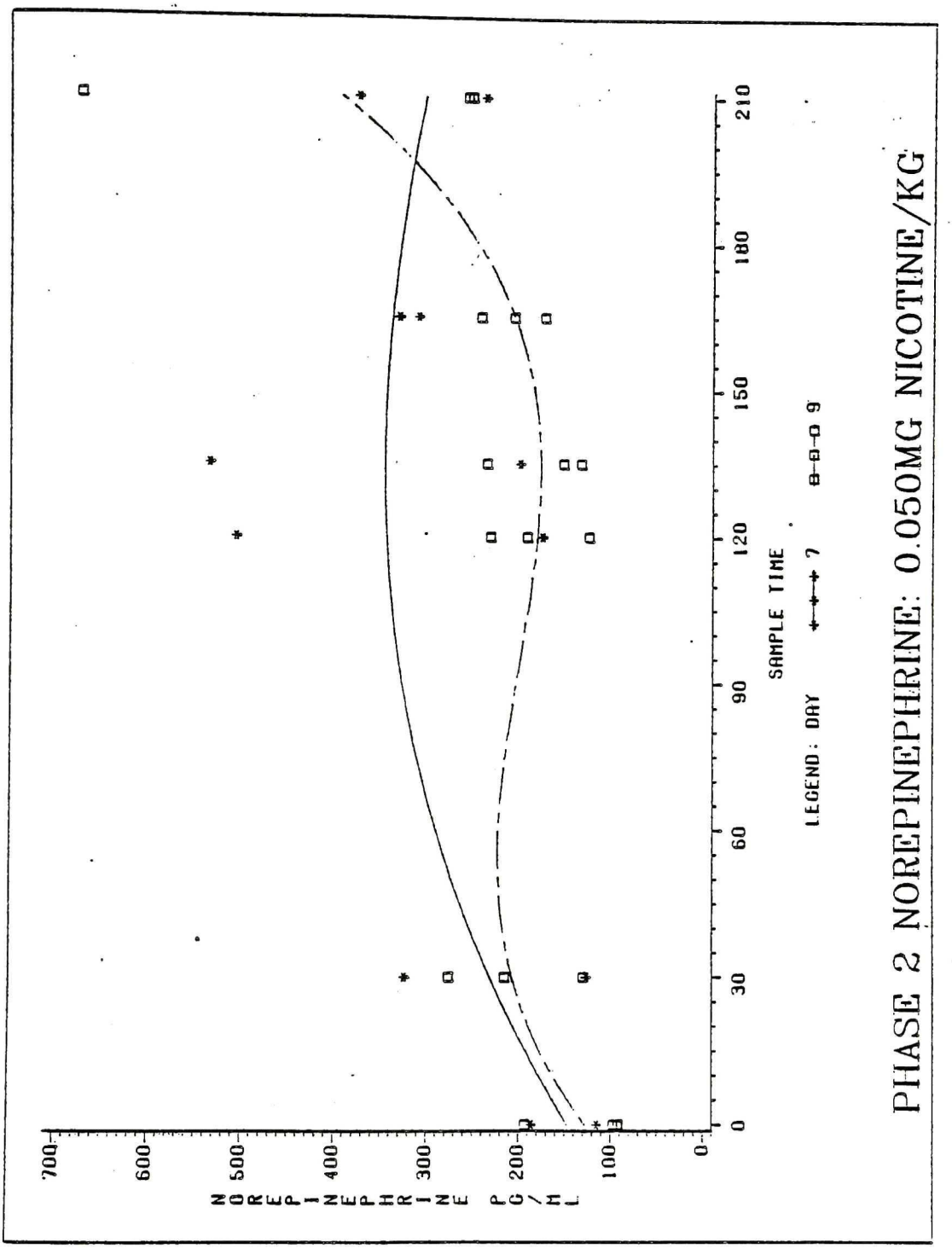
FIGURE 33

PHASE II: NOREPINEPHRINE RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 NOREPINEPHRINE: 0.050MG NICOTINE/KG

LIBRARY

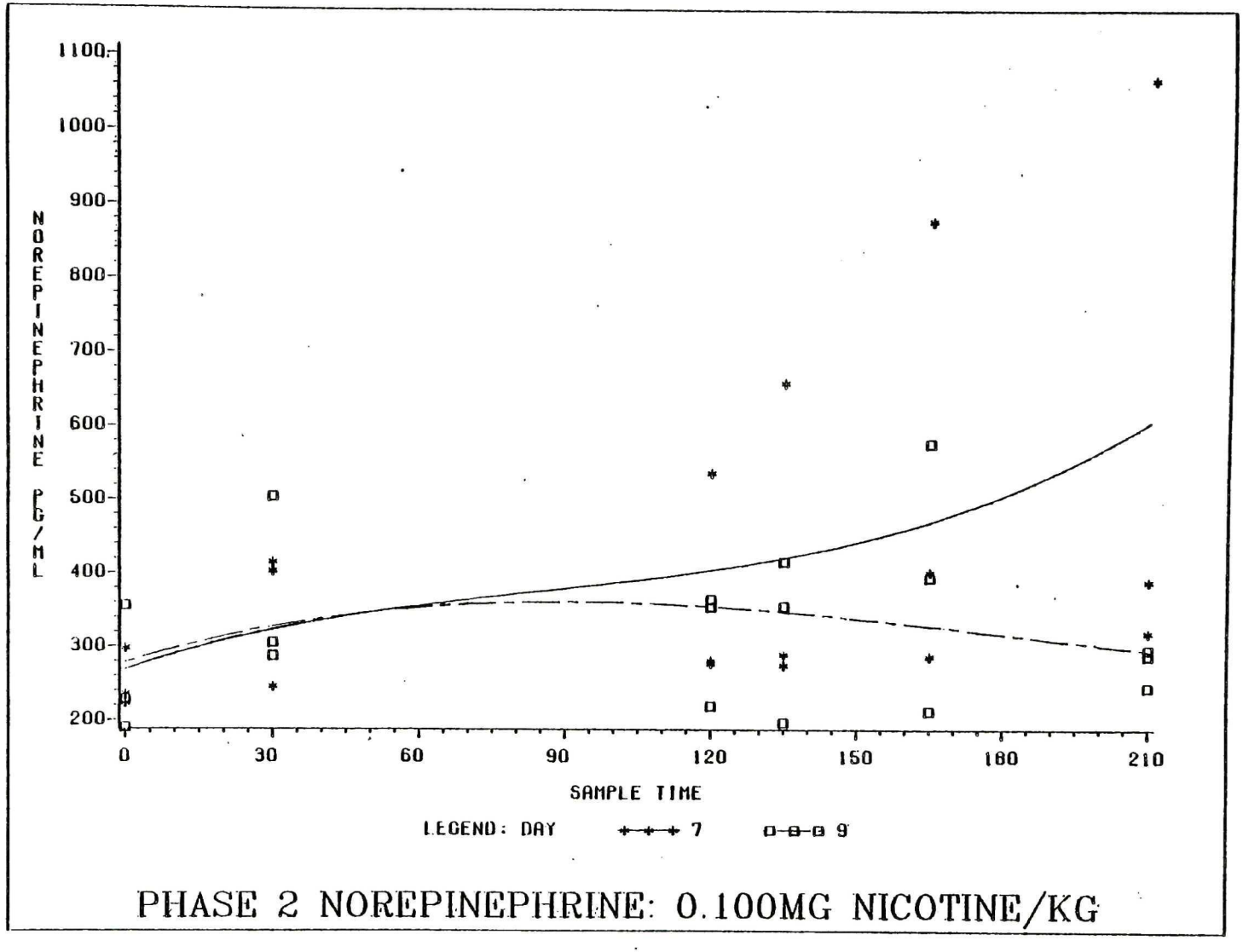
FIGURE 34

PHASE II: NOREPINEPHRINE RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 NOREPINEPHRINE: 0.100MG NICOTINE/KG

LIBRARY USE

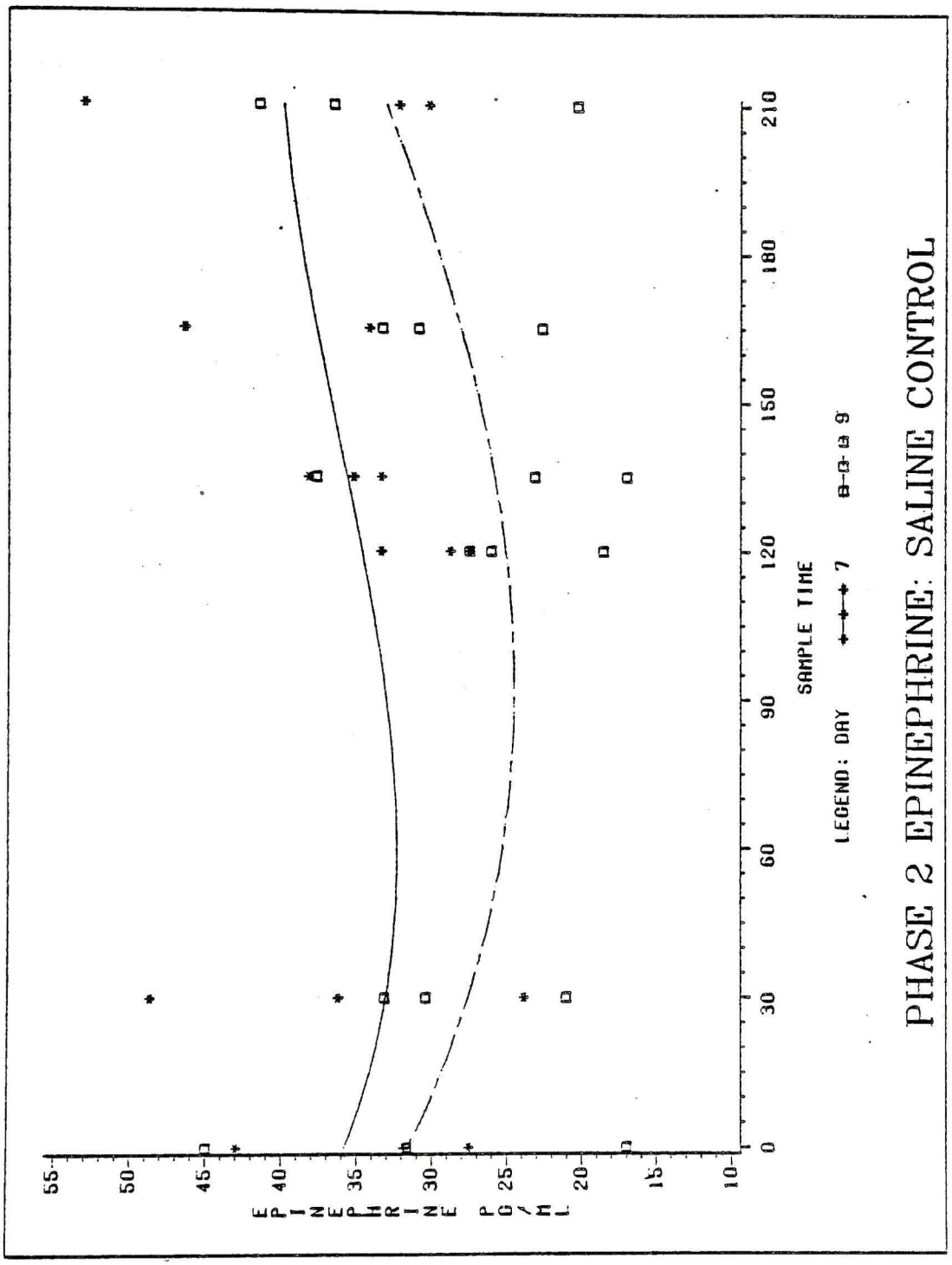
FIGURE 35

PHASE II: EPINEPHRINE RESPONSES FOR SALINE CONTROL CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES


PHASE 2 EPINEPHRINE: SALINE CONTROL
EPINEPHRINE PG/ML
55
50
45
40
35
30
25
20
15
10
0
30
60
90
120
150
180
210
SAMPLE TIME
LEGEND: DAY 7

LIBRARY U

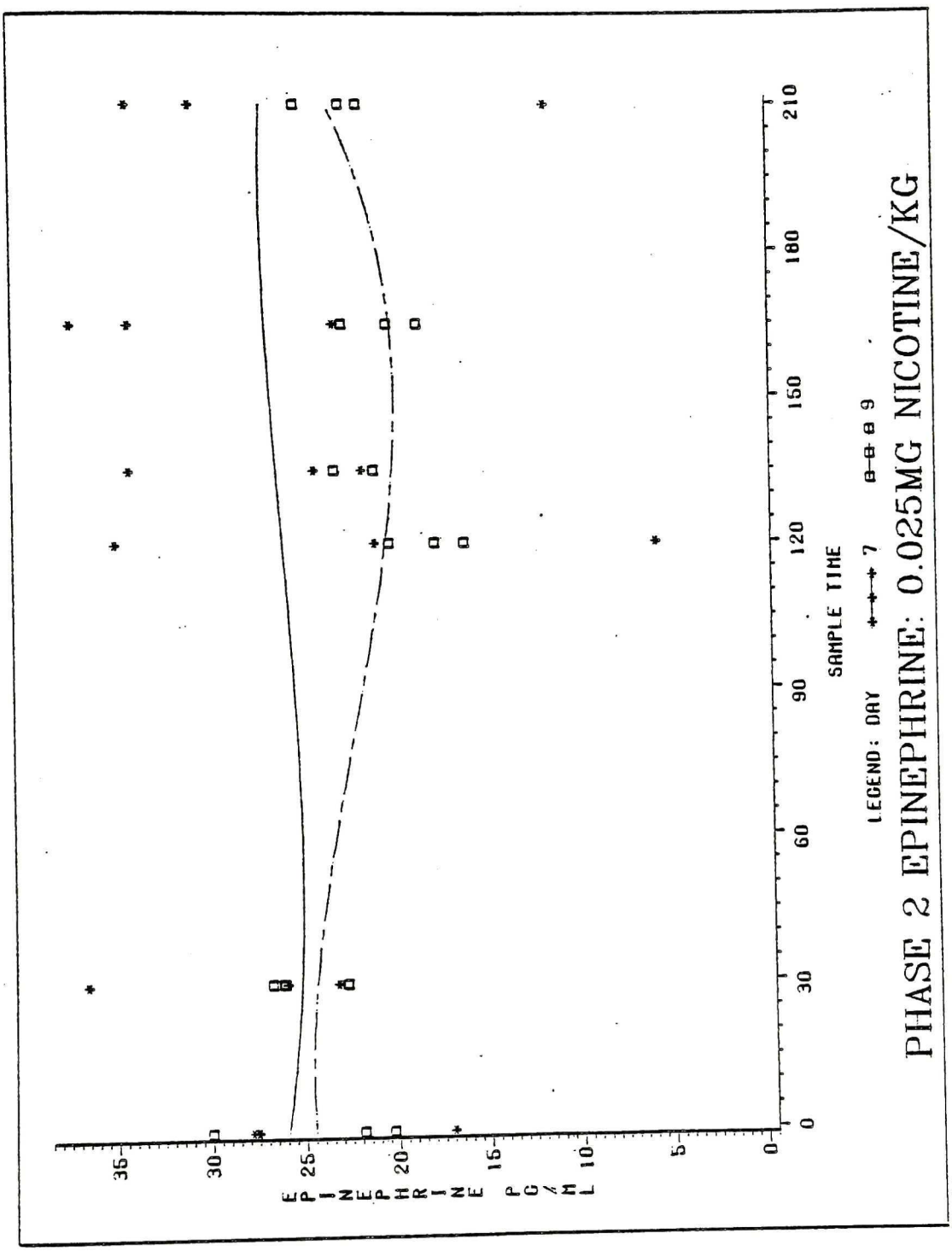
FIGURE 36

PHASE II: EPINEPHRINE RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 EPINEPHRINE: 0.025MG NICOTINE/KG

LIBRARY

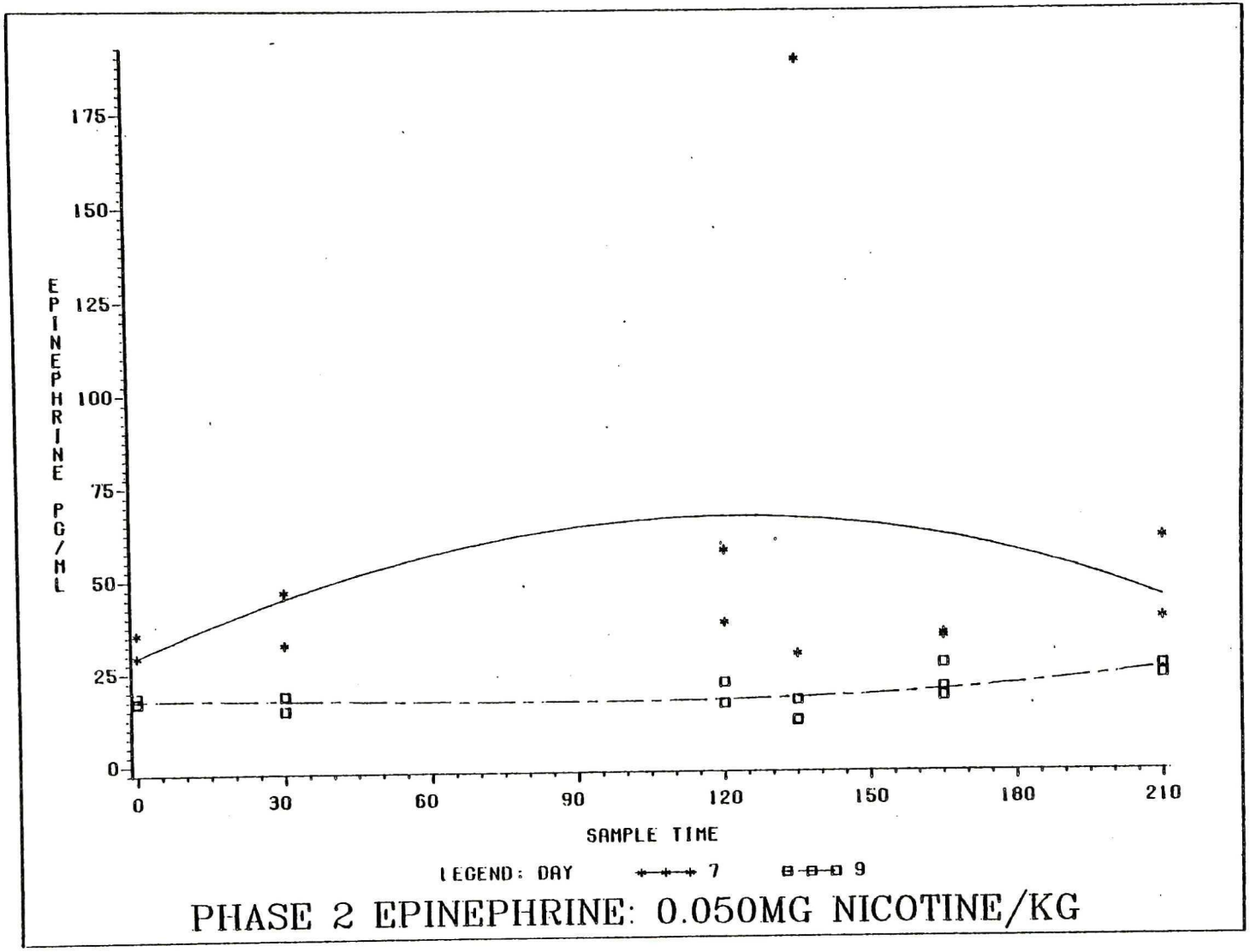
FIGURE 37

PHASE II: EPINEPHRINE RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 EPINEPHRINE: 0.050MG NICOTINE/KG

LIBRARY Use

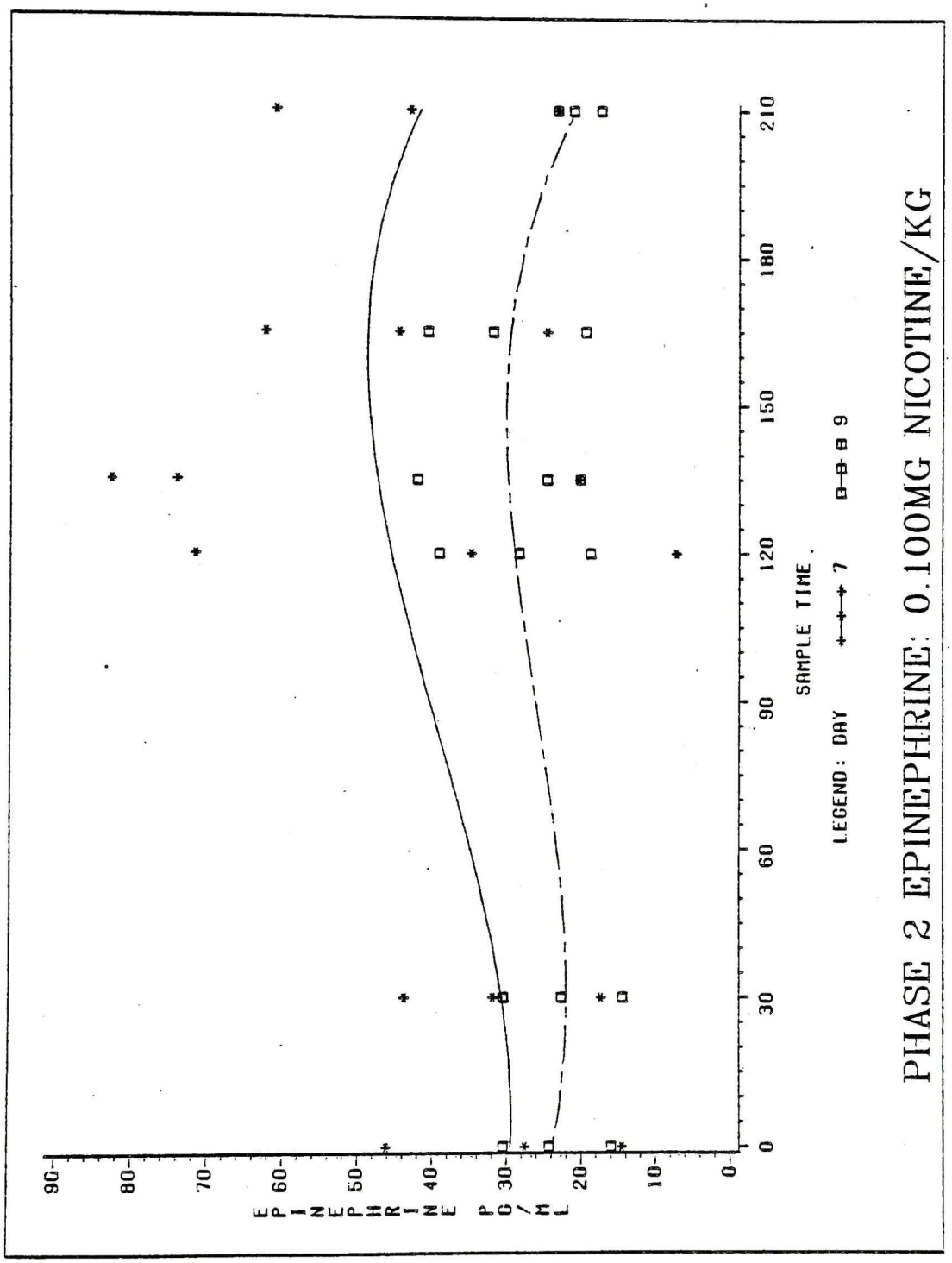
FIGURE 38

PHASE II: EPINEPHRINE RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 EPINEPHRINE: 0.100MG NICOTINE/KG

LIBRARY

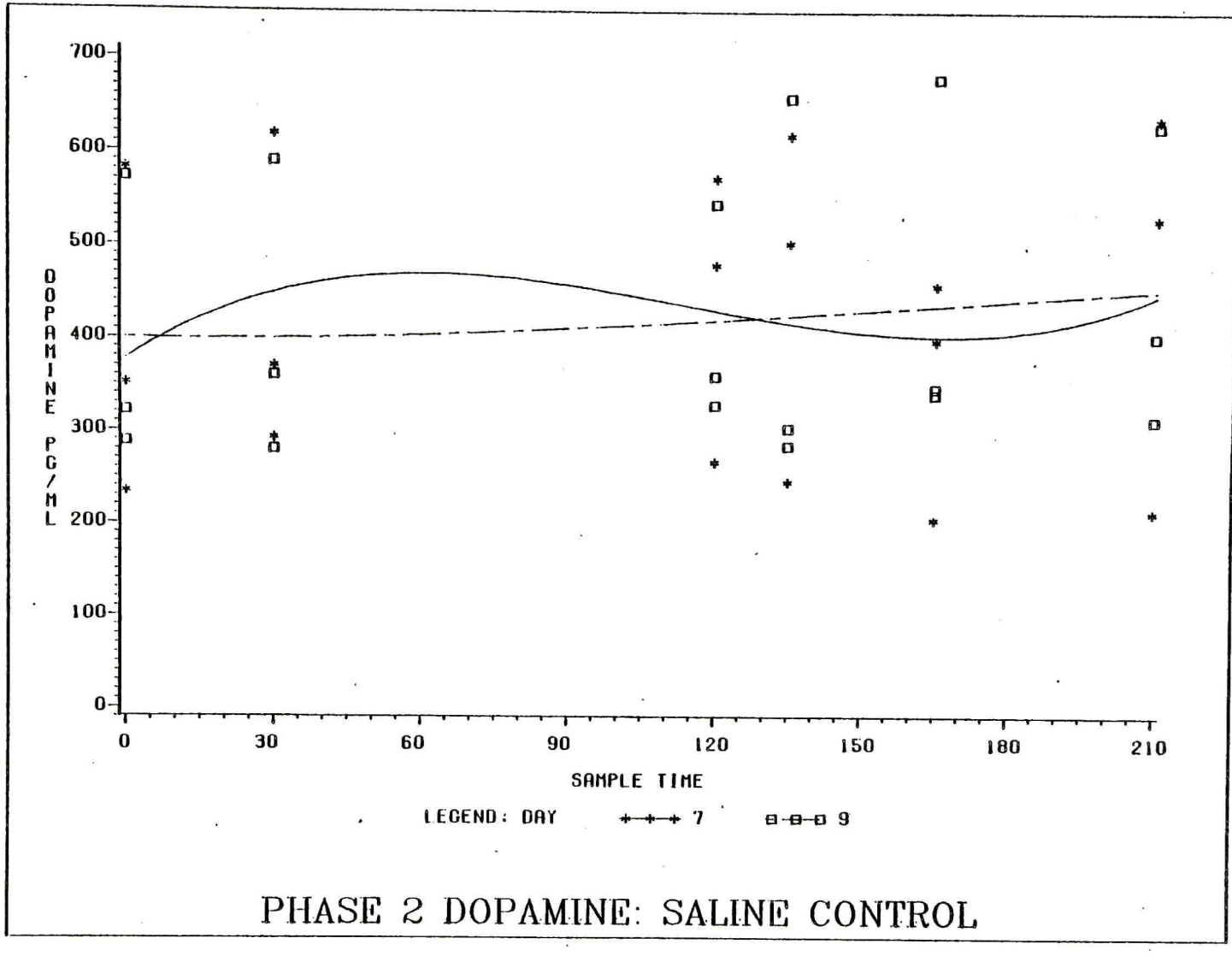
FIGURE 39

PHASE II: DOPAMINE RESPONSES FOR SALINE CONTROL CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 DOPAMINE: SALINE CONTROL

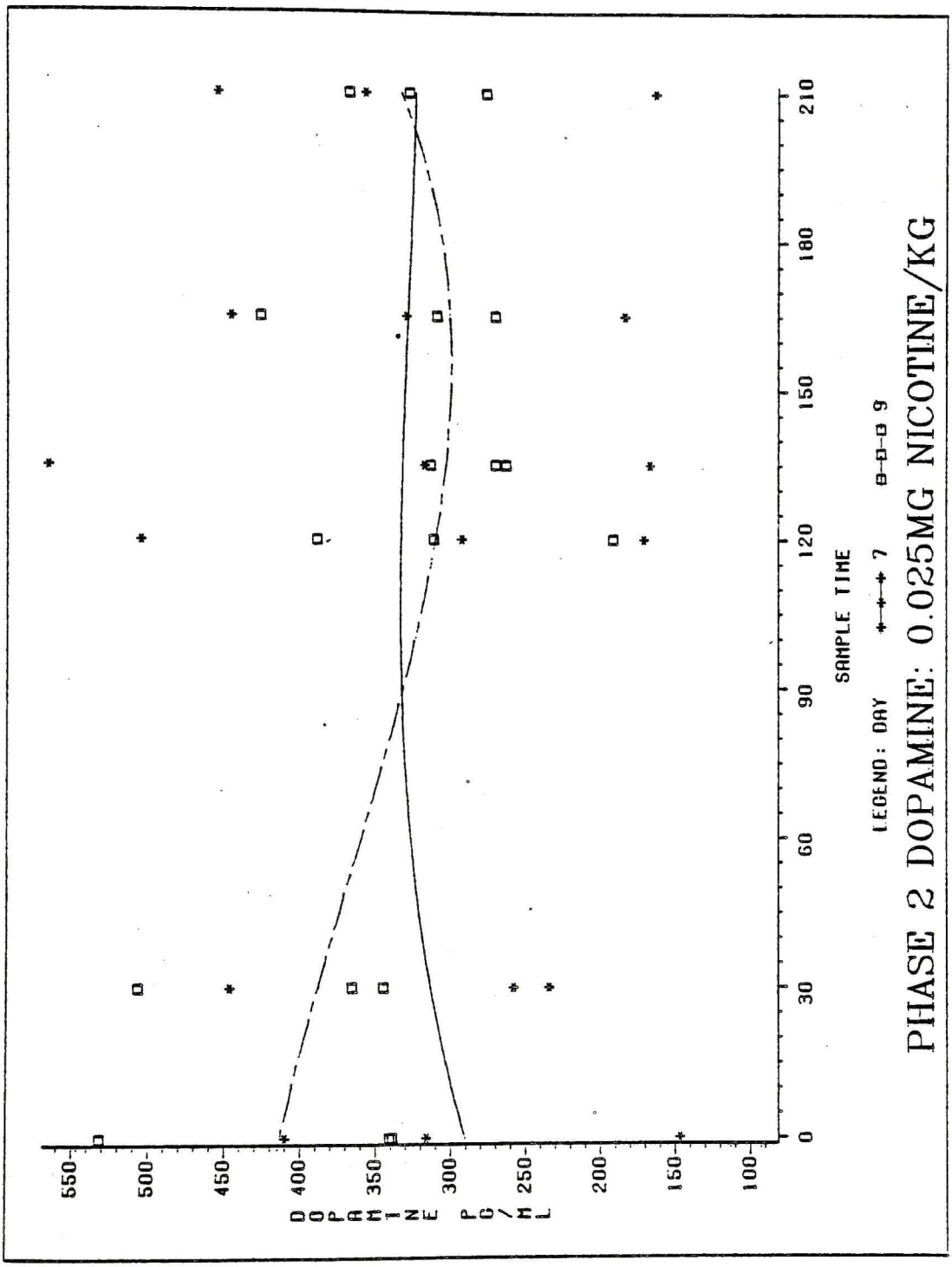
FIGURE 40

PHASE II: DOPAMINE RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 DOPAMINE: 0.025MG NICOTINE/KG

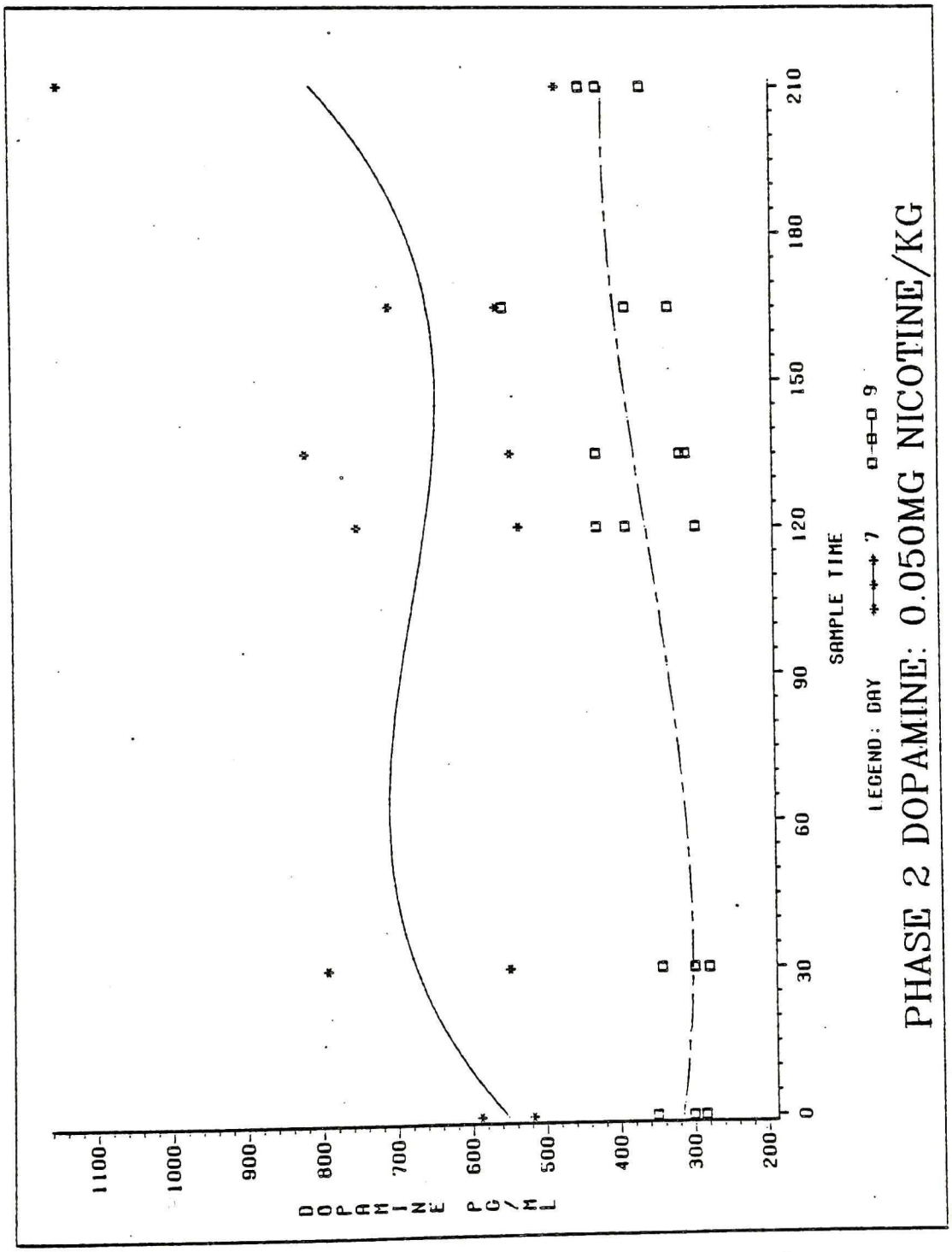
FIGURE 41

PHASE II: DOPAMINE RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 DOPAMINE: 0.050MG NICOTINE/KG

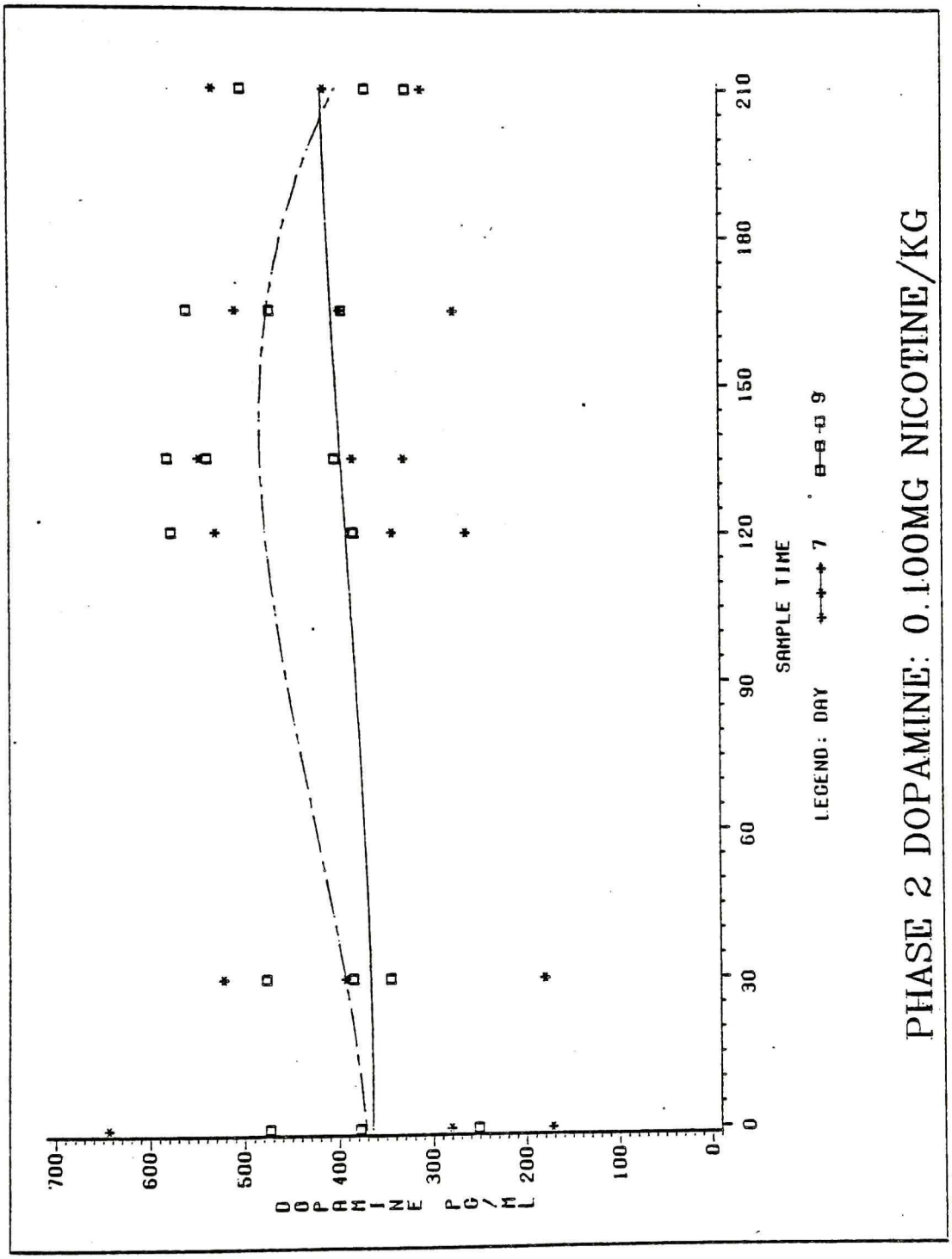
FIGURE 42

PHASE II: DOPAMINE RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 DOPAMINE: 0.100MG NICOTINE/KG

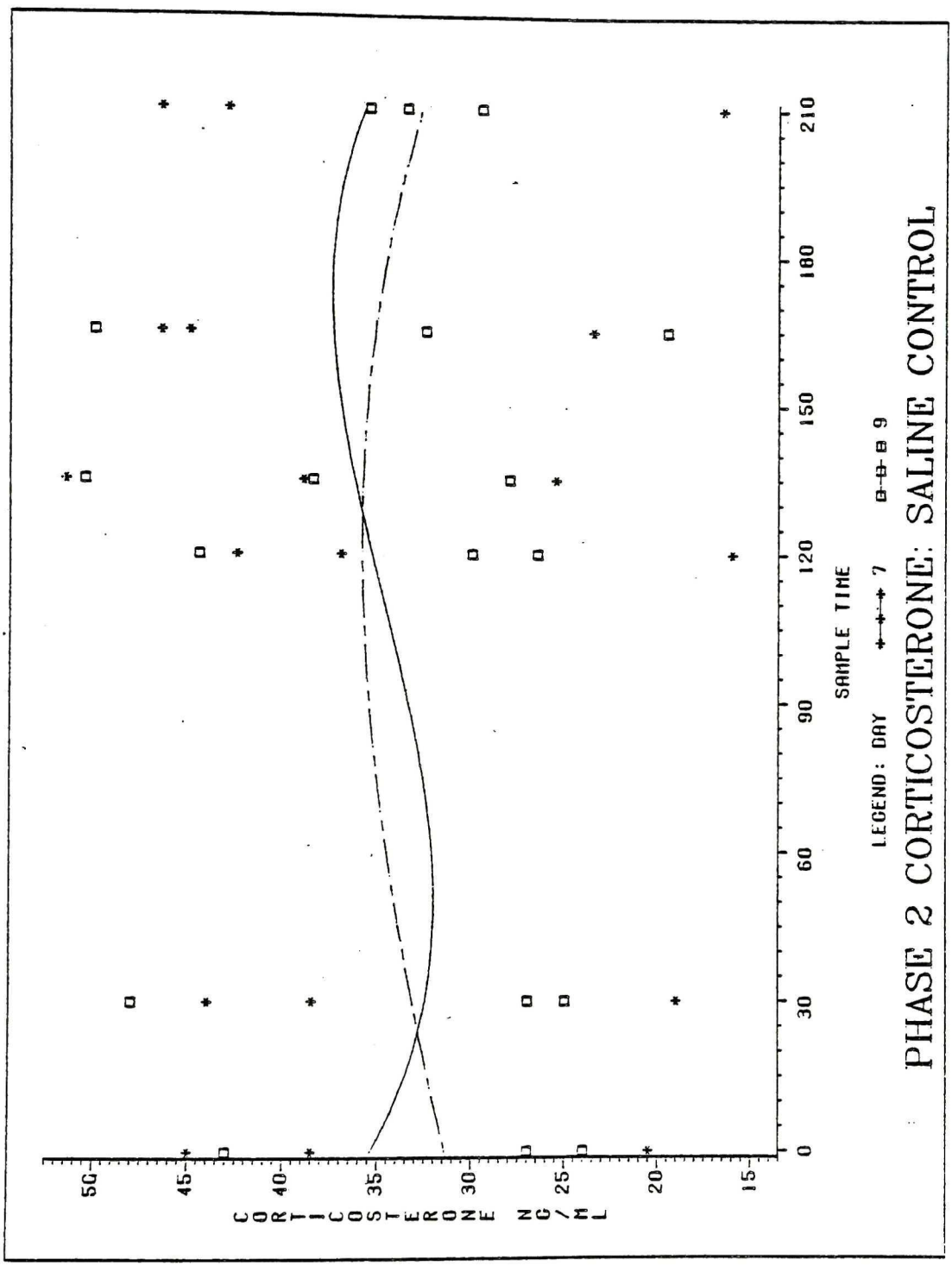
FIGURE 43

PHASE II: CORTICOSTERONE RESPONSES FOR SALINE CONTROL CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 CORTICOSTERONE: SALINE CONTROL

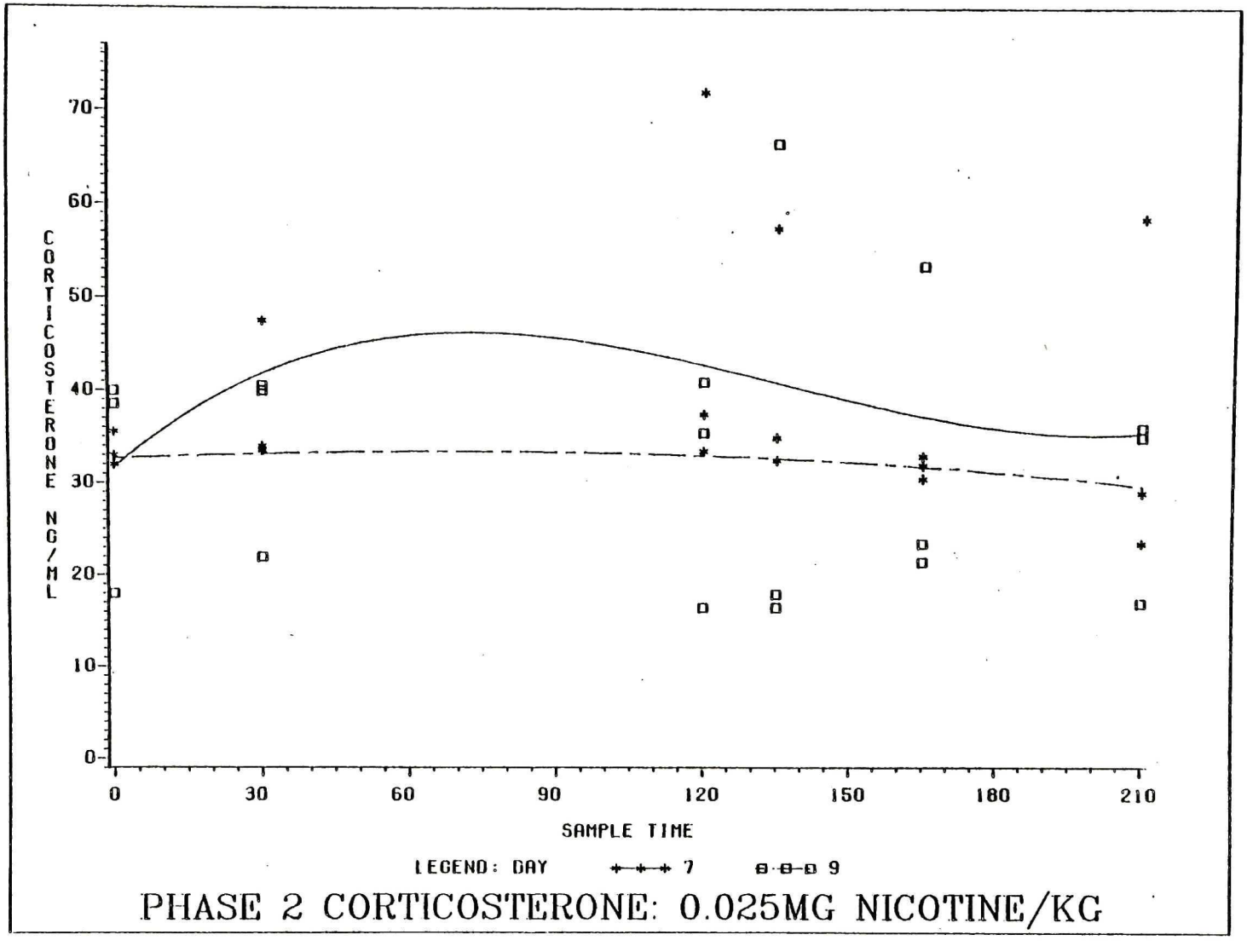
FIGURE 44

PHASE II: CORTICOSTERONE RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 CORTICOSTERONE: 0.025MG NICOTINE/KG

FIGURE 45

PHASE II: CORTICOSTERONE RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES

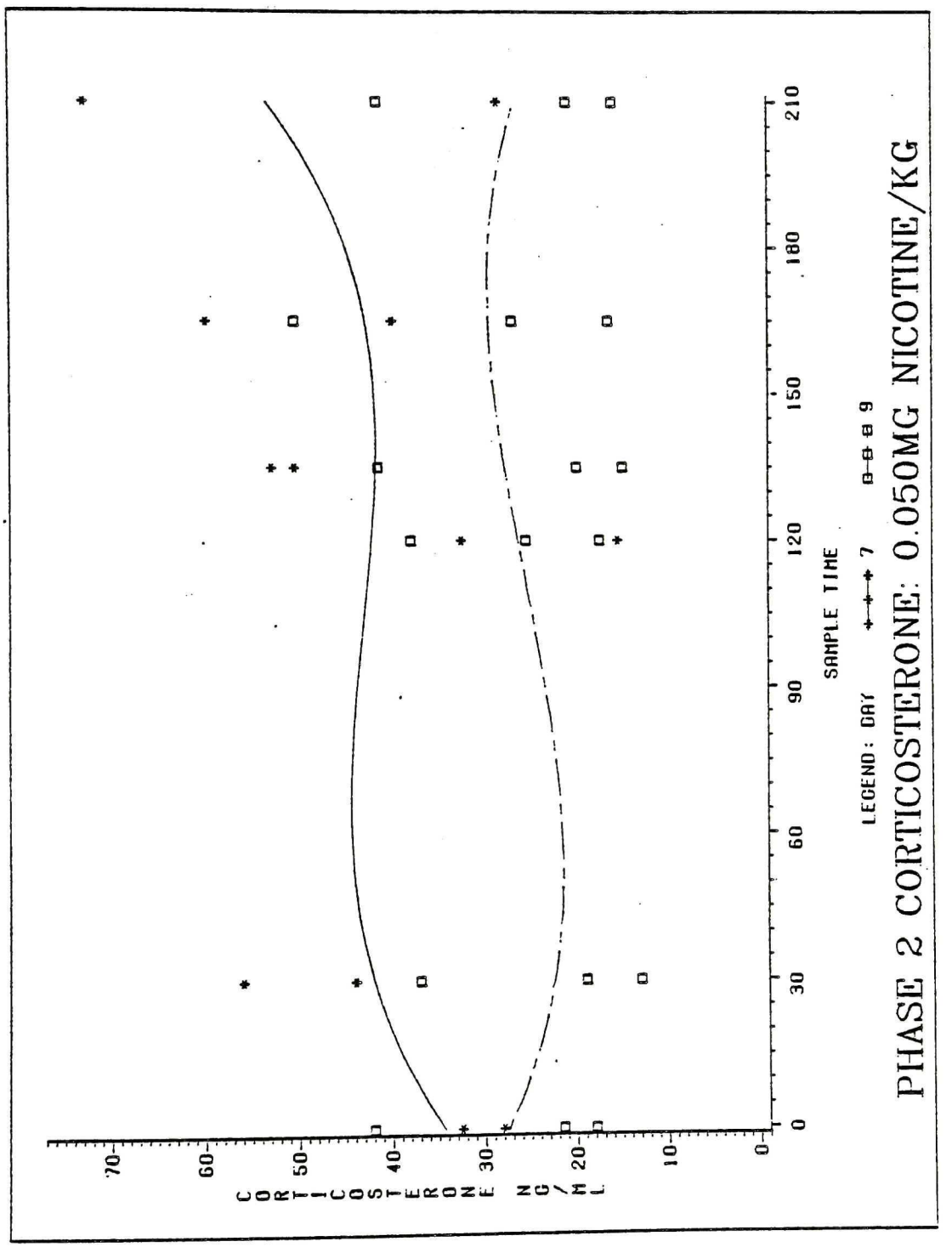

PHASE 2 CORTICOSTERONE: 0.050MG NICOTINE/KG
CORTICOSTERONE NG/ML
0
10
20
30
40
50
60
70
0
30
60
90
120
150
180
210
SAMPLE TIME
LEGEND: DAY 7 9

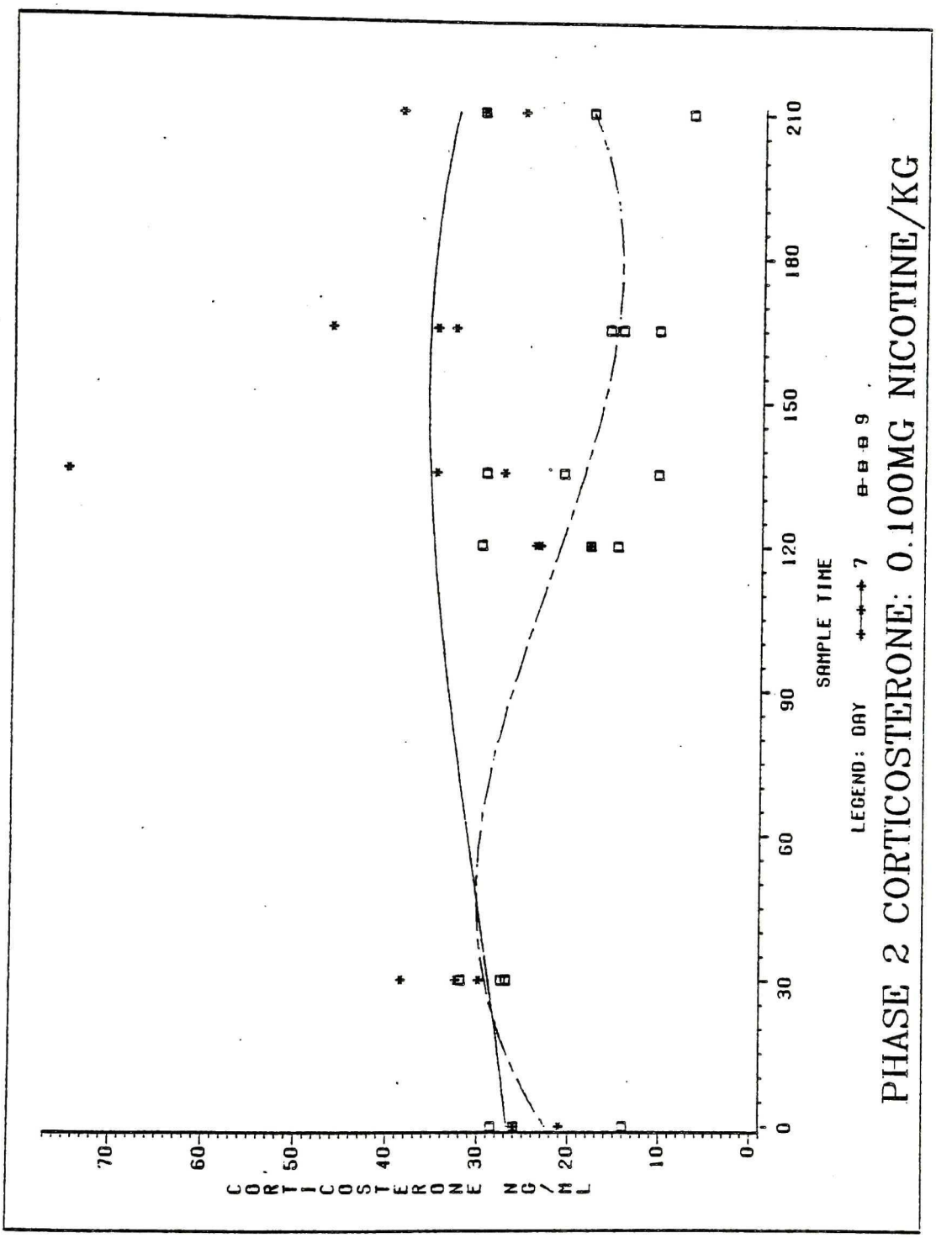
FIGURE 46

PHASE II: CORTICOSTERONE RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 CORTICOSTERONE: 0.100MG NICOTINE/KG

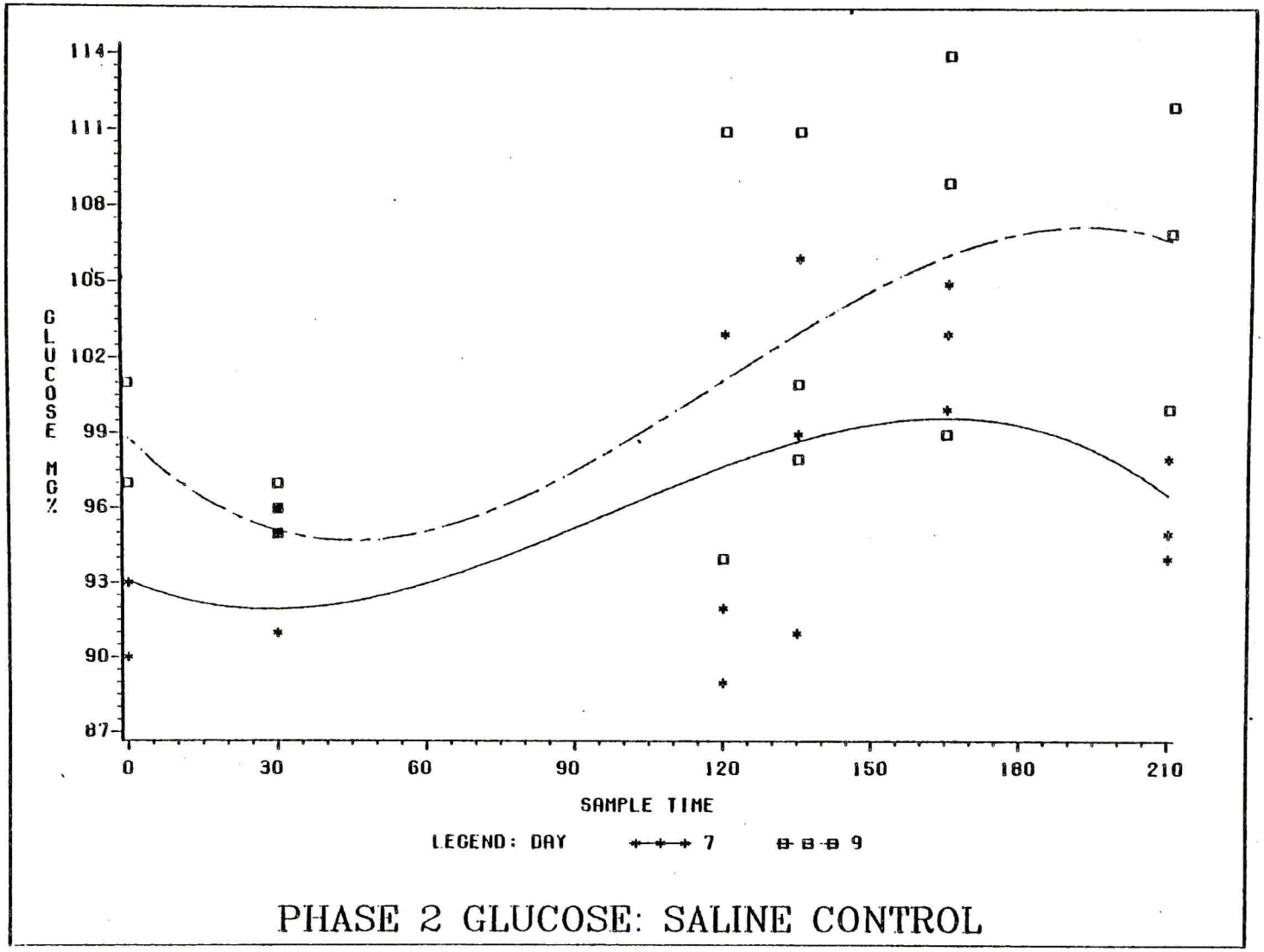
FIGURE 47

PHASE II: GLUCOSE RESPONSES FOR SALINE CONTROL CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 GLUCOSE: SALINE CONTROL

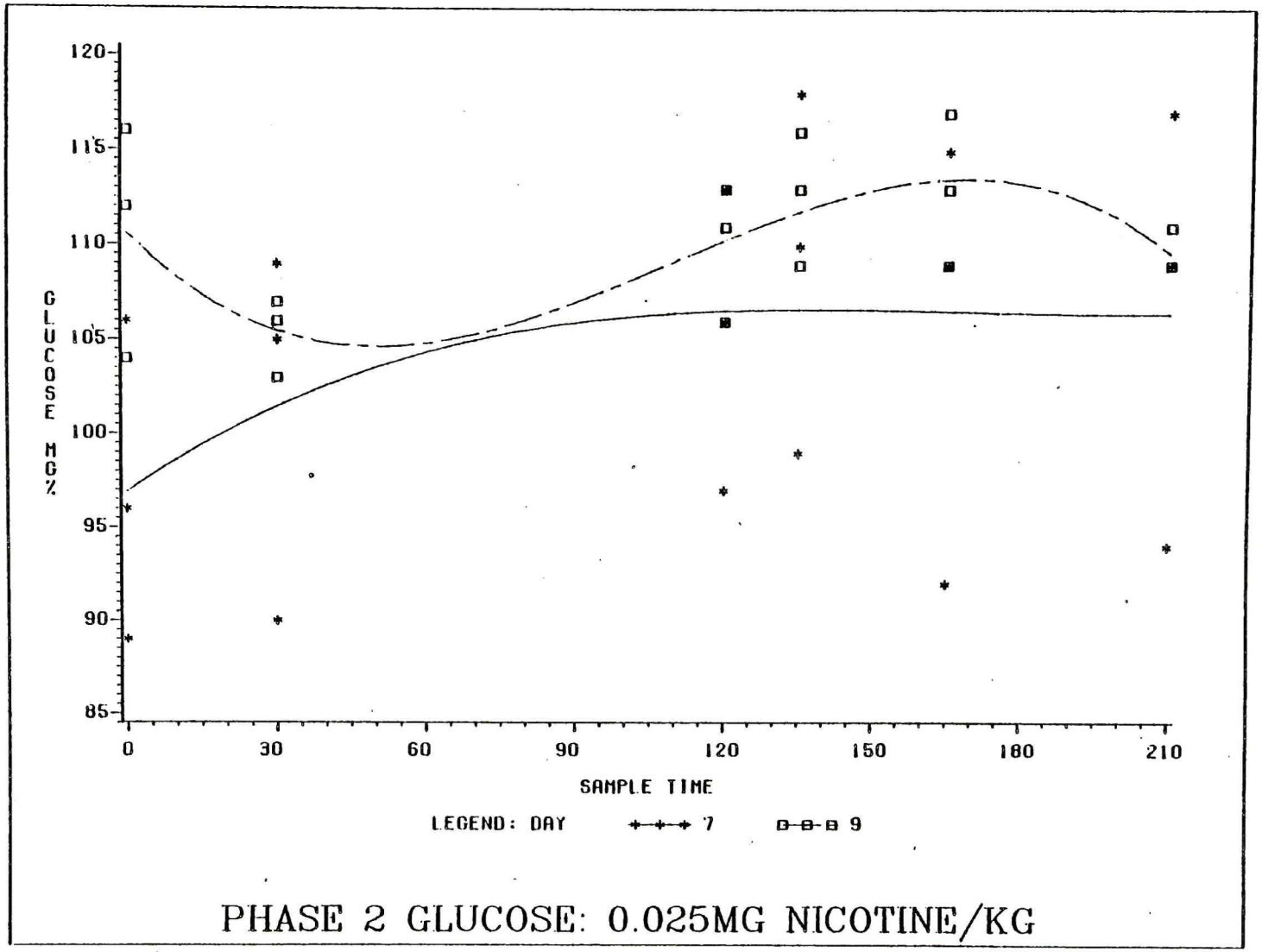
FIGURE 48

PHASE II: GLUCOSE RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 GLUCOSE: 0.025MG NICOTINE/KG

D206

FIGURE 49

PHASE II: GLUCOSE RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES

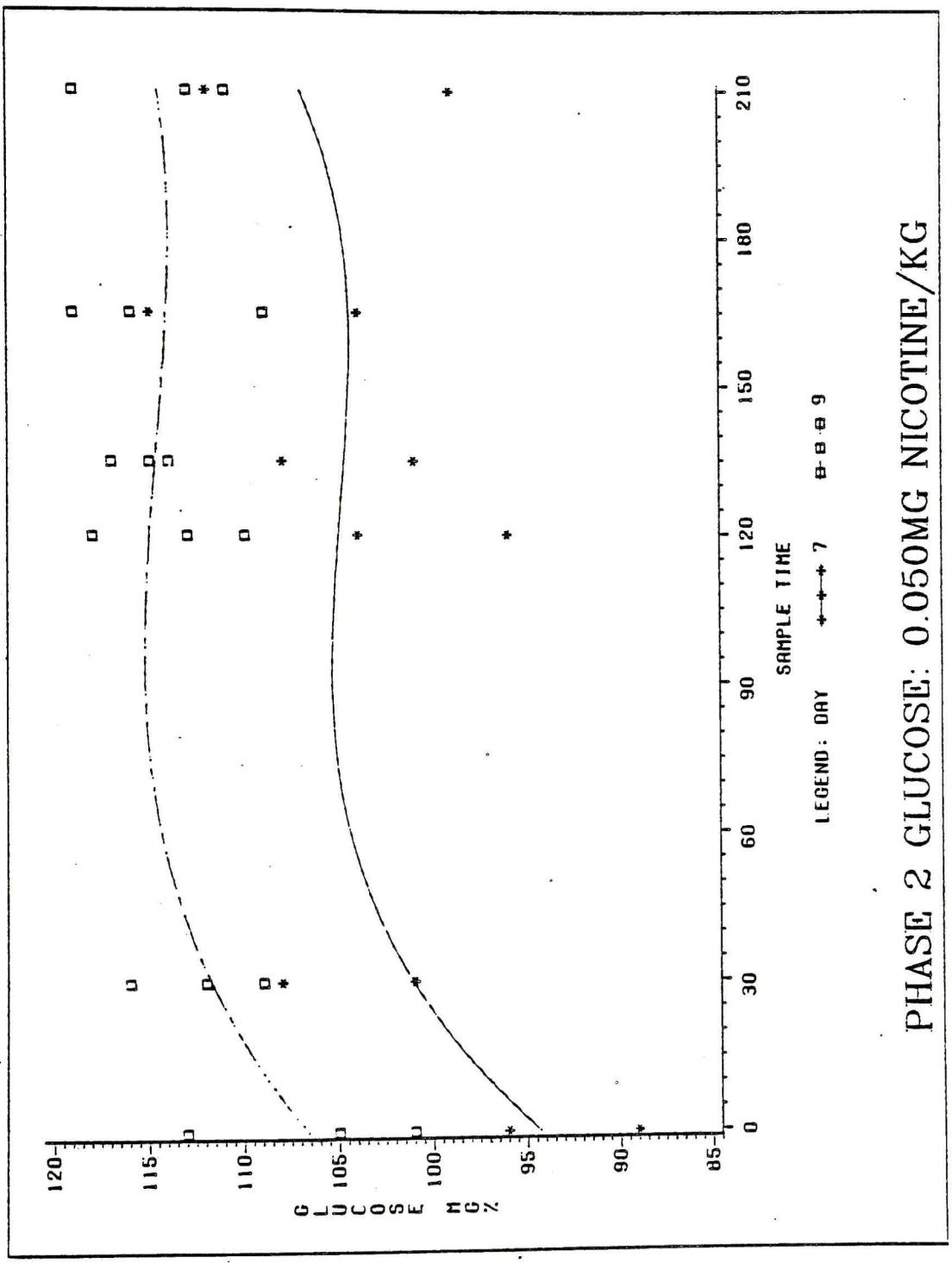

PHASE 2 GLUCOSE: 0.050MG NICOTINE/KG
GLUCOSE MG%
120
115
110
105
100
95
90
85
0
30
60
90
120
150
180
210
SAMPLE TIME
LEGEND: DAY 7 9

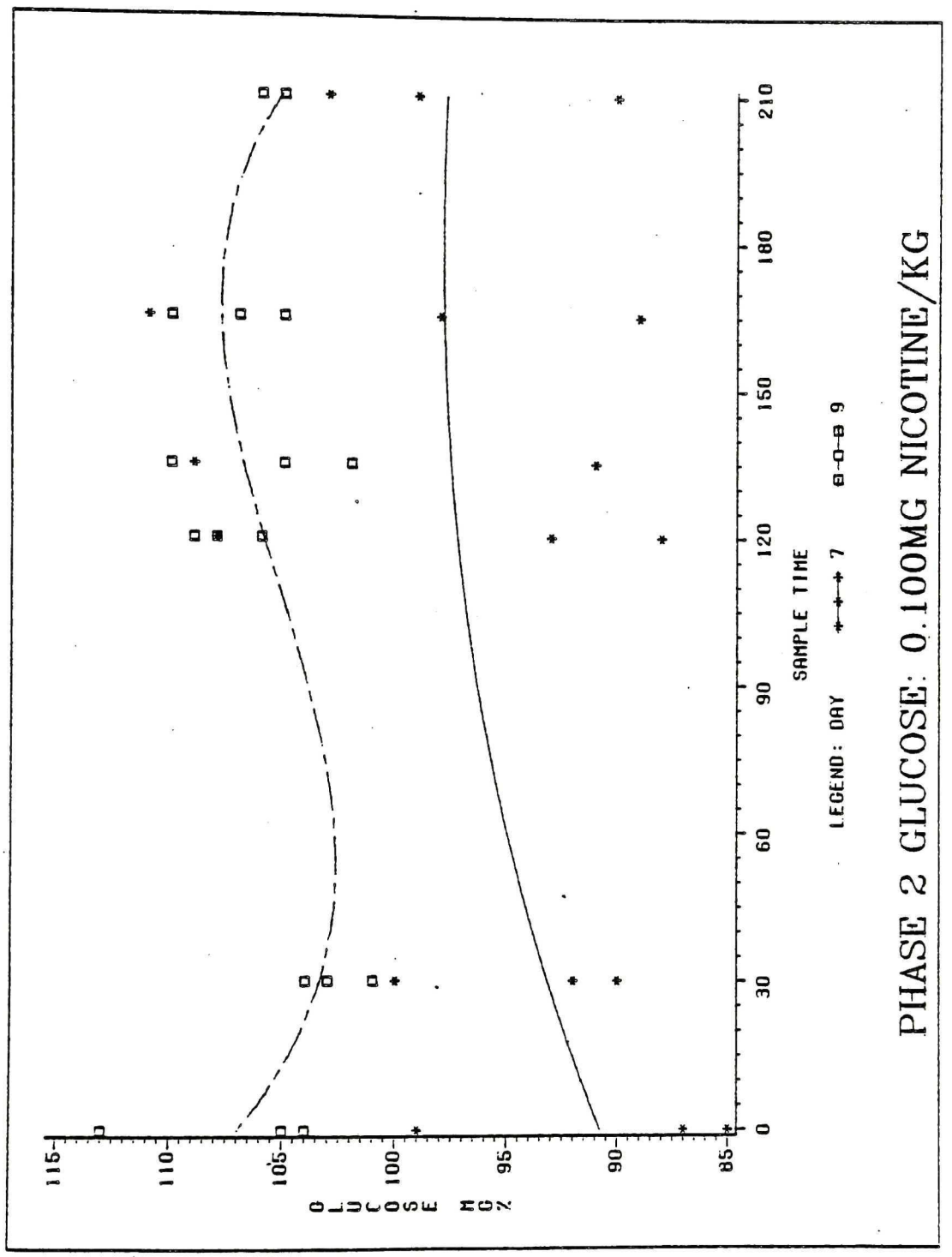
FIGURE 50

PHASE II: GLUCOSE RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 GLUCOSE: 0.100MG NICOTINE/KG

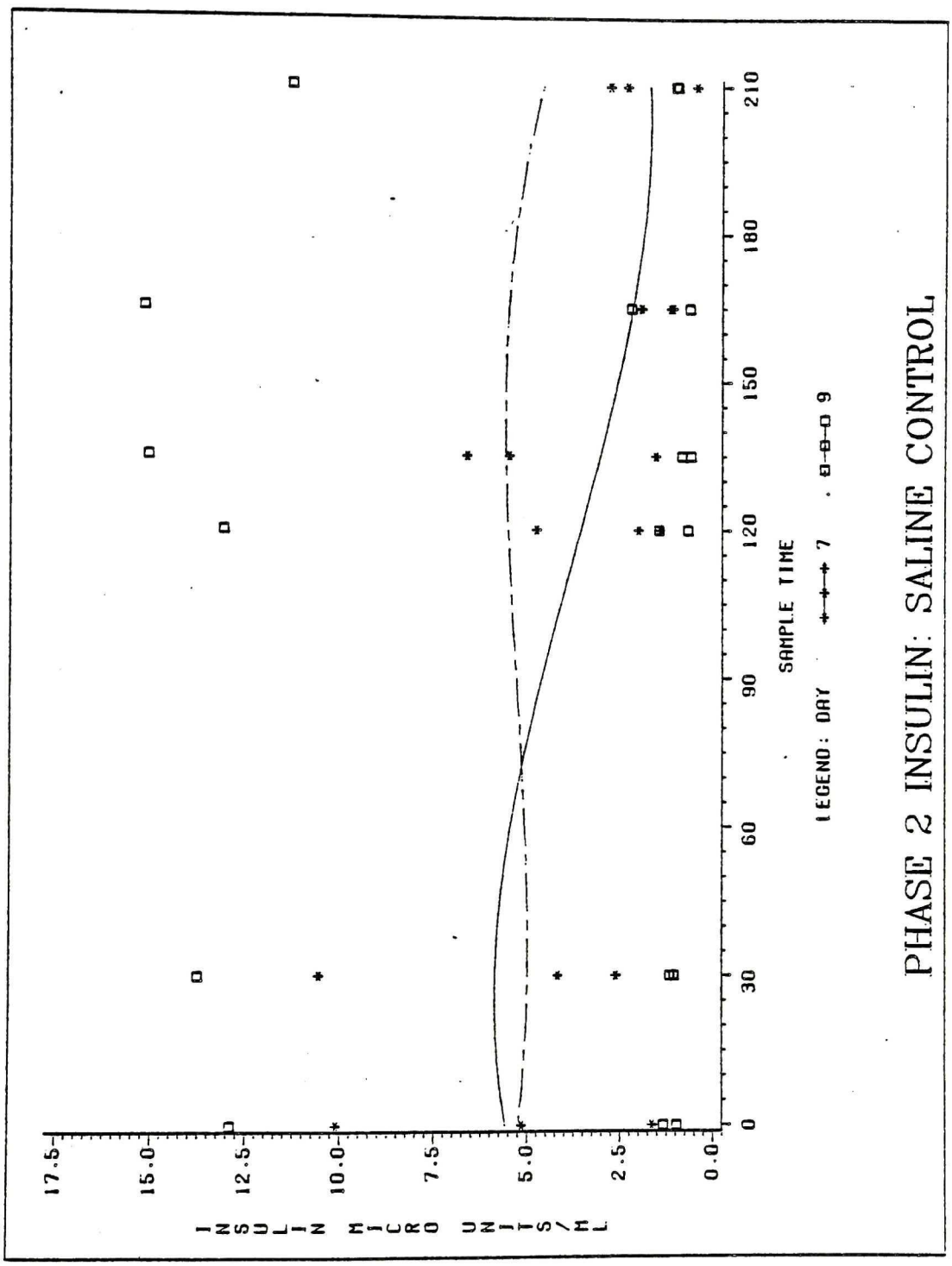
FIGURE 51

PHASE II: INSULIN RESPONSES FOR SALINE CONTROL CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 INSULIN: SALINE CONTROL

FIGURE 52

PHASE II: INSULIN RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES

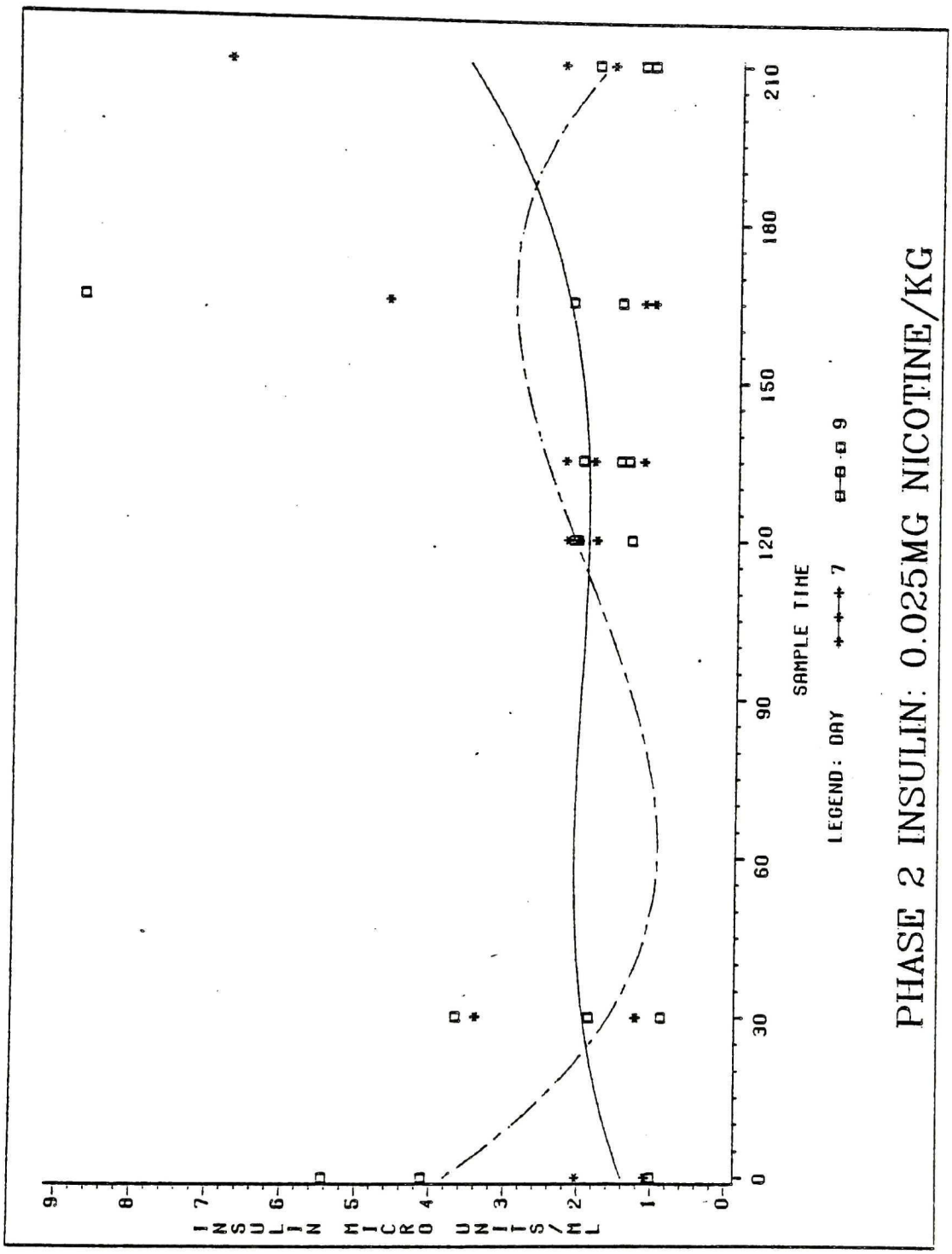

PHASE 2 INSULIN: 0.025MG NICOTINE/KG
INSULIN MICRO UNITS/ML
0
1
2
3
4
5
6
7
8
9
0
30
60
90
120
150
180
210
SAMPLE TIME
LEGEND: DAY 7 9

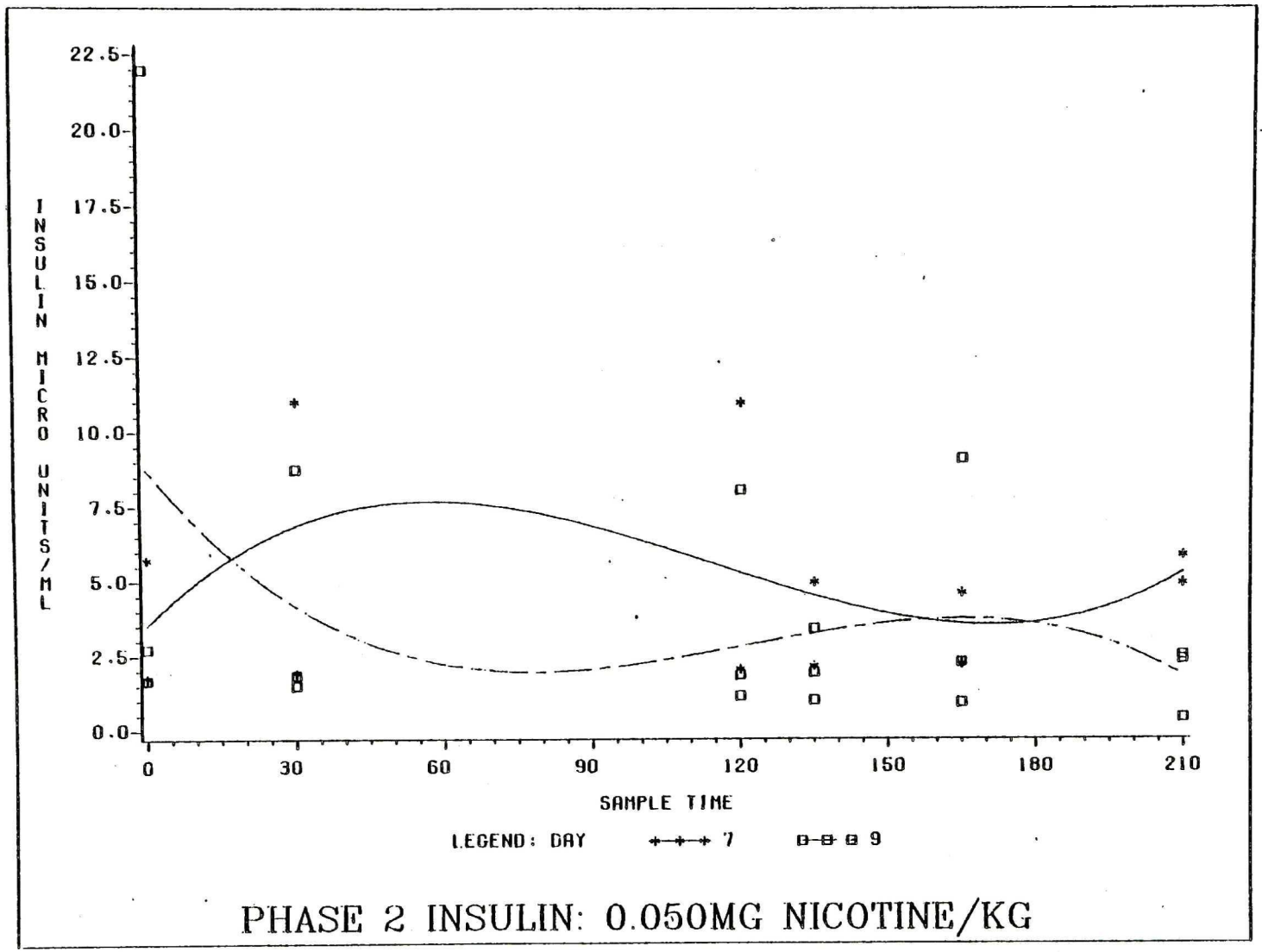
FIGURE 53

PHASE II: INSULIN RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 INSULIN: 0.050MG NICOTINE/KG

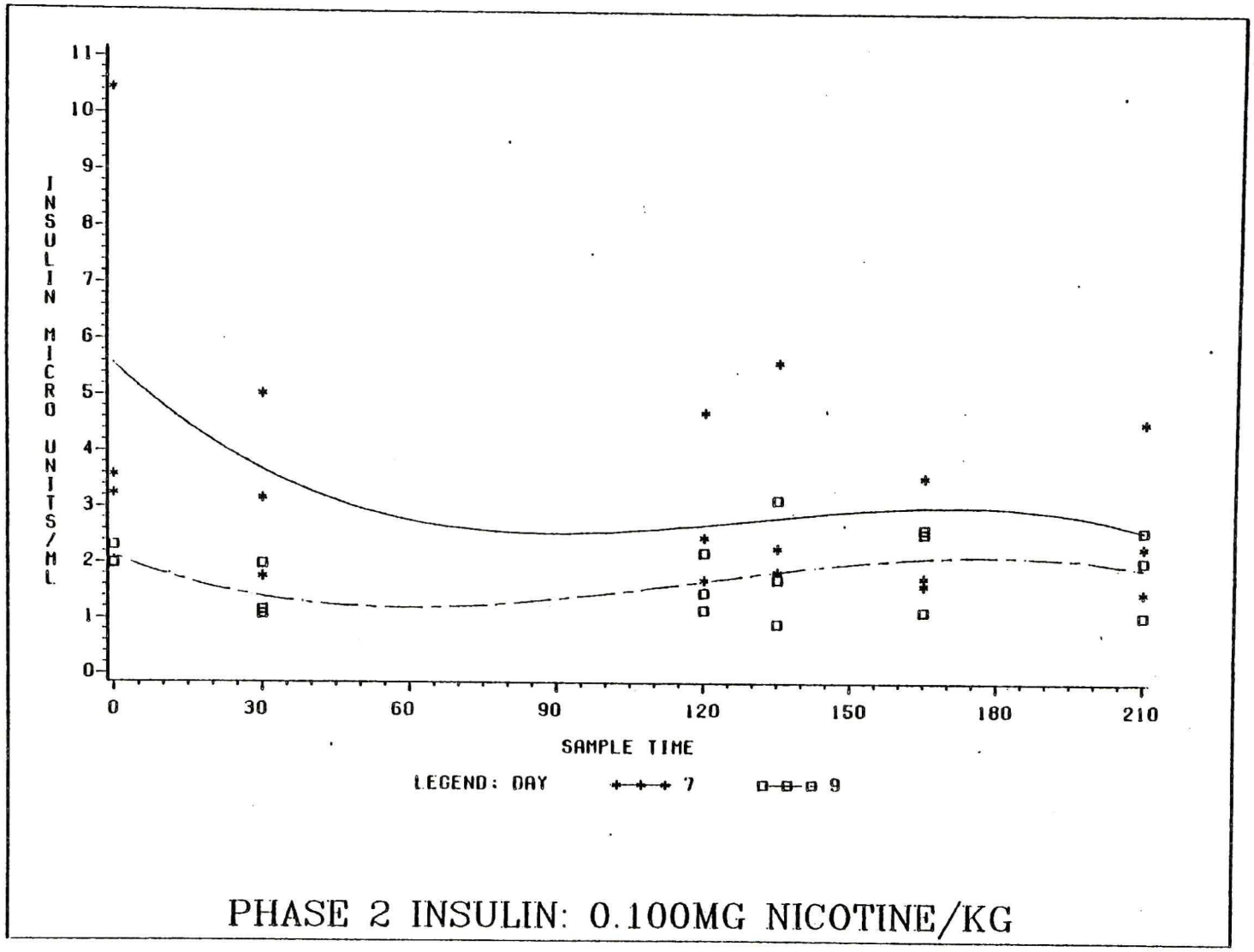
FIGURE 54

PHASE II: INSULIN RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION

LEGEND

DAY 7 = NICOTINE ADMINISTRATION

DAY 9 = TEST OF CONDITIONED DRUG RESPONSES



PHASE 2 INSULIN: 0.100MG NICOTINE/KG

FIGURE 55

PHASE III: NOREPINEPHRINE RESPONSES FOR SALINE CONTROL CONDITION

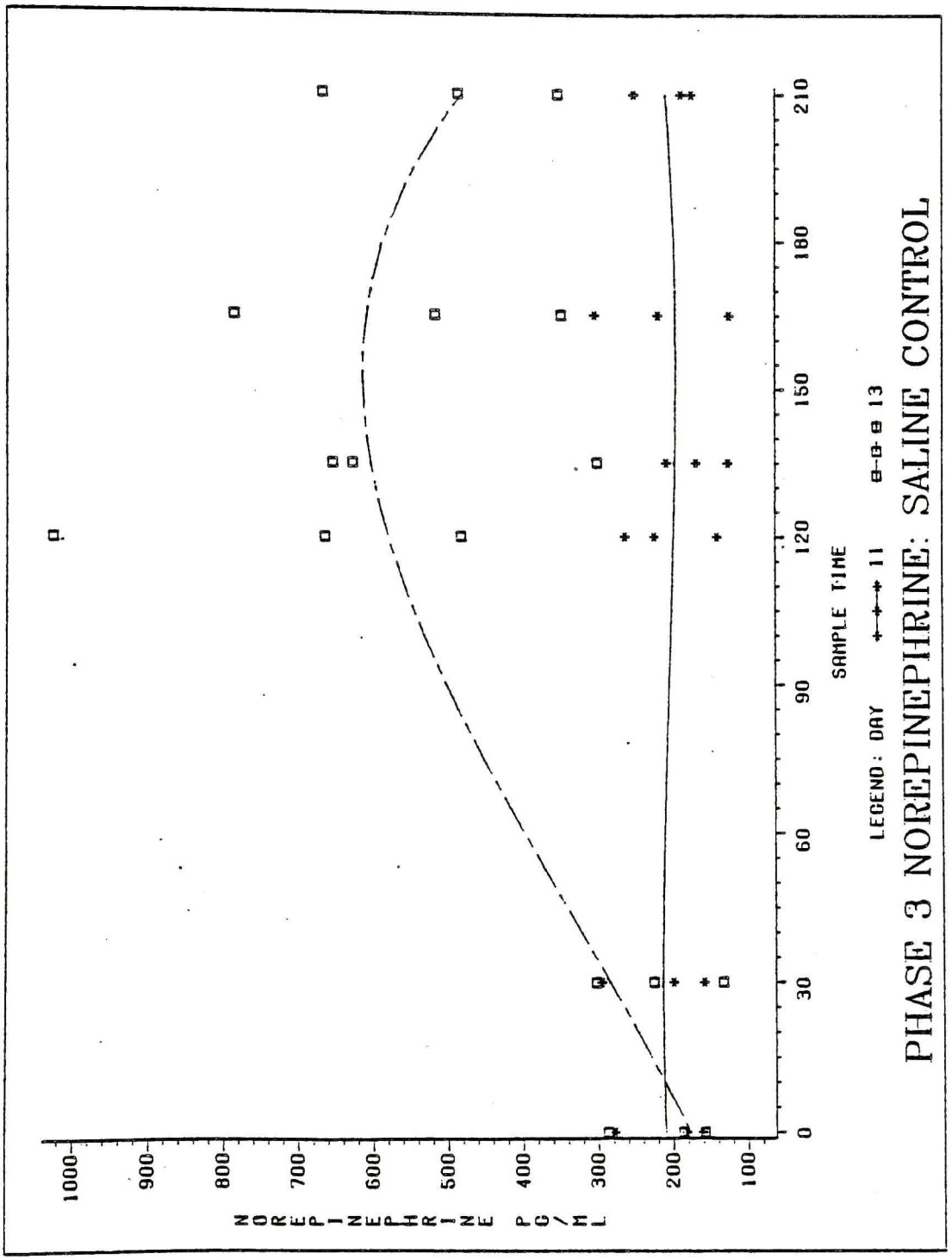
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 NOREPINEPHRINE: SALINE CONTROL

FIGURE 56

PHASE III: NOREPINEPHRINE RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION

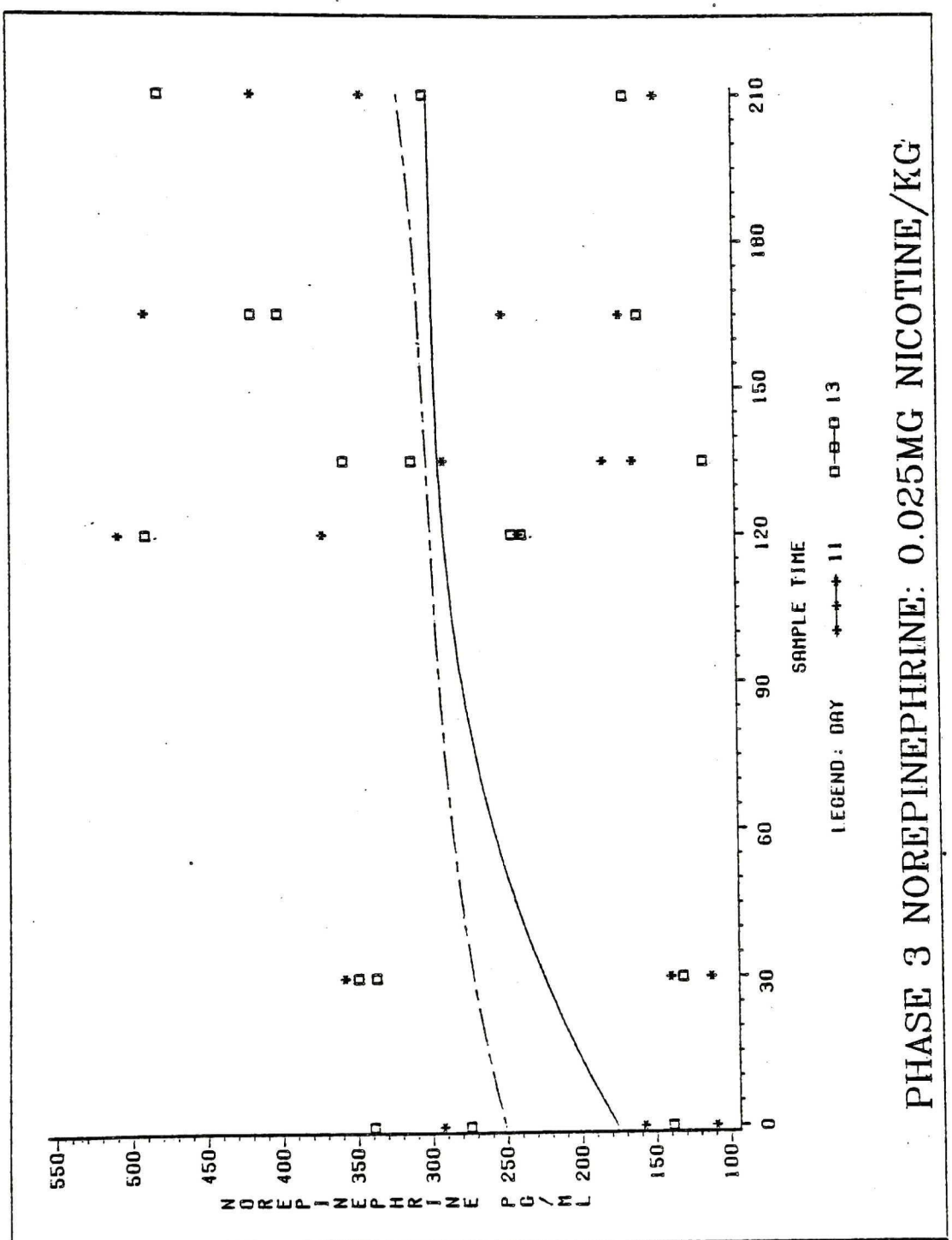
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 NOREPINEPHRINE: 0.025MG NICOTINE/KG

FIGURE 57

PHASE III: NOREPINEPHRINE RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION

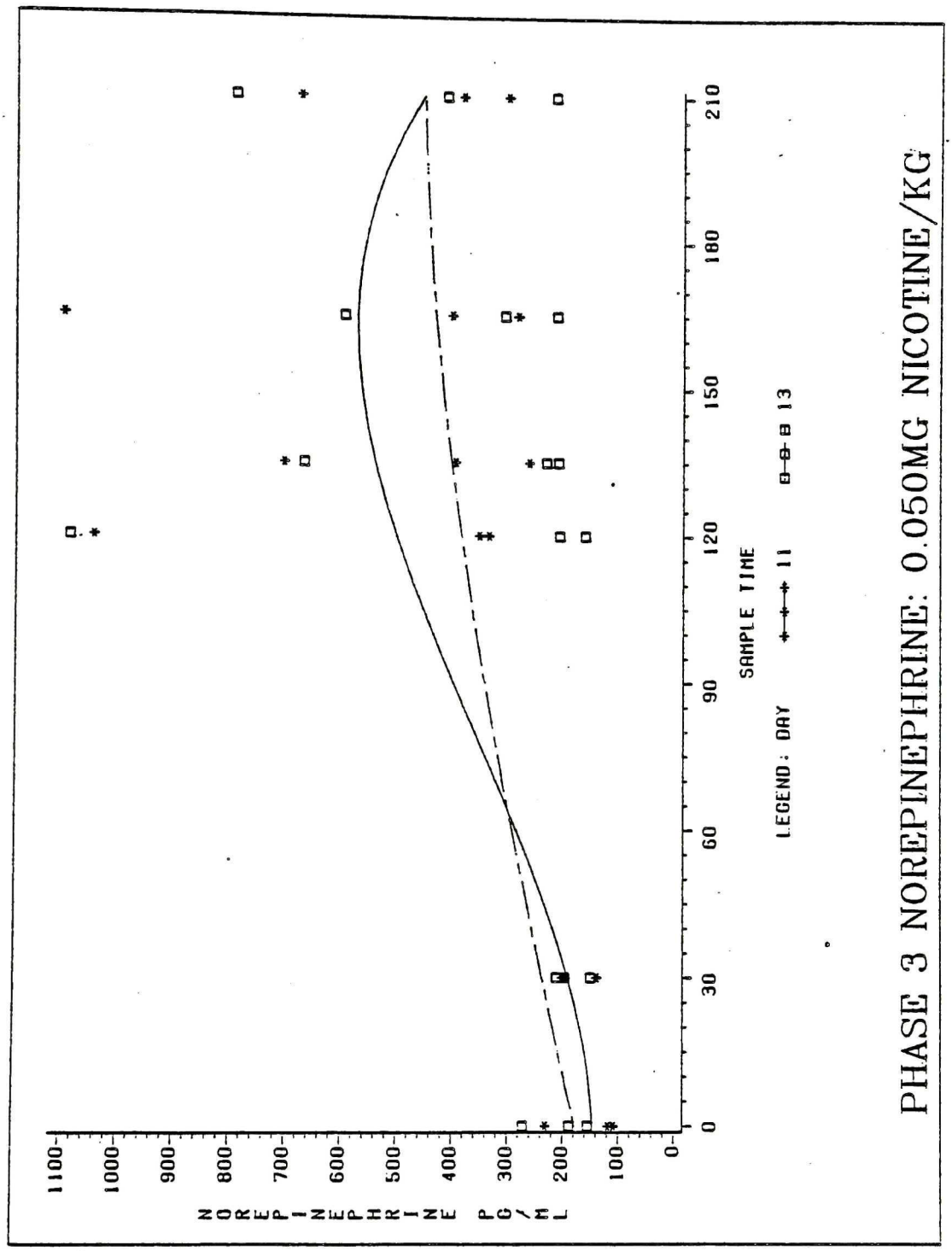
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 NOREPINEPHRINE: 0.050MG NICOTINE/KG

FIGURE 58

PHASE III: NOREPINEPHRINE RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION

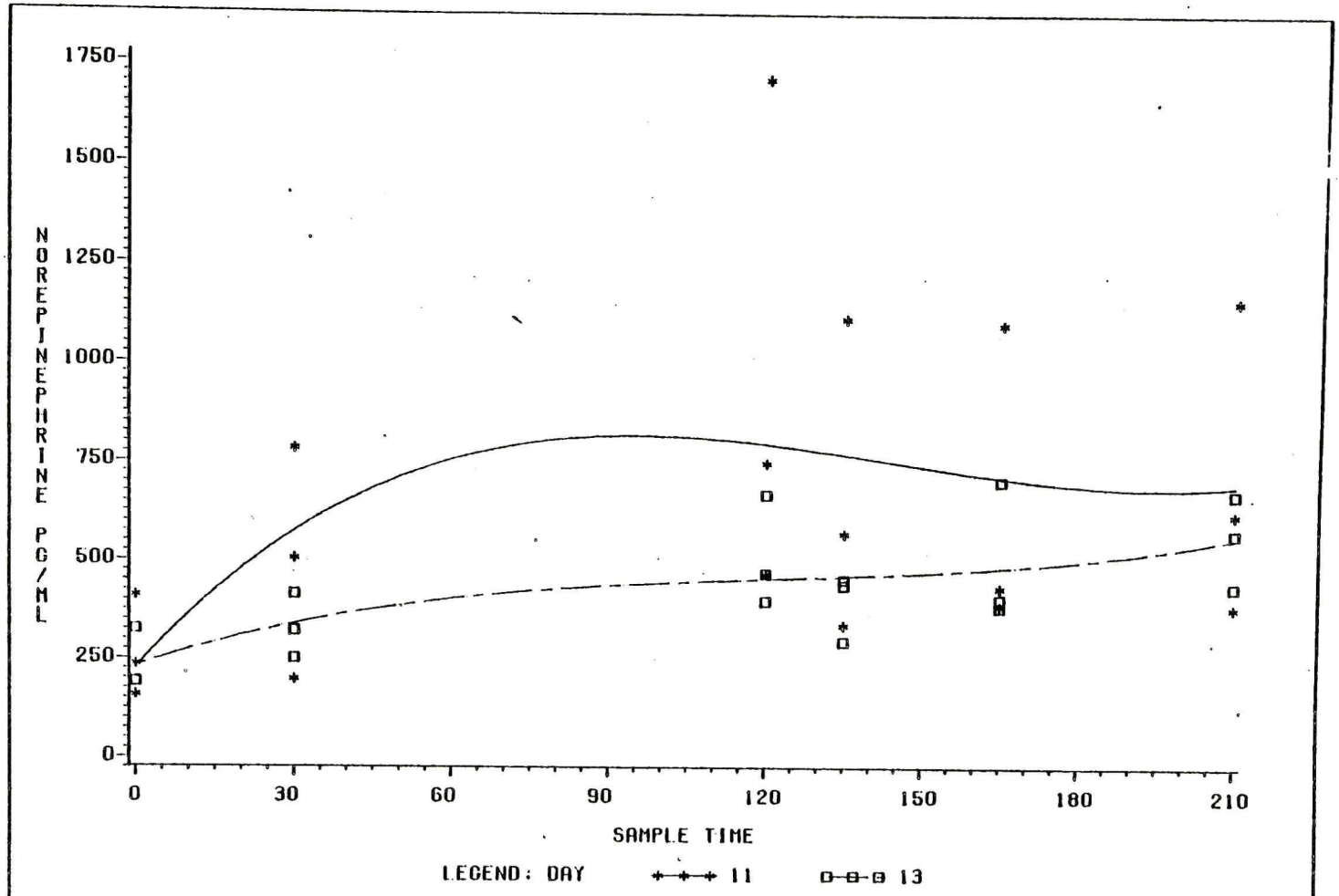
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 NOREPINEPHRINE: 0.100MG NICOTINE/KG

FIGURE 59

PHASE III: EPINEPHRINE RESPONSES FOR SALINE CONTROL CONDITION

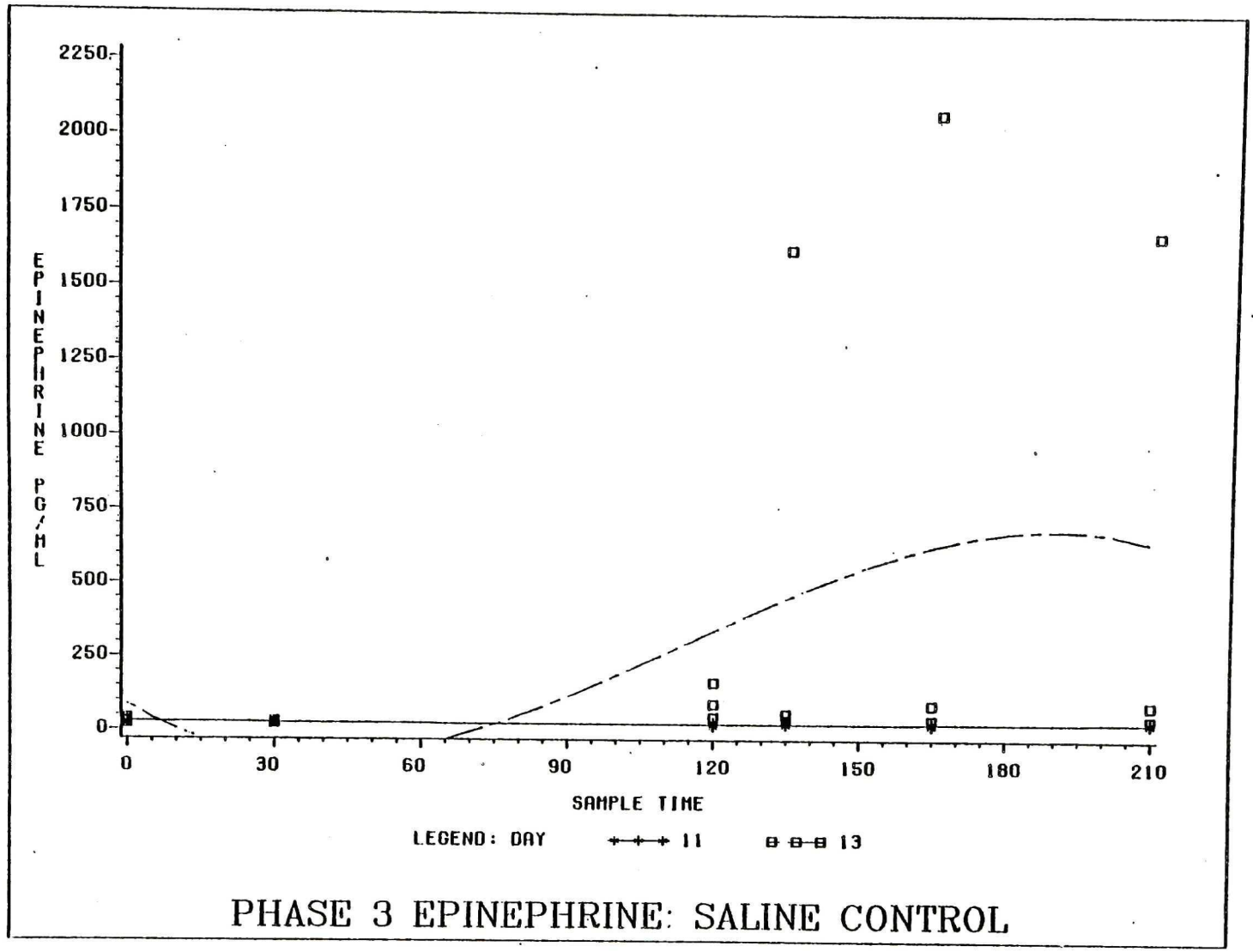
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 EPINEPHRINE: SALINE CONTROL

FIGURE 60

PHASE III: EPINEPHRINE RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION

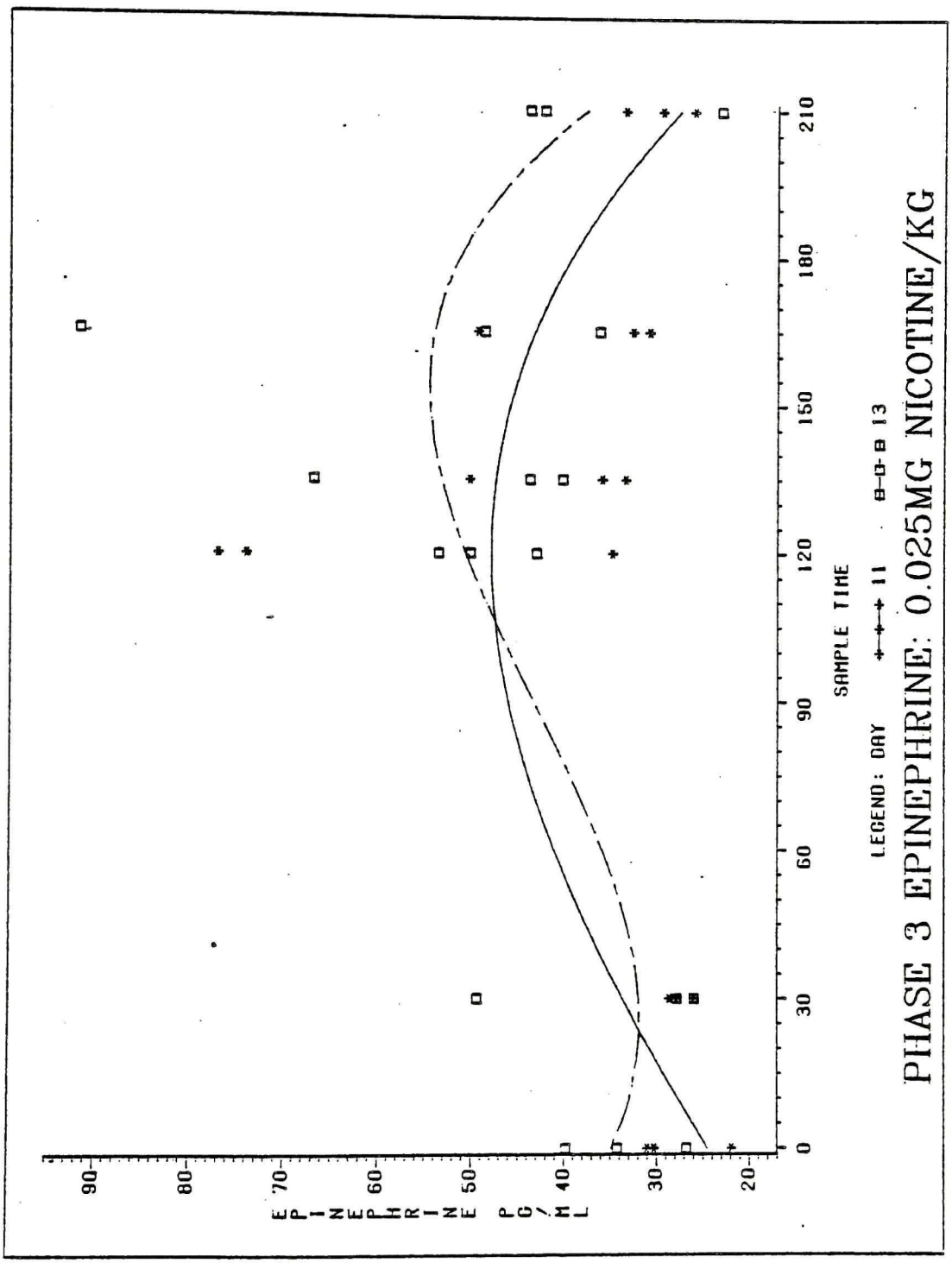
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 EPINEPHRINE: 0.025MG NICOTINE/KG

FIGURE 61

PHASE III: EPINEPHRINE RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION

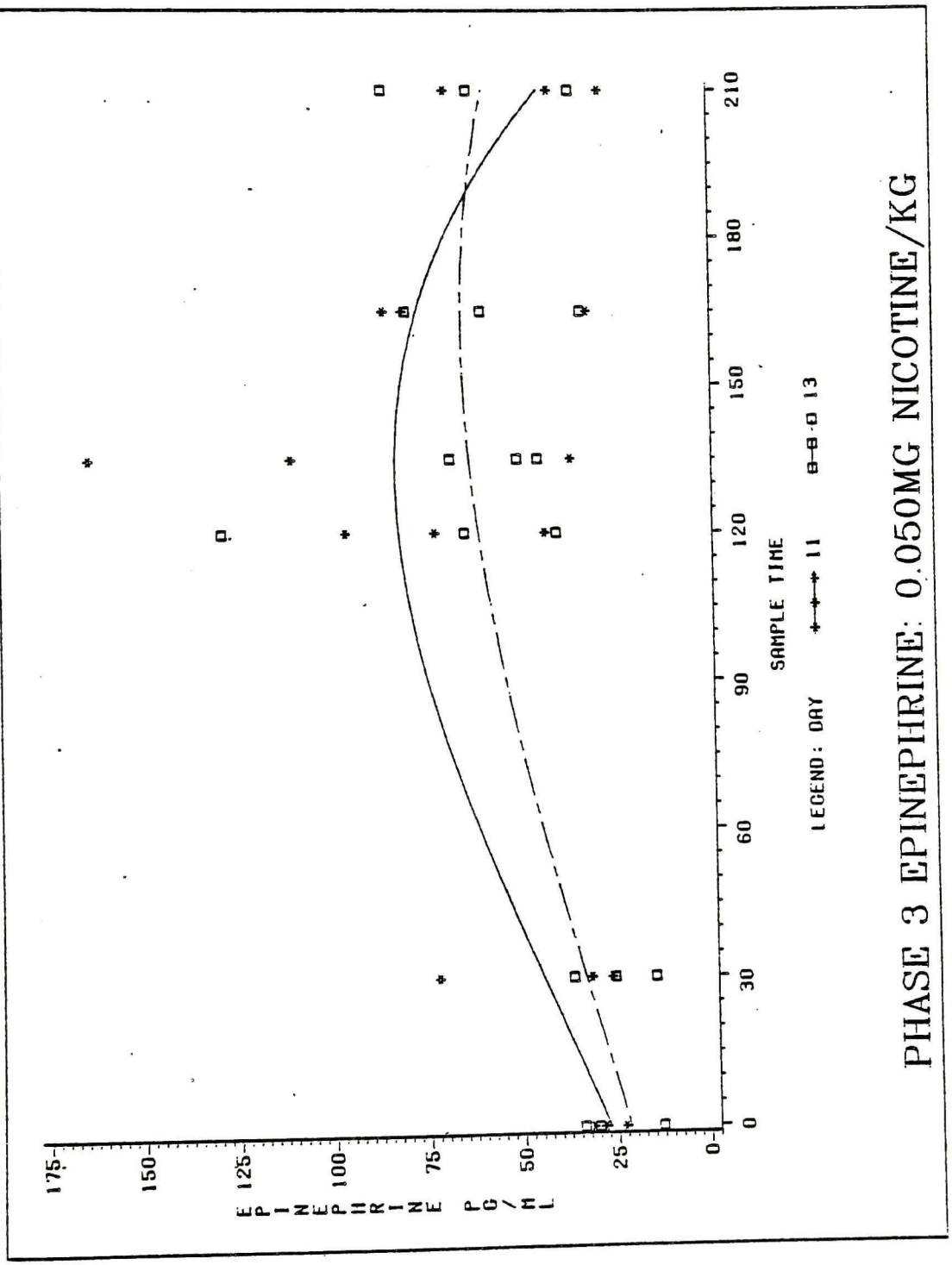
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 EPINEPHRINE: 0.050MG NICOTINE/KG

FIGURE 62

PHASE III: EPINEPHRINE RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION

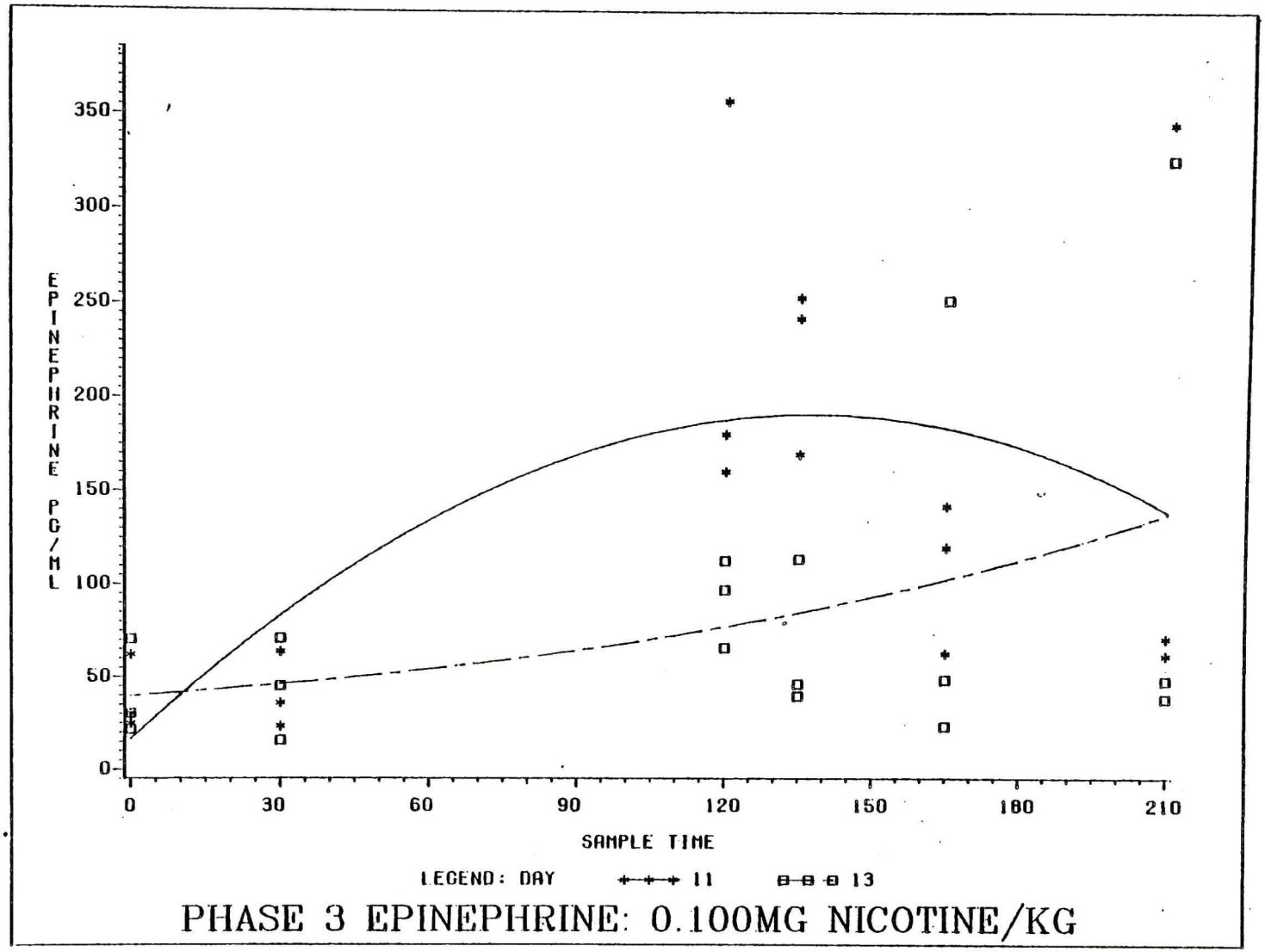
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 EPINEPHRINE: 0.100MG NICOTINE/KG

FIGURE 63

PHASE III: DOPAMINE RESPONSES FOR SALINE CONTROL CONDITION

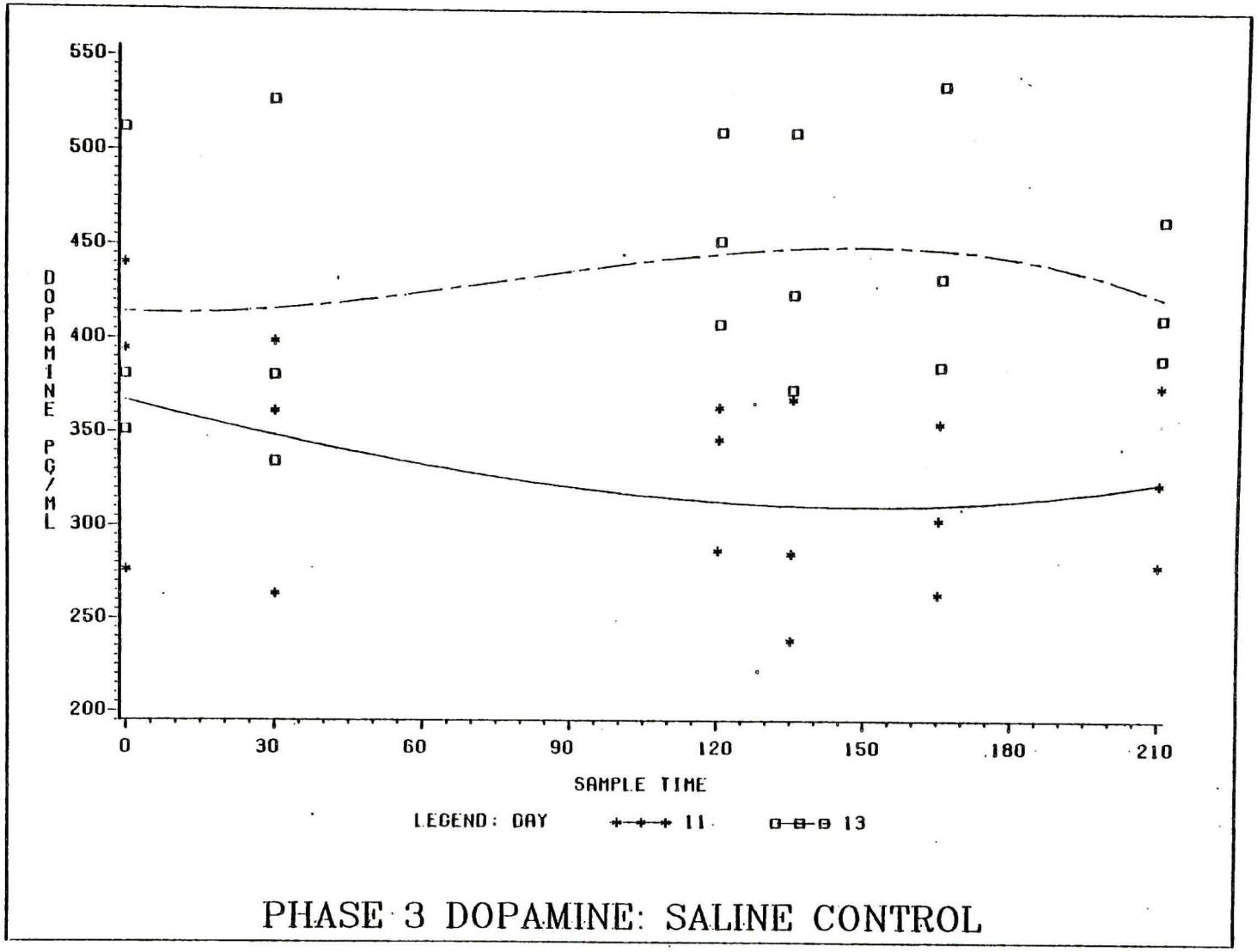
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 DOPAMINE: SALINE CONTROL

FIGURE 64

PHASE III: DOPAMINE RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION

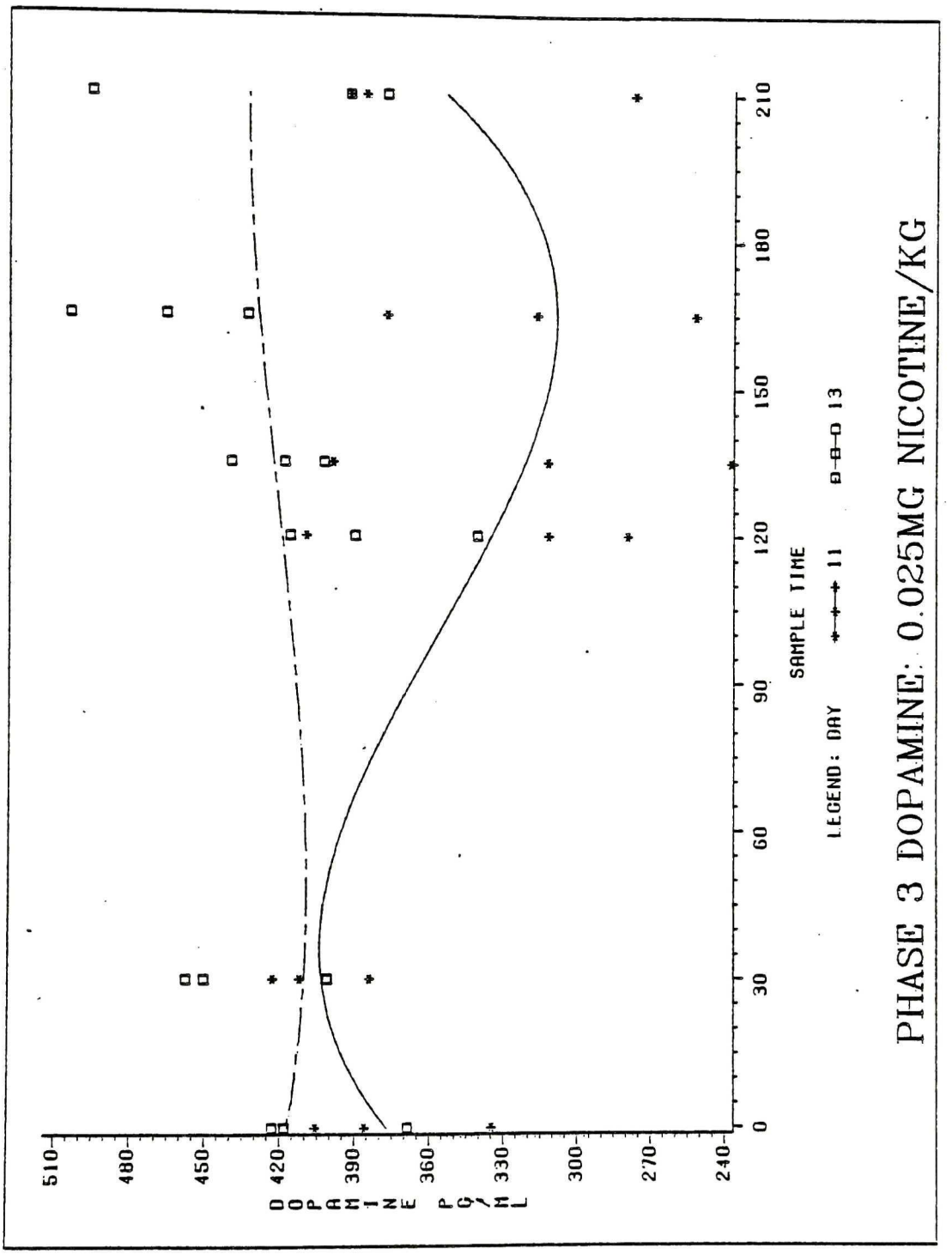
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 DOPAMINE: 0.025MG NICOTINE/KG

FIGURE 65

PHASE III: DOPAMINE RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION

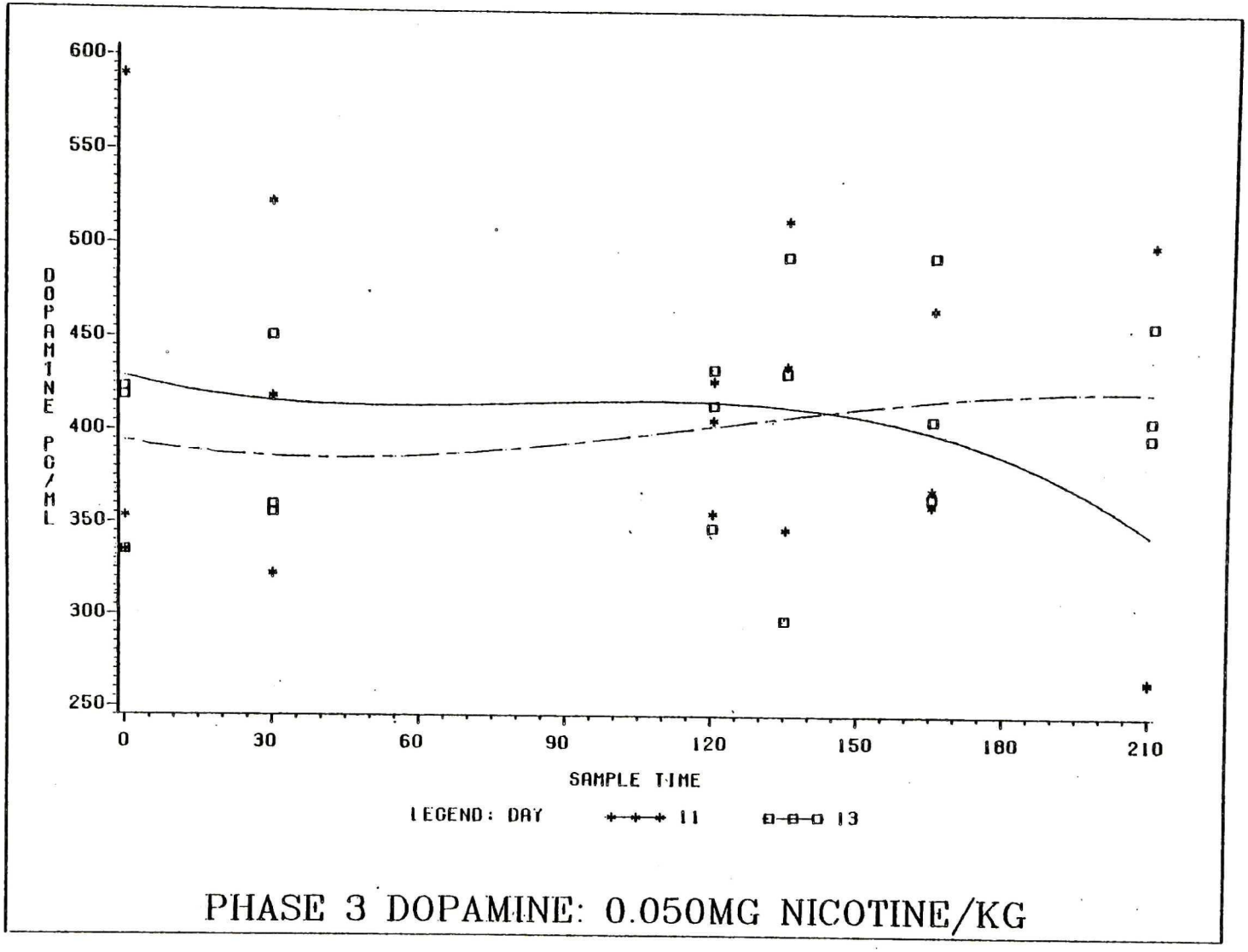
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 DOPAMINE: 0.050MG NICOTINE/KG

FIGURE 66

PHASE III: DOPAMINE RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION

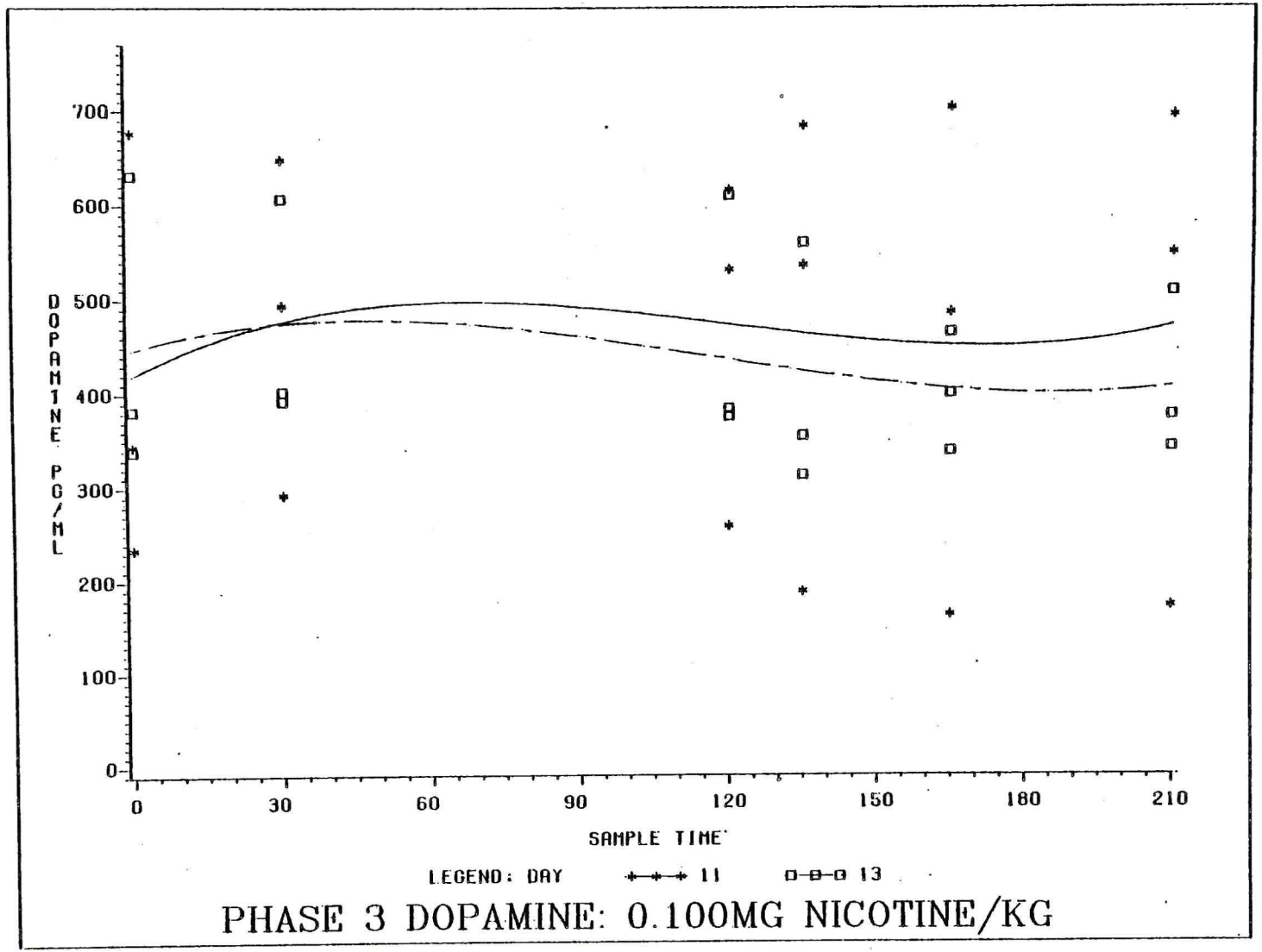
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 DOPAMINE: 0.100MG NICOTINE/KG

FIGURE 67

PHASE III: CORTICOSTERONE RESPONSES FOR SALINE CONTROL CONDITION

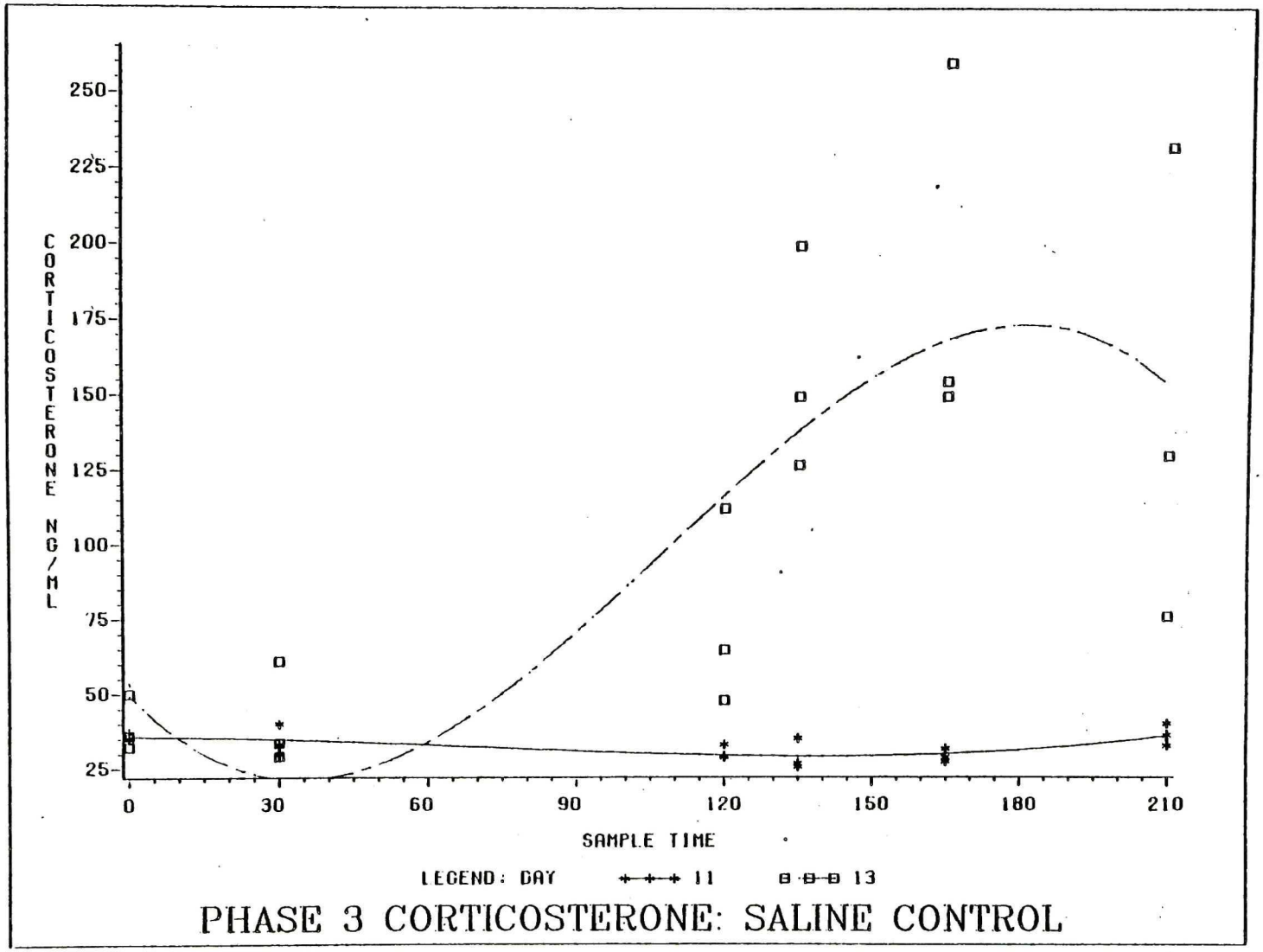
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 CORTICOSTERONE: SALINE CONTROL

FIGURE 68

PHASE III: CORTICOSTERONE RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION

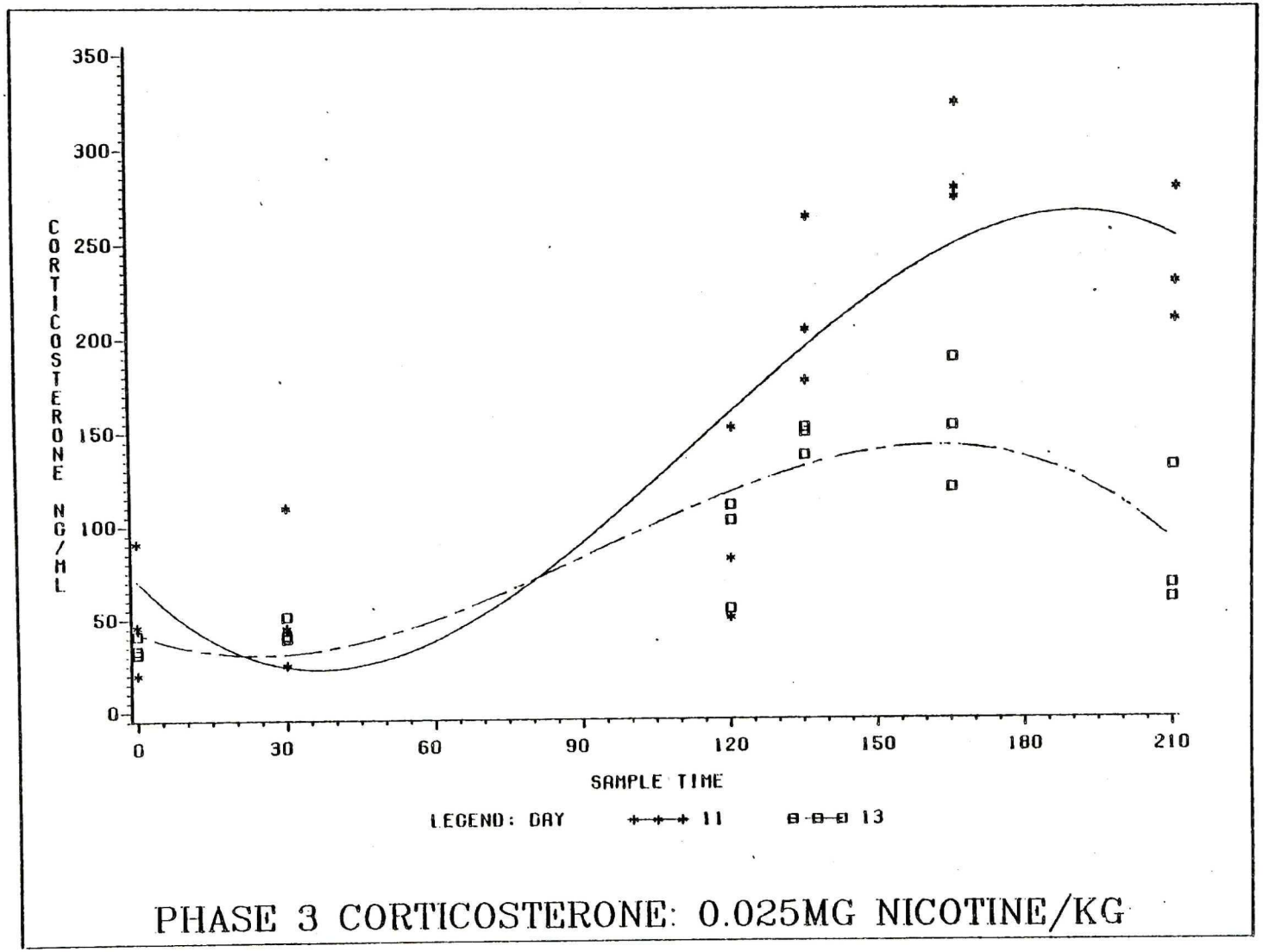
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 CORTICOSTERONE: 0.025MG NICOTINE/KG

FIGURE 69

PHASE III: CORTICOSTERONE RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION

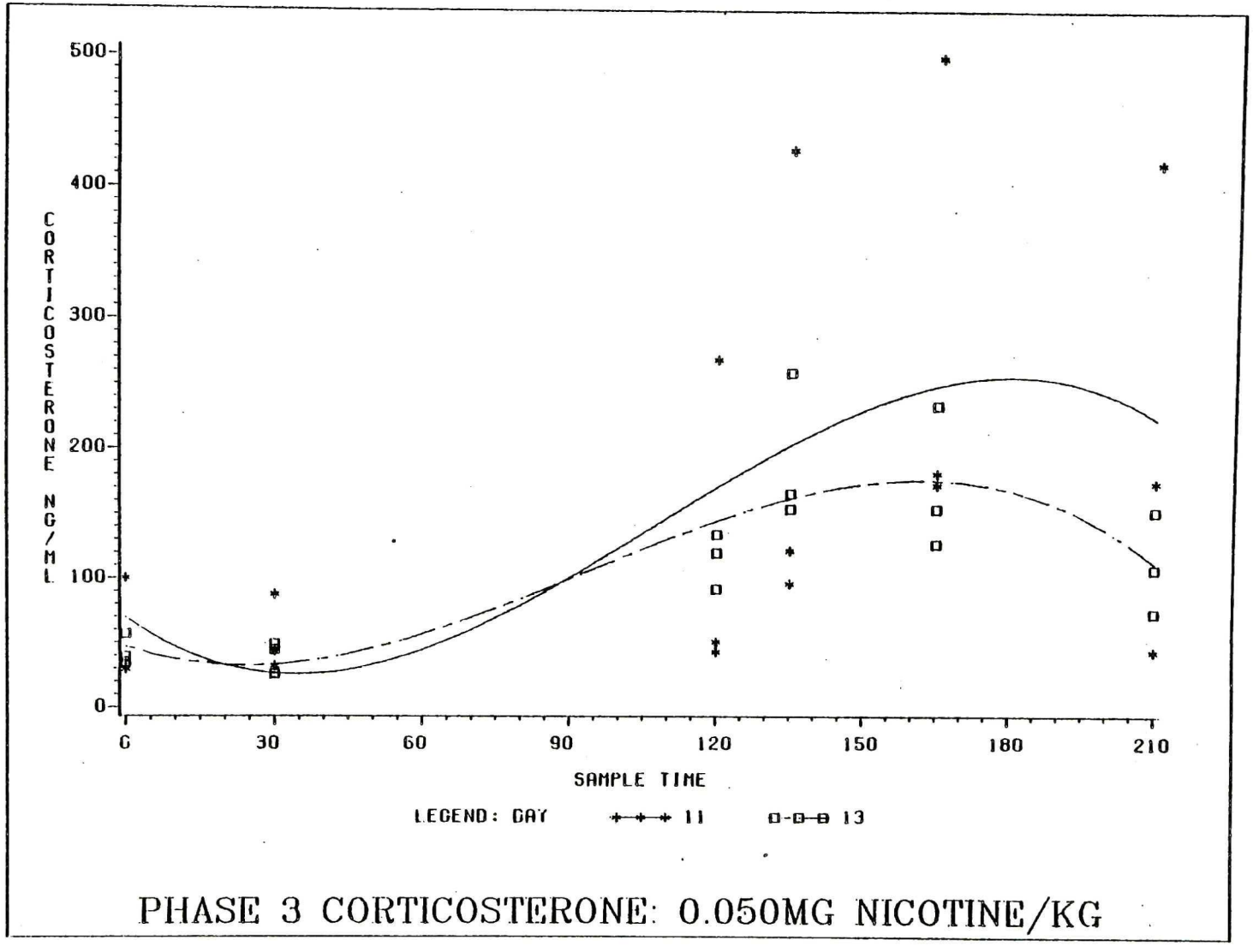
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 CORTICOSTERONE: 0.050MG NICOTINE/KG

FIGURE 70

PHASE III: CORTICOSTERONE RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION

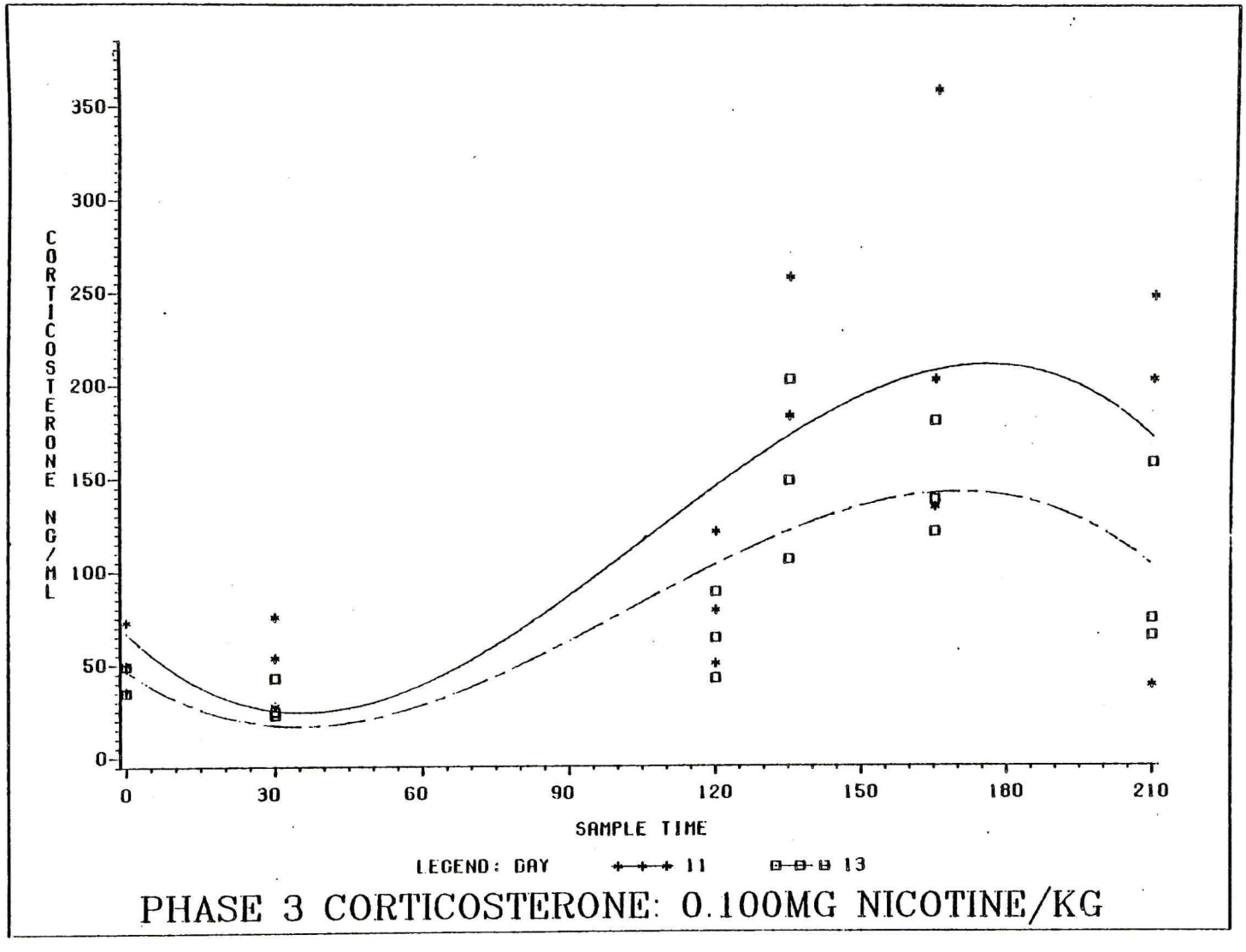
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 CORTICOSTERONE: 0.100MG NICOTINE/KG

FIGURE 71

PHASE III: GLUCOSE RESPONSES FOR SALINE CONTROL CONDITION

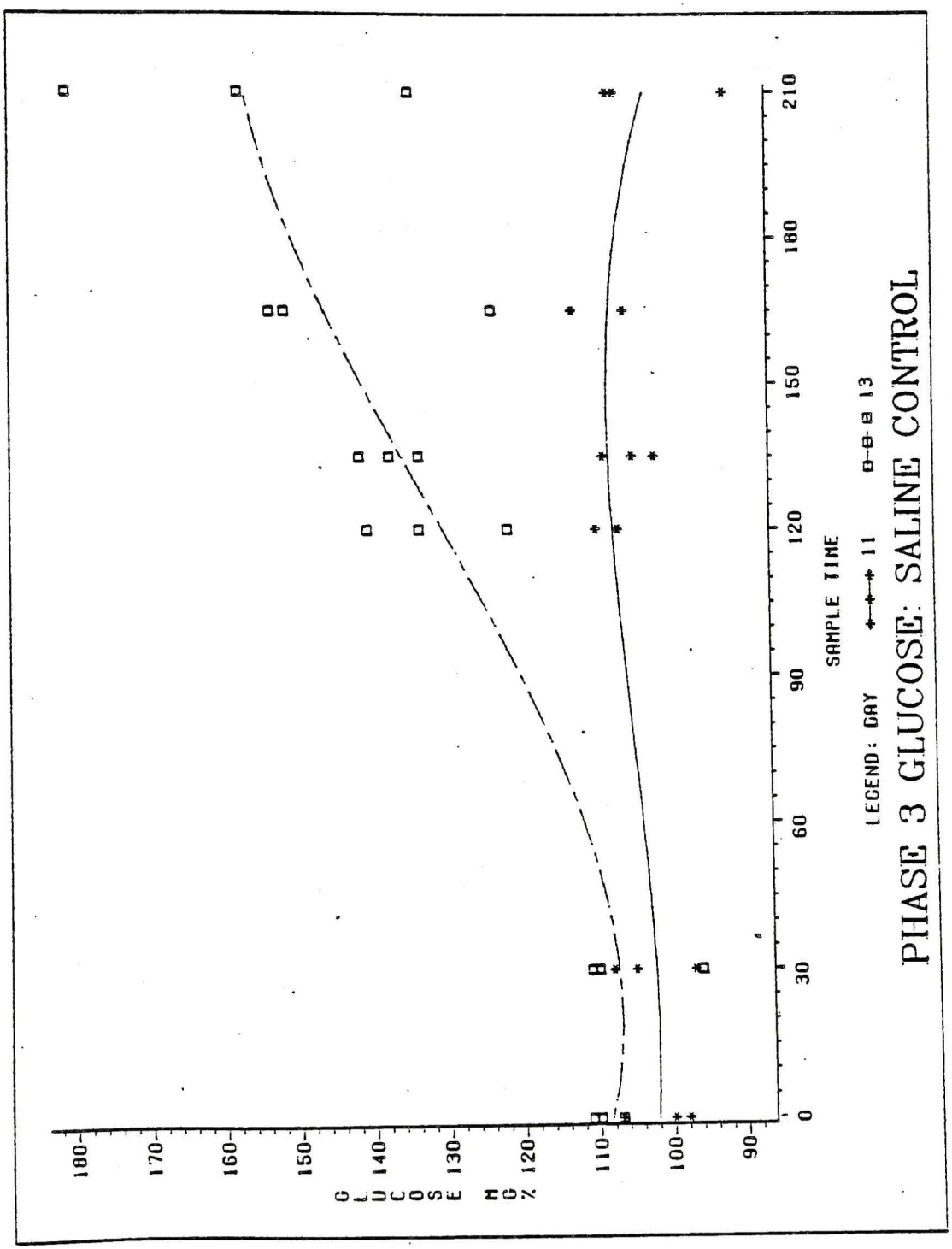
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 GLUCOSE: SALINE CONTROL

FIGURE 72

PHASE III: GLUCOSE RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION

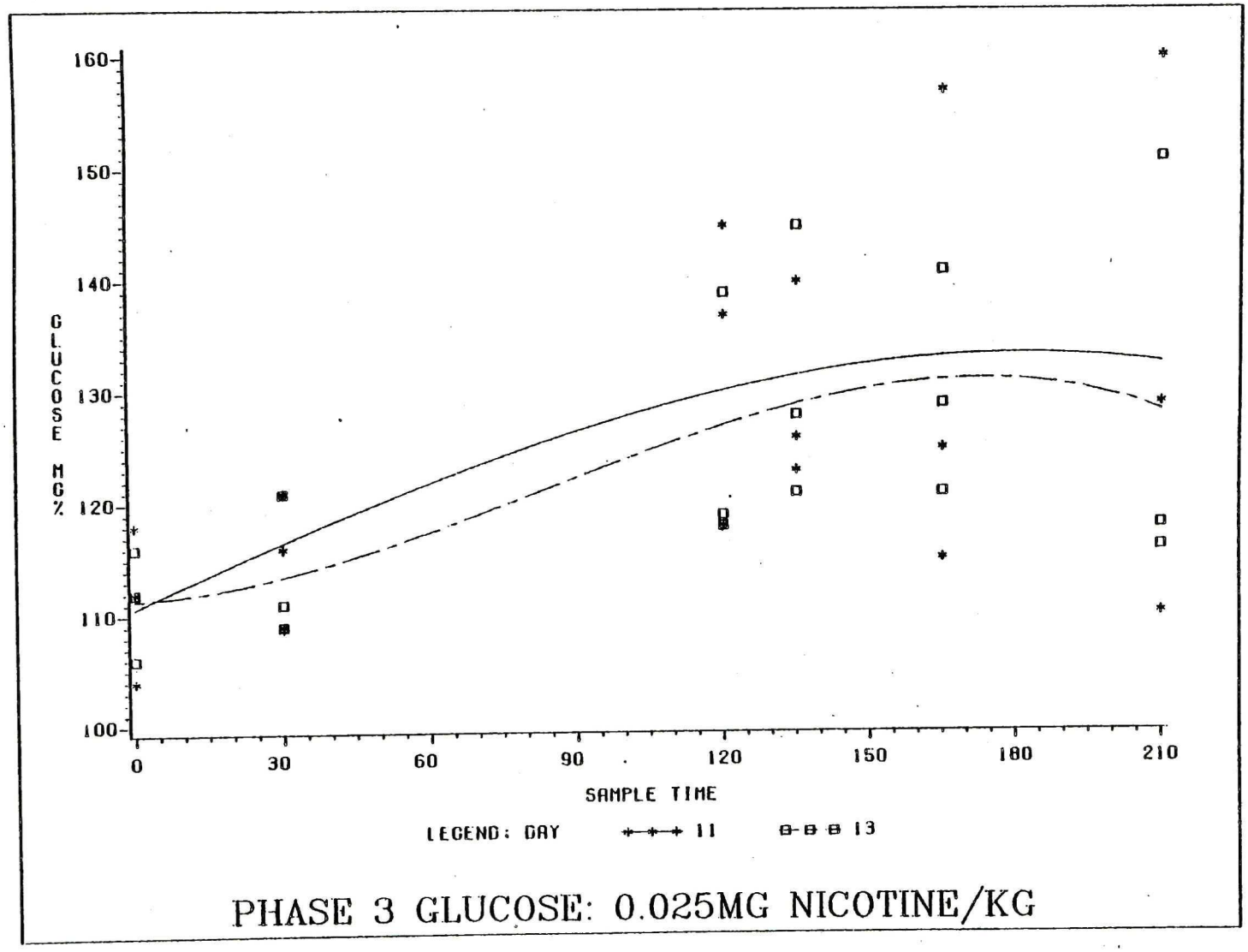
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 GLUCOSE: 0.025MG NICOTINE/KG

FIGURE 73

PHASE III: GLUCOSE RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION

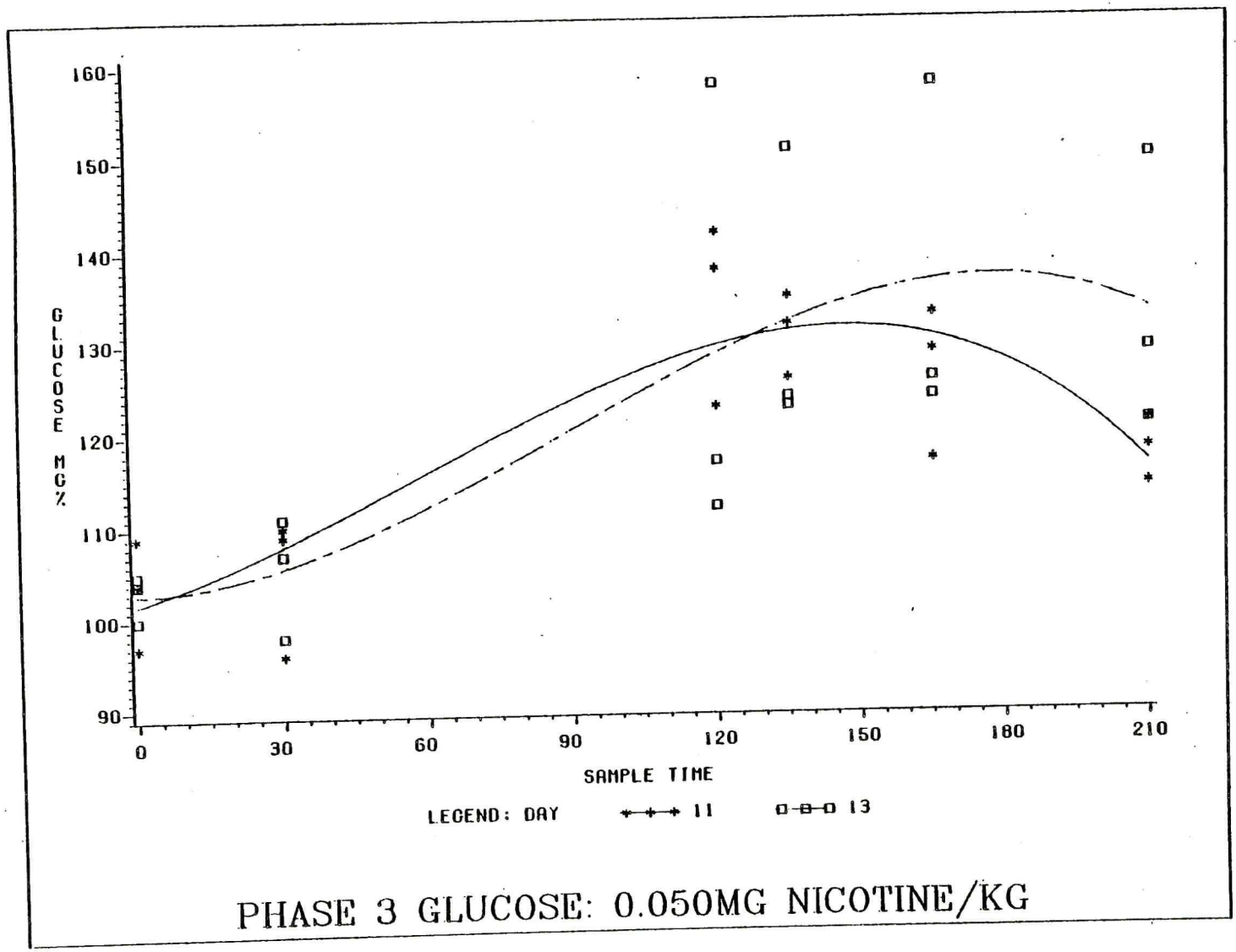
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 GLUCOSE: 0.050MG NICOTINE/KG

FIGURE 74

PHASE III: GLUCOSE RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION

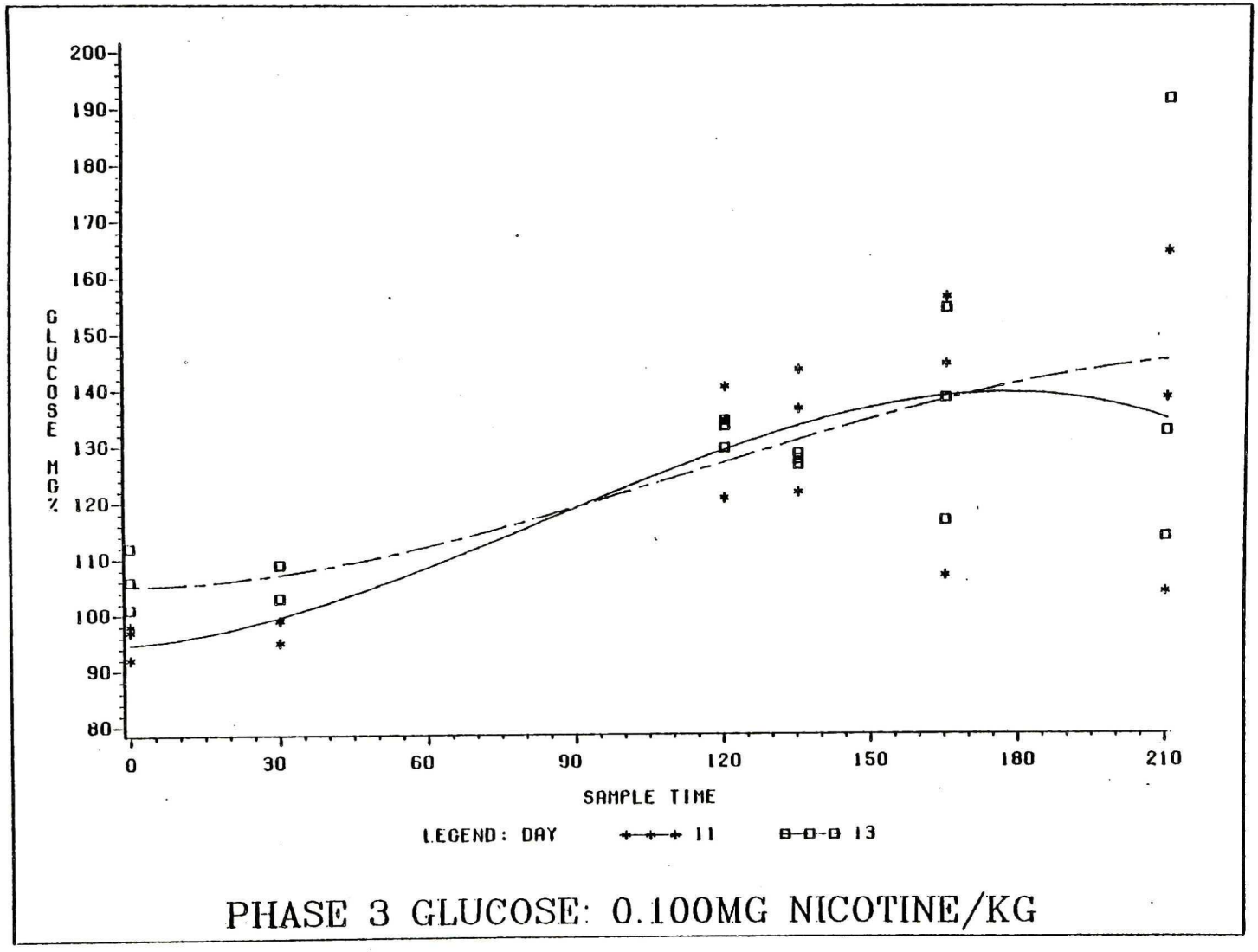
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 GLUCOSE: 0.100MG NICOTINE/KG

FIGURE 75

PHASE III: INSULIN RESPONSES FOR SALINE CONTROL CONDITION

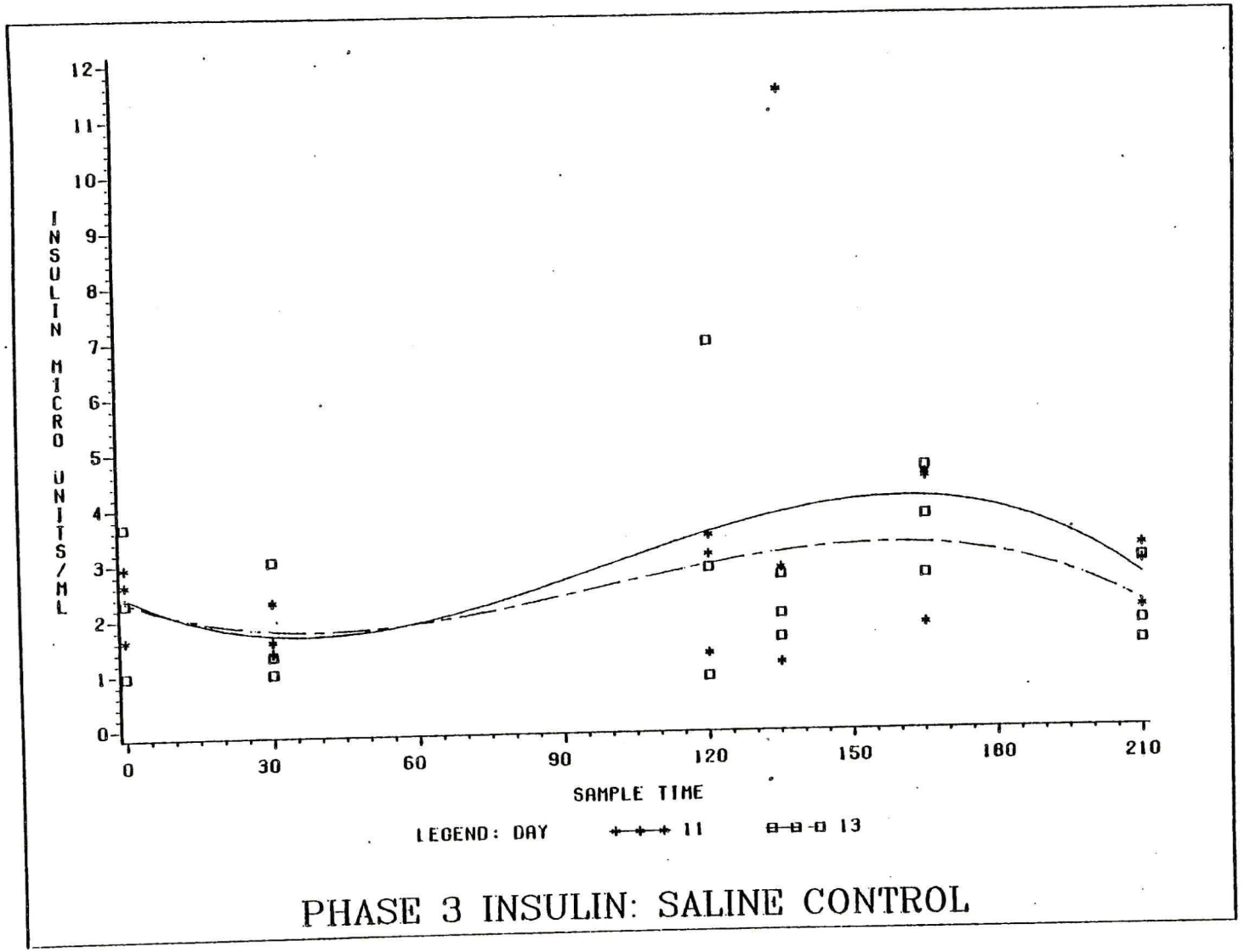
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 INSULIN: SALINE CONTROL

FIGURE 76

PHASE III: INSULIN RESPONSES FOR 0.025 mg NICOTINE/kg CONDITION

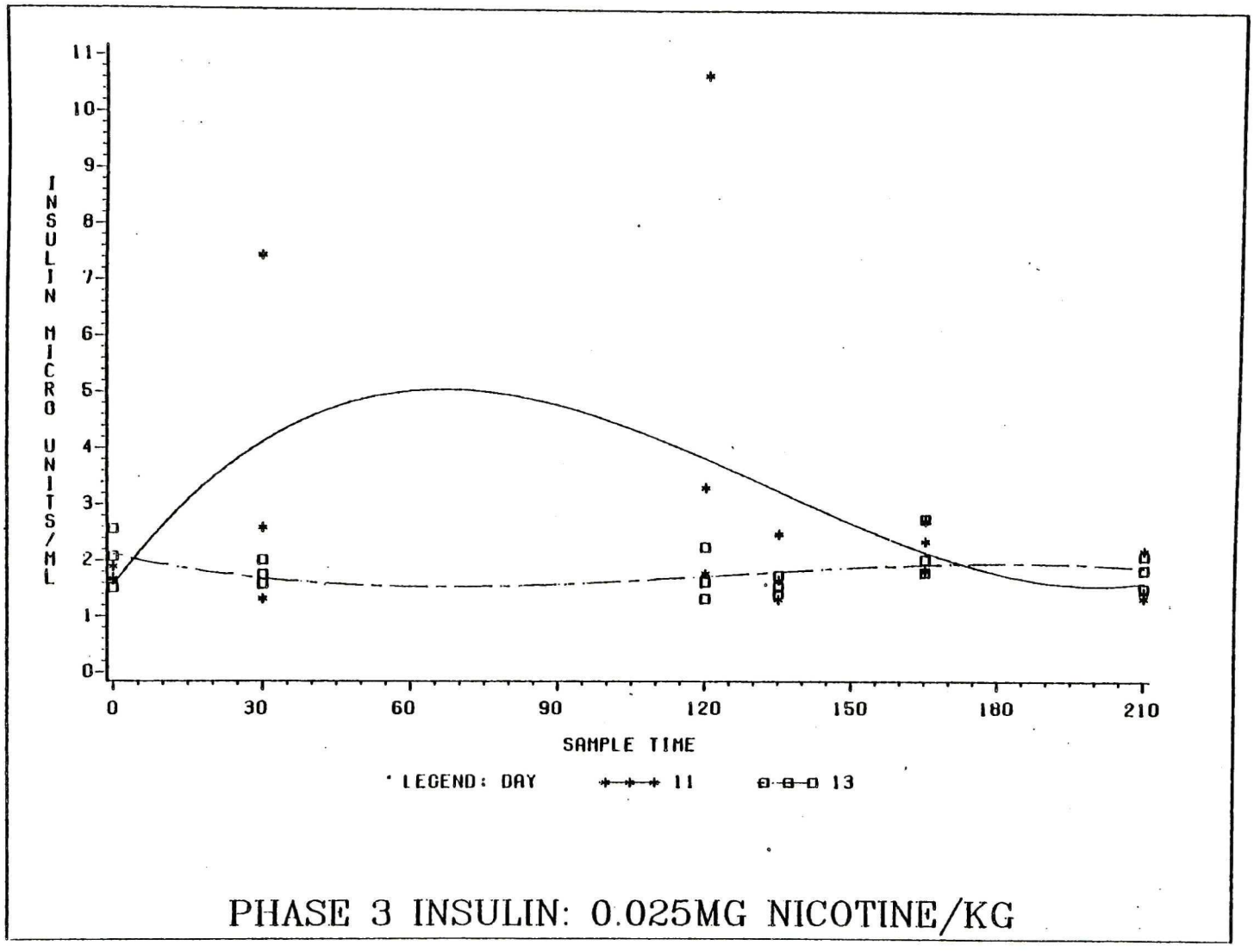
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 INSULIN: 0.025MG NICOTINE/KG

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FIGURE 77

PHASE III: INSULIN RESPONSES FOR 0.050 mg NICOTINE/kg CONDITION

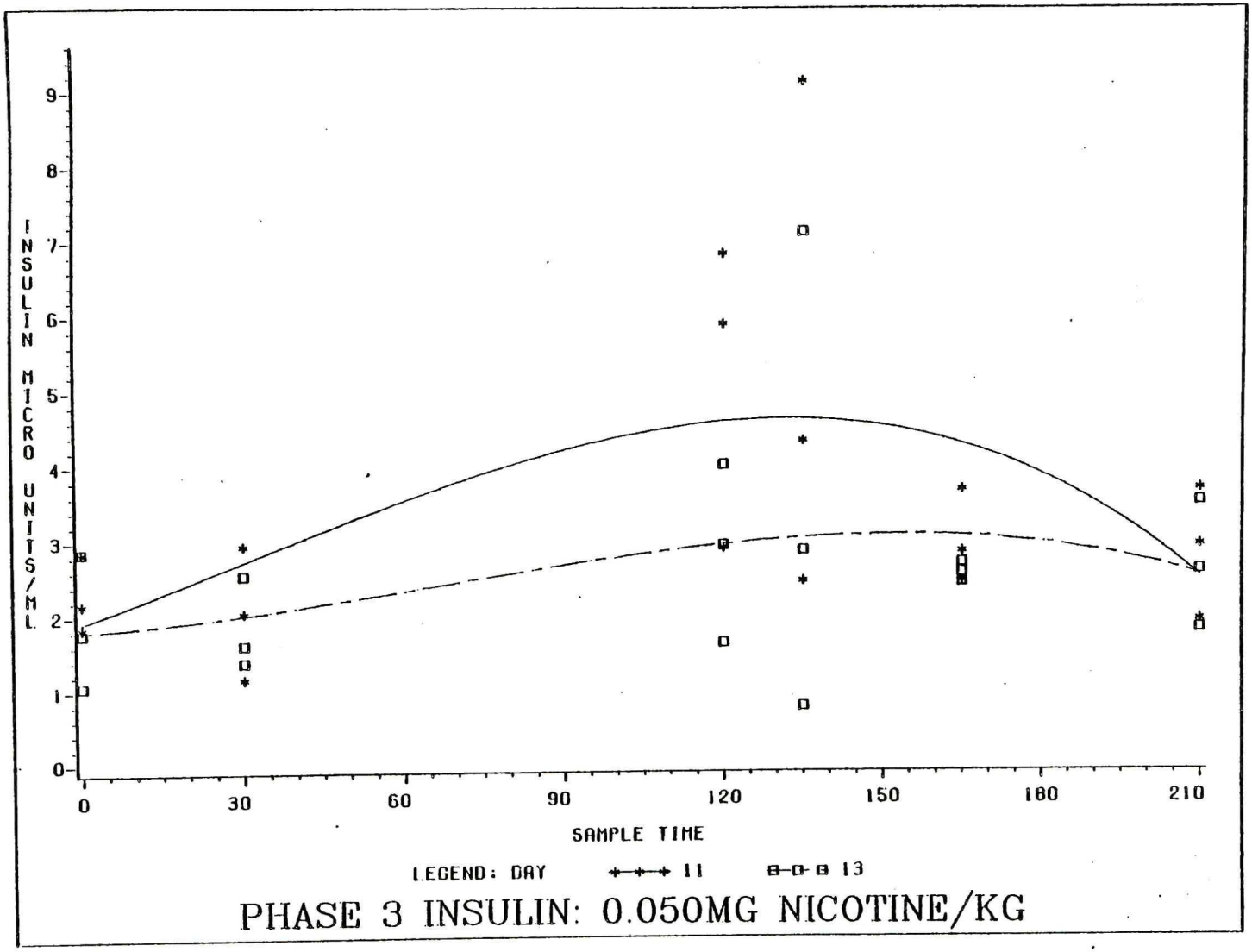
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 INSULIN: 0.050MG NICOTINE/KG

FIGURE 78

PHASE III: INSULIN RESPONSES FOR 0.100 mg NICOTINE/kg CONDITION

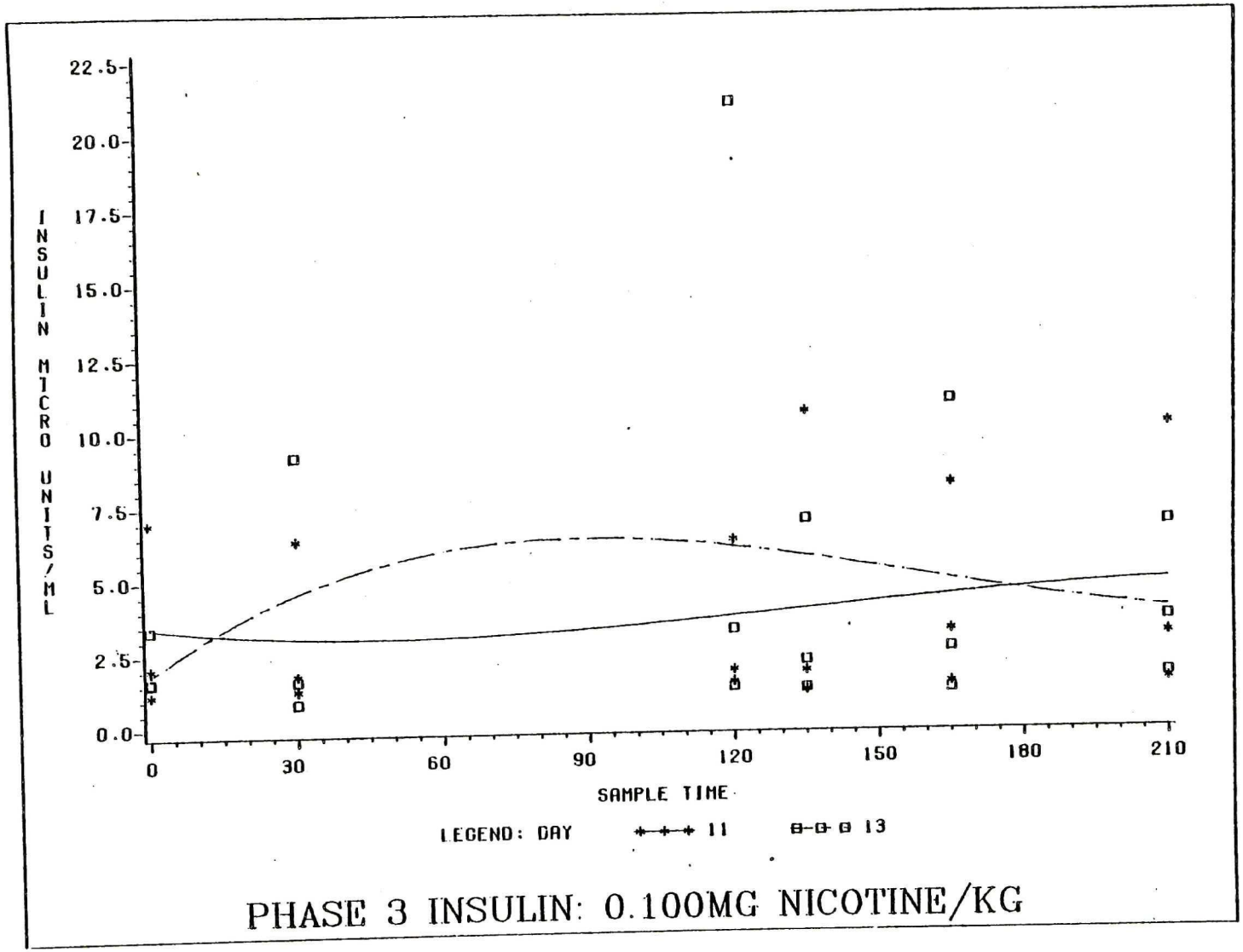
LEGEND

SALINE CONTROL DAY 11 = NO STRESS CONDITION

SALINE CONTROL DAY 13 = STRESS CONDITION

ALL NICOTINE DOSES DAY 11 = STRESS WITH NICOTINE

ALL NICOTINE DOSES DAY 13 = STRESS WITHOUT NICOTINE



PHASE 3 INSULIN: 0.100MG NICOTINE/KG

Reference Notes

1. Cox, B. Personal communication, April 1983.
2. Director, Shade Tobacco Growers Agricultural Association of Connecticut, Personal communication, March 1984.
3. Sylvario, D. and Lewis, E. Wyeth Laboratories, Inc., Philadelphia, PA 19101, Personal communication, January 1983.
4. Bigbee, H. and Boraski, E. Schering, Co. for Burns-Biotec Laboratories, Inc., Omaha, NE 68103, Personal communication, January 1983.

Footnotes

[1]At the present time some controversy exists regarding the duration of the half life of nicotine. The value of 30-60 minutes as presented in the text represents an average value which has been the predominately accepted standard during recent years. However, several recent investigations (Benowitz, Jacob, Jones & Rosenberg, 1982; Benowitz, Kuyt & Jacob, 1982; Kyerematen, Damiano, Dvorchik & Vesell, 1982) have reported two phases to the elimination of nicotine. The first phase of elimination pertains to changes in plasma concentrations of nicotine during initial drug distribution with a half life of 20-60 minutes. The secondary phase of elimination, which includes the elimination of nicotine from tissue stores, has a much longer half life of up to approximately 5 hours.

[2]Within the context of the present experiment no assumptions were made regarding the effects of increasing exposure to nicotine on nicotine induced responding (i.e., if the animals would display tolerance, habituation or sensitization to the effects of nicotine). However, previous research has demonstrated that tolerance to the lethal effects of a given acute dose of nicotine will develop following a few days of chronic exposure to a lower dosage (see review by Larson, Haag & Silvette, 1961). Therefore, it may be argued that the nicotine "experienced" animals which were used in phases II and III of the present study were probably endocrinologically and physiologically similar to chronic cigarette smokers.

[3]The study examined several endocrinological responses to the acute administration of nicotine, and changes in responding as a function of increasing prior exposure to nicotine (i.e., the dose and duration of prior exposure).

[4]A total of sixteen (16) animals were used in the study, twelve (12) animals successfully completed the two weeks of nicotine administration (three animals completed the study in each of the drug dosage conditions). The additional four (4) animals were surgically prepared with intravascular catheters but did not complete the study. Of these four animals, one was assigned to the high nicotine condition, two to the medium nicotine condition, and one to the low nicotine condition (the assignment of animals to the various drug dosages was conducted with the stipulation that an equal number of animals complete the study in each of the experimental conditions, i.e., the animals which died or otherwise prematurely failed to complete the study were subsequently replaced). The two animals receiving the medium nicotine dose did not complete the study due to failure of the intravenous and/or intra-arterial catheters (i.e., the catheters became clogged due to the formation of thrombi which prevented the withdrawal of blood specimens). The two remaining animals failed to complete the study due to their premature death. Premorbid observations and post-mortem examinations indicated that one animal (assigned to the low nicotine condition) died of a cerebral ischemic accident resulting from the migration of a thrombus which had formed around the tip of the arterial catheter, and that the second animal (assigned to the high nicotine condition) died of a massive

myocardial infarction with subsequent perforation of the myocardium resulting in the seepage of fluids into the pericardial sac and cardiac tamponade.

[5]New Zealand White rabbits (Oryctolagus cuniculus) were selected for use in the present study for the following reasons: a) the rabbit is a widely used research animal which has been well defined anatomically, biochemically and physiologically, b) the New Zealand White rabbit (and other strains of rabbits) has been used in previous research pertaining to the effects of cigarette smoking and nicotine (Larson, Haag & Silvette, 1961; Larson & Silvette, 1968, 1975), c) the rabbit is a relatively inexpensive research animal which is readily available, and is easy to handle and to house, and d) the rabbit has a large enough blood volume (estimated at approximately 80 ml/kg of body weight in the 3.0-3.5 kg animal) such that the removal of 18 ml of whole blood (the total volume of blood withdrawn during any one test day) should not have stressed the animal due to the development of hypovolemia and/or anemia.

[6]Under normal circumstances of food availability the wild rabbit is not known to eat any portion of the tobacco plant. In addition, while rabbits and many other animals (deer, wild and domestic dogs, raccoons, rodents, etc.) will enter the tobacco fields, none of these animals are known to eat the tobacco plant (personal communication with the Director, Shade Tobacco Growers Agricultural Association of Connecticut, March 1984).

[7]See Table on following page.

Pre- and Post-Experimental Body Weights by Drug Condition
(all values are in kg)

Drug Condition	Initial Experimental Body Weight	Mean, S.D.	Final Experimental Body Weight	Mean, S.D.
Control (Saline)	3.403		3.01	
	3.450		3.04	
	3.464		3.45	
		3.439		3.17
		0.032		0.245
0.025 mg Nic/kg	3.650		3.56	
	3.274		3.20	
	3.450		3.36	
		3.449		3.37
		0.201		0.180
Failed to Complete Experiment	3.514		3.20	
		3.465		3.33
		0.168		0.171
0.050 mg Nic/kg	3.877		3.55	
	3.148		3.11	
	3.624		3.36	
		3.549		3.34
		0.370		0.221
Failed to Complete Experiment	3.170		3.10	
	3.169		3.08	
		3.397		3.24
		0.334		0.208
0.100 mg Nic/kg	3.646		3.23	
	3.197		3.20	
	3.395		3.09	
		3.413		3.17
		0.225		0.074
Failed to Complete Experiment	3.524		3.40	
		3.440		3.23
		0.192		0.128

[8]The plasma concentrations of glucose and insulin and the sensitivity of tissues to insulin effects change with changes in body weight or obesity. Therefore, it was necessary that the mean body weight for all groups be comparable so as to decrease pre-experimental intergroup variability in glucose and insulin levels.

[9]In the present experiment no attempt was made to determine the actual plasma concentrations of nicotine and cotinine (the primary metabolite of nicotine) which were achieved following each drug infusion. However, on the basis of theoretical estimations (assuming that a relatively insignificant quantity of nicotine was metabolized and/or excreted during the twenty minute infusion period, and assuming that nicotine was approximately equally distributed throughout the total body water) it was predicted that the peak plasma concentration of nicotine which occurred following the initial drug infusion (on each nicotine administration day) should have ranged from 30 ng/ml - 120 ng/ml for the low and high doses of nicotine administered, respectively. While it is not always appropriate to compare the effects of identical doses of a drug in different species (i.e., due to differences in metabolism and excretion, tissue sensitivity, receptor affinity and other factors) (it may be more appropriate to compare the type and pattern of the drug induced responses when examining the effects of a drug in different species), the doses of nicotine used in this study should have produced peak plasma concentrations within the range of concentrations which have been reported in human cigarette smokers (Benowitz, Kuyt and Jacob, 1982; Feyerabend & Russell, 1978).

[10]Measurement of hematocrit was performed as follows: 1) after centrifugation of the blood sample, to separate cells from plasma, the volume of packed red blood cells and of the total sample was determined, and 2) the hematocrit was calculated as the ratio of the volume of red blood cells to the total sample volume, multiplied by 100.

[11]Anemia, as reflected by a low hematocrit, may interfere with normal physiological/endocrinological functioning.

[12]These results are not without some degree of ambiguity. In general, it was found that the administration of nicotine resulted in decreases in circulating levels of insulin. It was also observed that the drug administration procedure induced decreases in plasma insulin (this effect was observed in the responses of control animals which received infusions of physiological saline solution under conditions identical to those during the administration of the nicotine solutions). Insulin responses to the administration of nicotine displayed a somewhat shorter latency than was observed for the control subjects. Potentially more important, it was frequently observed that the administration of nicotine resulted in "spikes" in the release of insulin (i.e., 5-20 fold increases and decreases in the plasma concentration of insulin within a period of 15-30 minutes). These findings and previous empirical research suggest that nicotine (and possibly cigarette smoking) interferes with the normal regulation of insulin homeostatic mechanisms. The ramifications of nicotine induced disturbances in insulin and glucose regulation are not known and warrant future investigation.

[13]Glucose and insulin levels were also measured in samples obtained in Phase III of the procedure. Statistical analyses of these data are presented in Tables 17 and 18 (Parts I-IV, Appendix C, pages 97-106, respectively). The measurements were made for the following reasons: a) to corroborate findings (changes) observed in other systems, and b) to examine additional physiological responses to stress, per se.

It is widely accepted that the regulation of plasma glucose and insulin levels is at least in part controlled by the autonomic (sympathetic) nervous system. Activation of the sympathetic branch of the autonomic nervous system results in increased concentrations of plasma glucose and in decreased concentrations of insulin (this effect is due to an inhibition of the release of insulin from pancreatic islet cells). The homeostatic insulin regulatory system is also directly responsive to changes in plasma glucose concentrations (i.e., a change in plasma glucose levels will induce an increase or decrease in plasma insulin, so as to maintain or reinstate the normal homeostatic concentrations of plasma glucose).

As expected, application of the physical restraint stressor induced increases in plasma glucose concentrations among the control animals (see Figures 71-74, Appendix D, pages 250-257). Similar increases in plasma glucose were observed in the nicotine experienced animals when they were stressed (Stress without Nicotine). Nicotine in conjunction with the stressor produced effects which were similar to, or somewhat larger than, the responses induced by the stressor alone. Insulin responses were considerably more variable (see Figures

75-78, Appendix D, pages 258-265), suggesting that nicotine and restraint stress (activation of the sympathetic autonomic nervous system) interfered with normal insulin homeostatic responses.

These findings coincide with expectations and suggest that the restraint stressor was sufficiently intense to induce changes in the activity of the autonomic nervous system.

[14]The pattern of effects described was most evident in norepinephrine responses. Some evidence of similar, although smaller, changes was apparent in epinephrine and dopamine responses. See figures 5, 10 and 15 for the graphic presentation of these effects.

[15]Following euthanasia all of the experimental animals underwent a post-mortem examination. Initially, it was intended that these examinations be conducted in order to determine if any pathophysiological conditions were present which might have interfered with the normal activity of any of the systems being studied. However, during the first series of post-mortem examinations it was noted that there were gross morphological differences evident in the cardiac musculature of those animals which had been receiving nicotine as compared with corresponding tissues from animals in the saline control group. The morphological changes which were noted included a marked enlargement of the heart (this effect appeared to be most prominent in the ventricles) up to three times normal size (comparing the high nicotine group to the saline control group). It was subsequently decided that during each successive post-mortem procedure a tissue specimen (cardiac muscle) be taken and preserved for histologic examination.

The tissue specimens obtained were examined in the following manner: a) cross-sectional slices of the heart were made at the level of the mid-ventricle (i.e., halfway between the apex and the base of the heart), and b) the tissue sections were examined for variations in the thickness of the myocardial wall of the left and right ventricles (four measurements were taken of each section with the thickest and thinnest reported here).

Drug Condition	Right Ventricle	Left Ventricle
	(One unit = 30 microns)	
Control	36 - 60 units	122 - 155 units
0.025 mg Nic/kg	24 - 47 units	110 - 138 units
0.025 mg Nic/kg	28 - 52 units	115 - 148 units
0.050 mg Nic/kg	28 - 41 units	86 - 116 units
0.100 mg Nic/kg	23 - 33 units	93 - 127 units
0.100 mg Nic/kg	22 - 42 units	95 - 106 units

These data appear to suggest that the administration of nicotine resulted in a generalized degeneration of the cardiac musculature evidenced by thinning of the ventricular walls and dilation of the heart.

In addition, the extent of the degeneration observed appears to be positively related to the dose of nicotine administered (i.e., the degree of degeneration increased as the dose of nicotine increased). No other tissues examined were found to be consistently affected by the administration of nicotine.

The findings coincide with much of the previous literature which indicates an association between cigarette smoking and an

increased incidence of cardiovascular disorders. The results of the present study suggest that cardiac degeneration is (at least in part) directly related to the ingestion of nicotine. These findings have potentially important implications for the prevention and/or reduction of cardiovascular disorders in smokers. For example, the elimination or reduction of the nicotine content of cigarettes should reduce the incidence of premature morbidity and mortality among smokers due to cardiovascular disorders (i.e., sudden coronary death syndrome, ischemic heart disease and myocardial infarction).

It should be recognized that the conclusions stated above are based on the examination of a very limited number of animals and as such are still tentative. Extensive additional research is necessary in order to better delineate these findings and to examine the many implications involved for the prevention and treatment of smoking related cardiac disorders.

[16]Because no pituitary hormones were measured in this experiment it is not possible to make a positive assertion regarding this topic.

References

Ashton, H., & Stepney, R. (1982). Smoking: Psychology and Pharmacology. London: Tavistock.

Ashton, H., Stepney, R., & Thompson, J. W. (1979). Self-titration in cigarette smokers. British Medical Journal, 2, 357-360.

Ashton, H., & Watson, D. W. (1970). Puffing frequency and nicotine intake in cigarette smokers. British Medical Journal, 3, 679-681.

Balfour, D. J. K., Khullar, A. K., & Longden, A. (1975). Effects of nicotine on plasma corticosterone and brain amines in stressed and unstressed rats. Pharmacology Biochemistry and Behavior, 3(2), 179-184.

Baum, A., Grunberg, N. E., Lundberg, U., & Singer, J. E. (in press). Methods, problems, and results in the study of catecholamines and stress. In, C. R. Lake and M. G. Ziegler (Eds.), Norepinephrine: Clinical Aspects, Baltimore: Williams & Wilkins.

Baum, A., Grunberg, N. E., & Singer, J. E. (1982). The use of psychological and neuroendocrinological measurements in the study of stress. Health Psychology, 1, 217-236.

Beckett, A. H., Rowland, M., & Triggs, E. G. (1965). Significance of smoking investigations of urinary excretion rates of amines in man. Nature, 207, 200-201.

Beckett, A. H., & Triggs, E. G. (1967). Enzyme induction in man caused by smoking. Nature, 216, 587.

Benowitz, N. L., Hall, S. M., Herning, R. I., Jacob, P. III, Jones, R. T., & Osman, A.-L. (1983). Smokers of low-yield cigarettes do

not consume less nicotine. New England Journal of Medicine, 309, 139-142.

Benowitz, N. L., Jacob, P. III, Jones, R. T., & Rosenberg, J. (1982). Interindividual variability in the metabolism and cardiovascular effects of nicotine in man. Journal of Pharmacology and Experimental Therapeutics, 221(2), 368-372.

Benowitz, N. L., Kuyt, F., & Jacob, P. III. (1982). Circadian blood nicotine concentrations during cigarette smoking. Clinical Pharmacology and Therapeutics, 32, 758-764.

Benwell, M. E. M., & Balfour, D. J. K. (1982). Effects of chronic nicotine administration on the response and adaptation to stress. Psychopharmacology, 76, 160-162.

Bhagat, B. (1970). Influence of chronic administration of nicotine on the turnover and metabolism of noradrenaline in the rat brain. Psychopharmacologia (Berl.), 18, 325-332.

Bhagat, B., Kramer, S. Z., & Seifter, J. (1967). The effects of nicotine and other drugs on the release of H^3-norepinephrine and on endogenous norepinephrine levels in the rat brain. European Journal of Pharmacology, 2, 234-235.

Biscardi, A. M., & Wassermann, G. F. (1966). Release of catecholamines from adrenalinogenic and noradrenalinogenic tissue by the action of nicotine "in vitro." Archives International Pharmacodynamics, 159(2), 424-433.

Bizzi, A., Tacconi, M. T., Media, A., & Garattini, S. (1972). Some aspects of the effects of nicotine on plasma FFA and tissue triglycerides. Pharmacology, 7, 216-224.

Carruthers, M. (1976). Modification of the noradrenaline related

effects of smoking by beta-blockade. Psychological Medicine, 6, 251-256.

Clark, M. S. G. (1969). Self-administered nicotine solutions preferred to placebo by the rat. British Journal of Pharmacology, 35, 376.

Crowell, C. R., Hinson, R. E., & Siegal, S. (1981). The role of conditioned drug responses in tolerance to the hypothermic effects of ethanol. Psychopharmacology, 73, 51-54.

Cryer, P., Haymond, M. W., Santiago, J. V., & Shah, S. D. (1976). Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. New England Journal of Medicine, 295(11), 574-577.

Deneau, G. A., & Inoki, R. (1967). Nicotine self-administration in monkeys. Annals NY Academy of Science, 142, 277-279.

Eikelboom, R., & Stewart, J. (1982). Conditioning of drug-induced physiological responses. Psychological Review, 89(5), 507-528.

Eikelboom, R., & Stewart, J. (1981). Temporal and environmental cues in conditioned hypothermia and hyperthermia associated with morphine. Psychopharmacology, 72, 147-153.

Eikelboom, R., & Stewart, J. (1979). Conditioned temperature effects using morphine as the unconditioned stimulus. Psychopharmacology, 61, 31-38.

Eliot, R. S., Todd, G. L., Clayton, F. C., & Pieper, G. M. (1978). Experimental catecholamine-induced acute myocardial necrosis. In, V. Manninen & P. I. Halonen (Eds.), Advances in Cardiology, Vol. 25, Basel: S. Karger, AG.

Eneroth, P., Fuxe, K., Gustafsson, J. A., Hokfelt, T., Lofstrom, A., Skett, P., & Agnati, L. (1977a). The effect of nicotine on

central catecholamine neurons and gonadotropin secretion. II. Inhibitory influence of nicotine on LH, FSH and Prolactin secretion in the ovariectomized female rat and its relation to regional changes in dopamine and noradrenaline levels and turnover. Medical Biology, 55, 158-166.

Eneroth, P., Fuxe, K., Gustafsson, J. A., Hokfelt, T., Lofstrom, A., Skett, P., & Agnati, L. (1977b). The effect of nicotine on central catecholamine neurons and gonadotropin secretion. III. Studies on prepubertal female rats treated with pregnant mare serum gonadotropin. Medical Biology, 55, 167-176.

Eranko, O. (1955). Nodular hyperplasia and increase of noradrenaline content in the adrenal medulla of nicotine treated rats. Acta Pathologica et Microbiologica Scandinavia, 36, 210-223.

Eranko, O., Hopsu, V., & Raisanen, L. (1959). Effect of prolonged administration of nicotine on the medullary volume and the distribution of noradrenaline in the adrenals of the rat, the mouse and the guinea pig. Endocrinology, 65, 293-297.

Eysenck, H. J. (1973). Personality and the maintenance of the smoking habit. In, W. L. Dunn Jr., (Ed.), Smoking Behavior: Motives and Incentives, Washington, D.C.: Winston.

Feyerabend, C., & Russell, M. A. H. (1978). Effect of urinary pH and nicotine excretion rate on plasma nicotine during cigarette smoking and chewing nicotine gum. British Journal of Clinical Pharmacology, 5, 293-297.

Florey, C. DuV., Milner, R. D. G., & Miall, W. E. (1977). Serum insulin and blood sugar levels in a rural population of Jamaican adults. Journal of Chronic Diseases, 30, 49-60.

Frankenhaeuser, M., Myrsten, A. -L., Waszah, M., Neri, A., & Post, B. (1968). Dosage and time effects of cigarette smoking. Psychopharmacologia (Berl.), 13, 311-319.

Friedman, M. I., & Stricker, E. M. (1976). The physiological psychology of hunger: A physiological perspective. Psychological Review, 83, 409-431.

Fuxe, K., Agnati, L., Eneroth, P., Gustafsson, J. A., Hokfelt, T., Lofstrom, A., Skett, B., & Skett, P. (1977). The effect of nicotine on central catecholamine neurons and gonadotropin secretion. I. Studies in the male rat. Medical Biology, 55, 148-157.

Gilman, A. G., Goodman, L. S., & Gilman, A. (Eds.) (1980). The Pharmacological Basis of Therapeutics, (6 edition). New York: Macmillan.

Glauser, S. C., Glauser, E. M., Reidenberg, M. M., Rusy, B. F., & Tallarida, R. J. (1970). Metabolic changes associated with the cessation of cigarette smoking. Archives of Environmental Health, 20, 377-381.

Goldberg, S. R., & Spealman, R. D. (1982). Maintenance and suppression of behavior by intravenous nicotine injections in squirrel monkeys. Federation Proceedings, 41, 216-220.

Goldfarb, T. L., Gritz, E. R., Jarvik, M. E., & Stolerman, I. P. (1976). Reactions to cigarettes as a function of nicotine and "tar." Clinical Pharmacology and Therapeutics, 19, 767-772.

Goldfarb, T. L., Jarvik, M. E., & Glick, S. D. (1970). Cigarette nicotine content as a determinant of human smoking behavior. Psychopharmacologia, 17, 89-93.

Griffiths, R. R., Henningfield, J. E. (1982). Experimental analysis of human cigarette smoking behavior. Federation Proceedings, 41, 234-240.

Grunberg, N. E. (1982). The effects of nicotine and cigarette smoking on food consumption and taste preferences. Addictive Behaviors, 7, 317-331.

Grunberg, N. E., Bowen, D. J., & Morse, D. E. (in press). Effects of nicotine on body weight and food consumption of rats. Psychopharmacology.

Grunberg, N. E., & Morse, D. E. (in press). Cigarette smoking and food consumption in the United States. Journal of Applied Social Psychology.

Grunberg, N. E., Morse, D. E., & Barrett, J. E. (1983). Effects of urinary pH on the behavioral responses of squirrel monkeys to nicotine. Pharmacology Biochemistry and Behavior, 19, 553-557.

Haertzen, C. A., Kocher, T. R., & Miyasato, K. (1983). Reinforcement from the first drug experience can predict later drug habits and/or addiction: Results with coffee, cigarettes, alcohol, barbiturates, minor and major tranquilizers, stimulants, marijuana, hallucinogens, heroin, opiates and cocaine. Drug and Alcohol Dependence, 11(2), 147-166.

Hall, G. H., & Turner, D. M. (1972). Effects of nicotine on the release of H^3-noradrenaline from the hypothalamus. Biochemical Pharmacology, 21, 1829-1838.

Hanson, H. M., Ivester, C. A., & Morton, B. R. (1977). The effects of selected compounds on the self-administration of nicotine in rats. Federation Proceedings, 36, 1040.

Hanson, H. M., Ivester, C. A., & Morton, B. R. (1979). Nicotine self-administration in rats. In, N. A. Krasnegor (Ed.), Cigarette Smoking as a Dependence Process, NIDA Research Monograph 23, Washington, D.C.: Public Health Service, DHEW Publication No (ADM) 79-800.

Henningfield, J. E., & Griffiths, R. R. (1979). A preparation for the experimental analysis of human cigarette smoking behavior. Behavior Research Methods and Instrumentation, 11(6), 538-544.

Herman, C. P., & Kozlowski, L. T. (1979). Indulgence, excess and restraint: Perspectives on consummatory behavior in everyday life. Journal of Drug Issues, 9(2), 185-196.

Herning, R. I., Jones, R. T., Bachman, J., & Mines, A. H. (1981). Puff volume increases when low-nicotine cigarettes are smoked. British Medical Journal, 283, 187-189.

Hickey, R. J., & Harner, E. B. (1973). Ethological and biochemical interactions and their relationships to smoking. In, W. L. Dunn, Jr. (Ed.), Smoking Behavior: Motives and Incentives, New York: John Wiley & Sons.

Hill, P., & Wynder, E. L. (1974). Smoking and cardiovascular disease: Effect of nicotine on the serum epinephrine and corticoids. American Heart Journal, 87(4), 491-496.

Hokfelt, B. (1961). The effect of smoking on the production of adreno-cortical hormones. Acta Medica Scandinavia, 369, 123-124.

Hurvich, L. M., & Jameson, D. (1974). Opponent processes as a model of neural organization. American Psychologist, 29, 88-102.

Jaffe, J. H., & Kanzler, M. (1979). Smoking as an Addictive Disorder. In, N. A. Krasnegor (Ed.), Cigarette Smoking as a Dependence

Process, NIDA Research Monograph 23, Washington, D.C.: Public Health Service, DHEW Publication No (ADM) 79-800.

Jarvik, M. E. (1973). Further observations on nicotine as the reinforcing agent in smoking. In, W. L. Dunn Jr., (Ed.), Smoking Behavior: Motives and Incentives, Washington, D.C.: Winston.

Jarvik, M. E., Glick, S. D., & Nakamura, R. K. (1970). Inhibition of cigarette smoking by orally administered nicotine. Clinical Pharmacology and Therapeutics, 11, 574-576.

Jessor, R. (1978). Psychosocial factors in the patterning of drinking behavior. In J. Fishman (Ed.), The Bases of Addiction, Berlin: Dahlem Konferenzen.

Keppel, G. (1982). Design and Analysis: A Researcher's Handbook, second edition. Englewood Cliffs, NJ: Prentice Hall.

Kiser, J. C., Booher, W. T., & Watts, D. T. (1956). Blood epinephrine levels in dogs following the intravenous administration of nicotine. Archives International Pharmacodynamics, 3, 403-411.

Kovacic, B., & Robinson, R. L. (1970). Drug induced secretion of catecholamines by the perfused adrenal gland of the dog during nicotine blockade. Journal of Pharmacology and Experimental Therapeutics, 175, 178-182.

Kozlowski, L. T., & Harford, M. R. (1976). On the significance of never using a drug: An example from cigarette smoking. Journal of Abnormal Psychology, 85(4), 433-434.

Kozlowski, L. T., Jarvik, M. E., & Gritz, E. R. (1975). Nicotine regulation and cigarette smoking. Clinical Pharmacology and Therapeutics, 17, 93-97.

Kumar, R., Cooke, E. C., Lader, M. H., & Russell, M. A. H. (1977). Is

nicotine important in tobacco-smoking? Clinical Pharmacology and Therapeutics, 21, 520-529.

Kyerematen, G. A., Damiano, M. D., Dvorchik, B. H., & Vesell, E. S. (1982). Smoking-induced changes in nicotine disposition: Application of a new HPLC assay for nicotine and its metabolites. Clinical Pharmacology and Therapeutics, 32, 769-780.

Lang, W. J., Latiff, A. A., McQueen, A., & Singer, G. (1977). Self-administration of nicotine with and without a food delivery schedule. Pharmacology Biochemistry and Behavior, 7, 65-70.

Larson, P. S., Haag, H. B., & Silvette, H. (1961). Tobacco: Experimental and Clinical Studies. Baltimore: Williams & Wilkins.

Larson, P. S., & Silvette, H. (1968). Tobacco: Experimental and Clinical Studies (Supplement I). Baltimore: Williams & Wilkins.

Larson, P. S., & Silvette, H. (1975). Tobacco: Experimental and Clinical Studies (Supplement III). Baltimore: Williams & Wilkins.

Latiff, A. A., Smith, L. A., & Lang, W. J. (1980). Effects of changing dosage and urinary pH in rats self-administering nicotine on a food delivery schedule. Pharmacology Biochemistry and Behavior, 13(2), 209-213.

LeMagnen, J. (1980). The body energy regulation: The role of three brain responses to glucopenia. Neuroscience and Biobehavioral Reviews, 4 (Sup. 1), 65-72.

LeMagnen, J., Devos, M., & Larue-Achagiotis, C. (1980). Food deprivation induced parallel changes in blood glucose, plasma free fatty acids, and feeding during two parts of the diurnal

cycle in rats. Neuroscience and Biobehavioral Reviews, 4 (Sup. 1), 17-24.

Lucchesi, B. R., Schuster, C. R., & Emley, G. S. (1967). The role of nicotine as a determinant of cigarette smoking frequency in man with observations of certain cardiovascular effects associated with the tobacco alkaloid. Clinical Pharmacology and Therapeutics, 8, 789-796.

Maengwyn-Davies, G. D., & Thoa, N. B. (1973). Reactivity to biogenic amines of isolated aortic strips and electrically driven left atria from guinea-pigs after repeated treatment with nicotine. Archives International Pharmacodynamics, 202, 374-382.

Mansfield, J. G., & Cunningham, C. L. (1980). Conditioning and extinction of tolerance to the hypothermic effect of ethanol in rats. Journal of Comparative and Physiological Psychology, 94, 962-969.

Marshall, B. E., & Wollman, H. (1980). General anesthetics. In, Gilman, A. G., Goodman, L. S., & Gilman, A. (Eds.) The Pharmacological Basis of Therapeutics, (6th edition). New York: Macmillan.

Matsukura, S., Sakamoto, N., Takahashi, K., Matsuyama, H., & Muranaka, H. (1979). Effect of pH and urine flow on urinary nicotine excretion after smoking cigarettes. Clinical Pharmacology and Therapeutics, 25, 549-554.

Myrsten, A.-L., Post, B., Frankenhaeuser, M., & Johansson, G. (1972). Changes in behavioral and physiological activation induced by cigarette smoking in habitual smokers. Psychopharmacologia (Berl.), 27, 305-312.

Narita, S., Shibata, O., Waki, S., Egashira, K., & Suzuki, T. (1973). Adrenaline and noradrenaline secretion of the adrenal gland in conscious dogs in response to pilocarpine and nicotine. Tohoku Journal of Experimental Medicine, 110, 303-305.

Perlick, D. (1978). The withdrawal syndrome: Nicotine addiction and the effects of stopping smoking in heavy and light smokers. (Unpublished doctoral dissertation). New York: Columbia University, 1977. In, S. Schachter, Pharmacological and psychological determinants of smoking. Annals of Internal Medicine, 88, 104-114.

Physicians' Desk Reference, (34 edition). (1980). Oradell NJ: C. E. Baker, Jr. pub. for Litton Industries, Inc.

Pomerleau, O. F. (1981). Underlying mechanisms in substance abuse: Examples from research on smoking. Addictive Behaviors, 6, 187-196.

Pomerleau, O. F., Fertig, J. B., Seyler, L. E., & Jaffe, J. (1983). Neuroendocrine reactivity to nicotine in smokers. Psychopharmacology, 81, 61-67.

Poulos, C. X., Hinson, R. E., & Siegel, S. (1981). The role of pavlovian processes in drug tolerance and dependence: Implications for treatment. Addictive Behavior, 6, 205-211.

Rubin, R. P., & Warner, W. (1975). Nicotine-induced stimulation of steroidogenesis in adrenocortical cells of the cat. British Journal of Pharmac., 53, 357-362.

Russell, M. A. H. (1971). Cigarette smoking: Natural history of a dependence disorder. British Journal of Medical Psychology, 44, 1-16.

Russell, M. A. H., & Feyerabend, C. (1978). Cigarette smoking: A dependence on high-nicotine boli. Drug Metabolism Review, 8, 29-57.

Russell, M. A. H., Wilson, C., Feyerabend, C., & Cole, P. V. (1976). Effect of nicotine chewing gum on smoking behavior and as an aid to cigarette withdrawal. British Medical Journal, 2, 391-393.

Russell, M. A. H., Wilson, C., Patel, U. A., Cole, P. V., & Feyerabend, C. (1973). Comparison of effect on tobacco consumption and carbon monoxide absorption of changing to high and low nicotine cigarettes. British Medical Journal, 4, 512-516.

Sandberg, H., Roman, L., Zavodnick, J., & Kupers, N. (1973). The effect of smoking on serum somatotropin, immunoreactive insulin and blood glucose levels of young adult males. Journal of Pharmacology and Experimental Therapeutics, 184, 787-791.

Schachter, S. (1977). Nicotine regulation in heavy and light smokers. Journal of Experimental Psychology: General, 106(1), 5-12.

Schachter, S. (1973). Nesbitt's paradox. In, W. L. Dunn, Jr. (Ed.), Smoking Behavior: Motives and Incentives, New York: John Wiley & Sons.

Schachter, S., Kozlowski, L. T., & Silverstein, B. (1977). Effects of urinary pH on cigarette smoking. Journal of Experimental Psychology: General, 106(1), 13-19.

Schachter, S., Silverstein, B., Kozlowski, L., Herman, C. P., & Liebling, B. (1977). The effects of stress on cigarette smoking and on urinary pH. Journal of Experimental Psychology: General, 106(1), 24-30.

Schachter, S., Silverstein, B., & Perlick, D. (1977). Psychological and pharmacological explanations of smoking under stress. Journal of Experimental Psychology: General, 106(1), 31-40.

Schachter, S., & Singer, J. E. (1962). Cognitive, social, and physiological determinants of emotional state. Psychological Review, 69, 379-399.

Siegel, S. (1977). Morphine tolerance acquisition as an associative process. Journal of Experimental Psychology: Animal Behavior Processes, 3(1), 1-13.

Siegel, S. (1975). Conditioning insulin effects. Journal of Comparative and Physiological Psychology, 89, 189-199.

Siegel, S. (1972). Conditioning of insulin-induced glycemia. Journal of Comparative and Physiological Psychology, 78, 233-241.

Silverstein, B. (1978). An addiction explanation of cigarette induced relaxation. (Unpublished doctoral dissertation). New York: Columbia University, 1976. In, S. Schachter, Pharmacological and psychological determinants of smoking. Annals of Internal Medicine, 88, 104-114.

Silverstein, B., Feld, S., & Kozlowski, L. T. (1980). The availability of low-nicotine cigarettes as a cause of cigarette smoking among teenage females. Journal of Health and Social Behavior, 21(4), 383-388.

Silverstein, B., Kelly, E., Swan, J., & Kozlowski, L. T. (1982). Physiological predisposition toward becoming a cigarette smoker: Experimental evidence for a sex difference. Addictive Behaviors, 7(1), 83-86.

Slotkin, T. A., & Seidler, F. J. (1975). Acute and chronic effects of nicotine on synthesis and storage of catecholamines in the rat adrenal medulla. Life Sciences, 16, 1613-1622.

Solomon, R. L. (1980). The opponent-process theory of acquired motivation: The costs of pleasure and the benefits of pain. American Psychologist, 35(8), 691-712.

Solomon, R. L., & Corbit, J. D. (1973). An opponent-process theory of motivation: II. Cigarette addiction. Journal of Abnormal Psychology, 81(2), 158-171.

Solomon, R. L., & Corbit, J. D. (1974). An opponent-process theory of motivation: 'I. Temporal dynamics of affect. Psychological Review, 81(2), 119-145.

Spohr, U., Hofmann, K., Steck, W., Harenberg, J., Walter, E., Hengen, N., Augustin, J., Morl, H., Koch, A., Horsch, A., & Weber, E. (1979). Evaluation of smoking induced effects on sympathetic, hemodynamic and metabolic variables with respect to plasma nicotine and COHb levels. Atherosclerosis, 33, 271-283.

Stepney, R. (1981). Would a medium-nicotine low-tar cigarette be less hazardous to health. British Medical Journal, 283, 1292.

Stewart, J. E., Booker, W. M., & West, W. L. (1973). Chronic nicotine administration and norepinephrine synthesis in the isolated guinea pig heart. Archives International Pharmacodynamics, 201, 362-367.

Storms, M. D., & Nisbett, R. E. (1970). Insomnia and the attribution process. Journal of Personality and Social Psychology, 16, 319-328.

Su, C., & Bevan, J. A. (1970). Blockade of the nicotine-induced norepinephrine release by cocaine, phenoxybenzamine and desipramine. Journal of Pharmacology and Experimental Therapeutics, 175, 533-540.

Suzuki, T., Ikeda, H., Narita, S., Shibata, O., Waki, S., & Egashira, K. (1973). Adrenal cortical secretion in response to nicotine in conscious and anaesthetized dogs. Quarterly Journal of Experimental Physiology, 58, 139-142.

Taylor, P. (1980). Ganglionic stimulating and blocking agents, Chap. 10. In, Gilman, A. G., Goodman, L. S., & Gilman, A. (Eds.) The Pharmacological Basis of Therapeutics, (6th edition). New York: Macmillan.

Tjalve, H., & Popov, D. (1973). Effect of nicotine and nicotine metabolites on insulin secretion from rabbit pancreas pieces. Endocrinology, 92, 1343-1348.

Tsujimoto, A., Nishikawa, T., Dohi, T., & Kojima, S. (1974). Comparison of pharmacological responses to nicotine and release of catecholamines from the adrenals in dogs and monkeys. European Journal of Pharmacology, 26, 236-242.

Turnbull, M. J., & Kelvin, A. S. (1971). Cigarette dependence. British Medical Journal, 3, 115.

Turner, D. M. (1975). The role of adrenal catecholamines in the release of corticosterone and release of corticosterone and fatty acids by nicotine in the rat. Research Communications in Chemical Pathology and Pharmacology, 12(4), 645-655.

U. S. Department of Health, Education and Welfare. (1979). Smoking and

Health: A report of the Surgeon General, Washington, D.C.: Public Health Service, DHEW Publication No (PHS) 79-50066.

Wack, J. T., & Rodin, J. (1982). Smoking and its affects on body weight and the systems of caloric regulation. American Journal of Clinical Nutrition, 35, 366-380.

Watts, D. T. (1960). The effect of nicotine and smoking on the secretion of epinephrine. Annals NY Academy of Sciences, 90, 74-80.

Westfall, T. C. (1965). Effect of nicotine and nicotine analogues on tissue and urinary catecholamines in the rat. Acta Physiol. Scand., 63, 77-83.

Westfall, T. C., & Brasted, M. (1972). The mechanism of action of nicotine on adrenergic neurons in the perfused guinea-pig heart. Journal of Pharmacology and Experimental Therapeutics, 182, 409-418.

Westfall, T. C., & Brasted, M. (1974). Specificity of blockage of the nicotine-induced release of H^3-norepinephrine from adrenergic neurons of the guinea-pig heart by various pharmacological agents. Journal of Pharmacology and Experimental Therapeutics, 189, 659-664.

Westfall, T. C., & Watts, D. T. (1962). Effect of cigarette smoke on epinephrine secretion in the dog. Federation Proceedings, 21, 195.

Westfall, T. C., & Watts, D. T. (1963). Effect of cigarette smoke on epinephrine secretion in the dog. Proceedings of the Society for Experimental Biology and Medicine, 112, 843-847.

Westfall, T. C., Watts, D. T. (1964). Catecholamine excretion in smokers and nonsmokers. Journal of Applied Physiology, 19, 40-42.

Winternitz, W. W., & Quillen, D. (1977). Acute hormonal response to cigarette smoking. Journal of Clinical Pharmacology, 117, 389-397.

Woods, E. F., Richardson, J. A., Richardson, A. K., & Bozeman, R. F. Jr. (1956). Plasma concentrations of epinephrine and arterenol following the actions of various agents on the adrenals. Journal of Pharmacology and Experimental Therapeutics, 116, 351-355.

Woods, S. C., Decke, E., & Vasselli, J. R. (1974). Metabolic hormones and regulation of body weight. Psychological Review, 81(1), 26-43.

Woods, S. C., & Kulkosky, P. J. (1976). Classically conditioned changes of blood glucose level. Psychosomatic Medicine, 38, 201-219.

Woods, S. C., & Shogren, R. E. (1972). Glycemic responses following conditioning with different doses of insulin in rats. Journal of Comparative and Physiological Psychology, 81, 220-225.

Yanagita, T. (1973). An experimental framework for evaluation of dependence liability of various types of drugs in monkeys. Bulletin of Narcotics, 25, 57-64.