THE EFFECT OF ACUPUNCTURE WITH ELECTRICAL STIMULATION ON THE PLASMA ACTH AND CORTICOSTERONE LEVELS OF MORPHINE ADDICTED RATS AND MICE

電針療法對有嗎啡毒癮之大、小白鼠血漿內促腎上腺皮質激素及皮質固酮含量之效應

BY

YU-FUI TSANG

B.A. (HUMBOLDT STATE UNIVERSITY, CALIFORNIA, U.S.A.)

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF PHILOSOPHY IN BIOCHEMISTRY

JUNE 1978

DEPARTMENT OF BIOCHEMISTRY

THE CHINESE UNIVERSITY OF HONG KONG
We accept this thesis as conforming to the required standard of the degree of

MASTER OF PHILOSOPHY

Dr. W.-W. Tso
(Supervisor)

Prof. Ma Lin
(Chairman
Thesis Committee)

Prof. T.B. Lo
(External Examiner)

Dr. Y.M. Choy
(Departmental Examiner)

Dr. Walter K.K. Ho
(Departmental Examiner)
TO MY MOTHER COUNTRY

THE MAGNIFICENT CHINA
This thesis was based on the work of a project, directed by Dr. H.L. Wen, Professor L. Ma and Dr. W.-W. Tso on the Biochemical Basis of Acupuncture in the Treatment of Addiction. Part of the work has been presented in the 4th International conference on Alcoholism and Drug Dependence (Liverpool, England; 1978) by Wen et al. and in the Biochemical and Biophysical Research Communications by Choy et al. (in press). The manuscript on the results of the experimentation on rats of this thesis is in preparation and will be submitted for publication by Tso et al. shortly.
ACKNOWLEDGMENTS

I would like to express my sincere gratitude to Professor L. Ma and Dr. W.-W. Tso for their valuable guidance and discussions throughout the entire period of this investigation. My gratitude is also due to Prof. T.B. Lo, Dr. Y.M. Choy and Dr. Walter K.K. Ho for their willingness to serve as members of my committee.

Grateful thanks are also due to Dr. H.L. Wen who is one of the initiators of the project for his constructive advice.

To the late Mr. Fook-Son Ko, Mr. Wing-Tat Lee, the Lee Foundation of Singapore and Hong Kong, the Tak Shing Investment Co. Ltd. and the Y's Men's Club of Hong Kong, I thank for their interest and generous financial support to this project.

I would also like to express my appreciation to Mr. K.K. Au, Mr. K.P. Fung, Mr. K.C. Leung, Mr. H.K. Wong, Mr. T.T. Yip as well as all the staffs of the Biochemistry Department for their skillful assistance in many ways.
ABSTRACT

Acupuncture with electrical stimulation (AES) has been employed to alleviate withdrawal from morphine addicts in rehabilitation programs being practised in Hong Kong and Taiwan. It has been found that there are significant changes in plasma ACTH and cortisol levels in the patient at various stages in the rehabilitation program. The aim of this investigation is to establish an animal model for further biochemical studies.

Using rats to construct an animal model, a condition can be reached by intraperitoneal injection of morphine twice daily for a period of 2 weeks, followed by an injection of 0.01 mg of naloxone which gives a withdrawal syndrome comparable to that observed in addicted patients. This withdrawal syndrome can be alleviated by AES resemble the procedure practising in clinical rehabilitation program. Similar condition was found with laboratory mice. In general, the effect of AES brings about a suppression of the naloxone-precipitated withdrawal syndrome which is also demonstrated by a substantial reduction of the already elevated plasma ACTH and CS levels.

Experiments with chronic addicted rats abstained from morphine for 14 hours (whose withdrawal symptoms
are less extreme in magnitude than that induced by the injection of naloxone) show a depression of their plasma biochemical contents. On applying AES, this depressed plasma biochemical levels was restored.

The alleviation of withdrawals in both withdrawing classes with opposite changes in plasma biochemicals, an increase in natural withdrawal class and a reduction in naloxone-precipitated withdrawal class suggest a general regulating role played by AES.
# TABLE OF CONTENTS

**Page**

**INTRODUCTION** ................................................. 1

I. Aim of this Investigation ................................. 1

II. General Review ............................................. 2

   A. The pituitary-adrenal axis ............................. 2

   B. Effect of opiates on the pituitary-adrenal axis .... 9

   C. Morphine analgesia and acupuncture analgesia ....... 14

**MATERIALS AND METHODS** ..................................... 17

I. Methods Employed to Induce Morphine Addiction ......... 17

II. Behavior Assessment ......................................... 20

III. AES Treatment .............................................. 23

IV. Assay Methods ............................................... 28

   A. Determination of plasma ACTH ....................... 28

   B. Determination of plasma Corticosterone ............ 30

**RESULTS AND DISCUSSION** .................................. 35

Section A: Experiments with Rats ............................ 35

Section B: Experiments with Mice ............................ 63

**CONCLUSION** .................................................. 68

**REFERENCES** ................................................. 73
INTRODUCTION

I. AIM OF THIS INVESTIGATION

Apart from the conventional drug rehabilitation methods employing the narcotic antagonist therapy or the replacement therapy, acupuncture with electrical stimulation (AES) has been introduced to detoxify drug addicts in Hong Kong (Wen and Cheung, 1973 a&b) and Taiwan (Chen et al., 1976) since 1973. This simple, economical and relatively short term treatment has soon raised interest from different research teams, with the potential of being developed into the most successful method ever applied to rehabilitate drug addicts.

A previous study conducted by our group to follow the biochemical changes in blood and urine of heroin addicts treated by AES showed that after initial AES treatment, the plasma levels of Adrenocorticotropic hormone (ACTH) and cortisol in heroin addicts were reduced but not in the normal group (Harms, 1975; Ho et al., 1977b).

Numerous reports that acupuncture has been successfully applied to a great varieties of mammals including rat and rabbit strongly suggest that its biochemical effects may transcend animal origin (Wen et al., 1977; Lung et al., 1973; Research group...
of Acupuncture Analgesia, 1971). In this research, we have extended our observation to two laboratory animal types, namely rat (Sprague-Dawley Strain) and mouse (WHT Strain).

The first part of this investigation, the establishment of the animal model was done in collaboration with my colleague Mr. H.K. Wong. The behavioral study showed that addicted rats and mice treated with AES resulted in a reduction of the withdrawal scores. Plasma levels of ACTH and corticosterone (CS) measurements however, indicate that AES creates a regulating effect instead of unidirectional change in the biochemical level.

For the benefit of discussion, a general knowledge about the pituitary-adrenal axis, morphine addiction and acupuncture analgesia is revised in the following paragraphs.

II. GENERAL REVIEW
A. The pituitary-adrenal axis

ACTH has been isolated from sheep pituitaries and the complete structure of the polypeptide was identified by Li et al. (1954; 1955) in 1954 respectively. It is a single-chain polypeptide composed of 39 amino acids with molecular weight of approximately
4,500. The 1-24 amino acid of ACTH are similar in different species. Variations occur from 25-39 amino acid of ACTH among different species. The ACTH activity is found to be resided in the N-terminal 1-19 amino acid portion of the polypeptide chain (Li, 1962).

ACTH is secreted by chromophobe cells in the adenohypophysis (Siperstein and Miller, 1970). It is released into the circulatory system and carried to the adrenal cortex where it stimulates the release of glucocorticoids. ACTH is released upon stimulation of the adenohypophysis by corticotrophic releasing factor (CRF). According to Brodish (1972) there are two types of CRF, the hypothalamic CRF in the brain and the extrahypothalamic CRF in peripheral blood. The extrahypothalamic CRF is suspected to play a role in sustaining elevated level of ACTH during periods of prolonged severe stress.

The control mechanism of ACTH is fairly complicated and can be categorized into four mechanisms:

a. A diurnal rhythm maintained by biological clock in the temporal lobe: this is a programmed sequence of events under the central nervous system (CNS) control. There are episodic secretion within the whole day but the main secretion periods are during the 6-8 hour of sleep and first hour after awake.

b. A short feedback mechanism exerted by action of ACTH on the hypothalamus: this mechanism is important for
the fine adjustment of pituitary activity.

c. A close loop interaction of the corticosteroid-
ACTH-CRF completed by the negative feedback of

corticosteroid.

d. Stress causing excitatory neural inputs converged
on the hypothalamus resulting in ACTH secretion:
this can override the feedback control of gluco-
corticoids. Stress can be distinguished as neuro-
genic stress which is transmitted through CNS, and
systemic stress which is transmitted through humor-
al mechanism.

The integrated control of all these mechanisms
provides the organism with the ability to deal with re-
gular daily processes and to react against emergency
condition.

The biological properties of ACTH are quite div-
ersified and are still under investigation. The most
obvious effect is the action on the adrenals and the
release of corticosteroids. Li (1962) has summarized
the functions of ACTH, both in vivo and in vitro, and
are reproduced in Table 1.

Recent research reveals some interesting func-
tions of ACTH fragments and analogues in acquisition
and maintenance of conditioned behavior. Leshner and
Roche (1977) showed that ACTH treatment enhanced avoi-
<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Biological effects of ACTH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vivo</strong></td>
<td><strong>In vitro</strong></td>
</tr>
<tr>
<td>1. Increases the weight of adrenal glands</td>
<td>17. Suppresses increased capillary permeability induced by exudin</td>
</tr>
<tr>
<td>2. Repairs the adrenal of hypophysectomized rats</td>
<td>18. Causes an increase in liver fat in animals maintained with corticoids</td>
</tr>
<tr>
<td>3. Promotes corticoid production as estimated in the adrenal venous blood</td>
<td>19. Produces hypergranulation of the renal juxtaglomerular cells</td>
</tr>
<tr>
<td>4. Causes eosinopenia and thymic involution</td>
<td>20. Influences metabolism of cortisol</td>
</tr>
<tr>
<td>5. Increases metabolic rate of hypophysectomized rats</td>
<td>21. Stimulates corticosterone production in the rat adrenal and aldosterone production in the bullfrog adrenal</td>
</tr>
<tr>
<td>6. Causes eosinopenia in hypophysectomized-thymectomized rats</td>
<td>22. Causes melanophore expansion in skins of amphibians and reptiles</td>
</tr>
<tr>
<td>7. Enhances erythropoiesis in hypophysectomized animals</td>
<td>23. Releases nonesterified fatty acids from rat epididymal fat pad</td>
</tr>
<tr>
<td>8. Increases weights of sex accessory of hypophysectomized-castrated rats</td>
<td>24. Induces the uptake and oxidation of glucose by rat ventricle tissue</td>
</tr>
<tr>
<td>9. Maintains muscle glycogen in hypophysectomized animals</td>
<td>25. Exhibits incorporation of amino acids into adipose tissue protein</td>
</tr>
<tr>
<td>10. Prevents glycogen deposition in the liver</td>
<td>26. Influences metabolism of cortisol</td>
</tr>
<tr>
<td>11. Acts as a galactopoietic agent</td>
<td>27. Stimulates corticosterone production in the rat adrenal and aldosterone production in the bullfrog adrenal</td>
</tr>
<tr>
<td>12. Exerts antagonistic action to growth hormone</td>
<td>28. Causes melanophore expansion in skins of amphibians and reptiles</td>
</tr>
<tr>
<td>13. Causes an increase of liver fat in fasted animals</td>
<td>29. Releases nonesterified fatty acids from rat epididymal fat pad</td>
</tr>
<tr>
<td>14. Causes an elevation of serum-free nonesterified fatty acids</td>
<td>30. Induces the uptake and oxidation of glucose by rat ventricle tissue</td>
</tr>
<tr>
<td>15. Increases blood ketone bodies in fasted rats</td>
<td>31. Exhibits incorporation of amino acids into adipose tissue protein</td>
</tr>
<tr>
<td>16. Stimulates melanophore expansion in amphibians and reptiles</td>
<td>32. Influences metabolism of cortisol</td>
</tr>
</tbody>
</table>
dance retention no matter the hormone was injected to the rat prior to training, just following acquisition or 24 hours after acquisition. The facilitatory effect of ACTH treatment sustained for 24 hours but not 240. De Wied (1976) postulated that ACTH fragments affect the behavior by a temporary selective increase in the state of arousal in limbic midbrain structures, thereby increasing the motivational influence of environmental stimuli.

Another newly discovered property of ACTH is the exhibition of binding affinity to opiate receptors (Gispen et al., 1975; 1976; Terenius et al., 1975; Tarenius, 1976). Terenius et al. (1975) have confirmed that the N-terminal fragments of ACTH have an affinity for rat brain opiate receptors in vitro. The ACTH 4-10 fragment is crucial. These peptides are devoid of corticotropin activity and inhibit morphine induced analgesia. The peptides show a partial agonist-antagonist properties. The concentration giving 50% inhibition of the binding of 7,9-[^3]H]-dihydromorphine to the synaptic plasma membrane fraction is 10^-5 M which is much higher than morphine (10^-9 M). Taking this into consideration, Terenius concluded that the peptides are not likely to be important endogenous opiate receptor ligands in physiological condition;
and even though these fragments are present in sufficient concentration, their partial agonistic features suggest merely a modulatory role.

Guillemin et al. (1977) discovered in 1977 that both \( \beta \)-endorphin and ACTH are secreted concomitantly by the pituitary gland in normal rat. This gives rise to a suggestion both \( \beta \)-lipotropin and ACTH are derived from a common precursor. However a different result was reported by Krieger et al. in 1977 who found a different distribution pattern of \( \beta \)-lipotropin and of ACTH in discrete areas of bovine brain. This can either be due to independent synthesis, or differential distribution of degradative enzymes (or uptake sites) for these two peptides. What is the function of \( \beta \)-endorphin in periphery and how is it related to the function of concomitantly secreted ACTH still awaits further research work to disclose.

Corticosterone (CS) is the glucocorticoid secreted exclusively by rats and mice from the adrenal cortex. It is synthesized from cholesterol and belongs to the group of "C-21" steroid. Upon stimulation by ACTH, CS is secreted from the adrenal cortex into the circulatory system and is transported by binding to transcortin (\( \alpha \)-glubolin). There is very little free corticosteroid in plasma and the bound CS is physically
inactive. It serves as a reservoir for free CS. CS is metabolized in liver where it is conjugated into glucuronic acid and excreted in urine. The main function of CS is to regulate the energy utilization of cells and affects water, carbohydrate, protein and fat metabolism inside the body. It also plays a main role in resistance to stress. An adrenalectomized animal dies when exposed to even a minor stress.

CS secretion is triggered by ACTH and in return, CS has a negative feedback control on stress induced ACTH secretion. The mechanism is biphasic in nature (Dallman and Jones, 1973), acutely the rise of CS stimulated by increased ACTH levels acts through a rate-sensitive path to rapidly limit the duration of ACTH secretion while chronically, 2 or more hours after CS levels have been elevated, there is a decrease in the amount of ACTH secreted in response to stress. This second period of inhibition is independent of the CS levels. CS may also be involved in acquisition and maintenance of conditioned behavior. De Wied (1976) proposed that CS works together with ACTH and affects conditioned behavior by altering the arousal level in limbic midbrain structures to enhance discrimination and consequently the elimination of irrelevant behavioral responses. He suggests that neuropeptides related to ACTH play a basic role in
motivational, learning and memory processes while the pituitary-adrenal system, through the secretion of corticosteroids, carries a secondary modulatory function.

B. Effect of opiates on the pituitary-adrenal axis

Upon prolonged exposure to narcotic analgesics, physical dependence and tolerance will develop. Physical dependence is characterized by the abrupt termination of the drugs causing a predictable and reproducible syndrome to appear. Tolerance is defined as a necessity of ever-increasing doses of drug to achieve responses equal in magnitude to the initial effect. The narcotic analgesics composed of morphine, methadone, heroin and other chemically synthesized drugs with similar properties. There is another class of compounds which antagonized the action of the narcotics and is known as the antagonist. Naloxone is a pure antagonist. Between the pure agonists and antagonists, there are a number of agents which produce analgesia in man and can also inhibit or reverse the effects of one of the pure analgesics. Pentazocine and nalorphine are examples of drugs in this category (for structures, see Figure 1).

The appearance of severe withdrawal symptoms during cessation of drugs strongly implies the disturbance of the body by the action of the drugs. Re-
Fig. 1. Some Typical Examples of Pure Agonist, Mixed Agonist-antagonist and Pure Antagonist
<table>
<thead>
<tr>
<th>Type</th>
<th>Common Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGONIST</td>
<td>HEROIN</td>
<td><img src="image" alt="Heroin Structure" /></td>
</tr>
<tr>
<td>AGONIST</td>
<td>MORPHINE</td>
<td><img src="image" alt="Morphine Structure" /></td>
</tr>
<tr>
<td>WEAK AGONIST</td>
<td>METHADONE</td>
<td><img src="image" alt="Methadone Structure" /></td>
</tr>
<tr>
<td>MIXED AGONIST-ANTAGONIST</td>
<td>PENTAZOCYNE</td>
<td><img src="image" alt="Pentazocine Structure" /></td>
</tr>
<tr>
<td>ANTAGONIST CONTAMINATED WITH ONE AGONIST ACTIVITY</td>
<td>NALORPHINE</td>
<td><img src="image" alt="Nalorphine Structure" /></td>
</tr>
<tr>
<td>PURE ANTAGONIST</td>
<td>NALOXONE</td>
<td><img src="image" alt="Naloxone Structure" /></td>
</tr>
</tbody>
</table>

**FIG. 1**
cent research on identifying opiate-like substances such as $\beta$-endorphin, enkephalins (Cox et al., 1975; Hughes et al., 1975), and opiate receptors (Pert and Snyder, 1973; Kuhar et al., 1973) present in the brain has precipitated an idea that the exogenous opiates bind to the opiate receptors thus exerts an analgesic action. At the same time, the regular rhythm of different systems inside the body are disrupted (Christian, 1972; Gisburg and Cox, 1972; Walsh, 1972; Smith, 1972; Simon, 1972). Among different systems being affected, the pituitary-adrenal axis is one of them.

The effect of opiates on the pituitary-adrenal axis can be differentiated into an acute and chronic effect. It has long been found that a single injection of morphine in rats stimulates the secretion of ACTH and subsequently CS (Briggs and Munson, 1955; George and Way, 1955). This effect of morphine is dependent upon an intact pituitary-adrenal axis since it is abolished by hypophysectomy (George and Way, 1955). There is evidence indicating that morphine activation of ACTH secretion is mediated via a direct action on the rostral regions of the hypothalamus and median eminence (George and Way, 1959). In 1969, Lotti et al. (1969) employing the intrahypothalamic administration technique localized an area in the middle
region of the hypothalamus where focal injection of morphine (5-50 ug) produced a marked reduction of adrenal ascorbic acid and an elevation of plasma CS. In summary (George and Lomax, 1972), it appears that the acute administration of morphine may:

a. Produce a stress-like response by depletion of adrenal ascorbic acid or elevation of plasma corticosteroids.


c. Block the diurnal rise of plasma corticosteroids.

Chronic administration of morphine produces inhibition or tolerance to its effects on ACTH secretion and reduces adrenal corticosteroid secretion in man (Eisenman et al., 1961) and rats (Parole and Melchiorri, 1961; Ho et al., 1977b). Eisenman et al. (1962) found that morphine interfered with the early morning rise of plasma 17-hydroxycorticosterone. Moreover Cushman et al. (1970) and Kokka and George (1974) recently showed that stressful stimuli cause a significant increase of plasma CS suggesting the CNS response to stress is still functioning.

During withdrawal, either abrupt or naloxone-precipitated, there is an increase in both plasma ACTH and CS. The maximal rise in adrenal cortical
levels correlates well with the peak physiological
effects of the abstinence syndrome, approximately
48 hours after withdrawal (Eisenman et al., 1961).
In general, the effect of opiates on the pituitary-
adrenal axis is firmly recognized whereas how does
these changes take place is still an open question.

C. Morphine analgesia and acupuncture analgesia

Morphine has been employed as analgesic
centuries ago and is still one of the most potent
reagents for relieving intractable pain. Acupuncture
analgesia has only been developed in China during the
fifties. Since then a lot of work has been done
trying to correlate the analgesic effect of acupuncture
with that of morphine and to find out the mechanism
of analgesia. Recently Omura (1976) summarized the
similarities and differences between analgesia pro-
duced by morphine and acupuncture. The only signifi-
cant common effects between the two are analgesia,
euphoria, sedative effects for hyperactivity or
irritability and hyperglycemia effect. It was also
noted that the effect of acupuncture depends greatly
on the original condition of the body: with morphine,
blood pressure often decreases while with acupuncture
(O’Connor and Bensky, 1975), in subjects with normal
or high blood pressure, the blood pressure often
decreases; but, in those with low blood pressure, the change is a significant elevation towards normal. Acupuncture analgesia does not show any respiratory depression which is always accompanied with morphine analgesia. Moreover, analgesia produced by morphine acts on the whole body of the organism while acupuncture analgesia usually produces a localized effect. Another important characteristic of acupuncture analgesia is the necessity of an induction period for its action. This is most obvious when acupuncture is employed to treat drug addicts and in surgery.

In view of the recent discoveries of morphine-like peptides inside the brain, it is expected that the morphine action and the acupuncture effects may share some path in common (Lung et al., 1973; Research Group of Acupuncture Anaesthesia, 1974; Pomeranz and Chiu, 1976; Sjolund et al., 1977). Even though experimental results have actually been shown indicating that acupuncture analgesia can be blocked by administration of naloxone, a pure antagonist to morphine analgesics (Pomeranz and Chiu, 1976; Mayer et al., 1977), there are still many problems in establishing the interrelating pathway of the two analgesic effects. In China, acupuncture has long been employed beneficially to cure various kinds of sickness (O'Connor and Bensky, 1975; Wen and Chau,
1973; Shuaib and Haq, 1977; Yu and Lee, 1976; Tsuei et al., 1977). How can these effects be explained by a mechanism correlating morphine action and acupunctural effects is still not in sight. It is conceivable that analgesia is only part of the distinguish properties of acupuncture. Hopefully, a study of the AES action on morphine addicted animals may shed some light on the disclosure of the general mechanism of acupuncture. This will provide a better foundation for the investigation of other acupunctural effects not related to analgesic action.
MATERIALS AND METHODS

I. METHODS EMPLOYED TO INDUCE MORPHINE ADDICTION

Female rats of Sprague-Dawley Strain weighing 200-250 gm were used throughout the experiment. These rats were raised in a light (6:00-18:00 hour), temperature and humidity regulated environment. To prevent unnecessary stress generation, 10 rats were housed in each cage. Food and water were administered ad libitum. During the first part of the project, daily injection of morphine was used to addict the rats. This procedure is outlined in Table 2. The entire period of addiction was 8 weeks. Fourteen hours after the last administration of morphine, usually in the following morning, these addicted animals were employed for experimentation. In the latter part of the experiment, naloxone was introduced to precipitate the withdrawal syndrome, as suggested from our clinical observation. Under this condition, exceedingly shorter period, two weeks instead of eight, is adequate to create a suitable chronic morphine addiction state for AES treatment. For convenience, this modified schedule was followed ever since (Table 3).

Female mice of WHT Strain (British) weighing 25-30 gm were bred in our animal house under the same condition as described for the rats. These animals
<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Dosage in mg/Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>8:00 a.m.</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>8:00 p.m.</td>
<td>5</td>
</tr>
<tr>
<td>2nd</td>
<td>8:00 a.m.</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>8:00 p.m.</td>
<td>10</td>
</tr>
<tr>
<td>3rd</td>
<td>8:00 a.m.</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>8:00 p.m.</td>
<td>15</td>
</tr>
<tr>
<td>4th</td>
<td>8:00 a.m.</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>8:00 p.m.</td>
<td>20</td>
</tr>
<tr>
<td>5th</td>
<td>8:00 a.m.</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>8:00 p.m.</td>
<td>25</td>
</tr>
<tr>
<td>6th</td>
<td>8:00 a.m.</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>8:00 p.m.</td>
<td>30</td>
</tr>
<tr>
<td>7th</td>
<td>8:00 a.m.</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>8:00 p.m.</td>
<td>35</td>
</tr>
<tr>
<td>8th</td>
<td>8:00 a.m.</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>8:00 p.m.</td>
<td>40*</td>
</tr>
</tbody>
</table>

* The same dosage was used after the eighth day for about seven more weeks to prepare the rats for experimentation.
Table 3. Modified Morphine Addiction Schedule for Rats

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Dosage in mg/Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>9:00 a.m.</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>5:00 p.m.</td>
<td>5</td>
</tr>
<tr>
<td>2nd</td>
<td>9:00 a.m.</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>5:00 p.m.</td>
<td>10</td>
</tr>
<tr>
<td>3rd</td>
<td>9:00 a.m.</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>5:00 p.m.</td>
<td>20</td>
</tr>
<tr>
<td>4th</td>
<td>9:00 a.m.</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>5:00 p.m.</td>
<td>40</td>
</tr>
</tbody>
</table>

* The same dosage was used after the fourth day for about ten more days to prepare the rats for experimentation.
were rendered dependent on morphine by a subcutaneous implantation of 2 morphine pellets with an interval of 24 hours. The morphine pellets were prepared and implanted according to the methods of Hui and Roberts (1975). Each pellet contained 15 mg morphine hydrochloride. To ensure higher percentage of the experimental mice to be addicted, a screening procedure for responders was used (Simantov and Snyder, 1976). The animals were injected intraperitoneally with 10 mg/Kg naloxone (Endo Lab. Inc., N.Y.) 24 hours after the second implantation. Mice which showed jumping movement during a 15-minute observation period were selected and pellets were removed after this screening experiment. The jumpers were further "stabilized" for one week and rendered morphine dependent again by pellet implantation in exactly the same manner. The mice thus selected were found to show higher withdrawal scores on naloxone precipitation.

II. BEHAVIOR ASSESSMENT

In the first part of this research, special attention was paid to assimilate the clinical condition for heroin addiction in human subjects. Naloxone was not introduced in this period and withdrawal syndrome was merely due to abrupt abstinence from morphine for 14 hours. The withdrawal symptoms were
expressed as: teeth chattering, "wet dog" shakes (brief episodes of rapid repetitive shaking of the entire trunk, sometimes the animal shakes so violently that it may lose its balance), lacrimation along with salivation, ptosis, squealing on touch or "vocalization", diarrhea, and hyperactivity which is manifested by restlessness, increased exploratory behavior, rearing on hind legs, paw tremor, sniffing or digging and repeated attempts to escape from the container (Wei, et al., 1973; Cicero and Meyer, 1973). Of these various signs of withdrawal, "wet dog" shakes, teeth chattering, attempts to escape, abnormal posturing and diarrhea (Fig. 2) were among the most obvious and easily quantitized ones at the abstinence state in the rat. These parameters were thus used as a quantitative assessment throughout the behavioral studies.

It was found that naloxone-precipitated withdrawal system was generated at a more controllable manner than that from the natural morphine abstinence withdrawal. Unless specified, the behavior of the rats were observed for a 30-minute period before sacrifice for biochemical studies.

Withdrawal of addicted mice was induced by intraperitoneal injection of naloxone. Mice treated accordingly showed more repeated jumping and tremor
Fig. 2. The Abnormal Posturing of Rat Suffering from Withdrawal, the Rat also had Serious Diarrhea.
of the forelimbs (Fig. 3) while other symptoms such as hyperactivity are similar to the rats if not identical. This is in agreement with Maggiolo and Huidobro's observation (1961). The degree of withdrawal was assessed by counting the total scores of jumping, abnormal posturing, diarrhea and paw trembling in 15 minutes.

III. AES TREATMENT

Different size acupuncture needles of 2 inches long (guage 30) and 0.5 inch long (guage 34) were used for rats and mice correspondingly. Since the stimulation was generated electrically instead of manually, the acupuncture needles were slightly modified for this purpose (Fig. 4). The electrical stimulator (Biopulse Ltd., H.K.) generates a biphasic pulse with a frequency of 120 Hz. This is the same kind of stimulator employed in treating drug addicts (Wen and Cheung, 1973 a&b).

Morphine dependent rats were prepared for AES treatment a night before experimentation by having the acupuncture needles inserted into the conchae of both ears precleaned with an ethanol cotton swab. It was found that the point of insertion of the needle is not very critical and in general the conchae position was chosen which corresponds to the "lung" point.
Fig. 3. Jumping Behavior of Mice Resulted from Naloxone-precipitated Withdrawal
Fig. 4. Wiring Parts for AES Treatment

A, acupuncture needle; B, rubber fastener; C, plastic tube for insulation; D, plastic tube for connection; E, wire.
of human being. To ensure the needles do not slip during treatment, rubber fasteners were used. The animals with needles inserted into both ears required presumably an adequate period of rest to get used to their new features. They were then stabilized overnight in an undisturbed location. During this period, only water was supplied. In the next morning, the pointed end of the acupuncture needle in the AES treated group was connected to the stimulator mentioned above. Precautions were also taken to prevent poor connection as well as injury of the rat due to vigorous agitations and violent movements. During the early part of the research, a voltage of 3-4 volts was used. The voltage was found to be slightly higher than needed (see results) but creates in general similar results. To differentiate it from the lower voltage adopted in later experiments, this voltage was designated as high voltage. A lower voltage (0.5 or less) was used in later part of the experiments in rats. This low voltage was also used throughout all experimentation with mice. The rats receiving AES treatment were primed with AES for an hour prior to naloxone injection, and followed by AES treatment for another 30 minutes in the observation period (Fig. 5). Immediately after behavioral observation, the
Fig. 5. Rats under AES Treatment

The rat in the right hand tray was used as control (without AES treatment).
rats were decapitated and blood samples were collected in heparinized tubes and stored in an ice-bath for further investigations. Aliquots of the plasma samples were taken out for different biochemical assays.

Similar AES procedure was employed in mice except the needle insertion was carried out immediately, without a period of stabilization before the experiment. This modification was taken because mice reacted so violently to needle insertion that overnight "stabilization" will only increase the risk of damage to the needles as well as to the animals themselves. The priming period of mice receiving AES was 30 minutes. A 15-minute observation period was followed. Because of sample size, the plasma collected from the mice were pooled together for ACTH assay.

IV. ASSAY METHODS

A. Determination of plasma ACTH

Radioimmunoassay kits (Code IN.66) from the Radiochemical Centre, Amersham, England were employed for the determination of plasma ACTH in all animals. These kits showed high sensitivity in the determination of ACTH in plasma over a concentration range of 10^-4 to 4,000 pg/ml (Table 4). Since rats have a
Table 4. Precision and Accuracy of the ACTH Assay

<table>
<thead>
<tr>
<th>Number of control samples in each assay*</th>
<th>Mean ± S.D.</th>
<th>Precision (Percentage error at 95% confidence level)</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>219 ± 4.2</td>
<td>3.8%</td>
<td>109.5%</td>
</tr>
<tr>
<td>1</td>
<td>214</td>
<td>---</td>
<td>107%</td>
</tr>
<tr>
<td>3</td>
<td>229.7 ± 24</td>
<td>20.9%</td>
<td>114.9%</td>
</tr>
<tr>
<td>3</td>
<td>188 ± 29.5</td>
<td>31.4%</td>
<td>94%</td>
</tr>
<tr>
<td>3</td>
<td>221.3 ± 8.1</td>
<td>7.3%</td>
<td>110.7%</td>
</tr>
</tbody>
</table>

* Actual value of control sample is 200.
higher plasma ACTH level than clinical samples, 0.7 ml plasma is sufficient for the assay. The assay procedures are outlined schematically in Fig. 6. For each batch of samples, a standard curve was constructed. The labelled free ACTH was counted by a Gamma Counter (Nucleus, Inc., Oak Ridge, Tenn.). The percent standard error for total count of 10,000 is 2%. The level of ACTH in the sample is calculated by:

\[
\text{pg ACTH/mL plasma} = c \times \frac{5}{V} \times \frac{V}{700}
\]

where \( c \) = interpolated concentration from standard curve.

\( V = \) volume of unknown plasma sample (ml).

\( v = \) volume of extract solution used (ul).

B. Determination of plasma CS

Corticoids fluoresce when treated with concentrated sulphuric acid (Bauld et al., 1960; McLau-ghlen et al., 1958). Essentially, plasma CS was determined by the fluorometric method employed by Guillemin et al. (1959). The spectrofluorometer (Turner Model 430; Palo Alto, Calif.) used is capable to determine quantities of substance in nanogram level thus only 0.4 ml plasma sample is needed for each assay. The procedures of preparing
Sample (0.7 ml plasma)

- adsorb on to glass particles
  - supernatant
  - residue
    - wash with water, then IN HCl,
      eluted with 50% acetone
      - 1.5 ml eluant
    - dry down and redissolve in buffer
    - aliquots for RIA
      + antiserum
      - incubate for 16-20 hours at 4 C
        + labelled ACTH
      - incubate for 6-8 hours at 2-4 C
        + charcoal
      - aqueous
      - charcoal
      - discard
      - unbound ACTH counted
        by Gamma Counter

Fig. 6. Protocol Used In Preparing Plasma Samples For ACTH Radioimmunoassay
analysis sample are outlined schematically in Fig. 7. The precision and accuracy of these tests are monitored in Table 5.
Sample (0.4 ml plasma or less)
extract with 5 ml CH₂Cl₂
aqueous
discard
organic
evaporate to dryness
dissolve in ethanol:water (1:2, v/v)
remove aliquots for assay of CS content
+ conc. sulphuric acid:ethanol (7:3, v/v)
stand for 30 minutes
read fluorescence at 470/525 nm

Fig. 7. Protocol Used In Preparing Plasma Samples For Corticosterone Fluorometric Assay
<table>
<thead>
<tr>
<th>Number of control samples in each assay*</th>
<th>Mean ± S.D.</th>
<th>Precision (Percentage error at 95% confidence level)</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>65.4 ± 3.6</td>
<td>11%</td>
<td>91.7%</td>
</tr>
<tr>
<td>3</td>
<td>72.4 ± 4.3</td>
<td>11.9%</td>
<td>101.5%</td>
</tr>
<tr>
<td>2</td>
<td>75.9 ± 1.3</td>
<td>3.4%</td>
<td>106.4%</td>
</tr>
<tr>
<td>3</td>
<td>70 ± 5</td>
<td>14.3%</td>
<td>98.1%</td>
</tr>
<tr>
<td>5</td>
<td>67.5 ± 5.6</td>
<td>16.6%</td>
<td>94.6%</td>
</tr>
</tbody>
</table>

* Actual value of control sample is 71.4.
RESULTS AND DISCUSSION

Section A: EXPERIMENTS WITH RATS

Plasma ACTH and CS levels in chronic morphine addicted rats. Clinical observation from this group has demonstrated that the plasma ACTH levels in human drug addicts are lower than healthy normals. Experimental studies by Parole and Melchiorri (1961) with rats have suggested similar findings. In the laboratory animal chosen namely rats and mice, plasma ACTH and CS levels of control and morphine addicted rats were analyzed. A period of 8 weeks of addiction was chosen based upon the knowledge obtained from human subjects as well as behavioral observations. A comparison of these mean values depicted in Table 6 indicates that the plasma ACTH level of the morphine addicted rats is reduced by 41.7%. The test of significance, as calculated from the Student's t test shows a slightly higher p value, between 0.05-0.1. Taken into consideration that similar studies with mammals have shown ACTH depression in morphine addicted individuals (Parole and Melchiorri, 1961; McDonald et al., 1959; Ho et al., 1977a&b) and that the CS levels which are usually good reflection of the ACTH levels do demonstrate such a reduction. The results thus obtained should be regarded as consistent
Table 6. Plasma ACTH and Corticosterone Levels in Control and Morphine Addicted Rats

<table>
<thead>
<tr>
<th>Compound</th>
<th>Control</th>
<th>Morphine Addicted Rats</th>
<th>Test of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>ACTH (pg/ml)</td>
<td>37</td>
<td>454.7</td>
<td>262.7</td>
</tr>
<tr>
<td>CS (ug/100ml)</td>
<td>46</td>
<td>85.4</td>
<td>40.6</td>
</tr>
</tbody>
</table>

* Values in parentheses are units of concentrations.

# The significance level of the difference was evaluated by the Student’s t test.

@ 8 weeks of addiction, 4 hours after last dose of morphine injection.
with a reduction of plasma ACTH in addicted animals.

The plasma CS level in morphine addicted rats indicates significantly \((0.01 > p > 0.001)\) a reduction of 51.9%. Physiologically CS is released from the adrenal cortex upon stimulation by ACTH. Acute administration of morphine has been reported to elevate plasma CS level in rats (Briggs and Munson, 1955; George and Way, 1955). Upon chronic administration, however, adrenal cortical steroid secretion in rats and man gradually falls and presumably this change is recorded in a lower ACTH and hence lower CS levels. This is in good agreement with the above observation. These lower ACTH and CS levels are probably due to the inhibitory effect of morphine on the hypothalamus since most of the control mechanisms of ACTH secretion exert their actions there, and that the stimulatory effect of ACTH secretion by acute administration of morphine is also related to the function of the hypothalamus. Moreover this inhibition is insensitive to the close loop feedback interaction of the peripheral steroid levels as the lowered CS level cannot stimulate the secretion of ACTH (Dallman and Jones, 1973; Gann and Cryer, 1973; Feldman, 1973). However at this stage the ACTH secretion is still under the influence of many stress factors (Kokka and George, 1974).
Behavioral and biochemical changes in two periods of morphine abstinence. Experience obtained from clinical observations has suggested that the effect of AES is most satisfactory when it is applied to drug addicts before they have entered vigorous withdrawal state (Wen, H.L., personal communication). In the previous experiment, a 4-hour abstinence was arbitrary chosen. During this period, withdrawal symptoms had not yet developed. It was observed that a longer period, tentatively 14 hours, was necessary for more complete withdrawal symptoms such as "wet dog" shakes, teeth chattering, and diarrhea to occur. However, a comparison of the plasma ACTH and CS levels of these two rat groups has shown no significant difference (Table 7). These values are all lower than the normal individuals. Furthermore, if the mean ACTH value (181.6 pg/ml) at the 14 hour is selected to compare with the untreated control, a test of significance shows $p<0.001$. This is a further support of the conclusion drawn in the last section that chronic addicted rats has lower plasma ACTH levels.

Various reports have indicated that naloxone-precipitated withdrawal in rats as well as abrupt withdrawal in human show marked elevation in plasma CS levels (Wen, et al., 1977; Eisenman et al., 1961). To account for the low CS levels in both 4 and 14-
<table>
<thead>
<tr>
<th>Compound</th>
<th>4 hours</th>
<th>14 hours</th>
<th>Test of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>ACTH</td>
<td>6</td>
<td>265</td>
<td>114.6</td>
</tr>
<tr>
<td>(pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>6</td>
<td>41.1</td>
<td>15.6</td>
</tr>
<tr>
<td>(ug/100ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 8 weeks of addiction.
# Values in parentheses are units of concentrations.
@ The significance level of the difference was evaluated by the Student's t test; n.s., not significant.
hour periods, it is probable that the biochemical levels of these individuals are still under inhibitory control, with the 14-hour abstinent group closer to the moment of uprising since its behavior begins to score withdrawal symptoms. A further experiment employing naloxone to precipitate withdrawal in addicted rats, a methods recently applied clinically to induce more complete withdrawal, has revealed that this latter process, being more exhaustive, has triggered higher degree of withdrawal and accordingly a significant rise in plasma levels of ACTH and CS (Table 8). This suggests that the natural withdrawal occurred at the 14-hour period may very likely be at a mild withdrawal state. And that the primary chemical of action yet to be found has exerted its effect on the scoring of the behavioral withdrawal while both ACTH and CS, being secondary in nature, have their changes lagging behind.

**Effect of AES on naturally withdrawing rats.**

In order to animate the effect of AES on human morphine addicts, an experiment was designed to study the AES effect both behaviorally and biochemically on natural withdrawing rats. Table 9 and Fig. 3 report on these findings. There is a definite suppression of withdrawal symptoms with high statistical
Table 8. A Comparison of the Behavior and the Plasma Biochemicals in Morphine Addicted Rats
Showing Natural and Naloxone-precipitated Withdrawal*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>With Naloxone#</th>
<th></th>
<th>Test of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>S.D.</td>
<td>S.E.</td>
<td>N</td>
</tr>
<tr>
<td>Withdrawal Symptoms*</td>
<td>12</td>
<td>6.3</td>
<td>5</td>
<td>1.4</td>
<td>6</td>
</tr>
<tr>
<td>(scores/30 min.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACTH</td>
<td>18</td>
<td>181.6</td>
<td>114.5</td>
<td>27</td>
<td>12</td>
</tr>
<tr>
<td>(pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>18</td>
<td>53.5</td>
<td>22.5</td>
<td>5.3</td>
<td>12</td>
</tr>
<tr>
<td>(ug/100ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 8 weeks of addiction, 14 hours abstained from morphine.
# 0.2 mg naloxone per rat, i.p.
@ The significance level of the difference was evaluated by the Student's t test.
φ Score of withdrawal behavior is described in the text.
Table 9. A comparison of the Behavior and Plasma Biochemicals in Natural Withdrawal Rats with and without AES Treatment*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>With AES#</th>
<th></th>
<th>Test of Significance@</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>S.D.</td>
<td>S.E.</td>
<td>N</td>
</tr>
<tr>
<td>Behavior* (scores/30 min.)</td>
<td>10</td>
<td>12.9</td>
<td>8.3</td>
<td>2.6</td>
<td>10</td>
</tr>
<tr>
<td>ACTH (pg/ml)</td>
<td>18</td>
<td>181.6</td>
<td>114.5</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>CS (µg/100ml)</td>
<td>18</td>
<td>53.5</td>
<td>22.5</td>
<td>5.3</td>
<td>26</td>
</tr>
</tbody>
</table>

* 8 weeks of addiction, 14 hours abstained from morphine.
# High voltage, see text.
@ Evaluated by the Student's t test.
φ Individual served as control for itself.
Fig. 8. A Comparison of the Behavior and Plasma Biochemicals in Natural Withdrawal Rats with and without AES Treatment.

Data used for this figure are presented in Table 9. Number of samples is presented in parentheses above the bars.
FIG. 3
significance \((0.1 \leq p \leq 0.05)\). This suppression goes along with a significant rise in ACTH \((0.001 \leq p \leq 0.05)\) and CS \((0.01 \leq p \leq 0.001)\). It is of primary interest that while AES suppresses the natural withdrawal symptoms in rats, its mode of action, at the ACTH and CS level, is similar to that observed in a naloxone-precipitated withdrawal state. That is to say, the same hormonal increase result is found disregarded the animal's behavioral pattern, one at naloxone-precipitated withdrawal state and the other at suppression of withdrawal by AES.

An attempted explanation will have to consider the role played by these hormones to be secondary in nature. While a biochemical moiety as described in previous paragraphs dictates the withdrawal expression, the ACTH and CS levels only reflect the general state of the stress-related physiology of the animal. The dictator-biochemical moiety is in turn regulated by the chronic morphine addiction as well as adjusted by AES treatment. The net result of this dictator-biochemical controlled withdrawal effect is later exhibited in the hormonal level. It appears that the rate of the withdrawal (stress) state-related changes in hormonal levels is process dependent, with a rapid pickup (30 minutes) in an AES treatment and a slow development (14 hours) in a natural withdrawal condition.
It is conceivable that upon chronic morphine addiction, there is a hypothalamic inhibition of the basal secretion of ACTH which is insensitive to the feedback of low plasma CS level. At this state, however, a response to external stress is still functioning as manifested by an increase of ACTH upon stimulation. Two mechanisms have been suggested to explain the regulatory control of plasma ACTH level. They are namely an inhibitory effect by the hypothalamus and an excitatory effect by the external stress, with the latter exerting its action also through the hypothalamus. The stress resulted from abrupt morphine withdrawal is neurogenic and causes excitatory neural inputs to be converged on the hypothalamus. This stimulates the median eminence of the hypothalamus to secrete corticotropic releasing factor (CRF) and is transported through the hypophyseal-portal system to the anterior pituitary. The CRF then causes ACTH-secreting cells to release ACTH which in turn stimulates the adrenals to secrete CS. These elevated levels of plasma ACTH and CS are essential to prepare the organism to survive through an emergency situation induced by the stress (withdrawal syndrome in this case). In nature, this external stimulating effect can override the inhibitory effect due to chronic morphine addiction, and tentatively, can explain why an injection...
of naloxone elevates the plasma hormonal content. Other stressful stimuli such as cold exposure (Kokka and George, 1974) can also cause an elevation of ACTH.

Is AES treatment by itself an external stress-causing stimulus. The fact that AES treatment not only reduces the withdrawal symptoms in addicted rats but also raises their plasma ACTH and CS levels may open up the possibility of a dual function of the AES process. Primarily it regulates, reduces or corrects the withdrawal symptoms through the formerly postulated dictator-biochemical and secondarily the AES process itself exhibits a stress thus leading to an increase of plasma ACTH. In view of the fact that stress generation from experimented rats is hard to monitor or quantitate, a control experiment was devised to explore its likeliness. The results presented in Table 10 and Fig. 9 show no significant difference between the two groups of normal rats with or without AES treatment. This rules out the possibility of stress generated from AES treatment in normal rats. Nevertheless, the absence of hormonal change in normal rats compared to a change in withdrawal rats in the AES treatment indicates that no matter what the effect of AES is, it is very likely that it channels through morphine induced physiological state. Preferably this
<table>
<thead>
<tr>
<th>Compound*</th>
<th>Control</th>
<th>With AES</th>
<th>Test of Significance#</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>ACTH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(pg/ml)</td>
<td>37</td>
<td>454.7</td>
<td>262.7</td>
</tr>
<tr>
<td>CS</td>
<td></td>
<td>85.4</td>
<td>40.6</td>
</tr>
<tr>
<td>(ug/100ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values in parentheses are units of concentrations.

# The significance level of the difference was evaluated by the Student's t test, n.s., not significant.
Fig. 9. Plasma ACTH and CS Levels of Normal Rats

with and without AES Treatment

Data used for this figure are presented in Table 10. Number of samples is presented in parentheses above the bars.
FIG. 9
is taken to mean that the AES effect is not through a simple stress generating process but a more complicated pathway(s) involving a "regulation" of physiological and biochemical changes due to morphine addiction.

It has well been recognized that acupuncture has a general "regulatory" effect on a lot of diseases with a proper choice of stimulation at various acupuncture points (O'Connor and Bensky, 1975). In drug addict treatment, this "regulatory" effect covers a great variety of pathological, physiological and biochemical changes including those in the CNS (Jacquet and Lajtha, 1973), the endocrine system (Bruni et al., 1977), nucleic acid and proteins (Cox and Osman, 1970; Gisburg and Cox, 1972), metallic ions (Harris et al., 1977; Hitzemann et al., 1974), cyclic nucleotides (Nerali et al., 1975; Mehta and Johnson, 1975) as well as the neurotransmitters (Cheney et al., 1975; Carroll and Sharp, 1972; Leong Way, 1972). So, in the case of AES treated natural withdrawal rats, part of the "regulatory" effect is indicated by a restoration of normal level of plasma ACTH and CS levels. Tentatively this adjustment of ACTH level by AES can antagonize the inhibitory effect of morphine.
in normal rats. Naloxone was introduced clinically as a morphine antagonist for the precipitation of withdrawal symptoms in human addicts successfully (Hammond, 1971). Similar studies with rats have indicated that such a treatment in rats produces a more uniform and consistent behavioral withdrawal symptoms (see previous paragraph). To understand the nature of naloxone on the plasma biochemicals of addicted rats, an experiment was designed to study the same parameters in the normal rat counterpart. Table 11 lists the plasma ACTH and CS concentrations of the two normal rat groups with and without naloxone injection. This result is further represented in Fig. 10. It shows essentially no effect.

Effect of AES on naloxone-precipitated withdrawal in rats. It has been observed clinically that AES effectively alleviates the withdrawal symptoms precipitated by naloxone in morphine addicted patients (Wen and Cheung, 1973 a&b). In rats, a proper condition was chosen to give an optimal withdrawal response in addicted animals as judged by the extensiveness of the behavior of these addicted individuals. The dosage of naloxone used as well as the duration of addiction appear to be crucial for the effectiveness of the AES treatment. This is consistent with the observation
Table 11. Plasma ACTH and Corticosterone Levels of Normal Rats and Normal Rats Injected with Naloxone*

| Compound# | Control | | | | | With Naloxone | | | | Test of Significance@
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>S.D.</td>
<td>S.E.</td>
<td>N</td>
<td>Mean</td>
<td>S.D.</td>
<td>S.E.</td>
<td></td>
</tr>
<tr>
<td>ACTH (μg/ml)</td>
<td>37</td>
<td>454.7</td>
<td>262.7</td>
<td>43.2</td>
<td>7</td>
<td>309</td>
<td>125.2</td>
<td>47.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>CS (μg/100ml)</td>
<td>46</td>
<td>85.4</td>
<td>40.6</td>
<td>6</td>
<td>7</td>
<td>110.2</td>
<td>26</td>
<td>9.8</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* 0.2 mg/rat i.p.

# Values in parentheses are units of concentrations.

@ The significance level of the difference was evaluated by the Student's t test;
   n.s., not significant.
Fig. 10. Plasma ACTH and CS Levels of Normal Rats and Normal Rats Injected with Naloxone

Data used for this figure are presented in Table 11. Number of samples is presented in parentheses above the bars.
FIG. 10
that analgesia produced by acupuncture is blocked
by naloxone (Pomeranz and Chiu, 1976; Sjolund et al.,
1977). Clinical studies in progress have also indicated
that a better "detoxification" process has been
developed in treating drug addicts by carefully
monitoring the dosage of naloxone applied to achieve
a suitable withdrawal intensity in the patient for
more effective AES treatment (Wen, 1977). It was found
that rats addicted for 2 weeks, followed by a 22-hour
abstinence from morphine, gave proper and constant
withdrawal responses upon an intraperitoneal injection
of naloxone (0.01 mg/rat) and that this withdrawal
behavior can be successfully suppressed by AES
treatment at low voltage. Both high and low voltage
are as effective but the latter was chosen to eliminate
any complication due to possible electrical side effects.
Behavioral studies were done as described in Materials
and Methods except a shorter observation period
(15 minutes) was employed. The results are recorded
in Table 12 and depicted in Fig. 11.

To begin with, the behavioral and biochemical
levels of the naloxone-precipitated rats are higher
than that in the chronic morphine addicted rats (see
Table 8). AES reduces the behavioral responses to
42.2%, ACTH to 33% and CS to 76.2% correspondingly.
Table 12. A Comparison of Behavioral and Plasma Biochemicals in Naloxone-precipitated Withdrawal Rats with and without AES Treatment*  

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>With AES</th>
<th></th>
<th>Test of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>S.D.</td>
<td>S.E.</td>
<td>N</td>
</tr>
<tr>
<td>Withdrawal Symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(scores/15min.)</td>
<td>5</td>
<td>16.6</td>
<td>7</td>
<td>3.1</td>
<td>5</td>
</tr>
<tr>
<td>ACTH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µg/ml)</td>
<td>5</td>
<td>938.8</td>
<td>328.4</td>
<td>146.9</td>
<td>5</td>
</tr>
<tr>
<td>CS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µg/100ml)</td>
<td>5</td>
<td>118.5</td>
<td>14.7</td>
<td>6.6</td>
<td>5</td>
</tr>
</tbody>
</table>

* The rats were rendered morphine dependent by 2 weeks of i.p. injection. They were abstained from morphine for 22 hours. Withdrawal was precipitated by 0.01 mg naloxone/rat i.p., and observed for 15 minutes. Low voltage was used during AES treatment.
Fig. 11. A Comparison of the Behavior and Plasma Biochemicals in Naloxone-precipitated Withdrawal Rats with and without AES Treatment

Data used for this figure are presented in Table 12. Number of samples is presented in parentheses above the bars.
WITHDRAWAL SCORES IN 15 MIN

PLASMA ACTH LEVEL /µg/ml

CONTROL WITH AES

FIG. 11
This comparison clearly demonstrates that a suppression in the behavioral level can be qualitatively expressed by the plasma ACTH and CS levels. To account for this observation, the plasma ACTH, which in turn governs the plasma CS level, can play either one of the following roles: its level determines the extensiveness of the withdrawal syndrome, or its level is actually a direct indication of a chain of reactions initiated by the withdrawal syndrome. A more detail comparison of the behavioral responses and the plasma ACTH and CS levels at various stages of the experiment is shown in Fig. 12. In this case, even though application of AES treatment can alleviate both natural and naloxone-precipitated withdrawals with equal success, yet the changes of the plasma biochemicals are, in fact, in opposite directions. In natural withdrawing rats, the effect of AES is an increase in plasma ACTH and CS levels while in naloxone-precipitated withdrawal, the effect is a reduction. This finding suggests that both the biochemical levels as well as their bidirectional changes should not be considered as a main factor in generating AES beneficial effect.

It is more reasonable to accept a concept that AES plays a general regulatory role in the animal system and very likely it works through some dictator-
Fig. 12. A Comparison of the Behavior and Plasma Biochemicals at Various States in the Experiment

Number of samples is presented in parentheses above the bars. R, normal rats; RA, normal rats treated with AES (3-4 volts); RN, normal rats injected with naloxone (0.2 mg/rat, i.p.); M4, 8-week addicted rats, 4 hours after morphine; M, 8-week addicted rats, 14 hours after morphine; N, 2-week addicted rats, 22 hours after morphine, withdrawal precipitated by naloxone (0.01 mg/rat, i.p.); NA, same as N but treated with AES (0.5 volt).
FIG. 12
biochemical(s) yet to be found. This dictator-biochemical has a general function of "regulating" many mal-functions in different systems as described in previous paragraphs. As a result of the alleviation of the withdrawal symptoms and the "regulatory" effect, the plasma ACTH and CS regain their presumably normal levels.

Section B: EXPERIMENTS WITH MICE

Optimal naloxone dosage for AES alleviating withdrawal treatment. Experiments were done with mice to study the generality of the rehabilitation effect of AES on various animal types. As discussed in the previous section, the amount of naloxone is crucial for the quality of the AES effect. This becomes more important in a small body size animal such as mouse. Fig. 13 represents two withdraw-scoring curves in the presence and absence of AES treatment. A comparison of the values indicates that 10 mg/Kg body weight is the optimal naloxone dosage for expressing the AES effect. This amount of naloxone was thus used for further experimentation with mice.

Effect of AES on plasma biochemicals from naloxone-precipitated withdrawing mice. A study of
Fig. 13. Total Scores of Naloxone-precipitated Withdrawal

n: number of mice; *: statistically significant;
●—○—○: withdrawal scores without AES; □—□—□: withdrawal scores with AES.
In FIG. 13, the total withdrawal scores are plotted against the naltrexone dosage (mg/Kg body weight) for different groups indicated by * and **. The graph shows the range and variability of withdrawal scores for each dosage level.
the plasma ACTH in morphine-addicted mice reveals that it is lower (46.8 pg/ml) than the non-addicted control (114.7 pg/ml). This observation is in good agreement with the results obtained in rats as well as in man. Similarly, non-addicted control shows no significant plasma ACTH change upon an injection of naloxone. However, naloxone caused a ten-fold increase of plasma ACTH in morphine-addicted mice (46.8 to 446.6 pg/ml) as shown in Table 13. This increase is significantly reduced by AES to approximately the normal level (186.6 pg/ml).

Identical observations were obtained in human addicts and naloxone-precipitated withdrawing rats. Even though the natural withdrawal mice have not been tested, it seems most likely that the function of AES transcends animal origin and has a general regulating effect on withdrawal animals.
Table 13. Effect of AES on Plasma ACTH of Addicted Mice during Withdrawal

<table>
<thead>
<tr>
<th>Group</th>
<th>ACTH (pg/ml) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Addicted + naloxone</td>
<td>446.4 ± 236.5 (9)</td>
</tr>
<tr>
<td>Addicted + naloxone + AES</td>
<td>186.6 ± 143.2 (8)</td>
</tr>
</tbody>
</table>
CONCLUSION

The effects of AES have been successfully demonstrated in at least two aspects: a general analgesic effect and a regulatory effect in alleviating withdrawal symptoms from morphine addicts. This investigation has established two animal models for further studies on the regulating mechanisms and has demonstrated the resulting plasma hormonal changes at various stages of morphine addiction. In general, an AES effect requires a period of induction, \( \beta \)-30 minutes in the behavioral studies and presumably similar time interval in the plasma biochemical changes.

In view of the observation that the general analgesic effect can be blocked by naloxone and that morphine-like peptides are involved in the analgesic process, it is conceivable that a humoral pathway may take part in transducing the AES effect. A candidate of this humoral factor is very likely the \( \beta \)-endorphin. It was discovered that the \( \beta \)-endorphin, with a more potent opiate effect than morphine, is secreted from the anterior pituitary (Guillem et al., 1977; Krieger, et al., 1977). As distinct from enkephalin, a pentapeptide located in the nervous system which rapidly loses its morphine-like action in the blood, \( \beta \)-endorphin
is highly resistant to peptide degradative enzymes found in the circulatory system. Since analgesic effect can be observed in a recipient animal with blood cross-circulated from another animal under AES induced analgesia (Lung et al., 1973), it is expected that there is a stable functional biochemical moiety circulating in the blood and serves as a messenger to induce an analgesic effect on the recipient. This messenger type biochemical may very well be the humoral opiate factor and accordingly, the noticeable β-endorphin.

It is reported that ACTH and β-endorphin are both secreted concomitantly from normal rats (Guillenmin, et al., 1977). Our findings on the changes of plasma ACTH levels do not strongly substantiate the proposal that AES has an effect only in inducing ACTH thus β-endorphin secretion.

Experiments conducted by Mayer (1975) as well as Yaksh et al. (1976) have shown that focal electrical stimulation of the mesencephalic and periaqueductal central gray matter in the brain induces analgesia which is at most only partially reversed by naloxone. This finding suggests that other than the humoral pathway may be employed in inducing analgesia, tentatively, there is a "non-humoral" pathway. Consequently, this
"non-humoral" pathway should not be antagonized by naloxone. And the partial inhibition observed can be explained on the basis of the collaboration of both pathways. This is not unusual since in signal transduction, it has been documented that in bacterial chemotaxis, two reception processes are involved in the detection of one attractant, namely, glucose (Adler et al., 1973). One of the suggestions is that these two pathways, humoral and "non-humoral", contribute to different extents at different physiological states. In naloxone-precipitated withdrawal, AES exerts its alleviating effect through this "non-humoral" pathway with a reduction in the plasma ACTH and thus β-endorphin levels.

A primitive model which accounts for the above observations appears to include:

a. An inducer which can either be quantitatively or qualitatively charged to different extent by a natural withdrawal process or in the presence of naloxone. This charged inducer will govern the extensiveness of the withdrawal symptoms.

b. An intensified withdrawal will in turn trigger an increase in the plasma ACTH level of the addict. However this is withdrawal-process dependent. In a naloxone-precipitated
withdrawal, the effect is immediate while in a natural withdrawal, it takes a considerable time.

c. AES reduces the effect of the inducer in generating withdrawal symptoms. This reduction creates a "regulating" mechanism which sends the plasma ACTH back to normal level.

d. Apparently, there is an internal standard for normal ACTH level in each individual. Chronic addiction lowers this level while withdrawal eventually raises it. Both conditions are off normal. A regulating effect as mentioned in (c) exerts its influence on this system simply by removing the factor(s) which has upset the normal ACTH in either direction. This is in analogy to bringing a spring back to its most stable situation no matter the previous upsetting force is pulling or pushing.

The inducer moiety mentioned in the model can be as simple as a protein, tentatively a receptor-type protein. Presumably, an AES effect is to send off signal for inducing a conformational change in this "protein" to vary its affinity to its corresponding substrates. This variation governs the behavioral phenomenon. The signal can be as sub-
stantial as a small molecule which binds to the inducer. This model is very primitive and is specifically constructed to explain the major findings described in this thesis. Phenomena such as tolerance and physical dependence were not taken into consideration. In order to set up a more sophisticated model for the effect of AES on drug rehabilitation, information from the binding and action of opiate receptor, the secretion and general function of morphine-like peptides and a complete analgesia pathway would be helpful. Hopefully, future work may enrich our knowledge in these aspects.
REFERENCES


