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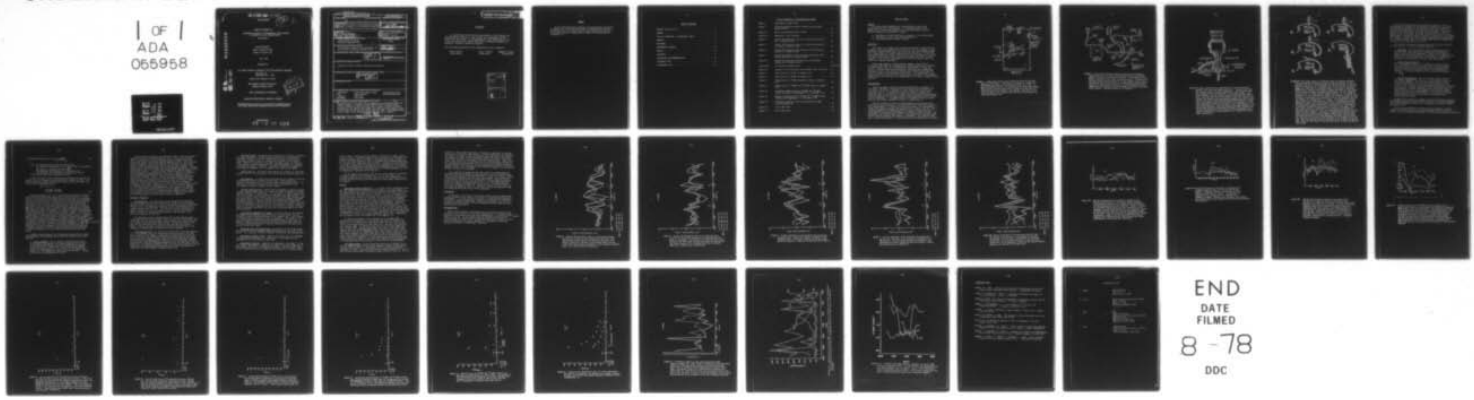

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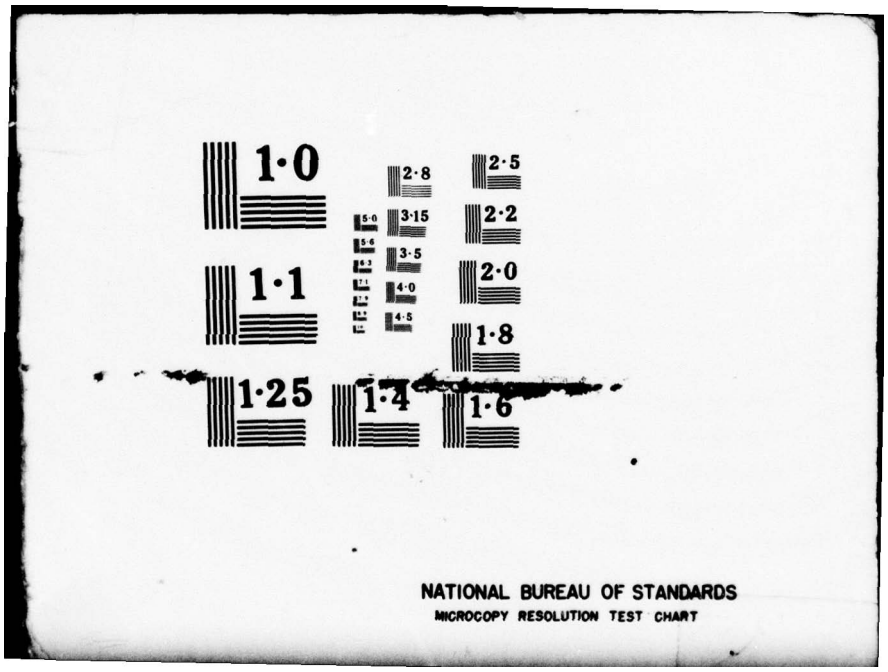
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REPORT NUMBER 007

Biochemical Analysis of Cerebrospinal Fluid Changes in Response to Drug Administration

Final Report

Barry Burns, Ph.D.
James L. Meyerhoff, M.D.
Robert W. Lennox, M.D.

May, 1978

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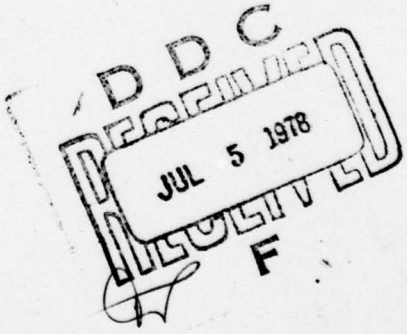

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amphetamine	blood brain barrier (BBB)	cerebrospinal fluid
CSF	blood pressure	sodium pentobarbital
primates	heart rate	
M.mulatta	drug abuse	

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The objectives of this study were to quantify the cerebrospinal fluid (CSF) biochemical changes in response to drug administration in rhesus monkeys. CSF was collected at hourly intervals over a period of months on several primates, and the samples were assayed for levels of neurotransmitter metabolites HMPG, HVA and the nucleotides C-AMP and C-GMP. Drug administration caused changes in the measured parameters (see body of report).

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FOREWORD

In conducting the research described in this report the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council.

The following personnel were supported by this contract

Barry Burns	Prin. Invest.	SS#462-72-3434
Dick Rabold	Technician	174-42-7011

SUMMARY

We have observed diurnal variations in the production rate (ml/hr) and composition of CSF from chronically instrumented rhesus monkeys. In addition, both of the above variables were influenced by the intravenous administration of amphetamine (1 mg/kg) and Na pentobarbital (10 mg/kg), I.V.

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BODY OF REPORT

PROBLEM

A systems-analysis approach has been undertaken to more fully characterize the total drug response. The following facets of the problem will be discussed separately and interrelationships stressed whenever supported by experimental evidence.

A. Validation of blood-brain-barrier integrity in the primate model.
B. Drug effects of CSF secretion and composition.
C. Cardio-respiratory response to drugs.

OBJECTIVES

Since there are a number of interlocking physiologic systems that participate in and perhaps modify drug responses in the intact animal, we have developed a unique primate model in which we are able to continuously monitor a large number of variables under controlled conditions. By utilizing long-term measurements of these variables over weeks and months, we are able to establish the normal diurnal patterns and compare these with the drug responses. Not only does each animal serve as his own control, but is the subject for repeated experiments.

Using rhesus monkeys, we have measured changes in CSF of cyclic nucleotides (AMP, GMP), HVA and HMPG during control periods and in response to certain abused drugs (d, l-amphetamines, pentobarbital, seconal and heroin). Additional physiologic parameters (blood pressure, temperature, respiration, heart rate) and overt behavior were monitored and correlated with CSF changes in neurotransmitter metabolites. The ultimate goal is to establish the central nature of the drug response to several of the commonly abused drugs in the hope that this information will be useful in human clinical medicine.

PREVIOUS WORK

We have developed a model for studying drug effects in primates. In this model, the monkey is maintained in an isolation booth provided with ventilation, white noise, lighting (12 hours on/off) and closed-circuit video-tape (Fig. 1). Catheters are surgically placed in cerebral subarachnoid space for CSF collection, femoral artery for pressure measurements, and in the femoral vein for drug infusion, blood sampling and central venous pressure monitoring. A continuous infusion of heparinized saline is maintained in the vascular catheters to prevent clotting (Fig. 2).

CSF is collected by a chronic system designed and tested under previous periods of this contract. The catheter for CSF collection is placed in the cisterna magna or in the basal cistern and connects to a head-mounted column which prevents movement and maintains strict asepsis (Fig. 3). CSF is collected remotely by a refrigerated (0°C) automatic fraction collector which operates continuously and changes the collection tube every hour. The hydrostatic collection pressure for CSF is determined by the vertical height of the fraction collector with respect to the monkey. The collection pressure is usually maintained at -10 cm water below the interauditory zero plane.

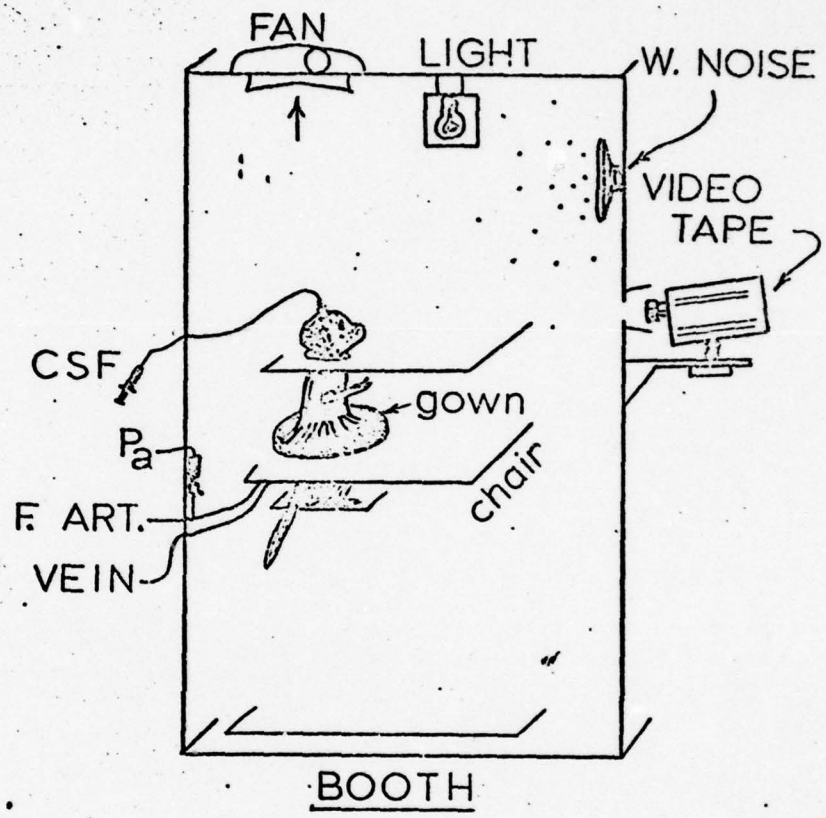

FIGURE 1. Experimental booth which houses the chair and primate during drug studies. The light is attached to a timer, and the monkey may remain in the booth for 24 hr at a time. A small transparent window is provided for the video tape camera. The arterial pressure gauge (P_a) is mounted on the booth in a fixed position with respect to the monkey. The chair is set on rails which permit removal from the booth horizontally for minor adjustments, etc.

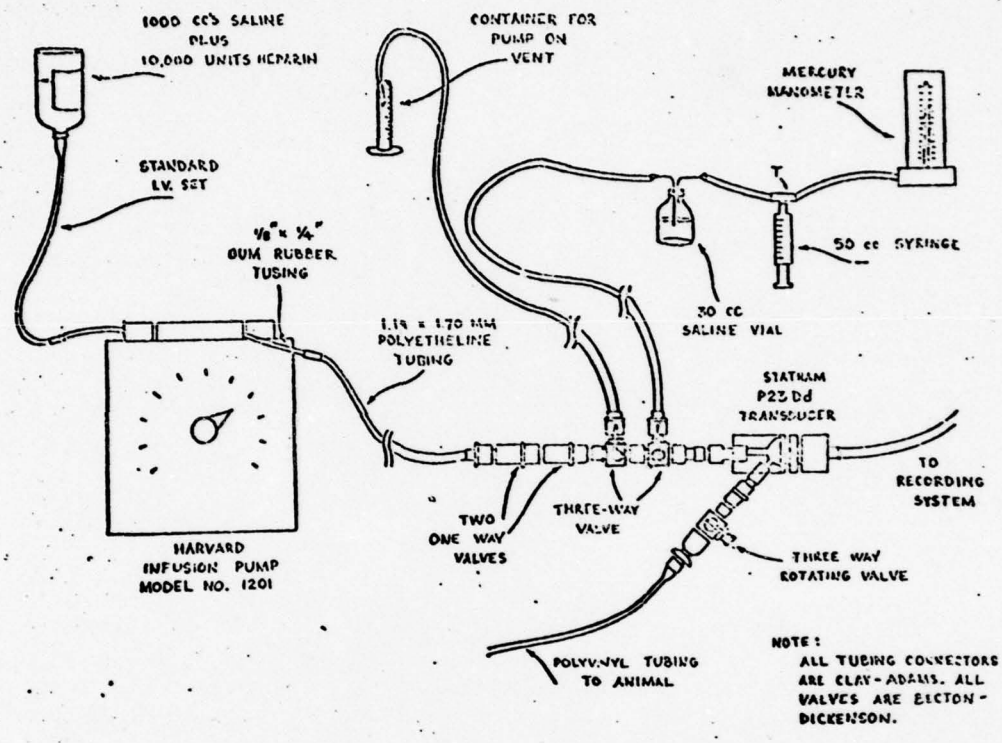

FIGURE 2. System for measuring arterial pressure and providing a constant infusion of saline through both arterial and jugular venous (not shown, but similar) catheters. The pressure transducer is mounted on the booth as shown in Fig. 4. We have tried various concentrations of Heparin in the infusion fluid, and at an infusion rate of 200 cc/day, a minimum Heparin concentration seems to be 1000 Units/liter saline. In some studies, we have infused lactated Ringers solution instead to maintain the plasma ionic balance. Drawing courtesy of D. Randall.

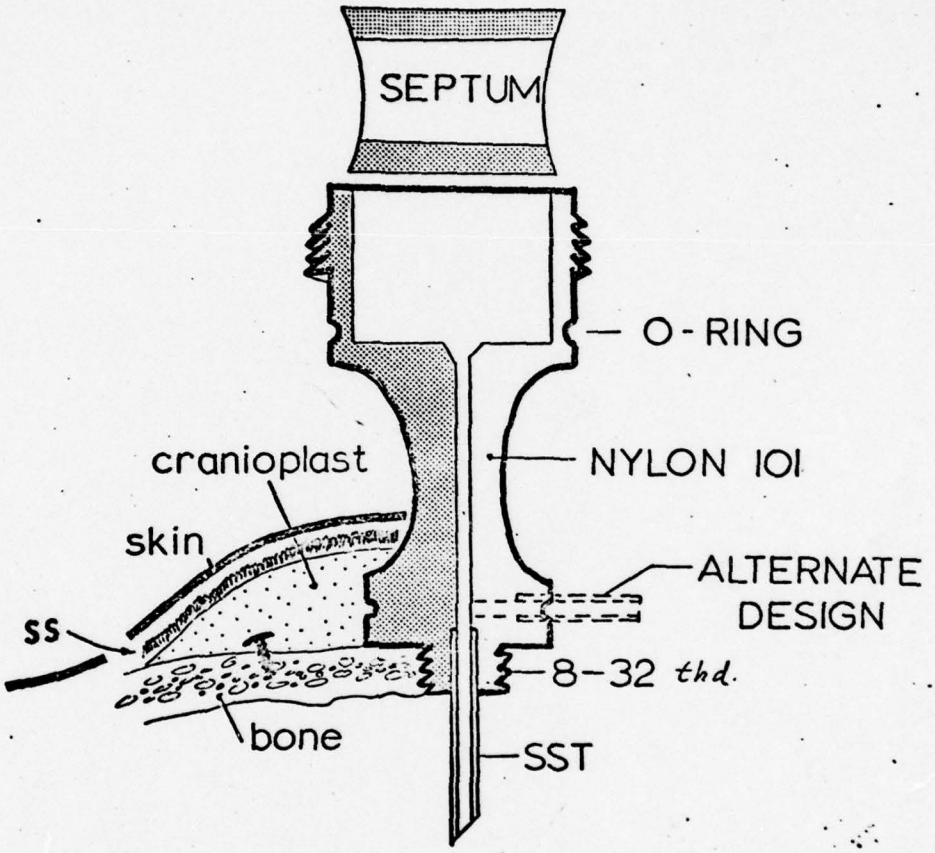

FIGURE 3A Nylon column which is head-mounted to provide continuous access to CSF while maintaining sterility. In most of our work the alternate design is used; connected to a sub-cutaneous catheter leading to the cisterna-magna or the basal cistern. The entire assembly (cap not shown) is steam autoclaved and implanted at least one week prior to placement of the CSF catheter. This reduces the bleeding from the surgery necessary for column placement at the time of sub-arachnoid space catheterization. A germ-tight seal between the skin and the column is provided by a circle of synthetic stroma (SS) attached to the cranioplast by silicone rubber adhesive. This has reduced the column infection rate to zero and prevented subcutaneous infections from reaching the CNS by progressing along the outside of the CSF catheter. The septum is pierced by a sterile needle with a length of tubing to begin sampling CSF.

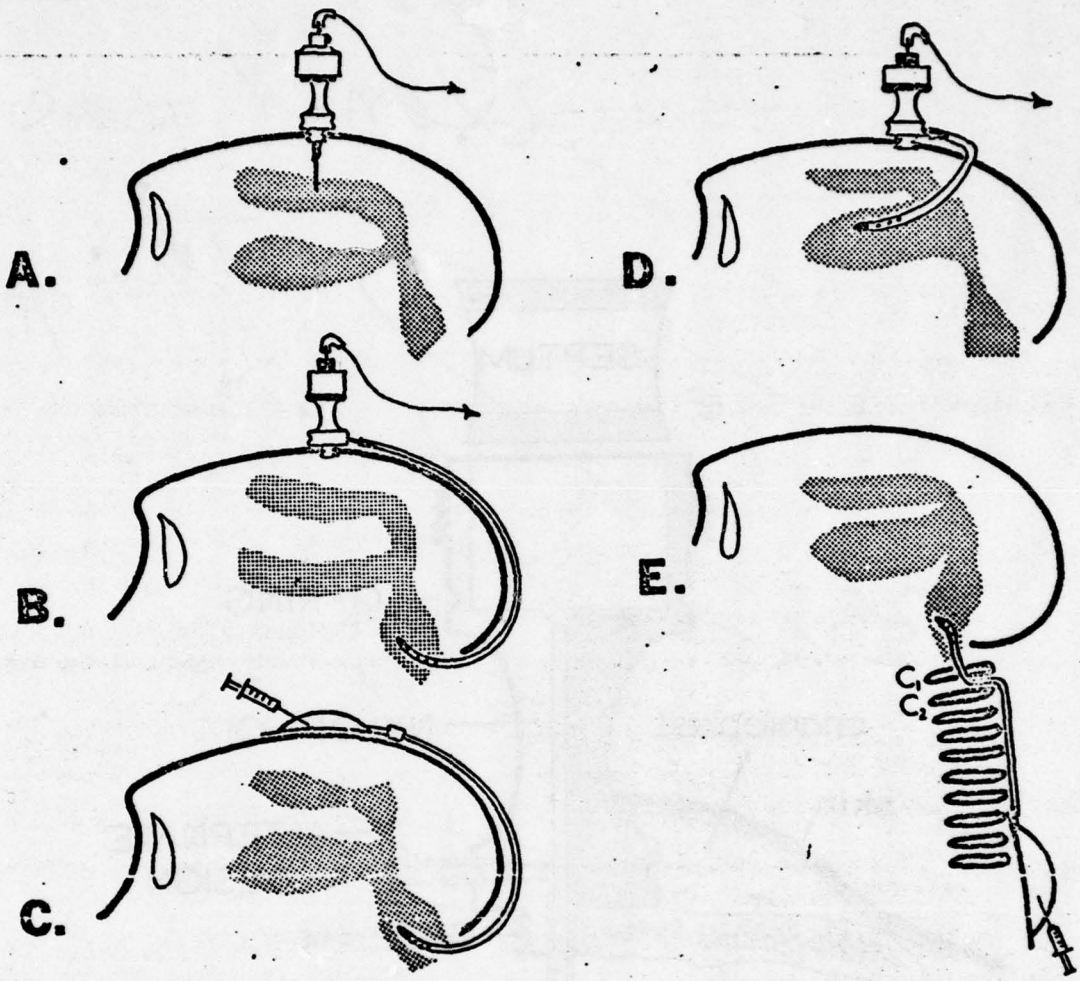

FIGURE 10 This figure depicts the various types of sampling techniques for obtaining CSF evaluated during this contract period. An approximate outline of the ventricular system from lateral to cisterna magna is represented by the shaded area. The column in (A) was designed for continuous or repeated access to the lateral ventricle. Whenever the sampling tube becomes clogged with debri or cell growth it is removed, cleaned, autoclaved and replaced. It will normally function for 1- 2 weeks between removals and for up to 4 months for continual use with intermittent removal and cleaning. Depth of penetration is controlled by an adjustable stop on the sampling tube and the septums are changed every 1- 2 weeks. (B) represents an alternate design with a side-hole exit to which is attached the tubing leading to either the cisterna magna or the lateral ventricle (D). The subcutaneous Ommaya reservoir is depicted in (C), connected to a cisternal catheter and positioned on the top of the head. Somewhat easier access is guaranteed if the reservoir is located on the lower back region as in (E), not requiring immobilization of the head to collect samples. Dead space is appreciable and the reservoirs must be manually flushed several times a day to ensure freedom from infection in the large dependent dead space and to enable the contents to accurately reflect changes in the composition of the CSF. The flushing also tends to blow small bits of debri or adherent cells from the side holes at the catheter tip in the CSF, but must be done slowly.

One of our primary goals was to quantitate changes in levels of neurotransmitter metabolities and additional substances as they relate to drug effects; therefore it was essential to show that the blood-brain-barrier (BBB) between the blood and CSF was intact. Since metabolites and other substances in the CSF are also present in varying amounts in the blood plasma, the drugs tested could result in a loss of BBB integrity, and then it would be possible for plasma levels of a dependent variable to affect CSF levels of rapid exchange between compartments. It is readily apparent that such an effect would seriously confound the interpretation of drug effects in the CNS.

The transfer of substances between blood and CSF can be considered to occur by any or all of the following mechanisms:

1. Bulk Flow: Bulk flow operates on existing hydrostatic pressure gradients, and requires free communication through gaps, spaces, or pores between compartments. Water and ions move together and no osmotic gradients are established. An example of this would be the unidirectional flow of CSF through the parasagittal arachnoid villi and spinal nerve roots into venous blood.

2. Simple Passive Diffusion.
This is described by Fick's law of diffusion in which the flux (J) of any molecular species is always down its electrochemical potential gradient and is proportional to the molecular diffusivity (D), concentration gradient (dC/dx) in the membrane of thickness "x", and the surface area (A) available for diffusion.

$$J = -DA \frac{dc}{dx} \qquad (1)$$

3. Facilitated Diffusion: This is a process in which the passive flux is augmented by a flux due to some molecular carrier in the exchange membrane. The total flux, which consists in this instance of the passive flux (J) and the facilitated flux (J_F), is also in the direction of the electrochemical potential of the diffusing species. Transport gradient of this type demonstrates saturation kinetics; however, facilitated flux (J_F) does not require energy (ATP) and is not sodium-dependent. Facilitated transport could be converted to active transport if the solute were actively detached from the carrier on one side of the membrane by an energy requiring process.

We would like to take just a moment to discuss the kinetics of facilitated diffusion because of the possible relevance to our recent experimental findings regarding diffusion of the norepinephrine metabolite (MHPG) from blood to CSF.

The flux facilitated only of a molecule whose transport is facilitated by a carrier of some sort has been found empirically to adhere to (Stein, '67) the following relationship, so long as the downstream concentration

is maintained effectively at zero: $J_F \simeq \frac{So \cdot Vmax}{So + K_m}$ (2)

where J_F= Forward unidirectional facilitated flux.
S_o= Substrate concentration at the "high" side of the gradient
Km= Substrate concentration when $V=\frac{Vmax}{2}$
Si= Substrate concentration at the downstream end
Vmax= Maximal rate of movement of substrate by carrier
A flux to this sort obeys saturation kinetics.

In the case where a finite downstream concentration of substrate is present (Si ≠ 0), one must then consider what is termed "net flux". Net flux is simply the algebraic sum of the forward and reverse fluxes, and is given by the following equation:

$$J_{Net} = J_F - J_R$$

$$= \frac{So\ Vmax}{So + Km} - \frac{Si\ Vmax}{So + Km} \quad (3)$$

This relationship in eq. 3 is a consequence of the fact that facilitated transport systems work equally well in either direction. If the carrier is saturated by high concentrations on both sides of the membrane, there will be no net flux, but there will be facilitated flux at maximal velocity in both directions. Facilitated flux is still taking place in this instance, but it is not resulting in any net movement of solute. This point is key to one possible interpretation of our data on labeled MHPG flux from blood to CSF. We found in these experiments that labeled MHPG moved from blood to CSF against the apparent concentration gradient. This finding suggested the presence of a carrier transport system for MHPG between blood and CSF. In facilitated transport systems, it is possible to demonstrate a phenomenon known as counter-transport, which is "apparent" uphill movement of a solute against its apparent concentration gradient. Plasma levels of MHPG are generally lower than CSF levels, therefore if MHPG is actively transported, the available evidence suggests that the higher concentration is in CSF (Sjoquist, Lindstrom, and Anggard, J. Neurochem., in press) and therefore the flux into CSF must be greater than the flux out. In comparison with active transport, the facilitated diffusion mechanism is simpler, requires no metabolic energy and operates on the existing concentration gradient, i.e., net flux would be out of CSF into the blood.

A complete understanding of the relationship between CSF and plasma MHPG presupposes some knowledge of the absolute concentrations at each location.

4. Active transport: This is another saturable process whereby the species transported can be moved independent of the existing concentration gradient, and which requires energy usually in the form at ATP. This process can be inhibited by metabolic poisons or sodium depletion. It is similar to facilitated diffusions, but the forward and reverse rates are not equal. (Iodide, thiocyanate, p-aminohippurate and others)

Several anions are known to be removed from CSF at rates far greater than the influx rate from blood (Davson, 1955). These ions have been shown to move against an electrochemical potential gradient into blood (Pollay and Davson, 1963; Davson and Hollingsworth, 1973). With the exception of thiocyanate, iodide and bromide, the substances actively transported by cerebral choroid plexus are the same as those secreted by the kidney tubules, implying perhaps some similar enzymatic pathways. Isolated choroid plexus will also actively take up quaternary ammonium bases (Tochino and Schanker, 1965 a). A similar uptake mechanism exists for biogenic amines (epinephrine, norepinephrine, etc.) and is competitively inhibited by the quaternary ammonium bases and eliminated by metabolic poisons (Tochino and Schanker, 1965 b). Studies of hexose transport have shown that glucose is actively taken up by choroid plexus (C'Saky and Rigor, 1964) and xylose concentrated by cortical tissue slices (Gilbert, 1965). Certain of the amino acids are concentrated by CNS active transport mechanisms (Caver, 1965; Tsukada, et al, 1963) which can be affected by DNP-an uncoupler of oxidative phosphorylation. In our own studies (Reports 01, 02) with primates, we have demonstrated that transport of homovanillic acid (HVA) and 5-hydroxyindole-acetic acid (5-HIAA) out of the CSF can be blocked in-vivo by I.P. sodium probenecid administration. Whether this transport of HVA and 5-HIAA may be active or simply facilitated is an interesting question, but one which we have not addressed.

METHODS OF PROCEDURE

CSF Collection. The technique has been developed during previous contract periods, and the newest modification consists of a refrigerated fraction collector mounted on the back of the primate booth on an adjustable vertical track. All samples are collected at 0°C, centrifuged prior to storage and maintained at -90°C. The fractionator holds a maximum of 50 samples, however only 24 are collected at once. The pressure at which CSF is collected is regulated by adjusting the vertical height of the collection apparatus on the booth.

The procedure involves catheterization of the sub-arachnoid space in the region of the cisterna magna or the basal cistern. We have been concerned that this CSF might not truly reflect changes occurring in more central regions of the peri-ventricular systems. It would be useful to compare the CSF obtained from the ventricle with that from the cisterna-magna. It may be possible to obviate the need for blocking drugs if the ependyma and choroid plexus in ventricle IV can be bypassed.

CSF Production Rate. The secretion rate for CSF is measured volumetrically. This method is valid so long as the outflow pressure at the catheter end distal to the monkey is maintained at constant pressure with respect to some arbitrary hydrostatic pressure reference point on the monkey. In previous acute studies, we have found a very close correlation between CSF production rates measured by the dilution of a non-diffusible indicator such as inulin, and that estimated volumetrically at the same time. The CSF production is an important parameter since it can be used to normalize the concentrations of neurotransmitter metabolites/ml of CSF, providing some insights into the actual amounts released per unit time.

Restraint System. The monkeys are maintained in a specially constructed booth, provided with internal lighting, white noises and ventilation. The monkey perches in a primate restraining chair and is fitted with a nylon gown to prevent access to catheter exit locations on the lateral flank. Behavior is monitored on an Ampex videotape system. All CSF and blood samples, pressure measurements and drugs administered are accomplished without the animals' awareness. Respiration is measured by means of a high frequency impedance device attached to the thorax of the monkey.

Chronic Devices. The chronic head-columns and catheters are described in Fig's 2 and 3. The dead-space of the CSF system is relatively small (0.1 cc).

Data Analysis. At present we are recording up to 8 channels of data on an ink-writing recorder acquired for this work. Chart records are analyzed by hand due to lack of suitable computer interfacing devices. All pressures are recorded in mm Hg and heart rate is monitored by a biotach computer coupler attachment for the recorder.

Arterial Blood Pressure. Femoral arterial pressure is measured through a 15 ga. silicone rubber catheter, treated with TDMAC and heparin to resist thrombus formation post operatively. In all animals, a femoral venous catheter is also placed to sample blood and administer drugs and medicines. The tip of the femoral venous catheter may be placed in the right atrium to monitor central venous pressure and evaluate right heart performance following drug administration. Pressure catheters are connected to Statham P23De transducers mounted on the booth at the level of the right atrium. The catheters are approximately 200 cm long and the system has a natural response in excess of 60 Hz with a damping ratio of 0.22% critical or better. All pressures are recorded continually each day, and calibrations made daily with a mercury manometer.

Post-Surgical Management of Monkeys. The distal ends of all catheters are brought out through the skin on the back or lateral flank, and exit the booth through a sound-proofed hole in the adjacent wall. Strict asepsis is carefully observed during all surgical procedures. All sensors, catheters and pressure transducers are gas sterilized prior to their use. To maintain catheter patency, continuous infusions of heparinized (1000 U/l) saline are maintained through all vascular catheters at a rate not exceeding 200 cc/day total fluids.

Respiratory Rate and Tidal Volume. Respiratory rate and tidal volume are calculated from the impedance pneumotach attached to the thorax of the monkey. It is possible to obtain a bipolar ECG from the same electrode pair.

Biochemical Analyses on CSF. Analyses on CSF for levels of C-AMP/GMP was done at Walter Reed by Dr. R. Lennox and J.L. Meyerhoff, who collaborated with us in all phases of this work.

Experimental Protocol. Surgeries are completed in two stages. In the first stage, the column is attached to the skull with a short length of silicone rubber tubing left sub-cutaneously (sealed). After at least 1 week, the sub-arachnoid catheter is implanted and attached to the tubing leading

to the column. Following the last surgery, a period of 1-2 weeks is permitted to elapse to establish baseline values for CSF levels and permit full recovery from the surgical anesthetic. The experimental drug is given I.V. without the animals awareness, and all parameters discussed above monitored on a 24 hour basis before and after the drug. A period of at least two days is allowed to elapse before repeating the drug experiment at the low doses. Longer intervening periods were permitted at the higher doses, the duration of which will depend on the plasma half-life of the drug (normally $T_{\frac{1}{2}} \simeq 7$ hr for amphetamine, depending on the blood pH).

The CSF samples were usually taken hourly; however when it is possible to perform assays on smaller volumes (0.5 - 1.5 cc) the samples are collected automatically at half hour intervals for the two hours prior to the drug and for four hours after the drug, all other samples taken at hourly intervals.

RESULTS

BLOOD-BRAIN-BARRIER PERMEABILITY. In separate studies designed to validate the integrity of the blood-CSF barrier (BBB) in our primate model, we have administered both diffusible and non-diffusible indicators in the blood and measured their appearance rates in the CSF. This is a key aspect of the previous work, since most of the substances of interest in CSF are also present to varying extents in the blood. The possibility of contamination of CSF from blood by bulk flow could exist if CSF pressure were negative with respect to brain capillary or cerebral venous pressure. This reverse sort of flow for CSF could only occur however under abnormal conditions which might result in loss of integrity for the blood-brain-barrier. Infection, changes in serum osmotic pressure, etc. are all able to cause leakage of solutes (water and ions) from plasma to CSF. We have tested the possibility of such leaks in several animals, both normally and following a drug (d-amphetamine, 1 mg/kg I.V.). The results demonstrate the intact nature of the blood-CSF barrier in our chronic preparation (Fig. 9,10). Amphetamine did not appear to alter the permeability of the BBB (Fig. 11).

In separate experiments on the permeability of the BBB to HMPG, a metabolite of norepinephrine, we did observe some apparent crossing from plasma to CSF in a monkey with a functioning BBB. We do not know at this time if the crossing was due to free label, or if the label was associated with authentic HMPG. We intend to check this by TLC of these samples for free vs bound label. While we did observe some label in CSF after plasma administration of labeled HMPH/HMPG-sulfate, this fractional amount crossing was not affected by d-amphetamine given intravenously (Figs. 12,13,14). This finding suggests that while there may be some crossing (perhaps a membrane carrier between plasma and CSF operating on the existing electrochemical gradient of permeant), it is not affected by giving the drug under study, and observed changes in CSF HMPG after amphetamine would not be due to contamination from the plasma pool of either free or conjugated HMPG.

CSF SECRETION RATE. We have found some indication of diurnal variations in hourly volumes of CSF produced using our constant outflow pressure collection method (Fig. 4). The volumes of CSF collected are pressure dependent to a point, and reducing the outflow pressure from zero to -10 cm H_2O, increases daily output and enhances hourly variations (Fig. 5). By col-

lecting at a more negative pressure, the effects of variations in cerebral venous tone in terms of volume changes in the CSF compartment are minimized. The effects of changes in arterial pressure and venous tone on the volume of CSF produced are only transient phenomena however, and cannot account for steady-state increases or decreases in the CSF secretion rate. Brain blood flow is most likely the rate-limiting factor in CSF production, and the volume fluctuations we observe may relate to variations in cerebral perfusion. We have observed some variations in CSF production which seem to be activity dependent (Figs. 6,7,8); presumably brain blood flow changes also, during the periods of activity or excitement.

It is possible to quantitate the total output of a neurotransmitter metabolite based on the amount of CSF produced in a given collection interval. We have used the hourly CSF production to correct for dilution effects, normalizing the dependent variable for volume changes. This correction is of course only valid if there is no additional removal mechanism operating between the CSF and blood or CSF and tissue. It is well known that weak acids, for example, are transported out of CSF by a saturable mechanism sensitive to prebenecid inhibition. Before we could be certain of the volume correction mentioned above, we would have to block any additional removal mechanisms during the course of the experiment.

CONCLUSIONS

On the basis of the work in this report, we feel that the monkey model we have developed, which permits several types of simultaneous physiologic measurements, will be expecially valuable in evaluating basis mechanisms of action of commonly abused drugs, antagonists and perhaps to screen new compounds for abuse potential or use as therapeutic agents capable of blocking the abused drug effects.

RECOMMENDATIONS

Much of the difficulty in performing work of this type related to the sensitivity and reliability of the assay procedure used. It would be highly desirable to develop more fully the assay methodology as a pre-requisite to studies similar to our own. We have demonstrated the technical feasibility of collecting CSF on a routine basis in unanesthetized animals, however the CSF levels of metabolites and parent compounds are remarkably low and place stringent requirements on measurement techniques.

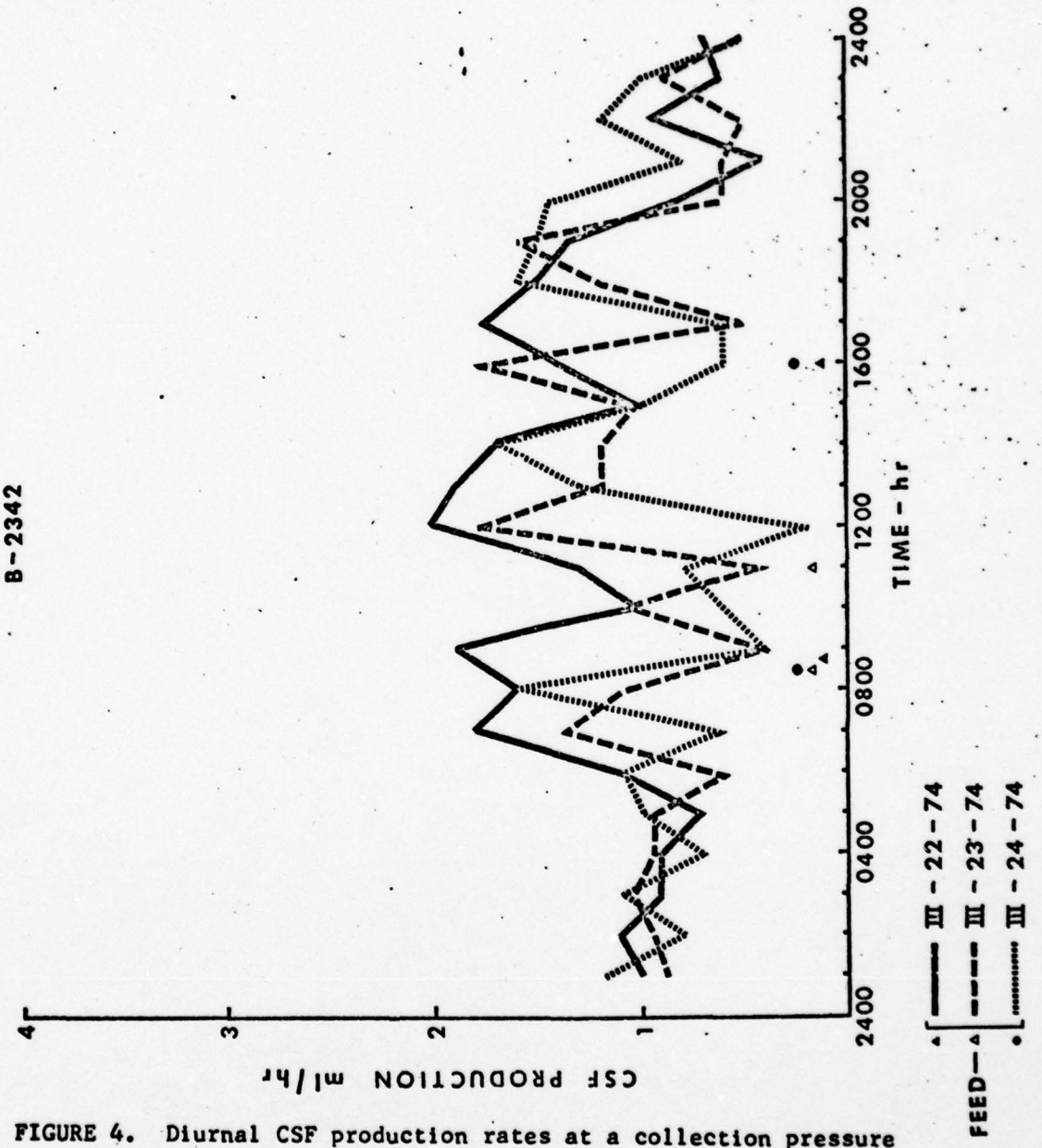

FIGURE 4. Diurnal CSF production rates at a collection pressure of zero with respect to the interauditory zero line. The CSF outflow is open-ended at the fraction collector (0°C) which is mounted at the back of the primate booth on a vertical track which permits adjustment of the collection pressure. Lights on at 0700 and off at 1900 hours.

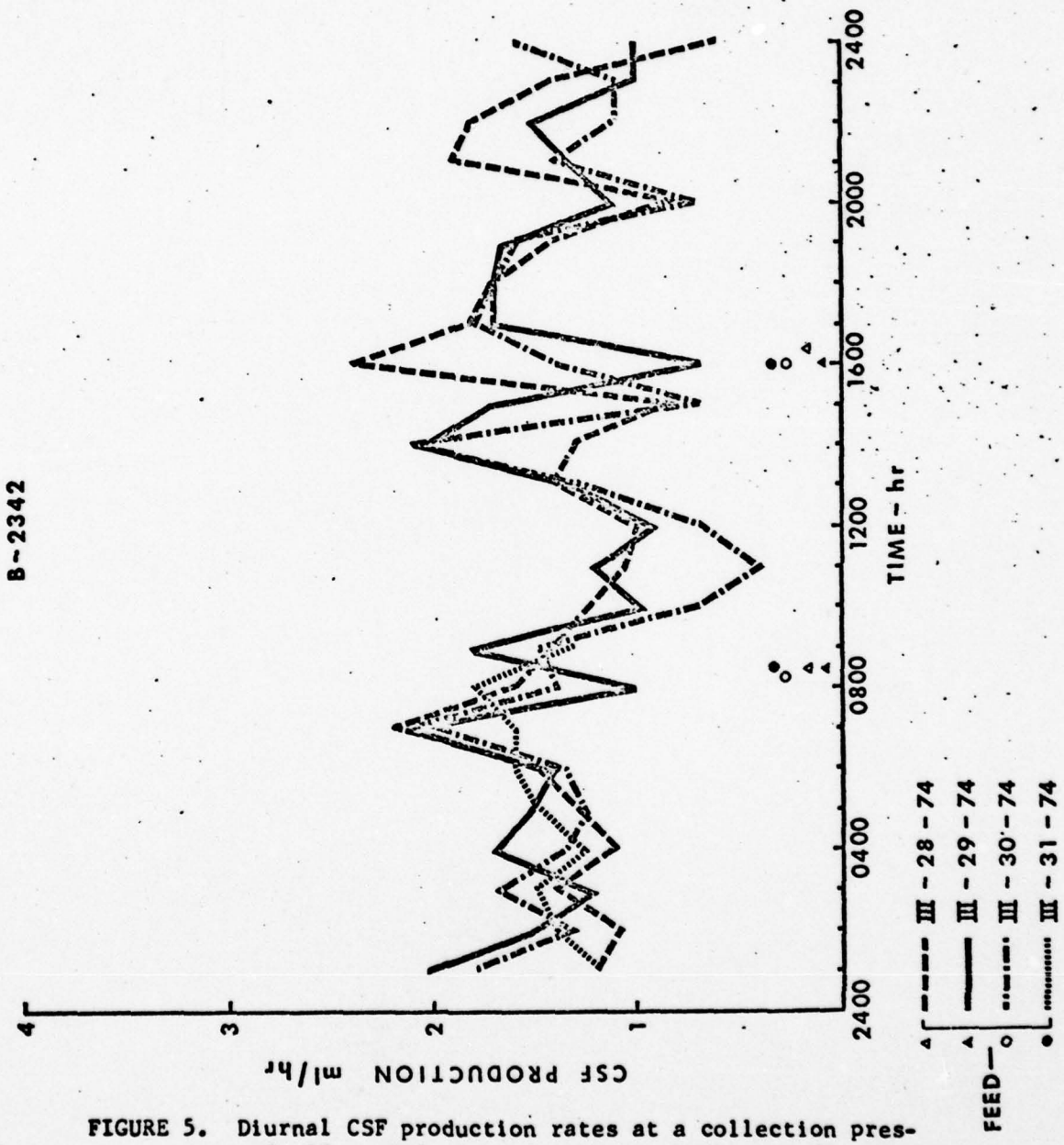

FIGURE 5. Diurnal CSF production rates at a collection pressure of -10 cm H_2O with respect to the interauditory zero plane of the monkey. The increase in volume over Fig. 4 may be due to CSF from spinal sources which were not available at the higher pressure or to increased capillary filtration. Lights on at 0700 and off at 1900 hours.

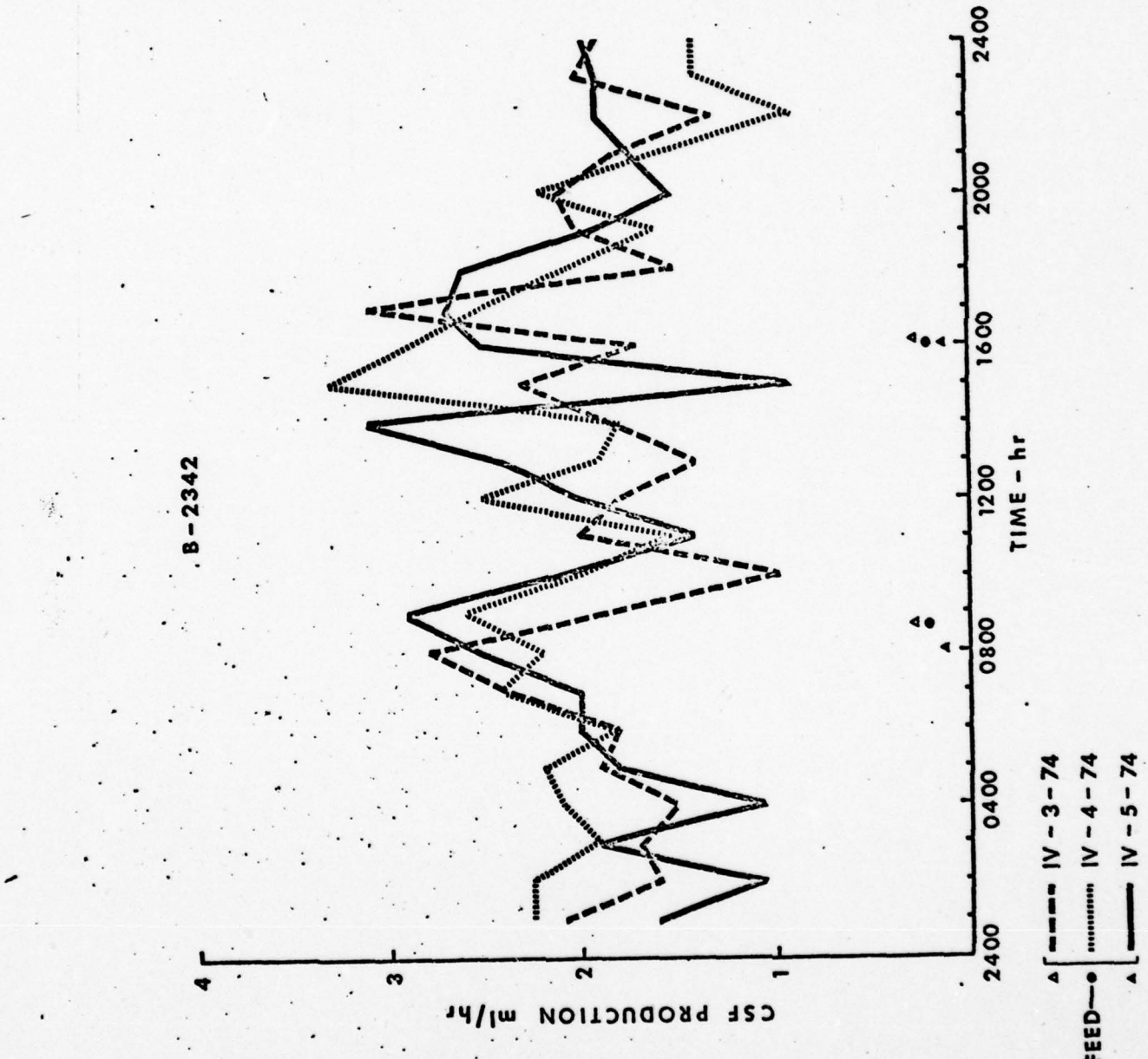

FIGURE 6. Diurnal variations in CSF secretion rate during the third week after surgery for catheter placement in subarachnoid space. Collection pressure -10 cm H_2O unless specified otherwise. Lights on at 0700 and off at 1900 hr.

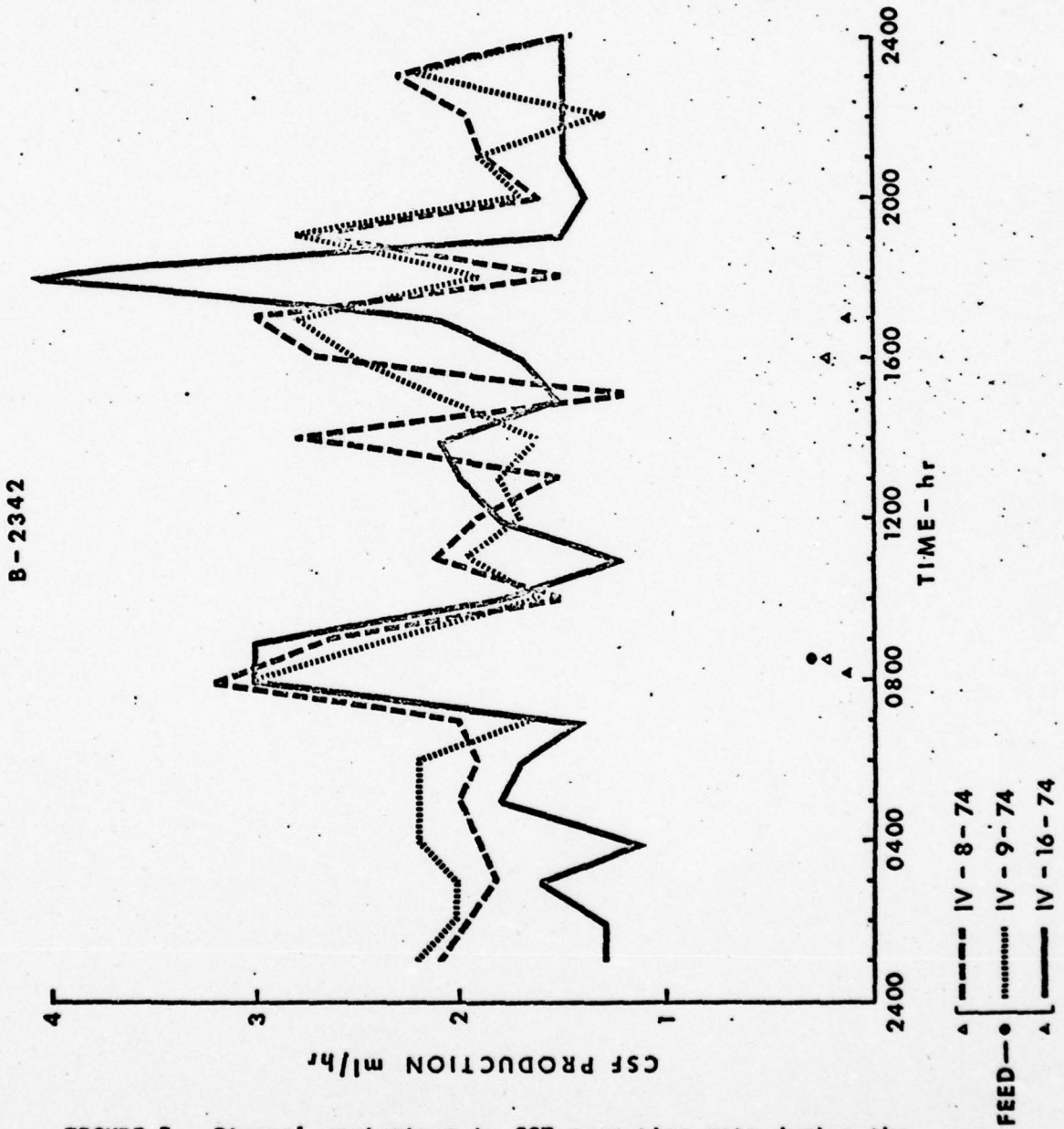

FIGURE 7. Diurnal variations in CSF secretion rate during the fourth and fifth weeks after surgery for placement of the CSF catheter. The two pronounced peaks in production are associated with daily feeding periods and lighting. Lights on at 0700 and off at 1900 hours.

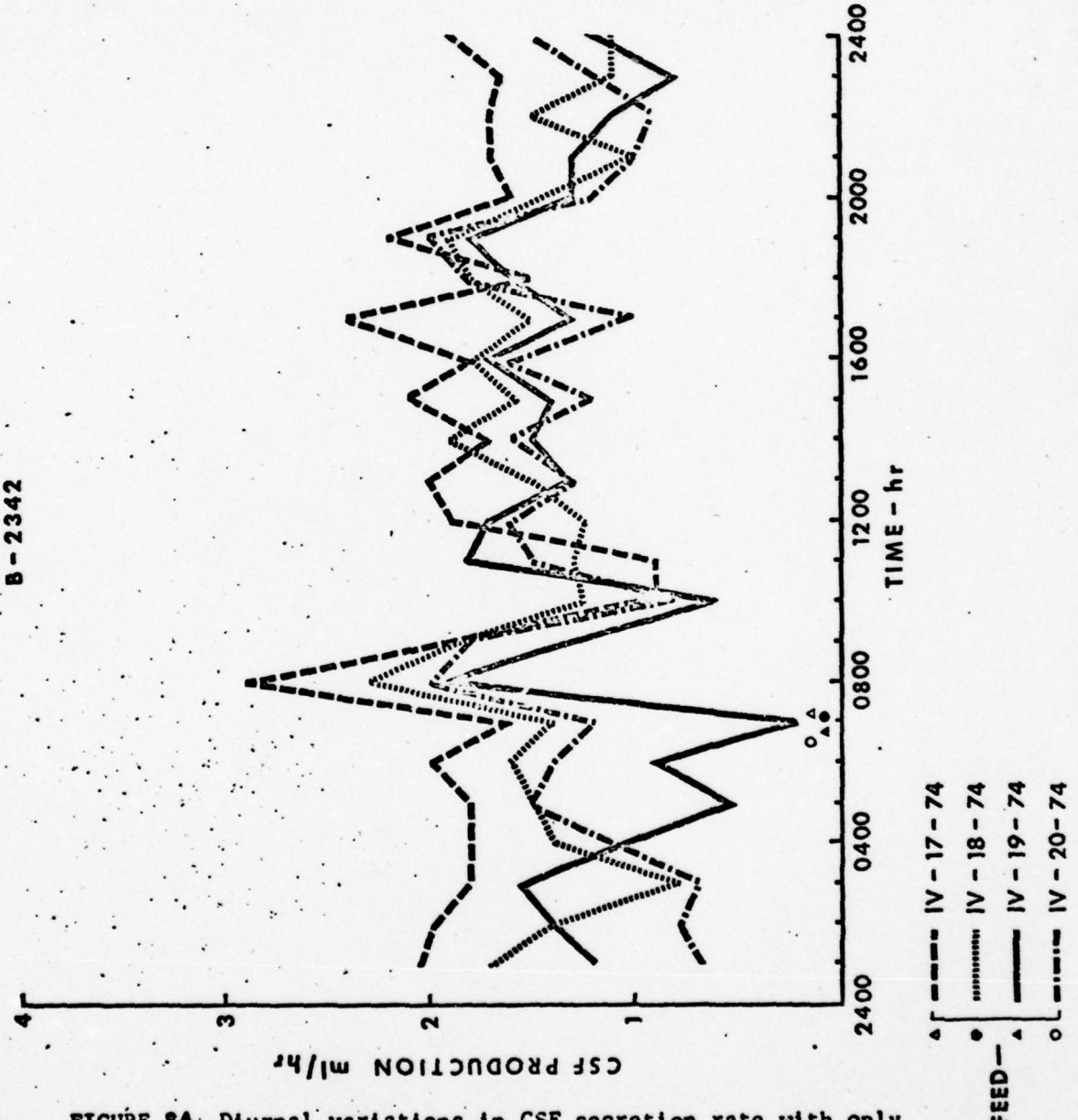

FIGURE 8A Diurnal variations in CSF secretion rate with only one feeding in the morning. There appears to be some entrainment the first day, but thereafter the 1600 peak is reduced. It is therefore possible to achieve fairly stable rates of CSF formation by omitting the evening feeding. Lights on at 0700 and off at 1900 hours.

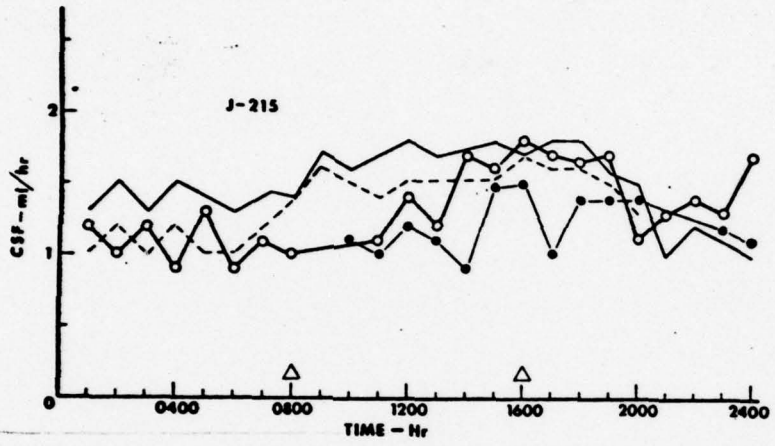

Figure 8β. Diurnal CSF production variations in primate J-215. CSF was collected hourly at constant outflow pressure (-3cm H_2O with respect to the interauditory zero plane) in the refrigerated fraction collector. There is a trend to increased volumes during the day, especially in the evening, at which times activity is also increased. Small triangles indicate the time at which the monkey is fed. At all other times the booth door is closed and the monkey undisturbed. Some of the time from 0800-1400 hours is usually spent lightly dozing, with activity (not shown) increasing in the evening until 1900 hours.

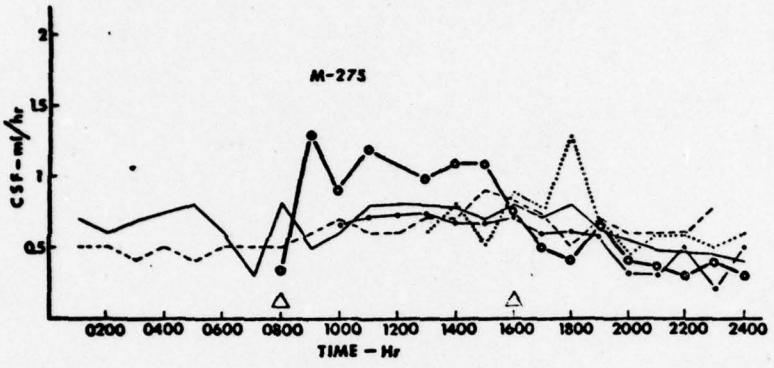

Figure 6C Diurnal CSF production variations in primate M-275. A similar trend to that in J-215 is seen in this monkey. Triangles represent feeding; at all other times the booth door is closed. On some days a complete 24 hour sample was not obtained due to catheter blockage. Whenever this occurred, the catheter was flushed with sterile ringers solution and the collection started again.

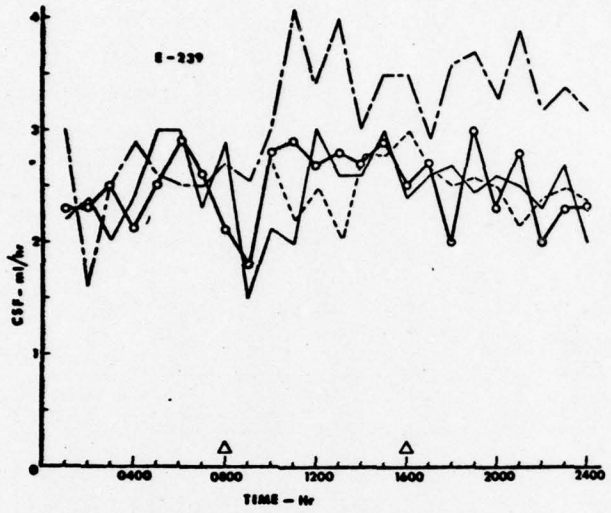

Figure 8D. Diurnal CSF production variations in primate E-239. The trend to a distinct diurnal variation was most apparent in a single 24 hour period, in which the greatest volumes of CSF were also collected. We suspect that this indicates lack of any temporary blockage or catheter compression for this period, so that representation of true diurnal fluctuations would be most pronounced for this one day. Similar trends however can be seen in the other 24 hour periods but they are not so distinct. Collection techniques as in Figure 8 and 9. Triangles represent feeding.

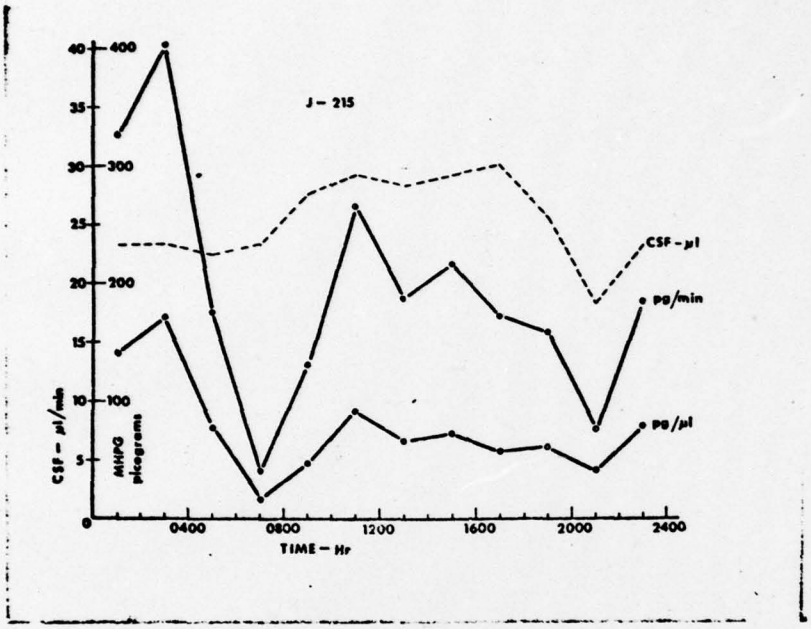

Figure 8E Diurnal variations in CSF MHPG levels in primate J-215. Here we have plotted the simultaneous CSF production rates and MHPG levels in the same CSF sample. For comparative purposes we have shown the uncorrected and corrected MHPG levels. In this instance the correction for CSF volume produced tends to magnify the diurnal variations in central MHPG output. There is an observable positive correlation here between the CSF secretion rates and MHPG levels. The hourly samples were pooled into two-hour intervals prior to analysis for MHPG, and the data are plotted at the mid-point of each two-hour interval.

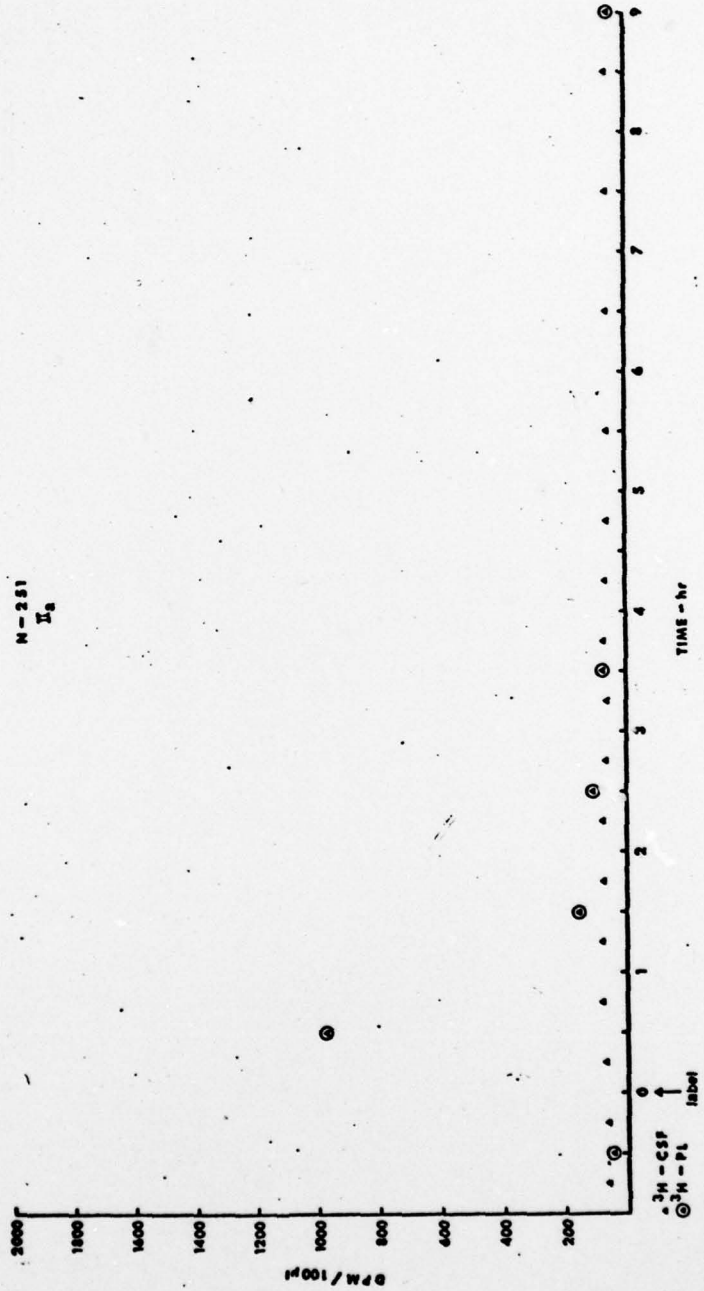

FIGURE 9. Lack of penetration of ^{3}H-polyethylene glycol (900 mw) into the CSF after intravenous injection of 10 µCi. CSF points represent the mid-point of the sampling intervals (30 min). The background activity prior to the label reflects the normal amount of radioactivity for this isotope. CPM's were converted to DPM's from a quench curve by computer in this and subsequent figures. Standard single and double label counting procedures were followed. Plasma (PL).

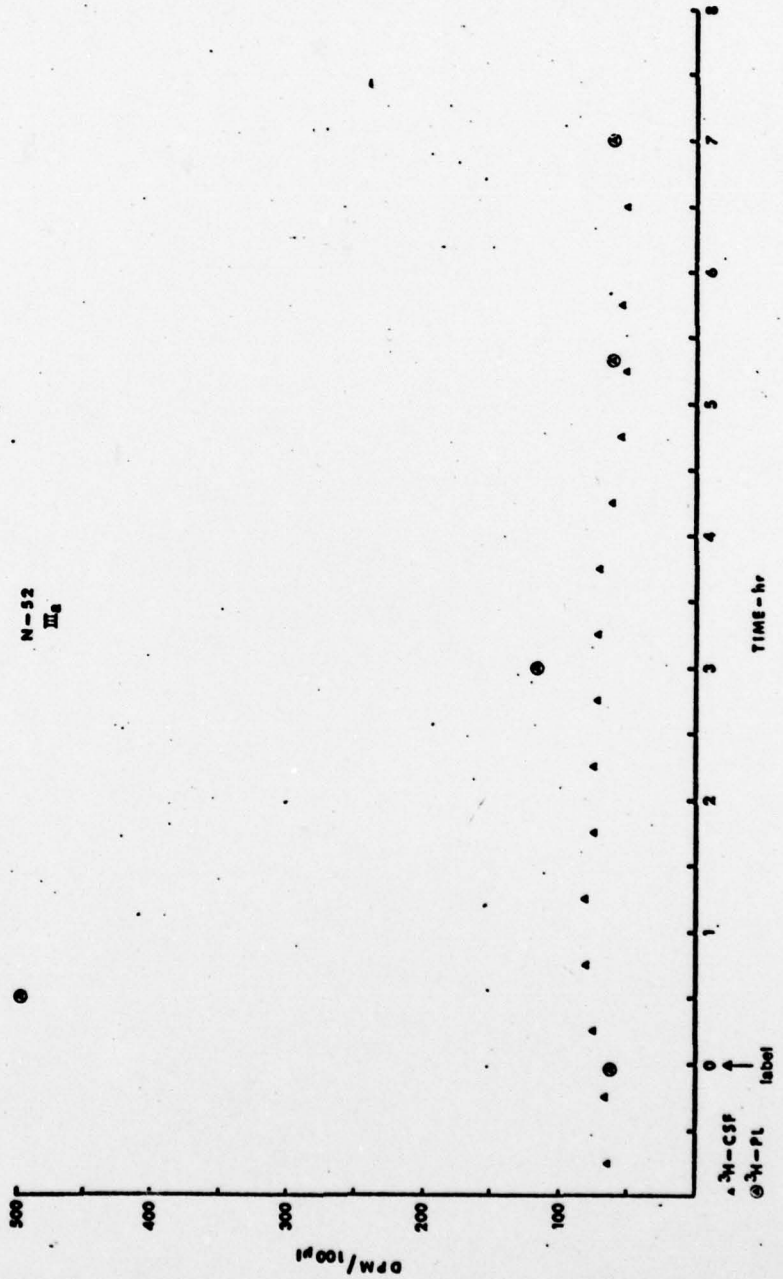

FIGURE 10. Lack of penetration of ^{3}H-polyethylene glycol (900 mw) into the CSF after intravenous injection of 10 μCi. Monkey No. N-52 shows essentially the same results as No. N-251 (Fig. 9). Scale on ordinate differs from that in Fig. 9, and reveals some very slight crossing, presumably representing the normal physiological "leaks" between CSF and blood.

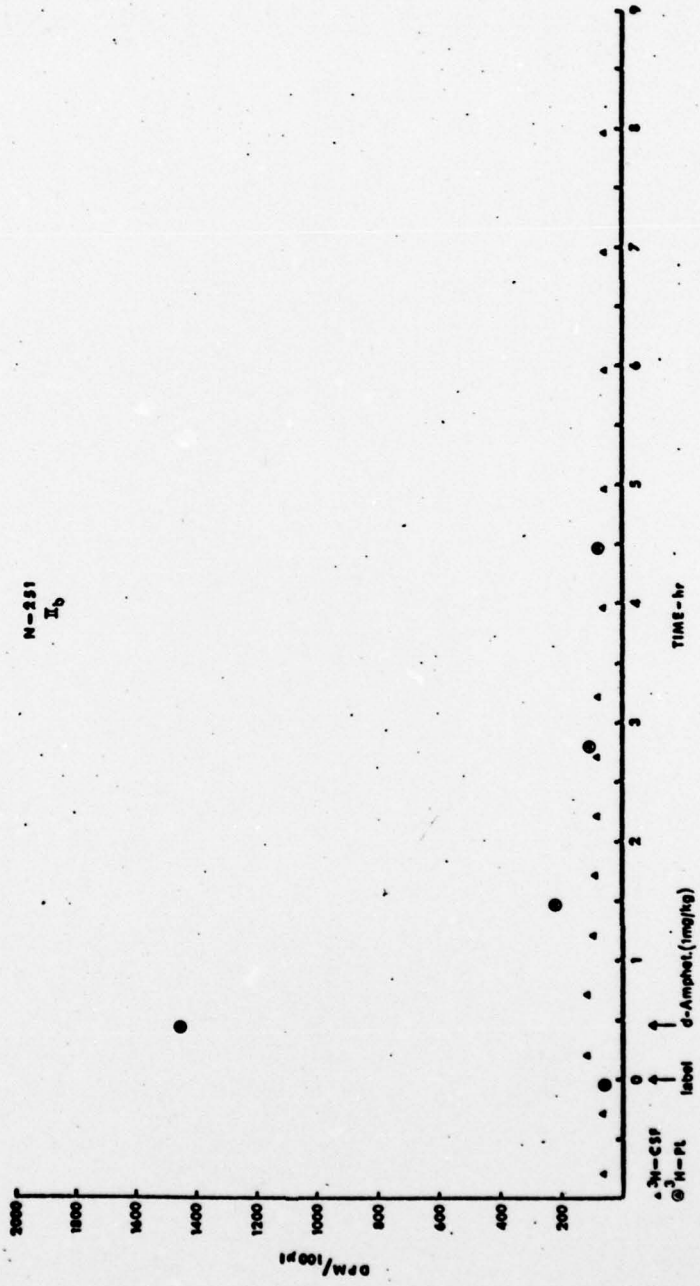

FIGURE 11. Repeat administration of ^{3}H-PEG to No. N-251, however in this experiment d-amphetamine is given 30 min after the label to examine possible drug-induced changes in BBB permeability. No increased crossing after the drug was observed. Ordinate scale as in Fig. 9. Both drug and label given I.V.

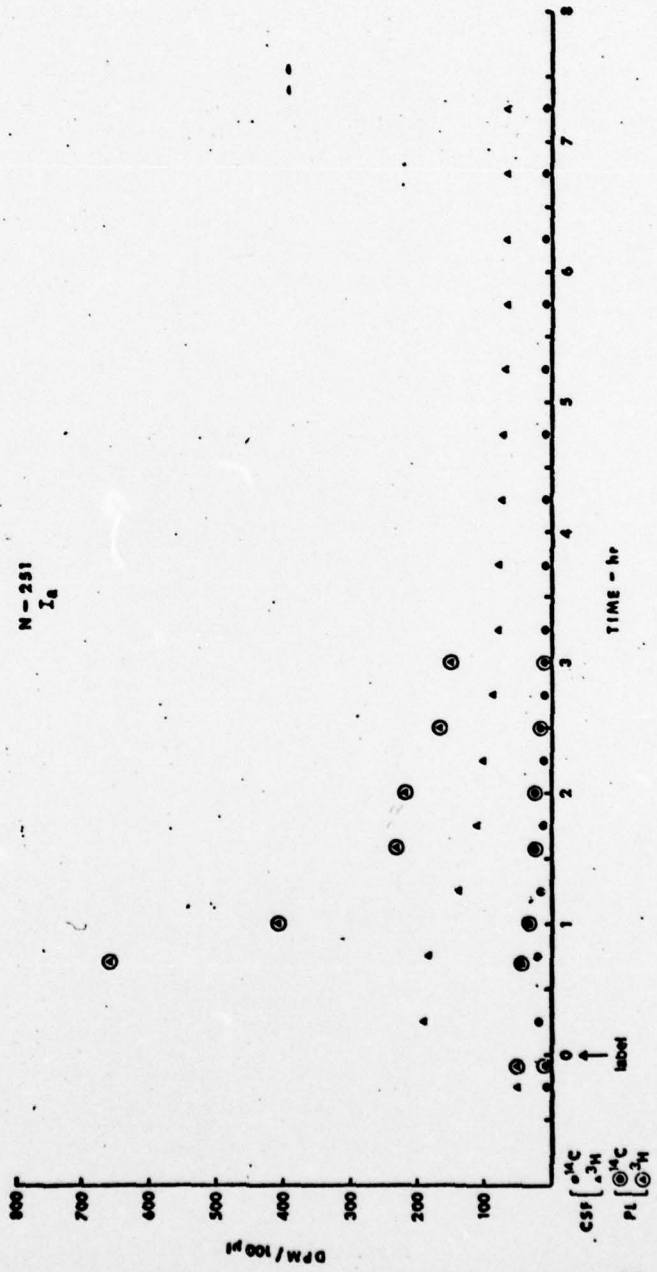

FIGURE 12. Intravenous administration of ^{3}H-HMPG and ^{14}C-HMPG sulfate. Some apparent crossing of HMPG into CSF is apparent, but we have not determined if this is free label or authentic HMPG yet. HMPG is not considered to be a non-diffusible indicator, and its transport may be carrier mediated between the CSF and blood plasma.

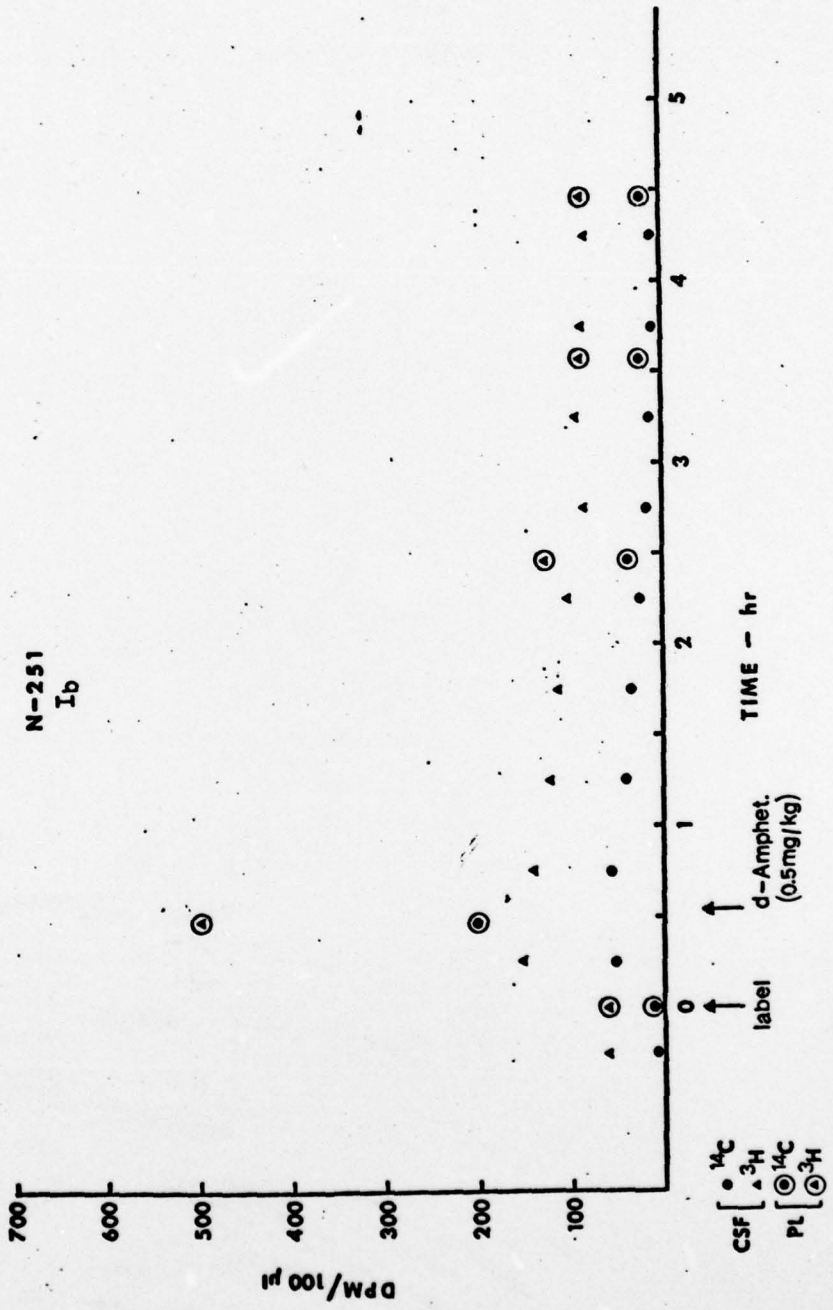

FIGURE 13. Administration of ^{3}H-HMPG and ^{14}C-HMPG sulfate I.V., followed by d-amphetamine I.V. 30 min later. The purpose of this experiment was to determine if the drug increased the crossing of label from plasma to CSF, which it did not. The ^{14}C background is normally lower than the ^{3}H.

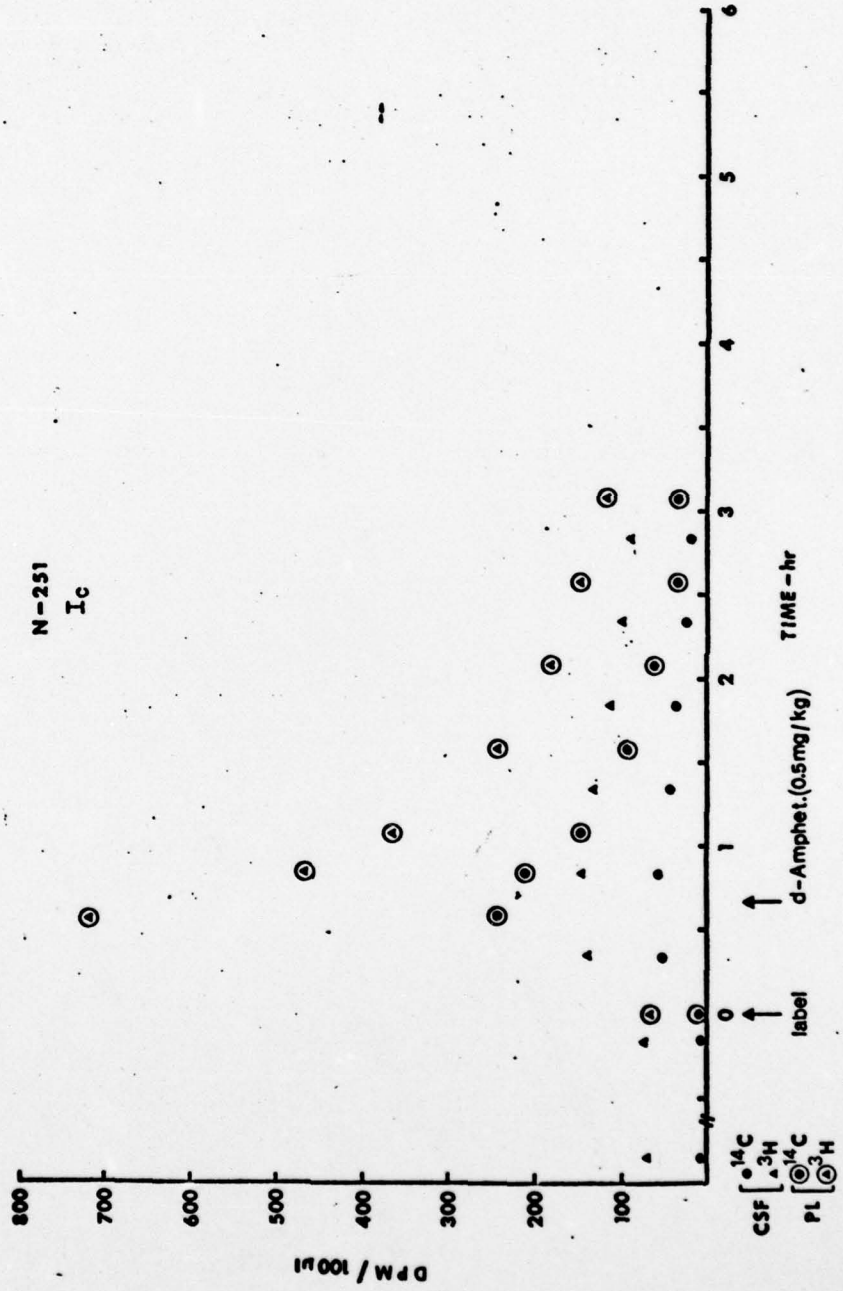

FIGURE 14. Repeat of the experiment in Fig. 13 on the same monkey. The results are identical, and indicate no increased crossing of label into the CSF from plasma following a moderate dose of d-amphetamine I.V.

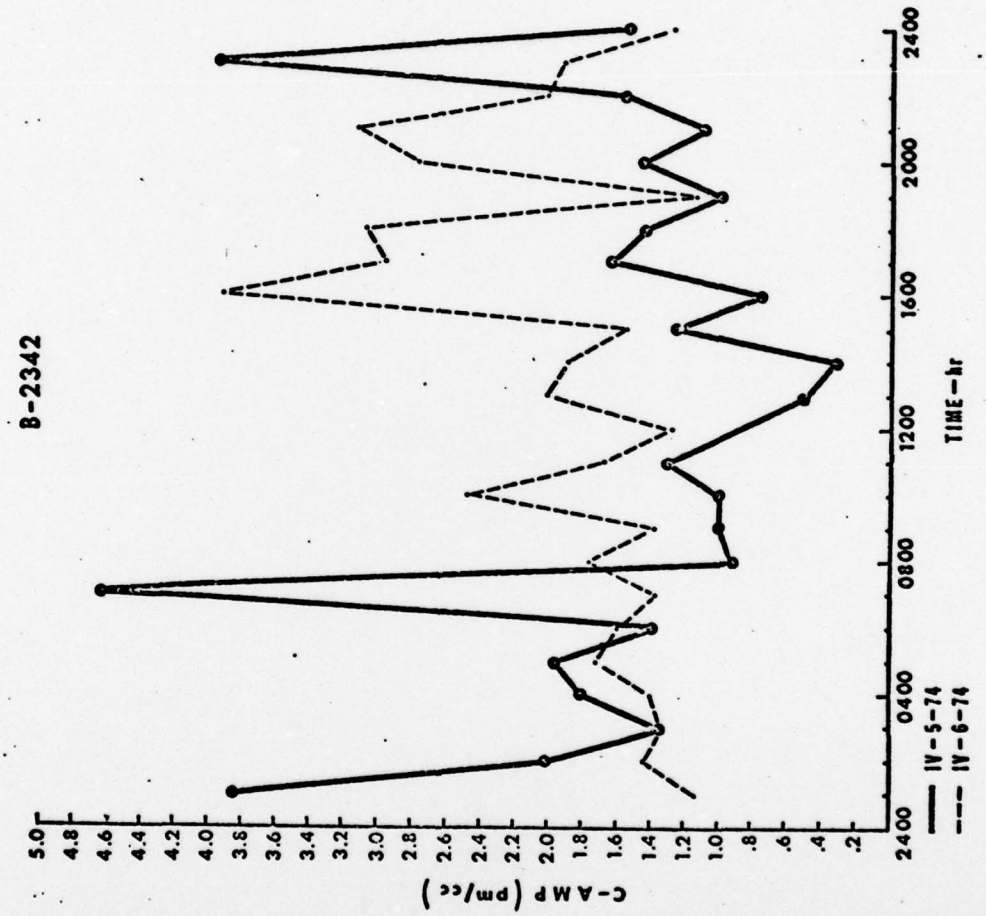

FIGURE 15. Preliminary results of the cyclic-nucleotide (AMP) assays on monkey CSF. Some diurnal variations are evident(random) using this radio-immunoassay, which is currently being modified by Dr. R. Lennox of the Neuroendocrine Division at Walter Reed. Preliminary results for C-GMP indicate a greater precision than for C-AMP (C-GMP results not available at this time). Data points represent the mean of duplicate analyses (SE not shown) and are plotted at the mid-point of the CSF collection interval.

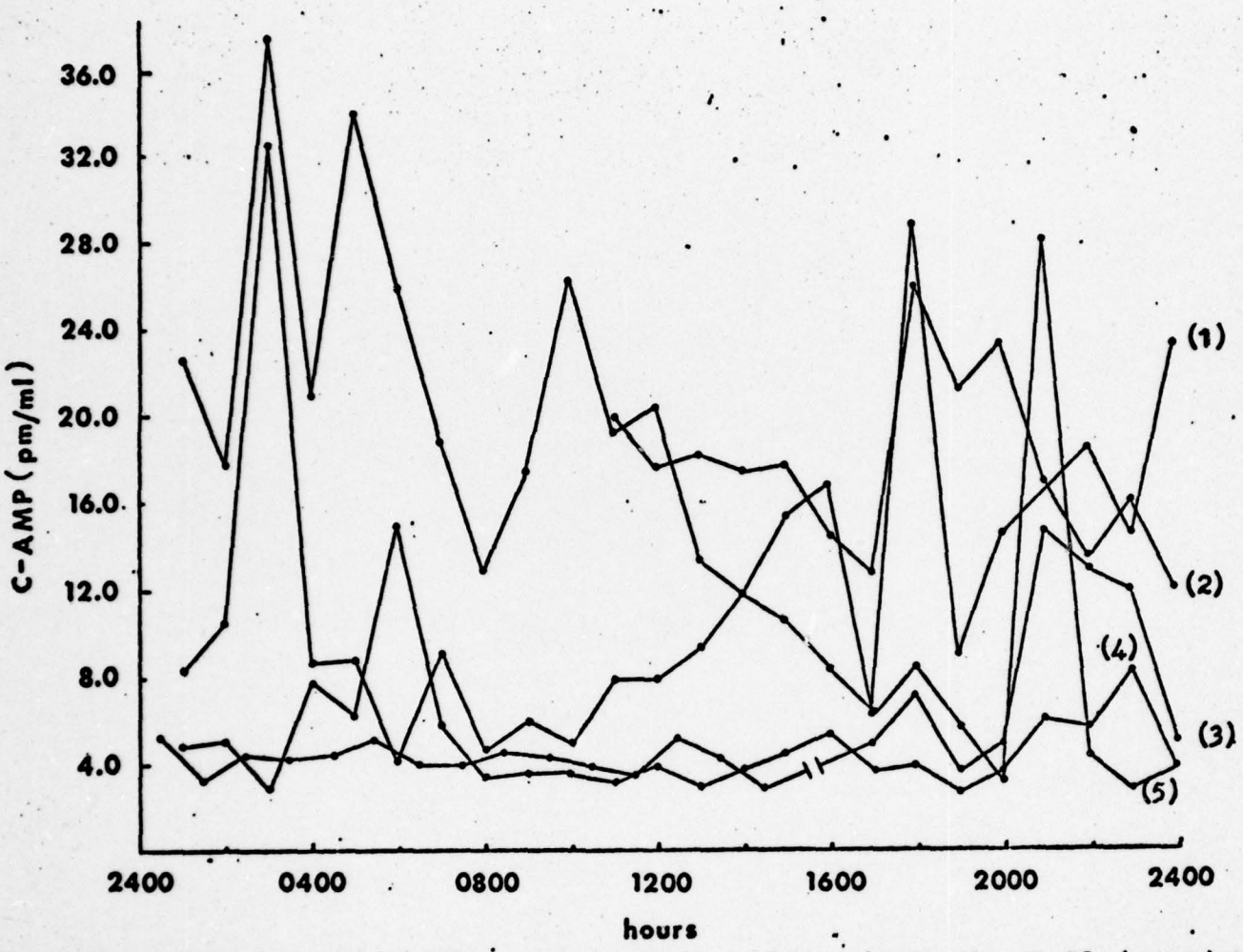

Figure 16. Variations in cyclic AMP on control days for primate No. N-52 (corrected for production volume of CSF and expressed per/ml for the hourly collection periods.

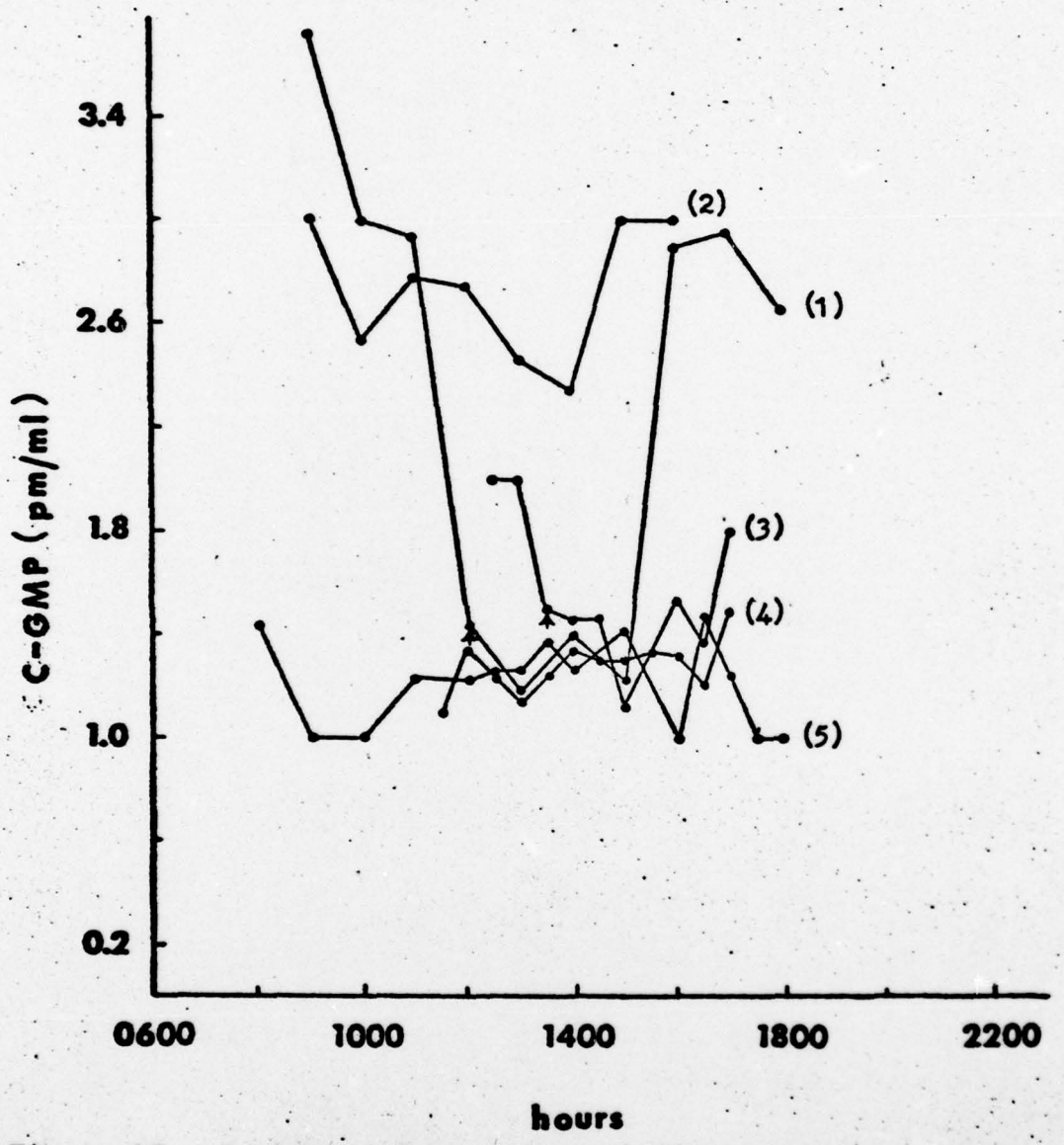

Figure 17. Control and experimental changes in cyclic GMP levels in primate N-251. At the arrows, Na pentobarbital was administered (ca. 10 mg/kg, I.V.) - the remaining curves represent control days. CSF levels normalized for total volume obtained during the collection period.

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