ENDOCRINE PROFILE IN HEROIN ADDICTS

By

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Dedicated to my son

JUNAID

CERTIFICATE

This thesis by Mr. Arshad Saadat is accepted in its present form by the Department of Biological Sciences, Quaid-I-Azam University, as satisfying the thesis requirements for the degree of Doctor of Philosophy in Biology (Endocrinology).

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LIST OF ABBREVIATIONS

µIU	micro International unit
µl	microliter
βLPH	Beta Lipotropin
µmol	micromole
ACTH	Adrenocorticotropic hormone
AFIP	Armed Forces Institute of Pathology
AMP	2-Amino2-methyl-1-propanol
ASP	Aspartate
ATP	Adenosine triphosphate
Ca^{++}	Calcium
cAMP	cyclic Adenosine monophosphate
CIA	Cemiluminiscence assay
CNS	Central nervous system
CPS	Counts per second
CRF	Corticotropic releasing factor
DHEA-SO_4	Dehydroxyepiandrosterone sulphate
E_2	Estradiol
EAA	Escitatory amino acid
Free T_4	Free thyroxine
FSH	Follicle stimulating hormone
g	gram
GABA	Gamma amino butyric acid
GH	Growth hormone
GLU	Glutamate
GnRH	Gonadotropin releasing hormone
GRF	Growth hormone releasing factor
GTP	Guanine triphosphate
ICV	Intracerebroventricular

IP3	Inositol 1,4,5 triphosphate
LAAM	L-α-acetylmethadol
LH	Luteinizing hormone
mol	Molar
M6G	Morphine-6-glucoronide
MAM	Monoacetylmorphine
mIU	milli International unit
ml	milliliter
mmHg	millimeter of Mercury
mmol	millimole
MW	Molecular weight
Na, K-ATPase	Sodium- potassium adenosine triphosphatase
NMDA	N-methyl-D-aspartate
NSB	Non specific binding
OXT	Oxytocin
PEG	Polyethylene glycol
Pi	inorganic phosphorus
pmol	picomole
PMT	Photomultiplier tube
POMC	Proopiomelanocortin
PRL	Prolactin
PTH	Parathyroid hormone
SEM	Standard error of mean
SHBG	Sex hormone binding globulin
T	Testosterone
T_3	Triiodothyronine
TCA	Trichloroaceric acid
TSH	Thyroid stimulating hormone
WHO	World Health Organization

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ABSTRACT

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A detailed study to determine the effects of chronic heroin addiction was conducted on a group of adult male addicts routinely admitted to rehabilitation centers in the District of Rawalpindi, Pakistan. The effects of heroin and the extent of damage, if any, prior to and following subsequent withdrawal therapy (detoxification treatment) were examined on a variety of physiological parameters especially the endocrine axes of pituitary with gonads, adrenal and thyroid. Determinations were carried out on serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), adrenocorticotropic hormone (ACTH), growth hormone (GH), thyroid stimulating hormone (TSH), testosterone (T), dehydroxyepiandrosterone sulphate (DHEA-SO_4), estradiol (E_2), sex hormone-binding globulin (SHBG), cortisol, free thyroxine (FT_4), triiodothyronine (T_3), insulin and glucose, parathormone (PTH) and gastrin. In addition, analysis was conducted to determine effects on volume of the ejaculate, sperm count, sperm motility and semen fructose level. Determinations were also made to measure activity of Na,K-ATPase in erythrocytes and leucocytes of the heroin addict.

A total of 60 male addicts periodically brought to the Rawalpindi General Hospital, Military Hospital Rawalpindi and Imran Detoxification Center, Rawalpindi for symptomatic detoxification were selected for the study. A total of 30 healthy subjects, matched for age and sex, were used as controls. Prior to selection for the study, the patients consented voluntarily for participation in the investigation. It was ascertained that none of the patients was critically ill. Information was obtained concerning route of drug intake, the amount of drug taken daily and that no drug other than heroin was used by them during the past six months. The patients ranged between the ages of 25-30 yr (mean age: 27.7 $\pm$ 1.08 yr) and admitted use of the drug for the past 2-16 yr (mean duration: 7.3 $\pm$ 0.46 yr). The daily intake of 30% pure street heroin was 3-5 g (mean 3.66 g/day). The mode of drug intake was inhalation of vapors (51 patients) smoking (8 patients) and sniffing (1 patient). The last dose taken was 1-2 hours prior to entry in the rehabilitation centers. Blood, urine and semen samples were taken soon after entry of the patients to the Centers. The urine samples were used to determine presence of heroin and

its metabolites. The mean blood pressure of the patients at the time of entry was 112/70±1.06/1.03 mm Hg. Blood and semen samples were taken again following symptomatic detoxification treatment (withdrawal therapy) on the 10^{th} and the 30^{th} day of drug withdrawal.

The results show that the serum level of FSH increases while that of LH decreases significantly in the addicts. Prolactin also increases significantly. Of the male gonadal hormones, serum T is depressed but neither DHEA-SO_4 nor E_2 undergo significant change in the addicts. Increase in serum SHBG parallels the change in testosterone. Both ACTH and cortisol levels in the serum also undergo significant depression. The level of cortisol is altered both in the morning and evening but the diurnal rhythm remains unaffected by heroin. Growth hormone, insulin, glucose and gastrin also increase significantly. Free thyroxine (FT_4) and PTH levels show decrease. In contrast, no change could be detected in serum T_3 and TSH. Determination of the above parameters 10 and 30 days after drug withdrawal revealed that all of the above changes are reversible. Delayed recovery (30^{th} day of detoxification) from the damaging effects of heroin was recorded for FSH, LH, T, SHBG, ACTH, cortisol and FT_4. Early recovery (10^{th} day of detoxification) was noted for PRL, GH, insulin, glucose and PTH.

The most lasting alteration due to heroin addiction occurred in sperm count, motility of sperms and fructose level in the semen. A drastic decrease in these seminal factors was recorded. No modification could be detected in the volume of the ejaculate. The patients failed to recover from these effects of heroin even after 30 days of detoxification therapy. Thus heroin exerted irreversible influence on seminal integrity as far as can be argued on the basis of 30-day limit of detoxification treatment.

The activity of Na,K-ATPase in the addicts remained unaltered in the erythrocytes but showed a significant increase in the leucocytes. The enzyme activity in the leucocytes was restored to the control level on the 15^{th} day of withdrawal therapy.

The data presented show that the majority of physiological aspects examined undergo significant alteration due to chronic heroin addiction. The endocrine system most noticeably affected by heroin is the pituitary-gonadal axis apparently resulting in

irreversible alterations in sperm count, sperm motility and seminal fructose. This observation provides affirmation of the self reported loss of libido by the majority of the addicts participating in this investigation.

Analysis of the data by separating into short duration and long duration chronic addiction revealed that PRL, ACTH, cortisol and all semen parameters undergo far greater impairment due to heroin. Even recovery from the effects of the drug following detoxification is blocked in the longer duration addicts (9-16 yr).

It is important to note at the outset some of the unavoidable constraints of conducting a study on human subjects like the one presently under consideration. Among the most critical of these are (1) the dosage of street heroin, (2) its purity, and (3) the timing of the last dose taken. Admittedly total reliance has been placed on the assurances given by the addicts regarding the timing of last dose, and on published information on the average purity of street heroin. Any further standardization of these conditions could not be done in view of the ethical considerations.

However, it is evident from reversibility of the changes in most of the physiological parameters that timely rehabilitation steps can restore normalcy in the life of the addicts.

INTRODUCTION

INTRODUCTION

Use of herbs and plant materials as a means of cure of disease has been as old as human history. Innumerable plants have been discovered over centuries which have medicinal properties. Modern research has harnessed a variety of active plant ingredients, refined them and even synthesized many through pharmaceutical innovation for use as drugs. Certain classes of these are remedies for disease, others influence the central nervous system. Many of the centrally acting drugs are known to relieve pain, have psychotomimetic effects, even influence the mind and produce euphoria. A vast array of new psychotropic agents including sedatives, hypnotic and anxiolytic drugs have come in medical use. They are safe as long as used under medical supervision and advice. Unfortunately, however, when these are used unwittingly by humans for pleasure, it constitutes drug abuse. Prolonged abuse results in addiction with ruinous consequences for the individual, the family and the society at large. Heroin, the subject of the present investigation, is an opioid drug obtained from the poppy plant *Papaver somniferum*. This plant yields opium which has been eaten or smoked for generations in many parts of the world. The term opioid refers to all narcotic analgesics, both natural and synthetic, which possess morphine-like activity. When used for pleasure and in the absence of medical care or self control, they are frighteningly addictive.

Until 1979, heroin addiction was unknown in Pakistan. Coincident with the Afghan crisis in the region and since then it has been on the increase here, perhaps due to its easy availability (Gallardo, 1984). According to National survey of drug abuse by Pakistan Narcotics Control Board (Anonymous, 1993), this country has been hit by this drug epidemic in the worst possible manner. By 1994, the estimated numbers of heroin addicts had reached 1.53 million (Fayyaz, 1994).

Although much work has been done on the physiological, behavioral and social consequences of heroin use in Europe and North America, little research has been done in this context in Pakistan. This together with the gravity of heroin addiction and its dire socio-cultural repercussions provided the primary motivation for undertaking the present

investigation. The work was further facilitated by the opportunity of access to heroin addicts brought to local hospitals and detoxification centers for remedial therapy.

Opium contains more than 20 distinct alkaloids. In 1806, Serturner, a German pharmacist, isolated a pure active alkaloid from opium and named it morphine, after Morpheus, the Greek god of dreams. The alkaloids constitute about 25% of opium by weight and can be divided into two distinct chemical classes, phenantherenes and benzylisoquinilines. The principal phenantherenes are morphine (10% of opium), codeine (0.5%) and thebaine (0.2%). The principal benzylisoquinolones are nosacine (6.0%) and paraverine (1%), a smooth muscle relaxant (Jaffe and Martin, 1975).

Heroin is a derivative of morphine and prepared by acetylating it at the 3 and 6 positions either with acetic anhydride or more easily with acetyl chloride. Both hydroxyl groups of morphine are esterified. The chemical name of heroin is 3,6-Di-O-acetylmorphine hydrochloride monohydrate (C21 H23 NO5,HCl H2O). Its Mol wt. is 423.9 and melting point is 229°C to 233°C. It is also called, Diamorphine hydrochloride, Diamorph hydrochloride, Diacetylmorphine hydrochloride and Heroin hydrochloride (Dollery, 1991).

Pure heroin is a white powder easily soluble in water (1 g in 2 ml of water at 25° C) and has a characteristic bitter quinine-like taste. The pH of a 0.01 mol solution is 5.2. Heroin is somewhat denser powder than morphine, and is more potent on weight basis. It hydrolyzes to morphine acetate in boiling water and also hydrolyzes on prolonged exposure to air, turning pink and emitting an acetic or vinegar taste. All illicit heroin dealers in Pakistan can produce heroin base which is commonly referred to as smoking heroin. This heroin is usually brown in color having on the average 60 - 80% purity. It is not water soluble and its fumes are inhaled by "burning" it on tin foil or smoked by stuffing it into cigarettes. Many, but not all, illicit heroin chemists in Pakistan can produce the water soluble heroin hydrochloride, commonly called injectable heroin. It has 80 - 95% purity. This heroin is usually white, chalky or light tan in color. Injectable heroin is 4 - 5 times more expensive than the smoking heroin. Most of the heroin

available at street level in Pakistan is brown in color and contains only 30 - 40 % pure heroin (Sheikh, 1994). In Pakistan, heroin is often not what it is purported to be as it is "cut" (diluted) with cheap contaminants having heroin-like properties to deceive the addicts. The commonly used chemicals for this purpose may enhance the bitter taste of heroin and its vasodilator effects. It is usually diluted in Pakistan with such substances as phenobarbitone, quinone, hair removing powder and aspirin, while abroad inert materials such as talc, lactose, baking soda, starch, sucrose and inositol form the bulk of the contaminants along with amphetamines, strychnine, quinine, barbiturates, aminopyrine and methapyrilene (Dimijian, 1974). Consequently, the resulting unit for local purchase is a "bag" containing 90 mg of the material, of which an average of only 3 mg is heroin (Colasanti and Martin, 1994). It is usually sold in 1/2 gm to 5 gm packets containing 30 - 40 % heroin (Sheikh, 1994).

Heroin users may change their customary methods of drug administration from time to time, from the perspective of harm reduction. Methods that avoid intravenous administration have been preferred (Strang, 1993). In Pakistan the common method is smoking. Some of the tobacco is taken out from the cigarettes and replaced by heroin, then it is smoked like ordinary cigarette. So it can be used anywhere without anyone knowing about it. Inhalation is also fairly common, and 78% of the heroin addicts apply this technique. It is more efficient than smoking as upto 70 - 80% of heroin is utilized (Sheikh, 1994).

Several types of opioid receptors have been identified from definable functional domains at various sites in the nervous system and other tissues by radio-ligand binding, autoradiographic and immunohistochemical techniques (Martin *et al.*, 1976; Pert and Synder, 1973; Simon, 1973; Gilbert and Martin, 1976). These receptors are called Mu (μ), Kappa (k), Delta (δ), Sigma (σ) and Epsilon (ε) (Goodman *et al.*, 1983). With the development of more specific ligands, subtypes of μ, δ, and k - receptors have also been identified (Pasternak , 1988; Goldstein and Naidu, 1989). There are two functional domains within a receptor, a "ligand-binding domain" and an "effector domain", based on the specific three dimensional structure. All of the opioid receptor types are linked either to second messenger system or directly to ion-channels (Simond, 1988) by a group

of GTP- binding proteins known as G proteins (Gilman, 1987; Ross, 1989).The mu-receptor and the delta receptor are coupled through G protein to adenylate cyclase or inositol 1,4,5 triphosphate (IP3) or to potassium ion channels (Berridge, 1993). Activation of the mu receptors and the delta receptors opens the potassium channels, thereby decreasing calcium ion conductance. Activation of the k-receptors results in the closing of calcium channels. The decreased calcium conductance is believed to be responsible for decreased transmitter release, including acetylcholine, norepinephrine, dopamine, serotonin and substance P (Woolverton and Johnson, 1992). It is postulated that several of these transmitters are involved in the action of opioids, since no single transmitter system can account for all the effects of the opioids (Heijna, 1992). The opioids and opiopeptins (endorphin, enkephalin, dynorphin) have been shown to inhibit the release of excitatory transmitters from these primary efferents (Koyuncuoglu, 1995).

Heroin does not itself bind to the opiate receptors, but works through its active metabolites, morphine and 6-monoacetylmorphine. It is deacetylated by tissue esterases, mainly in liver, to 3 or 6 monoacetylmorphine (MAM), which enters brain followed by morphine. Heroin is readily absorbed following injection, oral or nasal administration, rapidly hydrolyzed to 3 or 6 monoacetylmorphine in the blood and then slowly metabolized to morphine which is its major active metabolite. Normorphine is also formed to a minor extent. All of these metabolites may be conjugated with glucuronic acid in the liver. Upto 80% of the administered dose is excreted in the urine in 24 hours mainly as morphine-3-glucuronide, while about 5-7% of the dose is removed as free morphine; 1% as 6 monoacetylmorphine, 0.1% as unchanged drug. The heroin metabolites are also excreted in the bile but to a very negligible extent (Moor and Hand, 1988).

It is generally believed that addiction to diamorphine (heroin) is more easily acquired than addiction to morphine because it produces intense euphoria and fewer side effects (constipation, nausea, vomiting). The pharmacological properties of morphine and heroin are same with the exception that heroin is more potent and effective due to greater penetrability into the brain where its effects are likely mediated by morphine and 6-

acetylmorphine (Dewey and Brase, 1991). Plasma half live of heroin is three minutes while that of morphine is 2-3 hr (Moore and Hand, 1988).

MAJOR EFFECTS OF HEROIN

A variety of depressant and stimulant effects of heroin are known (Dewey and Brase, 1991; Reisine and Pasternak, 1996).

Depressant effects

1. Suppression of pain (analgesia)
2. Drowsiness and decreased mental alertness (sedation)
3. Decreased respiration
4. Decreased myocardial oxygen demand
5. Suppression of cough (antitussive)
6. Decreased peristalsis
7. Inhibition of fluid and electrolyte accumulation in intestinal lumen.
8. Decreased immune response
9. Inhibition of emetic center.
10. Slight decrease in body temperature.

Stimulation effects

1. Euphoria
2. Constriction of pupil (miosis)
3. Stimulation of chemoreceptor trigger zone.
4. Increased tone of intestinal smooth muscles
5. Increased tone of sphincter of Oddi, increased biliary pressure
6. Increased tone of detrusor muscles
7. Increased tone of vesicular sphincter
8. Increased release of prolactin and antidiuretic hormone.
9. Proconvulsions with overdose.
10. Constipation owing to:
    a. Prolongation of stomach emptying time
    b. Decreased propulsive contractions of the small intestine and Increased tone of the large intestine slowing down transit, and
    c. Increased tone of the anal sphincter and inattention to normal sensory stimuli for defecation.
11. Increased bile duct pressure and increased sphincter tone, leading to difficulty in micturition.
12. Increased tone of the vesicular sphincter tempered by a reduced volume of urine production.

The phenomena of tolerance and withdrawal are believed to be a result of neuroadaptive changes in the brain. These changes are part of a homeostatic process which counteract the acute pharmacological effects and occur when a drug is administered. Tolerance is reversible, the normal degree of sensitivity being achieved 2-3 weeks after withdrawal. With repeated administration of morphine or its surrogates, there is a gradual loss in their effectiveness resulting in tolerance for the drug. To reproduce the original response, a large dose must be administered. With the development of tolerance, physical dependence occurs, and continued administration of the drug becomes necessary to prevent characteristic withdrawal or abstinence syndrome. The mechanism of development of tolerance and physical dependence is not related to pharmacokinetic factors but is a true cellular adaptive response that is associated with changes in second messenger system related to Ca^{++} flux, adenyl cyclase inhibition and G protein synthesis. Chronic exposure and tolerance to opioids is associated with an elevation of intracellular Ca^{++} content - unlike acute exposure which often causes a decrease. The effect appears to be related to a change in the receptor's ability to associate with G coupling proteins, increased level of G proteins and an-upregulated cAMP system. In addition, the number of the receptor may be reduced by internalization and by their decreased synthesis (Reisine and Pasternak, 1996). It is postulated that some aspects of withdrawal are attributed to higher cAMP levels. Drug-induced supersensitivity and alteration in intracellular Ca^{++} concentration may also involve alteration in G proteins (Johnson and Fleming, 1989). Reisine and Pasternak (1996) have hypothesized that chronic opioid administration triggers the activation of endogenous antiopioid peptides by changes in gene expression. When the subjects experience euphoria induced by opioids, it is correlated with decreased brain glucose metabolism, especially in the cortical area (Jaffe, 1995).

According to Koyuncuoglu (1995) the mechanism underlying the development of physical dependence upon opiates may involve four main biological processes.

1. The inhibition by opiates of L-asparaginase and glutaminase; the enzymes producing the excitatory amino acid (EAA), aspartate (ASP) and glutamate (GLU).

2. The blockade by opiates of biological processes occurs by the stimulation of the ASPergic/GLUergic receptors, especially the N-methyl-D-aspartate (NMDA) subtype.

3. The upregulation of the NMDA receptors and the superactivation of the biological and biochemical processes due to the blockade by the opiates.

4. The central and peripheral prompt adaptation of the individual to the lesser production of the neurotransmitter ASP and GLU, and to the blockade of functions elicited by NMDA-receptor stimulation.

The decrease in the biological functions in terms of the inhibition of ASP and GLU production and the blockade of biological processes can lead to an upregulation and supersensitivity of the receptors whose endogenous ligands are released by activation of NMDA receptors. As is known, these endogenous ligands include catecholamines, serotonin, GABA, histamine, acetylcholine and dopamine. The ASPI/GLUergic receptors, not blocked by opiates anymore, respond to EAAs (excitatory neurotransmitter amino acids) normally or possibly more strongly than is normal. EAAs cause the release of endogenous ligands. Together with other actions, ASPI/GLUergic receptor stimulation creates the well known abstinence syndrome (yawning, lacrimation, rhinorrhoea, perspiration, goose flesh, muscle tremor, dilated pupils, anorexia, joint and muscle ache, restlessness, insomnia, emesis, diarrhea, craving, rejection of smoking). Gil-Ad *et al* (1985) concluded from their study that lack of endorphins is a factor in the development of withdrawal symptoms in opiate addiction and that the mechanism (such as action of clonidine to relieve these symptoms) involves stimulation of the release of beta-endorphins and suppression of noradrenergic activity in the locus ceruleus, similar to that of opiates. Sarnyai and Kevase (1994) suggested that oxytocin (OXT) inhibits the development of tolerance to morphine, heroin, beta-endorphin and enkephalin. Oxytocin also inhibits the development of cross-tolerance between the predominantly mu-agonist heroin and the predominantly delta agonist enkephalin in mice. Oxytocin inhibits the

development of opiate tolerance, dependence and self-administration. Therefore oxytocin may act as neuromodulator of dopaminergic neurotransmission in the limbic-basal forebrain structures to regulate the adaptive CNS processes leading to drug addiction.

Physical dependence is observed when the cessation of administration of a substance (abstinence) causes physical signs of withdrawal. As the physical sign of withdrawal generally represents physiological actions opposite to the acute action of heroin (e.g. constipation), the corresponding physical withdrawal sign is diarrhea. In the case of short-acting drugs, such as heroin, the first symptoms may be seen within 6-12 hr after the last dose of the drug.

SYMPTOMS FOLLOWING WITHDRAWAL OF HEROIN

A variety of withdrawal symptoms have been observed in the heroin addicts (Dewey and Brase, 1991; Jaffe, 1995).

6 - 12 hrs.

Drug seeking (purposive behavior), restlessness, lacrimation, rhinorhea, sweating, yawning, anxiety, dysphoria.

12 - 24 hrs

Restlessness or broken sleep for several hours (Yen), feeling more miserable after awakening, irritability, tremors, dilated pupils, anorexia, gooseflesh (piloerection, cold turkey).

24 - 72 hrs

Increased intensity of above signs, weakness, depression, nausea and vomiting, intestinal cramps, diarrhea, alternate chills and flushes, various aches and pains (aching of bones, back and muscles), increased heart rate and blood pressure, involuntary movement of arms and legs (twitching of muscles and kicking movement of lower extremities, " Kicking the habit"), dehydration, possible electrolyte imbalance, low grade fever.

After 72 hrs

Above symptoms of autonomic hyperactivity alternating with brief periods of restless sleep, gradually decreased intensity until the addict feels better in 7-10 days, yet exhibiting strong craving for the drug, some mild signs still detectable for upto 6 months.

Cross-tolerance is an extremely important characteristic of the opioids. The patients tolerant to heroin are also tolerant to other agonist opioids and their congeners, and this forms the basis of detoxification. The principles of detoxification are the same for all drugs; to substitute a longer acting, orally active, pharmacologically equivalent drug for the drug abused, stabilizing the patient on the substitute and then gradually withdrawing it. Without treatment, the syndrome usually runs its course in 7-10 days. It may take many months for full physiologic equilibrium to be restored (Fultz and Senay, 1975). The usual procedure is to prescribe a drug which has a slower action (less addictive) than the street drug. Methadone or raw opium ("Afune") is prescribed in place of heroin.

Currently, the opioid drugs most often used to ameliorate the severity of withdrawal is oral methadone (Farrel *et al.*, 1994). A longer acting drug LAAM (L-α-acetylmethadol) has also been used recently. Bupernorphine (partial mu-agonist opioid) has been recently introduced for the treatment of opioid dependence (Strain *et al.*, 1994). Clonidine, naltrexone, naloxone, acetorphan are α 2- agonists have been shown to suppress some elements of the opioid withdrawal syndrome (Brambilla *et al.*, 1984; Kleber *et al.*, 1987). Propranolol, a beta blocking drug, prevents the euphoric action of heroin and may alter the patterns of compulsive craving for it (Grosz, 1972). Propoxyphene salts are also used to detoxify the heroin addicts (Jaffe, 1995).

Acupuncture-stimulated release of endogenous opioids is utilized to alleviate opioid withdrawal in some countries (Katims *et al.*, 1992; Jaffe, 1995). Grossman (1985) observed that changes in cerebrospinal fluid met-enkephalin and beta-endorphin after acupuncture may be involved in the effectiveness of this therapy in the

treatment of heroin withdrawal and severe pain. The proposed treatment methods in Pakistan are:

1. Symptomatic treatment
2. Replacement therapy
3. Gradual withdrawal

The treatment goal at all local centers throughout the country is detoxification through total abstinence (cold turkey with symptomatic treatment) since methadone and other heroin antagonists are not available (Sheikh, 1994). It is claimed that detoxification is usually accomplished in most cases in 10-15 days but a former addict is liable to relapse both psychologically and socially (Sell *et al.*, 1995). After detoxification, psychotherapy is badly needed to keep the patient abstinent, because brief detoxification alone is generally followed by relapse (Vaillant, 1988).

The harm of heroin addiction can extend to all physiological systems. A number of endocrine abnormalities have been recognized in the heroin addicts. Receptors for opiates are distributed throughout the central nervous system (CNS), being present in the highest concentration in the limbic system (frontal and temporal cortex, amygdala and hippocampus), striatum, midbrain, laminae I, II, IV and V of the dorsal horn in the spinal cord, thalamus and hypothalamus (Goodman and Pasternak, 1985). There is a high density of opioid receptors in the median eminence of the hypothalamus (Atweh and Kuhar, 1983) where the anterior pituitary-regulating substances are released into the portal capillary system, and the modulation of pituitary hormone secretion by endogenous and exogenous opiates appears to take place (Clement-Jones and Besser, 1983). The opiates specifically affect the pituitary physiology (Pert and Synder, 1973; Yen *et al.*, 1985). Pituitary gland volume becomes larger in the heroin addicts than in the controls (Teoh *et al.*, 1993).

The Available literature on the effect of heroin and related opioids shows that the central nervous system particularly the hypothalamic-pituitary axis is one of the most vulnerable targets of these drugs. The target system responsive to the pituitary system and known to be most conspicuously affected by the opioids is the gonads and its accessory organs. The serum levels of the follicle stimulating hormone (FSH) and luteinizing hormone (LH) have been shown by most workers to be adversely affected (Tepperman, 1973; Martin *et al.*, 1973; Brakke *et al.*, 1974; Tolis *et al.*, 1975; Cicero *et al.*, 1976a, 1977; Afrasiabi *et al.*, 1979; Mendelson *et al.*, 1980; Lafisca *et al.*, 1981; Smith and Gilbeau, 1985; Singer *et al.*, 1986; Khan *et al.*, 1991; Mena-Valdivia *et al.*, 1995). There is substantial evidence that serum prolactin also undergoes significant alteration in the addicts (Tolis *et al.*, 1975; River *et al.*, 1977; Dupont *et al.*, 1977; Chan *et al.*, 1979; Cushman, 1980; Lafisca *et al.*, 1981; Spagnolli *et al.*, 1987a, b; Ragni *et al.*, 1988; Ahmed and Ahmed, 1993). Regarding gonadal functions, the most consistent abnormality in the heroin addicts is impotence, decreased libido and problems pertaining to the ejaculate. Serum testosterone (T) and dehydroepiandrosterone sulphate (DHEA SO_4) but neither progesterone nor estradiol (E_2) have been demonstrated to alter significantly in majority of studies done in the past (George, 1971; Azizi *et al.*, 1973; Cushman and Kreek, 1974; Mendelson *et al.*, 1975; Cicero *et al.*, 1976b; Bolleli *et al.*, 1979; Mendelson and Mello, 1982; Ragni *et al.*, 1988; Ahmed and Ahmed, 1993; Pedrazzoni *et al.*, 1993). In assiciation with serum testosterone, sex hormone-binding globulin (SHBG) which has a high affinity for testosterone than estradiol, is also modified following heroin intake (Vermeulen *et al.*, 1969; Burke and Anderson, 1972; Wang *et al.*, 1978). The above mentioned alterations together with the observed reduction in seminal vesicular fructose in the semen, the sperm count and sperm motility (Cicero *et al.*, 1975; Katz *et al.*, 1982; Ragni *et al.*, 1985, 1988; Singer *et al.*, 1986) point out that the hypothalamo-pituitary-gonadal axis is one of the most drastically affected systems.

The other physiological parameters known to be adversely influenced by heroin addiction include adrenocorticotropic hormone (ACTH) (Hellman *et al.*, 1975; Ho *et al.*, 1980; Glass, 1982; Mutti *et al.*, 1992; Schurmeyer, 1995), growth hormone (GH) (Ghodse and Reed, 1984; Hashiguchi *et al.*, 1996a,b), serum thyroxin (T_4) (Redding *et al.*, 1966; Webster *et al.*, 1973; Azizi *et al.*, 1974; Bakke *et al.*, 1973; Afrasiabi *et al.*,

1979; Glass, 1982), insulin/glucagon and glucose metabolism (Ipp *et al.*, 1978, 1980; Giugliano *et al.*, 1987; Hashiguchi *et al.*, 1995), the stomach hormone, gastrin (Unvas, 1969; Jaffe and Martin, 1975; Magee, 1975) and parathormone (Pedrazzoni *et al.*, 1993).

It is noteworthy in relation to the above described evidence that the current literature contains sufficient conflict in the data for nearly all of the physiological parameters studied to date in various laboratories.

As already stated, endogenous opioids and opioid receptors exist in the central nervous system. Heroin and related drugs exert primary effect at this level including effects on neurotransmitter release at synapses. Calcium ion influx and subsequent inhibition of adenosine triphosphatase (ATPase) are well known as a trigger for neurotransmitter release (Visi, 1978). In this regard, various drugs have been shown to inhibit the activity of Na,K-ATPase which increases the release of various transmitters (Skou, 1965; Schwartz *et al.*, 1975). Other drugs inhibit such release due to stimulation of Na,K-ATPase (Visi, 1978). Past attempts to analyze effects of exogenous opioids on the activity of this enzyme in a variety of tissues have provided at best inconsistent information (Jain *et al.*, 1974; Desaiah and Ho, 1977; Maeda *et al.*, 1988)

The current level of heroin usage in Pakistan provides compelling reasons to carry out detailed investigations to assess the level of possible damage caused by the drug in the addicts. The present work was thus undertaken with this objective in mind, also keeping in view the unique differences in socio-economic background of local drug users compared to those in the western world. More importantly, there are also differences in respect to drug potency, drug intake techniques, nature of drug diluents and detoxification methods. The street heroin used by the local addicts is of much higher potency than used elsewhere. The dilution of the drug in Pakistan is done with chemically active substances whereas in the west usually inert materials are used. Also, the majority of the studies done in Europe and North America are based on subjects using injectable heroin. In Pakistan brown street heroin is generally smoked or inhaled. Furthermore, detoxification methods practiced elsewhere are also quite different from those used in Pakistan. In the west, substitution therapy is done by applying methadone or opiate agonists are administered, whereas in Pakistan symptomatic treatment is the rule. In the light of these circumstances, the present study was planned to evaluate the effects of street heroin on

serum levels of several pituitary hormones (PRL, FSH, LH, ACTH, GH, TSH), selected male gonadal steroids, thyroid hormones, parathormone, insulin and glucose and gastrin (a stomach hormone) in chronic addicts. Special attention was given to seminal fructose and analysis of the ejaculate for sperm count and sperm motility. In addition possible alterations in Na,K-ATPase activity were studied using blood cells which plays vital role in homeostasis and immunity respectively. Erythrocytes and leucocytes have been extensively used for human studies mainly because of easy access and their rugged nature and can sustain the *in vitro* experimental maneuvers easily. Erythrocytes are atypical cells (non-nucleated), whereas leucocytes serve as a good representative model for most of the body cells. The extent of effects of heroin was assessed in the addicts subjected to withdrawal therapy (detoxification treatment). In addition, the data obtained for total subjects were analyzed to check if duration of addiction has any effect on the level of impairment prior to or after withdrawal therapy.

MATERIALS AND METHODS

MATERIALS AND METHODS

Subjects

A total of 60 male patient, aged 25-30 yr (average 27.7 ± 1.08 yr) were selected for this study. These patients were admitted at various times to the psychiatric ward at Rawalpindi General Hospital (RGH), Military Hospital (MH) and Imran Detoxification Center at Rawalpindi for detoxification treatment. All patients were informed about the purpose of the investigation and their consent was obtained before taking samples. Samples were taken within 24 hours of their admission to the hospital. The patients were requested to provide information concerning the route of drug administration, amount of the drug taken daily and duration of the addiction. None of the patients was critically ill and none had severe disturbance of consciousness. The patients admitted use of the drug for 2-16 yr (average 7.3 ± 0.46 yr). Prior to their inclusion in the study, urine samples were obtained and presence of heroin and its metabolites was ascertained by chemiluminecence assay (CIA). The addicts denied use of any drug other than heroin during at least three months prior to their admission to the hospital.

Loss of libido and decreased potency were reported by 52 out of 60 patients and 18 patients have had hepatitis in the past but were asymptomatic at the time of their entry to the hospital. The mode of heroin use in 51 patients was by inhaling vapors of volatilized heroin on tin-foil ("chasing the dragon" method). In eight patients, the intake was by smoking cigarettes and in the remaining case by sniffing. The daily intake ranged between 3 to 5 g/day (average 3.66 g/day) of 30% pure street heroin. The average blood pressure in these addicts was 112/70 ± 1.06/1.03 mmHg (Mean ± SEM). At the time of laboratory testing, all patients were alert and afebrile with stable vital signs and in no acute distress. Their last dose of heroin was taken 1-2 hr prior to testing.

The data obtained was analyzed on the basis of total length of addiction as well as by splitting the subjects into two groups on the basis of duration of addiction. Group I comprised 44 addicts of 2-8 yr duration and group II consisted of 16 addicts of 9-16 yr

duration. This was done to assess whether duration of addiction has any influence on the possible effects of the drug or reversibility of effects following detoxification therapy.

Thirty normal healthy subjects aged, 24-30 yr (average 27.2 ± 1.06 yr) served as controls. None of them showed signs of impaired liver, kidney or endocrine functions as determined by preliminary tests. None had ever taken narcotic drugs as confirmed by negative opiate screening in urine. Average blood pressure of the controls was 121/81 ± 1.07/1.09 mm Hg (Mean ± SEM).

ENDOCRINE STUDIES AND SEMEN ANALYSIS

Sampling techniques

All subjects were fasted overnight and blood and semen samples were taken in the morning (between 6-9 am) for analysis of all the parameters to be examined. Samples were also collected in the same evening (between 6-9 p.m.) to check possible alteration of diurnal rhythm of cortisol in the addicts. Heroin was taken off as soon as the basal blood and seminal samples (basal analysis) were collected. The patients were treated symptomatically for heroin withdrawal. Thereafter, it was ensured under the vigilant control at the hospital wards that the patients had no access to the drug. Blood, seminal and urine samples were again obtained on 10th and 30th day after heroin withdrawal, keeping the patients fasting overnight and using the same times for sample collection as for basal analysis. The patients with positive urinary tests for heroin catabolites were excluded from the study.

Blood samples measuring 10 ml/patient were collected in prechilled tubes. Each tube received 5000 units of Trasylol (500 units per ml of blood). The samples were immediately centrifuged at 4°C, serum was separated, labelled, registered and stored aliquoted (1 ml) at - 70°C in the freezer until the time of analysis.

Seminal samples were collected by masturbation after 6-7 days of abstinence and were examined for physical and microscopic abnormalities. The semen samples were allowed to liquefy at 37°C for 15 minutes before an aliquot was withdrawn for routine sperm count and motility examination. All semen specimens were assessed against the

limits of normality set down by the World Health Organization (1992) i.e. sperm count <20 million/ml as oligozoospermia, < 40% with forward motility as asthenozoospermia and < 50 % with normal morphology as teratospermia.

Seminal plasma was separated by centrifugation at 700x g for 15 minutes and stored aliquoted at -70°C in sterile tubes containing fluoride. Seminal fructose assay was performed immediately according to the method of Haltmann *et al.* (1956).

Parameters Studied

Determination of free thyroxin (FT_4), triiodothyronine (T_3), thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), testosterone (T), estradiol (E_2), dehydroxyepiandrosterone sulphate (DHEA-SO_4), sex hormone-binding globulin (SHBG), growth hormone (GH) and parathyroid hormone (PTH) was carried out by automated chemiluminescent immunoassay method (Bobson, 1991). The analysis of adrenocorticotropic hormone (ACTH) and gastrin was done by double-antibody RIA and that of insulin by coat-a-count RIA. Semen count was done using Horwell fertility chamber. Sperm motility was assessed according to WHO criteria (1992) and fructose was determined photometrically. Intracellular sodium, potassium and Na,K-ATPase were determined in erythrocytes and leucocytes. Protein and phosphorus estimation in the blood cells was carried out to express ATPase activity as µmol inorganic phosphorus (pi) per g protein per hour.

Reagents

All chemicals and reagents were analytical grade and purchased from Sigma (USA), Merck (Germany) or BDH (England). The kits for microalbumin for protein determination were purchased from Spinreact (Spain), for glucose estimation from Cromatest (Spain) and for phosphorus measurement from Menarini Diagnostic (Italy). Immulite kits for assays of free T_4, total T_3, TSH, FSH, LH, prolactin, testosterone, E_2, PTH, DHEA-SO_4, SHBG, cortisol, growth hormone and for urinary opiates and RIA kits for ACTH, insulin and gastrin were purchased from Diagnostic Product Corporation (USA).

Principal of automated chemiluminescent apparatus "IMMULITE"

The immulite system consists of a benchtop, random access immunoassay analyzer (Bobson 1991) built around a proprietary test unit that provides for rapid and efficient washing of a 0.25 inch (6.4 mm) polystyrene bead. The antibody-coated bead is held captive within the bar-coded test unit, which serves as the reaction vessel for all incubations, washes and signal development. The patients sample and the alkaline phosphatase (Bovine calf intestine) conjugated label are simultaneously introduced into the test unit and incubated for 30 or 60 minutes, depending on the assay at 37°C with intermittent agitation. During this time the substance to be analyzed in the sample competes with the enzyme- labelled ligand for the limited number of antibody binding sites on the bead. After incubation of the sample with alkaline phosphatase-labelled reagent, rapid separation and efficient washing of the bead and test unit are achieved by spinning the test unit at high speed about its vertical axis (centrifugal wash). The fluid contents are instantaneously and completely transferred to a coaxial sump chamber, which is integral with the test unit. Four or more discrete washes can be processed sequentially and with uniform timing, leaving the bead with no residual unbound label. The bound label is then quantified with a dioxetane substrate that produces light upon hydrolysis. The basic test procedure is outlined below.

Test procedure

The samples in the bar-coded carriers are loaded onto a continuous conveyer. Each is followed by bar-coded test units, in any order, for the test desired on that sample. The test units are conveyed into the analyzer for bar-code identification, and then into the incubation carousel. The pipettor adds sample and labelled reagent. The reagent carousel accommodates up to twelve resident assays.

After incubation with intermittent shaking at 37°C for 30 or 60 minutes (depending on the assay), the test units are shuttled to the spin wash station, where separation of bound and free label takes place. Substrate is then added, and the test units are transferred to the luminometer. After another 10 minute incubation at 37°C, the signal

reaches a maximum, and the photon counts are measured with a photomultiplier tube (PMT). Automatic attenuation of the light signal increases the dynamic range of the measuring system 100-fold, thereby enabling accurate measurement at both extremely high and extremely low concentration. Counts per second (cps) are converted to analyte concentration using stored master curves that are periodically adjusted with a pair of adjusters, which are loaded and processed just like the samples. Adjusters are processed in quadruplicate. Slope and intercept are calculated for the straight line determined by a plot of these observed average signals against the "assigned" (Master Curve) signals for each adjuster.

The chemiluminescent reaction

The alkaline phosphatase conjugate (reagent) is bound to the bead (within the test unit) during the immunological reaction. The amount of alkaline phosphatase captured is proportional or inversely proportional to the concentration of the analyte in the patient sample. Once the test unit has been washed, a luminogenic substrate solution consisting of a phosphate ester of adamantyl dioxetane in an (AMP) 2-amino-2 methyl-1-propanol buffer with enhancers and preservatives is added to the test unit, which is then transferred onto the luminometer chain. Ten minutes later, the test unit arrives in front of the photomultiplier tube (PMT), where the light generated (prolonged glow) by the luminogenic reaction is measured. Specifically, in the luminogenic reaction, the substrate (an adamantyl dioxetane phosphate) is dephosphorylated into an unstable anion intermediate by the alkaline phosphatase conjugate captured on the bead. The unstable intermediary emits a photon upon decomposition. The amount of light emitted is directly proportional to the amount of bound alkaline phosphatase.

Alkaline phosphatase

Dioxetane phosphate----------Unstable dioxetane----------Light(hv)

Label

Individual chemiluminescent assays:

The following determinations were carried out using the chemiluminescent assay (immunoassay or immunometric assay) (automated chemiluminescent assay system "immulite" from Diagnostic Products Corporation, USA).

a) Free thyroxine (FT_4)

Immulite free T_4 is a solid-phase competitive analog, sequential chemiluminescent immunoassay with 60 minutes incubation. First the patient sample and ligand-labelled T_4 analog are introduced into the test unit containing monoclonal murine anti T_4 antibody, and incubated for 30 minutes at 37°C. They compete for a limited number of antibody binding sites on the bead. Unbound analog is then removed by centrifugal wash. Then alkaline-phosphatase labelled anti-ligand is introduced and further incubated for 30 minutes, before substrate addition.

Light emitted is inversely proportional to FT_4 concentration in the sample.

Working range is 1.93 to 77.2 pmol/l.

Volume required is 10 µl serum.

b) Total tri-iodothyronine (T_3)

T_3 is a solid-phase, chemiluminescent immunoassay. Bead is coated with monoclonal murine antibody for T_3. Incubation time is 30 minutes at 37°C.

Light emitted is inversely proportional to T_3 concentration in the sample.

Working range is 0.54 to 9.2 nmol/l.

Volume required is 25 µl serum.

c) Thyroid stimulating hormone (TSH)

It is a solid phase, two site chemiluminescent immunometric assay with 60 minutes incubation. Bead is coated with monoclonal murine anti-TSH antibody.

Light emitted is directly proportional to TSH concentration in the sample

Working range is 0.002 to 75 µIU/ml.

Volume required is 75 µl serum.

d) Follicle stimulating hormone (FSH)

It is a solid phase, two-site chemiluminescent immunometric assay with 30 minutes incubation. Bead is coated with monoclonal murine anti-FSH antibody.

Light emitted is directly proportional to FSH concentration in the sample.

Working range is 0.1 to 170 mIU/ml

Volume required 50 µl serum.

e) Luteinizing hormone (LH)

It is a solid phase, two-site chemiluminescent immunometric assay with 30 minutes incubation. Bead is coated with monoclonal murine anti-LH antibody.

Light emitted is directly proportional to LH concentration in the sample.

Working range is 0.7 to 200 mIU/ml.

Volume required is 50 µl serum.

f) Prolactin (PRL)

Immulite prolactin is a solid phase, two-site chemiluminescent immunometric assay with 30 minutes incubation. Bead is coated with murine monoclonal anti-prolactin antibody.

Light emitted is directly proportional to prolactin concentration in the sample.

Working range is 10.6 to 3180 mIU/l.

Volume required is 25 µl serum.

g) Testosterone (T)

It is a solid-phase ligand labelled, competitive chemiluminescent enzyme immunoassay. Bead is coated with polyclonal rabbit anti-testosterone antibody. The patient sample and the ligand-labelled testosterone are simultaneously introduced into the test unit, and incubated for 30 minutes at 37°C. The testosterone in the sample competes with the ligand-labelled testosterone for antibody binding sites on the bead. The unbound material is then removed by a centrifugal wash. Then alkaline phosphatase-labelled anti ligand is introduced and incubated for further 30 minutes.

Light emitted is inversely proportional to the testosterone concentration in the sample.

Working range is 0.1 to 20 ng/ml.

Volume required is 20 µl serum.

h) Estradiol (E_2)

Immulite estradiol is a solid-phase, chemiluminescent immunoassay with 60 minutes incubation. Bead is coated with polyclonal rabbit anti-estradiol antibody.

Light emitted is inversely proportional to estradiol concentration in the sample.

Working range is 20 to 2000 pg/ml.

Volume required is 25 µl serum.

i) Dehydroepiandrosterone (DHEA - SO_4)

It is a solid-phase chemiluminescent immunoassay with 30 minute incubation. Bead is coated with a polyclonal rabbit anti DHEA SO_4 antibody.

Light emitted is inversely proportional to DHEA SO_4 concentration in the sample

Working range is 30 to 1000 µg/l.

Volume required is 5 µl serum.

j) Sex hormone-binding globulin (SHBG)

It is a solid-phase, two-site chemiluminescent immunometric assay with 30 minutes incubation. Bead is coated with monoclonal murine anti-SHBG antibody.

Light emitted is directly proportional to the SHBG concentration in the sample.

Working range is 3 to 180 nmol/l

Volume required is 10 µl of prediluted serum sample (1-21).

k) Cortisol

It is a solid-phase chemiluminescent immunoassay with 30 minute incubation. Bead is coated with polyclonal rabbit anti-cortisol antibody.

Light emitted is inversely proportional to cortisol concentration in the sample.

Working range is 1 to 50 µg/dl

Volume required is 10 µl serum.

l) Growth hormone (GH)

It is a solid-phase, two-site chemiluminescent immunometric assay. The patient sample and alkaline phosphatase-conjugated rabbit polyclonal anti-HGH antibody are incubated for 30 minutes. Bead is coated with monoclonal murine anti-HGH antibody

Light emitted is directly proportional to HGH concentration in the sample.

Working range is 0.13 to 104 mIU/l

Volume required is 50 µl serum.

m) Parathormone (PTH)

Immulite intact PTH is a solid-phase two-site chemiluminescent immunometric assay with 60 minutes incubation. Bead is coated with affinity purified goat polyclonal anti-PTH antibody.

Light emitted is directly proportional to PTH concentration in the sample.

Working range is 0.1 to 263 pmol/l

Volume required is 50 µl serum.

Quality control

Immunoassay tri-level internal control Con4, Con5 and Con6 (low, normal and high) Lot 015 from DPC (USA), were used to check the assay performance and monitor the continued applicability of the stored master curve.

Individual Radioimmunoassays:

Assays for ACTH and gastrin were performed by double antibody procedure of RIA, while that of insulin was performed by coat-a-count procedure of RIA. The kits for

these assays were purchased from DPC (USA). The radioimmunoassay technique is capable of measuring the reaction between antigen and antibody. Labelled antigen (Ag*) and unlabelled antigen (Ag) compete for binding to the antibody. Keeping in view the avidity of the antibody, both the labelled and the unlabelled antigens must be the same under these conditions. The probability of the antibody binding the labelled antigen is inversely proportional to the unlabelled antigen concentration.

Quality control

Immunoassay tri-level internal control Con4, Con5 and Con6 (low, normal and high) Lot 015 from DPC (USA), were used to check the assay performance.

a) Gastrin

Lyophilized gastrin antiserum was reconstituted by adding 10 ml distilled water. Lyophilized iodinated gastrin was also reconstituted by adding 10 ml distilled water. Ready-to-use gastrin calibrators containing 0, 25, 50, 100, 200, 600, 1200 pg/ml gastrin were set up (Table 1). Second antibody and dilute polyethylene glycol (PEG) were mixed in a vial and kept ready for use as precipitating solution. Individual tubes, each designated as total counts (T), non-specific binding (NSB), maximum binding (A) and standards B through G were prepared in duplicate (totals 18 tubes). Duplicate tubes were also prepared for serum and internal controls.

Table 1. Layout of calibrator tubes for gastrin.

Calibrators	A	B	C	D	E	F	G
Concentration pg/ml	0	25	50	100	200	600	1200

A 200 µl aliquot of the zero calibrator A was pipetted into the NSB and A tubes. An equal amount (200 µl) of each of the remaining calibrators B through G was

transferred into correspondingly labelled tubes. The tubes prepared for the serum and internal control also received 200 µl of the sample. After addition of 100 µl of ^{125} I gastrin to all tubes, the holding rack was shaken gently (Shaker by Diagnostic Product corporation, USA). This was followed by addition of 100 µl of gastrin antiserum to all tubes except the NSB and T tubes. The tubes were vortexed and incubated for two hours at room temperature. All tubes were vortexed again after addition of 1.0 ml of well mixed, cold precipitating solution. The tubes were then centrifuged (refrigerated centrifuge Hitachi, Japan) for 15 minutes at 3000 x g. The supernatant in each tube was decanted and counted for one minute in a gamma counter (Gambyt CR20, Diagnostic Product Corporation, USA).

b) Adrenocorticotropic hormone (ACTH)

One vial of lyophilized ACTH antiserum was reconstituted by adding 10 ml of distilled water. One vial of lyophilized iodinated ACTH (^{125}I ACTH) was also reconstituted by adding 10 ml of ACTH diluent. Seven calibrators containing 0, 12, 35, 65, 160, 530, 1250, uIU/ml ACTH were prepared (Table 2). The 0 calibrator was reconstituted by adding 3 ml distilled water while the remaining calibrators received 1 0 ml distilled water each. A vial of precipitating solution consisting of goat anti-rabbit gamma globulin (GARGG) and dilute polyethylene glycol (PEG) in saline was kept ready for use. Eighteen polypropylene tubes in duplicate were placed in a rack and marked as Total counts (T), non-specific binding (NSB), maximum binding (A) and standards B through G (see below). Additional tubes, were prepared also in duplicate each for serum and internal controls.

Table 2. Layout of calibrator tubes for ACTH.

Calibrators	A	B	C	D	E	F	G
Concentration µIU/ml	0	12	35	65	160	530	1250

A 100 µl aliquot of the zero calibrator A was pipetted into the NSB and A tubes. An equal amount (100 µl) of each of the remaining calibrators B through G was transferred to correspondingly labelled tubes. The tubes designated as serum and internal control tubes received 100 µl of the sample. The tubes were vortexed following addition of 100 µl of ACTH antiserum to all except the NSB and T tubes. The tubes were incubated for 60 minutes at room temperature. After addition of 100 µl of ^{125}I ACTH all tubes were vortexed again and incubated for at least 16 hours at 4° C. Vortexing was resumed after adding 1.0 ml of cold precipitating solution to individual tubes. The tubes were then centrifuged for 15 minutes at 3000 x g. The supernatant was decanted and discarded. The precipitate was saved for counting for one minute in a gamma counter.

c) Insulin

Polypropylene tubes coated with antibodies to insulin were prepared. A vial of the concentrate consisting of iodinated insulin (^{125}I Insulin) was prepared by adding 100 ml of distilled water, which was mixed by gentle inversion. Seven lyophilized calibrators were prepared in duplicate containing 0, 5, 15, 50, 100, 200, 400, µIU/ml insulin (Table 3). The 0 calibrator was reconstituted with 6 ml distilled water while the remaining calibrators received 3 ml distilled water. Four plain polypropylene tubes were designated T(total counts) and NSB (nonspecific binding) in duplicate. Fourteen insulin Ab-coated tubes A (maximum binding) and standards B through G were set up in duplicate. Additional Ab-coated tubes, were prepared in duplicate for internal control and serum samples.

Table. 3 Layout of calibrator tubes for insulin.

Calibrators	A	B	C	D	E	F	G
Concentration µIU/ml	0	5	15	50	100	200	400

A 200 µl aliquot of the zero calibrator A was transferred to the NSB and A tubes. An equal amount (200 µl) each of the remaining calibrators, the internal control and the serum samples were pipetted into the bottom of the tubes. Every tube received 1.0 ml of ^{125}I insulin. After vortexing the tubes were incubated for 18-24 hours at room temperature. The supernatant was decanted and the precipitate was counted for one minute in a gamma counter.

d) Glucose

Glucose was measured using glucose kit (Chromatest, Spain). The method involves reaction of standard (5.6 mmol/l) or sample with a working reagent containing Ampyrone (0.4 mmol), Glucose oxidase (≥3000U), Peroxidase (≥500U), Phenol (2.5 mmol) and phosphate buffer (50 mmol) with a pH of 7.5 at 37°C. Following incubation of the reactant (Table 4) for 10 minute at 37°C the absorbance of the resulting complex was read at 500 nm against a blank on a spectrophotometer.

Table 4. Volume and ratio of blank, standard and sample for analysis of glucose.

Content	Blank	Sample	Standard
Sample	.....	0.02 ml	.....
Standard	.....	.....	0.02 ml
Reagent	2.5 ml	2.5 ml	2.5 ml

The concentration of glucose was calculated as follows.

$$\text{Glucose (mmol/l)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Standard concentration}$$

Semen Analysis

Volume, sperm count and motility

The volume of the semen was measured using a graduated pipette. Semen count was estimated by withdrawing a drop of liquefied semen with a dropper and placing it in the center of the Horwell fertility chamber (ARH counting chamber, Laboratory clinical supplies, London, England), and covered with the cover slips provided with the fertility chamber. The sperms were counted using 10 x objective giving a magnification of 100 using the four corner squares and the middle square of the chamber. The total number was obtained by adding all counts and multiplying by one million. The sperm count thus obtained was regarded as the total sperm count. Three such counts per sample were made to obtain an average value. Sperm motility was assessed according to the WHO criteria for motility (WHO 1992). Pus cells in the semen were counted as the number of cells per high power field (40 x 10) of the Horwell fertility chamber.

Fructose level in semen

Fructose in semen decomposes rapidly. Therefore the assay for fructose was performed before the seminal plasma was frozen at -70°C. Prior to fructose determination, the seminal plasma was deproteinized and other interfering substances were removed by Somogyi filtrate preparation. When the sample so obtained is heated in strong hydrochloric acid in the presence of resorcinol, fructose forms a pink color which can be detected photometrically (Quicklab chemistry analyzer by Ames, Germany). Deproteinization of the seminal plasma was carried out according to the schedule shown in Table 5.

Table 5. Deproteinization schedule for seminal plasma.

Contents	Standard	Sample
Distilled water	2.0 ml	2.0 ml
Standard	0.1 ml	- - - - -
Ejaculate	- - - - -	0.1 ml
Zinc sulfate solution (348 mmol/l)	1.0 ml	1.0 ml
Sodium hydroxide (0.5 mol/l)	1.0 ml	1.0 ml

After thorough mixing, the individual tubes were placed in a boiling water bath for 2 minutes, allowed to cool in cold water and centrifuged at 1500 x g for 5 minutes. The following schedule (Table 6) was used for determination of fructose.

Table 6. Schedule for determination of fructose in seminal plasma.

Contents	Blank	Standard	Sample
Distilled water	0.5 ml	- - - - -	- - - - -
Standard mixture (11.1 mmol/l)	- - - - -	0.5 ml	- - - - -
Analyte supernatant	- - - - -	- - - - -	0.5 ml
Resorcinol solution (9.09 mmol/l)	1.0 ml	1.0 ml	1.0 ml
Hydrochloric acid (9.42 mol/l)	4.0 ml	4.0 ml	4.0 ml

After through mixing the tubes were placed in a boiling water bath for exactly 6 minutes and allowed to cool in cold water. The standard and test samples were transferred into cuvettes and measured immediately against the blank. The fructose concentration was calculated as follows.

$$\text{Concentration of semen fructose (mmol/l)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Std. Conc}$$

Determination of Na,K-ATPase Activity

Subjects

The content of intracellular sodium, potassium and Na,K-ATPase activity were measured in samples of erythrocytes and leucocytes. For this purpose, 17 male subjects (aged 25-30 yr, average 27.3 ± 1.00 yr) were selected from the heroin addicts admitted to Imran Detoxification Center, Rawalpindi, for detoxification less than 24 hr prior to start of sampling. The drug abuse background, health history and details for sample collection prior to and after withdrawal treatment (basal and post treatment samples) were the same as described on page 15. The noteworthy differences were duration of prior drug abuse (2-10 yr., average 6.1 ± 0.60 yr.) and the time of sample collection after withdrawal of the drug (i.e. 15th day). Ten healthy subjects (25-30 yr, average 27.5 ± 1.02 yr) served as controls (see page 17 for details)

The data obtained was analyzed on the basis of total length of addiction as well as by splitting the subjects into two groups on the basis of duration of addiction. Group I comprised 12 addicts of 2-7 yr duration and group II consisted of 5 addicts of 8-10 yr duration. This was done to assess whether duration of addiction has any influence on the possible effects of the drug or reversibility of effects following detoxification therapy.

Collection of blood

Blood samples were collected for separating erythrocytes and leucocytes. Blood was taken from antecubital vein by venepuncture into 20 ml plastic capped tubes containing 3 units of lithium heparin per ml blood. The blood samples were used for both erythrocyte and leucocyte separation and measurement of electrolyte and enzyme activity.

Protein estimation

Protein estimation was performed using microalbumin kit (Spinreact, Spain). In this method, a solution (reagent 1) of pyrogallol red (50 mmol/l) and sodium molybdate (0.04 mmol/l) mixed in a 1:1 ratio is reacted with standard albumin/globulin (0.2 g/l) or sample. The reaction gives a red complex following incubation of the reactants (table 7) for ten minutes at room temperature. The color is directly proportional to the protein concentration. The extinction is measured at 598 nm against a blank on a spectrophotometer (Quicklab chemistry analyzer by Ames, Germany).

Table 7. Volumes and ratios of blank, standard and sample for protein analysis.

Content	Blank	Standard	Sample
Standard	- - - - -	50 µl	- - - - -
Sample	- - - - -	- - - - -	50 µl
Reagent 1	3.0 ml	3.0 ml	3.0 ml

Protein concentration was calculated as follows.

$$\text{Microalbumin (g/l)} = \frac{\text{Extinction of Sample}}{\text{Extinction of Standard}} \times \text{Standard Conc (g/l)}$$

Phosphorus estimation

Phosphorus was measured using Menagent Phosphofix (Menarini diagnostic, Italy). The method involves reaction of standard (5 mg/dl inorganic phosphorus) or sample with a working reagent containing ammonium molybdate (0.60 M) and sulphuric acid (0.60M) in the presence of adequate tensio-active substance. Following incubation of the reactants (Table 8) for 5 minute at 37°C the absorbance of the resulting complex was read at 340 nm against a blank on a spectrophotometer.

Table 8. Volumes and ratios of blank, standard and sample for analysis of phosphorus.

Content	Sample	Standard	Blank
Working reagent	2.00 ml	2.00 ml	2.00 ml
Standard	- - - - -	0.02 ml	- - - - -
Sample	0.02 ml	- - - - -	- - - - -
Distilled water	- - - - -	- - - - -	0.02 ml

The concentration of phosphorus was calculated as follows.

$$\text{Phosphorus (mg/dl)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Standard Conc.}$$

(A) Separation of erythrocytes

Blood (3 ml) was centrifuged at 1500 g for 10 minutes at room temperature. The plasma and buffy layer was removed by suction with a plastic pasture pipette. The erythrocytes were washed in $MgCl_2$ solution (287 mmol/l), which was kept at 4°C and centrifuged for 5 minutes at 1500 x g. The wash was repeated twice. After washing, 400 µl aliquot of erythrocytes was transferred into microcentrifuge tubes (500 µl capacity) in

triplicate. The samples were centrifuged at 15000 x g in a microcentrifuge (Hitachi, Japan) for 30 minutes. The tubes were immediately cut into two pieces, 2 mm below the plasma level and the portion with plasma was discarded. The cut tubes were stored at -20°C in sealed plastic tubes.

Measurement of intracellular sodium, potassium and water

At the time of measurements each tube containing the cells was cut into two segments (20 mm and 30 mm) after discarding the 3 mm end portion. One of the segment (20 mm) was used for sodium and potassium measurements. It was weighed (Sartorius Analytical Balance, Germany) and dropped into 10 ml of $LiNO_3$ solution (15 mmol/l) and vigorously shaken to release the contents into the solution. The individual erythrocyte samples were centrifuged at 1500 x g and the concentration of sodium and potassium was measured by flame photometry (Corning clinical flame photometer, 410C, UK). The flame photometer was used in a manner that the samples were measured directly without dilution step. A standard calibration curve was prepared using the following working standards prepared from stocks of 40 mmol/l and 200 mmol/l NaCl and KCl respectively in 15 mmol/l $LiNO_3$.

	Working standard	
	NaCl	KCl
1.	0.1 mmol/l	0.5 mmol/l
2.	0.2 mmol/l	1.0 mmol/l
3.	0.3 mmol/l	1.5 mmol/l
4.	0.4 mmol/l	2.0 mmol/l

The other segment (30 mm) was used to calculate the water content of the erythrocytes. The erythrocytes were allowed to run out into preweighed Auto Analyzer cups. The cups were weighed and placed in an oven for drying at 56°C for 24 hours. The final weight of the cup was taken for calculating the intracellular water content.

Measurement of Na,k-ATPase activity

Na,K-ATPase activity was measured using a freeze-thaw technique (Huang and Askari, 1975). Packed erythrocytes (2.0 ml) were taken into a plastic tube and immersed in methanol/solid CO_2 until the samples were frozen. The samples were thawed at 37°C in a water bath (Memmert, Germany). The freezing and thawing was repeated twice. After the final thaw, 2.0 ml distilled water was added to the erythrocytes. The enzyme activity was measured using 6 plastic capped tubes, each containing 2.0 ml of an incubation medium comprising NaCl (100 mmol/l), KCl (15 mmol/l), $MgCl_2$ (3 mmol/l), ATP (2 mmol/l), EDTA (1 mmol/l) and Tris/HCl buffer (80 mmol/l) with a pH of 7.4 at 37°C. Unlabelled ouabain (10 mmol/l in 5% ethanol) was added to 3 of the tubes giving a final concentration (after the addition of erythrocyte sample) of 100 µmol/l. To each of the tubes, 0.5 ml of erythrocyte sample was added. The individual samples were incubated at 37°C in a shaking water bath (Diagnostic Product Corporation, USA) for 2 hr. The tubes were shaken thoroughly after every 15 minutes. Then the reaction was stopped by adding 1.0 ml of 12% Trichloroacetic acid (TCA) to each tube. The samples were centrifuged at 500 x g for 5 minutes and the supernatant was removed and used to measure phosphorus immediately. To the precipitate was added 5 ml of a solution containing $NaCO_3$ (200 mmol/l) and NaOH (200 mmol/l)) to dissolve the proteins. The samples were stored overnight and appropriately diluted in the above solution before measuring the protein content. The enzyme activity was expressed as the difference between release of inorganic phosphate (Pi) from ATP in the absence and presence of ouabain and expressed as µmol of Pi/g Protein/h.

(B) Separation of Leucocytes

Leucocytes were separated by the method of Baron and Ahmed (1969) with minor modifications. A dextran reagent was prepared by adding 6g of Dextran (mol mass 252000 Da) per dl of a solution consisting of 115 mmol/l NaCl, 6 mmol/l KCl, 24 mmol/l $NaHCO_3$, 1.8 mmol/l $CaCl_2.7H_2O$, 1.0 mmol/l $NaH_2PO_4.2H_2O$, 5.5 mmol/l glucose and phenol red indicator (pH 7.5 at 37°C). The reagent was mixed with heparined venous blood in a ratio of 1 : 4. The tubes containing the mixture were gently inverted 2-3 times

to ensure that dextran solution had mixed with the blood. The air bubbles at the top were aspirated with the help of a plastic pasture pipette. The leucocytes were allowed to sediment for 20-25 min at room temperature. The supernatant was taken carefully and centrifuged at 250 x g for 5 minutes at 25°C. After decanting the supernatant, the leucocytes were lyzed hypotonically by adding 3 ml of distilled water for 10-15 s. The sample was whirlimixed, and then 1 ml of solution consisting of NaCl (460 mmol/l), KCl (24 mmol/l), $NaHCO_3$ (96 mmol/l), $CaCl_2.2H_2O$ (7.2 mmol/l), $MgSO_4.7H_2O$ (3.2 mmol/l), $NaH_2PO_4.2H_2O$ (4 mmol/l), Glucose (20.2 mmol/l) and phenol red indicator (pH 7.5 at 37°C) was added and mixed. The sample was again centrifuged for 3 minutes at 200 g at 25°C. The supernatant was discarded and the sides of the tube were cleaned with tissue paper. The cells were re-suspended for various measurement as follows:

1. In $MgCl_2$ solution (287 mmol/l) for cell electrolyte assessment. This was done to rid the leucocytes of trapped extracellular Na or K.
2. In distilled water for Na,K-ATPase activity assay.

Measurement of intracellular sodium, potassium and water

The cells were dried to a constant cell mass in an oven at 110°C for 30 minutes, then weighed again to give dry cell mass. This enabled the intracellular water content to be calculated. The cell button was then mixed with 5 ml of a solution containing 0.1 mmol/l HNO_3 and 15 mmol/l $LiNO_3$ and kept at 56°C for 24 hr. Sodium and potassium were then measured by flame photometry. The flame photometer was used in a manner that the samples were measured directly without dilution step. A standard calibration curve was prepared using the following working standards prepared from stock of 100 mmol/l each of NaCl and KCl in 15 mmol/l $LiNO_3$.

NaCl	KCl
0.1 mmol/l	0.1 mmol/l
0.2 mmol/l	0.2 mmol/l
0.3 mmol/l	0.3 mmol/l
0.4 mmol/l	0.4 mmol/l

Measurement of Na,K-ATPase Activity

Na,K-ATPase activity was measured by the method of Baron and Khan (1985) The leucocyte pellet was lyzed hypotonically in 2.25 ml of distilled water for 15 minutes followed by 750 µl of Tris/HCl buffer (40 mmol/l, pH 7.4). Na,K-ATPase activity was measured in the same incubation medium consisting of NaCl (100 mmol/l), KCl (15 mmol/l), ATP (5 mmol/l), $MgCl_2$ (7 mmol/l), EDTA (1 mmol/l) and Tris/HCl buffer (50 mmol/l) with a pH of 7.2 at 37° C. The lyzed leucocyte sample (500 µl), was added to 2 ml of the incubation medium in each of the six tubes used for duplicate measurement of the enzyme activity. Ouabain (25 µl) at a final concentration of 100 µmol/l in aqueous ethanol (9 mmol/l) was added to two of the above tubes containing leucocyte sample. To the rest of the four tubes, 25 µl of aqueous ethanol (9 mmol/l) was added (to compensate for the ethanol added to the tubes containing ouabain). In two of these tubes (without ouabain), the reaction was immediately stopped by adding 1 ml of 12% TCA and centrifuging the samples at 500 x g for 5 minutes. The reaction in the rest of the four tubes was carried out at 37°C for 60 minutes in a shaking water bath. The samples were whirlimixed every 10 minutes during the incubation and were occasionally stirred with the help of a plastic Pasteur pipette. The reaction was stopped by adding 1 ml of 12% TCA and the samples were centrifuged at 500 g for 5 minutes. Inorganic phosphate was measured by menagent phosphorus kit. To each sample, 5 ml of an alkaline solution containing 200 mmol/l each of $NaCO_3$ and NaOH was added and kept for 12-24 hr before measuring protein concentration. Na,K-ATPase activity was calculated as the difference between inorganic phosphate (Pi) released by the action of leucocyte samples on ATP in the presence and absence of ouabain. Total ATPase was calculated as the difference between the samples without ouabain and the one in which the reaction was immediately stopped.

Statistical Analysis

Statistical analysis of all data was performed using Student's t-test for unpaired observations. Values were expressed as means ± standard error of the mean. A comparison was considered significantly different when p was <0.05.

RESULTS

RESULTS

Gonadotropins (FSH, LH)

In the healthy controls, the mean serum FSH (Fig. 1) and LH (Fig. 2) values were 4.28 ± 0.24 mIU/ml and 6.12 ± 0.38 mIU/ml respectively. In the addicts the mean FSH level before detoxification was 9.58 ± 0.62 mIU/ml, being significantly higher ($p < 0.001$) compared to the controls. After detoxification, the serum FSH level dropped significantly below the levels of the addicts ($p < 0.05$) on the 10th day but still remained higher than the value for the control subjects ($p < 0.01$). Further decrease below the level of the addicts on the 30th day brought the mean value down to 5.24 ± 0.38 mIU/ml which was now statistically indistinguishable from that of the controls (Fig. 1). Thus detoxification restored the serum FSH to the control level with a delay (i.e. 30th day).

The level of LH in the addicts (3.53 ± 0.30 mIU/ml) was significantly ($p < 0.001$) below the control level indicating depression of LH by heroin (Fig. 2). Detoxification did not bring about a significant change by the 10th day of treatment. The observed deviation from the situation for the addicts was statistically non-significant ($p > 0.05$). On the 30th day, however serum LH increased significantly to near control level (6.03 ± 0.35 mIU/ml, Fig. 2). Although the effect of heroin on LH was opposite to that of FSH, detoxification occurred again with a delay.

Prolactin (PRL)

The mean basal prolactin level (Fig. 3) was drastically elevated in the addicts (214.5 ± 13.1 mIU/ml), the difference being highly significant ($p < 0.001$) compared to the healthy control value (99.6 ± 3.87 mIU/ml). Prolactin level dropped significantly below the level of the addicts and statistically matched the value for the control (97.9 ± 6.7 mIU/ml) as early as the 10th day and was maintained comparably even on the 30th day (100.0 ± 7.39 mIU/ml) after detoxification (Fig. 3).

Testosterone (T)

The mean serum testosterone level in the healthy subjects was 19.4 ± 0.86 nmol/l while in the addicts it decreased significantly ($p<0.001$) to 12.3 ± 0.61 nmol/l (Fig. 4) indicating that heroin exerts adverse effect on this hormone. Although the level rose (13.5 ± 0.65 nmol/l) above the level of the addicts on the 10th day after detoxification, the difference between the two remained statistically insignificant and complete recovery from the effect of heroin was still not achieved by the 30th day of withdrawal treatment. The mean testosterone level of 15.3 ± 0.64 nmol/l was statistically significant from the control value (Fig. 4). These results show some resistance of serum testosterone to full recovery after detoxification.

Sex Hormone-binding globulin (SHBG)

In the controls, the SHBG level was 19.7 ± 1.28 pmol/l while in addicts it was 40.7 ± 2.77 pmol/l prior to detoxification treatment, the difference between the two being significant ($p <0.001$). Heroin evidently caused a sharp increase in SHBG (Fig. 5). A significant decrease ($p<0.01$) below the level of the addicts occurred following detoxification therapy on the 10th day (29.1±2.04) but it was only on the 30th day that SHBG level (24.2 ± 1.73 pmol/l) became statistically similar to that of the controls indicating delayed recovery from the effects of the heroin (Fig. 5).

Dehydroxyepiandrosterone ($DHEA.SO_4$) and Estradiol (E_2)

No significant difference was observed between the controls and the addicts (either before or after detoxification) in the serum $DHEA.SO_4$ and estradiol (E_2) levels (Figs. 6, 7). In the healthy controls, their levels were 209 ± 4.32 µg/dl and 39.3 ± 1.71 pmol/l respectively. In the heroin addicts, these were 210.55± 5.48 µg/dl and 37.9 ± 2.98 pmol/l respectively showing that the addiction had no effect on these hormones. Detoxification therapy did not bring about any noteworthy deviation from these values even on the 30th day post treatment.

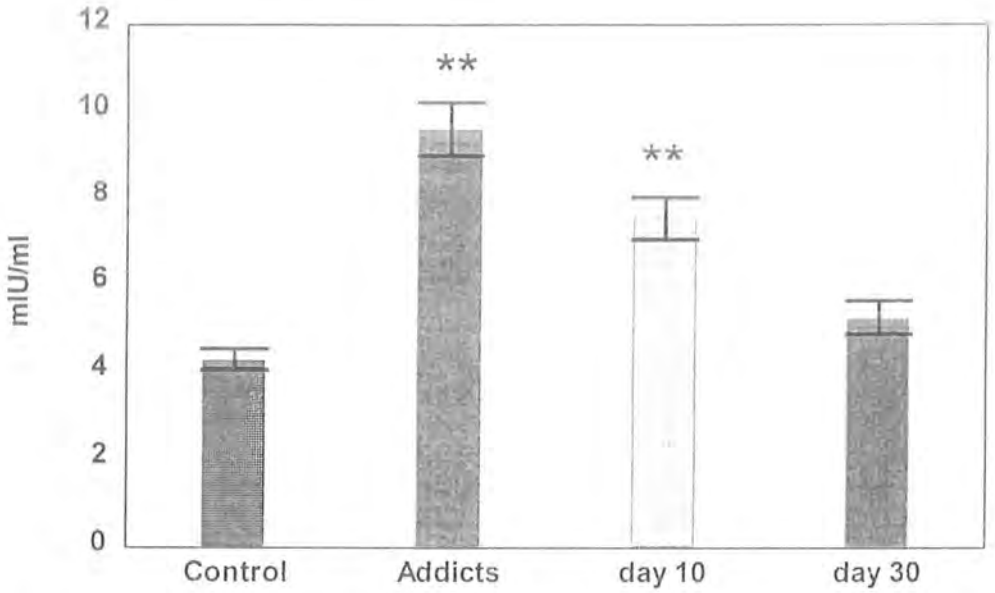

Fig. 1 Serum FSH levels (mean ± SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex.
** ($p<0.001$) compared to control group.

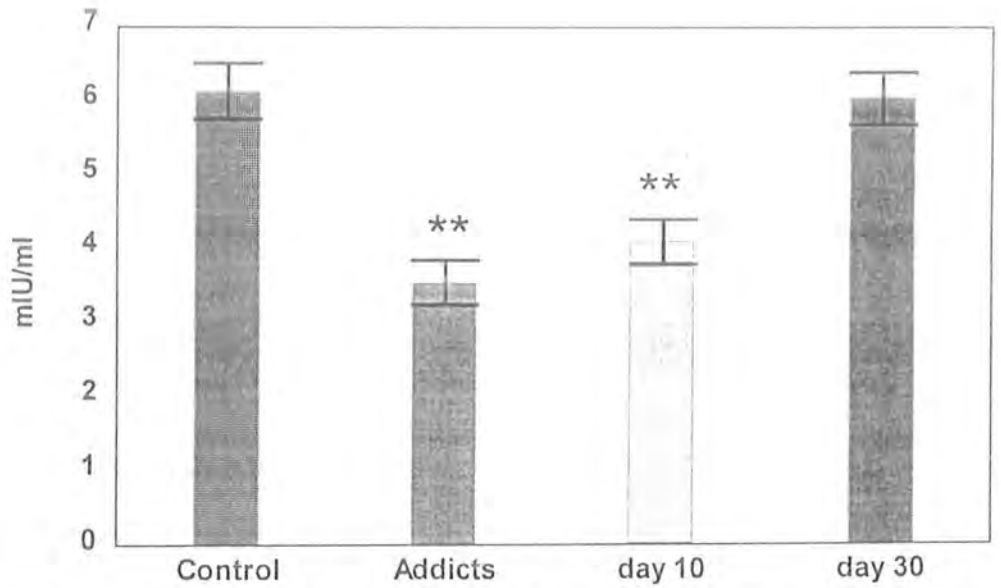

Fig. 2 Serum LH levels (mean ±SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex.
** ($p<0.001$) compared to control group.

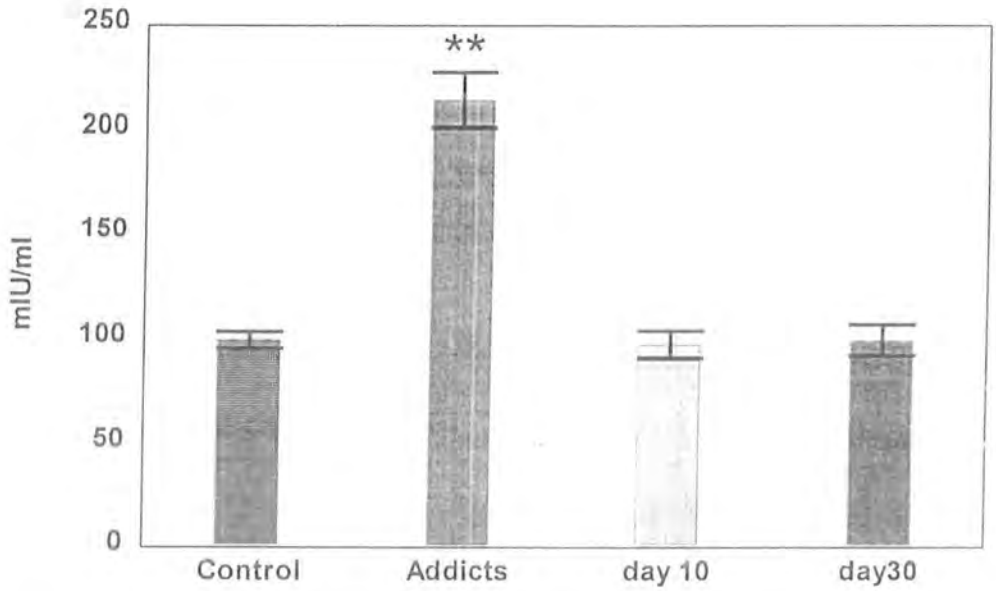

Fig. 3 Serum Prolactin levels (mean ± SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex. ** ($p<0.001$) compared to control group.

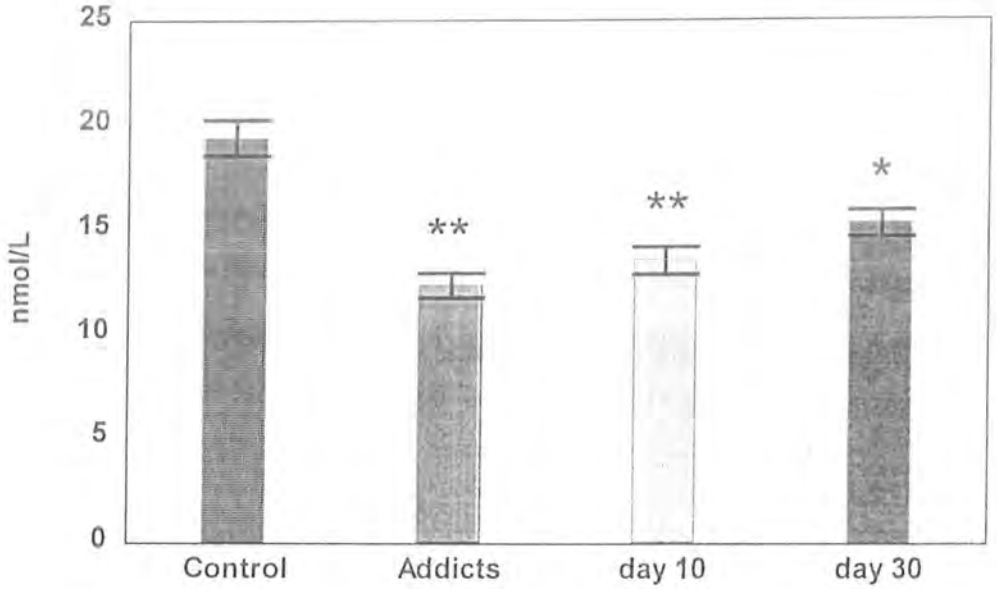

Fig. 4 Serum Testosterone levels (mean ± SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex. ** ($p<0.001$) * ($p<0.01$) compared to control group.

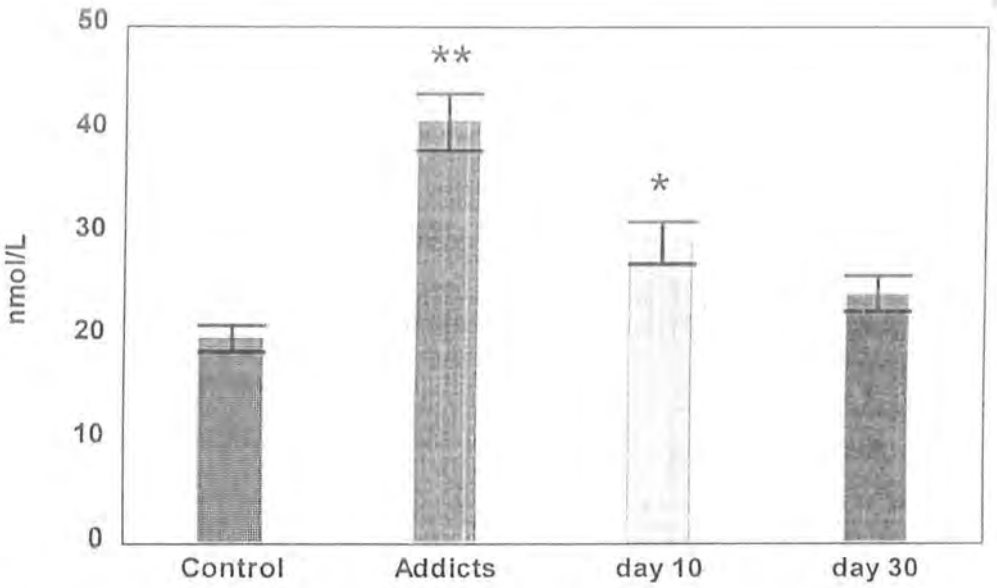

Fig. 5 Serum SHBG levels (mean ± SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex. ** ($p<0.001$) * ($p<0.01$) compared to control group.

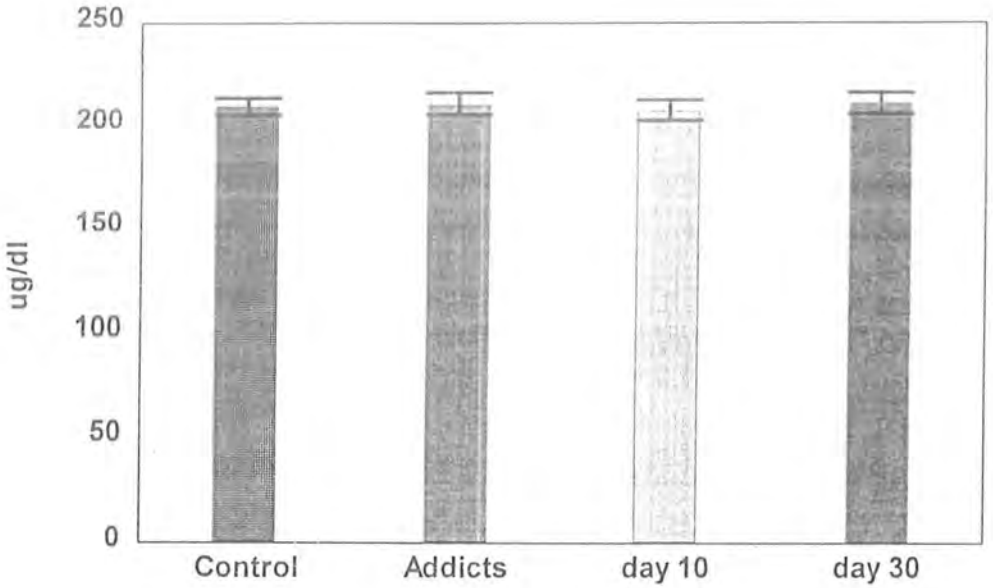

Fig. 6 Serum DHEA.SO4 levels (mean ± SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex.

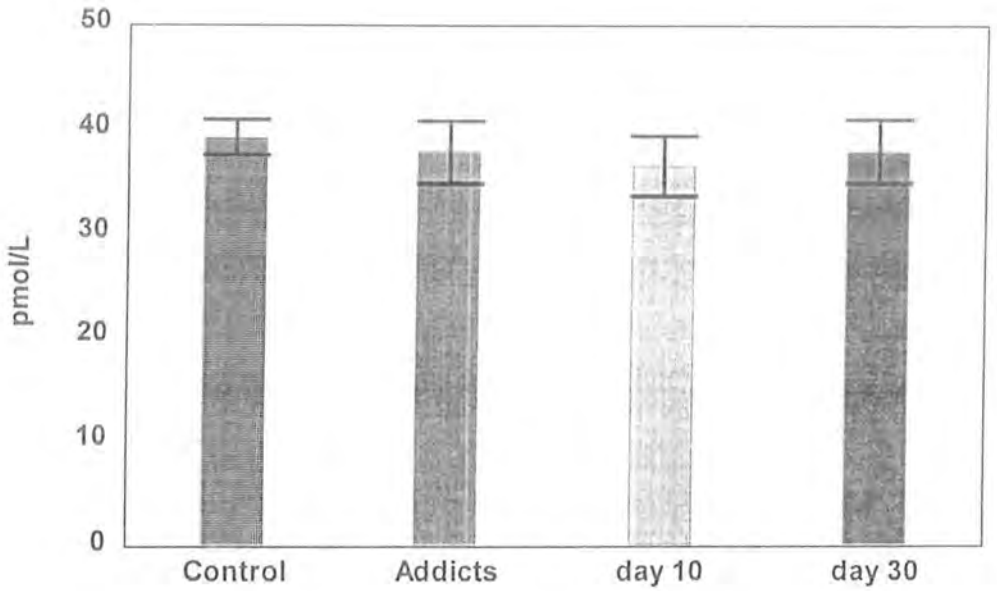

Fig. 7 Serum Estradiol (E2) levels (mean ± SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex.

Cortisol

Cortisol level showed a diurnal rhythm in both the healthy controls and the addict group (Fig. 8). In both cases, the morning level was substantially higher (17.8 ± 0.73 and 12.3 ± 0.42 ug/dl respectively) compared to the evening levels (6.58 ± 0.38 and 4.87 ± 0.30 ug/dl respectively). Although this diurnal rhythm remained clearly unaffected by heroin addiction, the drug depressed cortisol significantly ($p<0.001$) below the control level in the morning as well as in the evening (Fig. 8). Withdrawal therapy had no significant effect on the 10th day of treatment at either time of the day. Whereas the morning/evening values increased above the level of the addicts, the difference with the control was still marked indicating lack of complete recovery. Complete recovery from the effect of the drug was evident only on the 30th day of post-detoxification, when the cortisol levels in the morning and evening increased significantly (morning: $p<0.001$, evening: $p<0.01$) above the respective values for the addicts and became statistically similar to those for the control subjects.

Adrenocorticotropic hormone (ACTH)

The basal ACTH level was significantly lower in the addicts than in the controls (6.06 ± 0.63 Vs 11.78 ± 0.79 pg/ml; $p<0.001$) (Fig. 9). Thus heroin exerts drastic effect on this hormone. Detoxification resulted in significant increase above the level of the addicts($p<0.001$) on both the 10th (7.98 ± 0.54 pg/ml) and the 30th day (10.9 ± 0.59 pg/ml), the two values becoming nearly comparable to that of the control value. Thus the recovery from the effect of heroin occurred early.

Growth hormone (GH)

Growth hormone was drastically influenced by heroin addiction (Fig. 10). A significant ($p<0.001$) rise was recorded in the addicts before detoxification (4.01 ± 0.22 mIU/l) when compared with the healthy controls (1.31 ± 0.06 mIU/l). Detoxification therapy brought the levels significantly below the level of the addicts to near control value as early as the 10th day of treatment, which was maintained on the 30th day as well (Fig. 10).

Free Thyroxine (FT_4)

Free T_4 levels (12.6 ± 0.43 pmol/l) in the heroin addicts were significantly lower ($p < 0.001$) as compared to the healthy controls (16.6 ± 0.47 pmol/l). Following detoxification, free T_4 level on the 10th day increased significantly above the level of the addict (14.5 ± 0.37 pmol/l, $p<0.001$) but was still statistically distinguishable from that of the controls ($p < 0.01$). It returned to near normal level only on the 30th day (15.92 ± 0.38 pmol/l), the difference with the healthy controls on the 30th day being insignificant (Fig. 11). The results indicate that detoxification improves the condition of the addicts with a delay.

Triiodothyronine (T_3)

There was no significant difference in total T_3 between the healthy controls (mean 1.97± 0.04 nmol/l) and the addicts either before (2.01 ± 0.04 nmol/l) or after detoxification (Fig. 12). The mean value of T_3 on the 10th day was 1.97 ± 0.03 nmol/l and on the 30th day 2.01 ± 0.04 nmol/l.

Thyroid stimulating hormone (TSH)

In the case of TSH also, there was no difference between the controls (1.60 ± 0.12 mIU/l) and the heroin addicts (1.62 ± 0.11 mIU/l). The value of the TSH following detoxification on the 10th day (1.60 ± 0.04 mIU/l) or on the 30th day (mean 1.63 ± 0.09 mIU/l) were statistically similar to those of the addicts and the controls (Fig. 13).

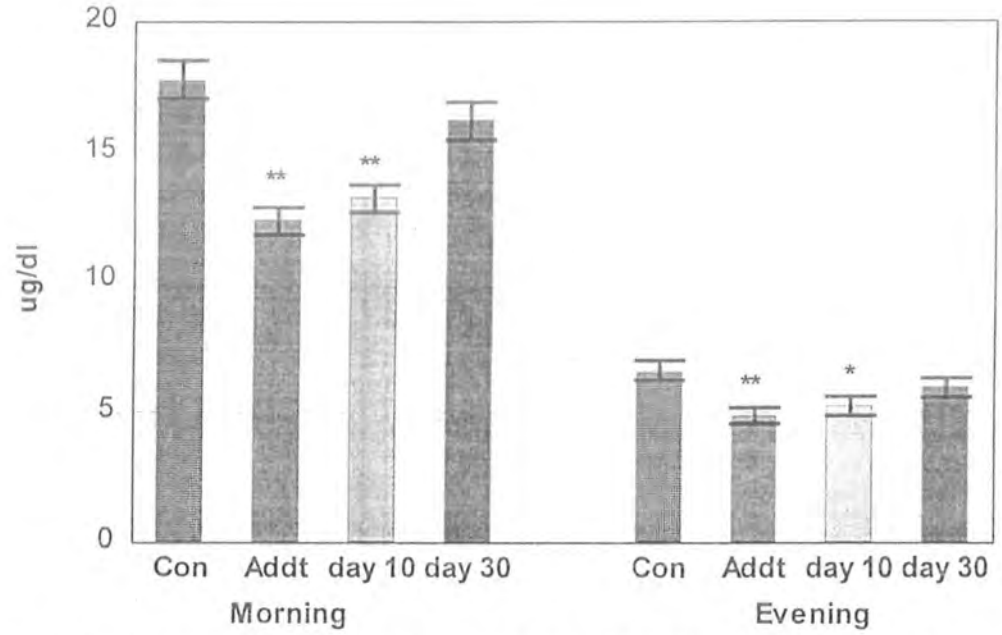

Fig. 8 Serum Cortisol (morning and evening) levels (mean + SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex. ** (p<0.001) * (p<0.01) compared to control group.

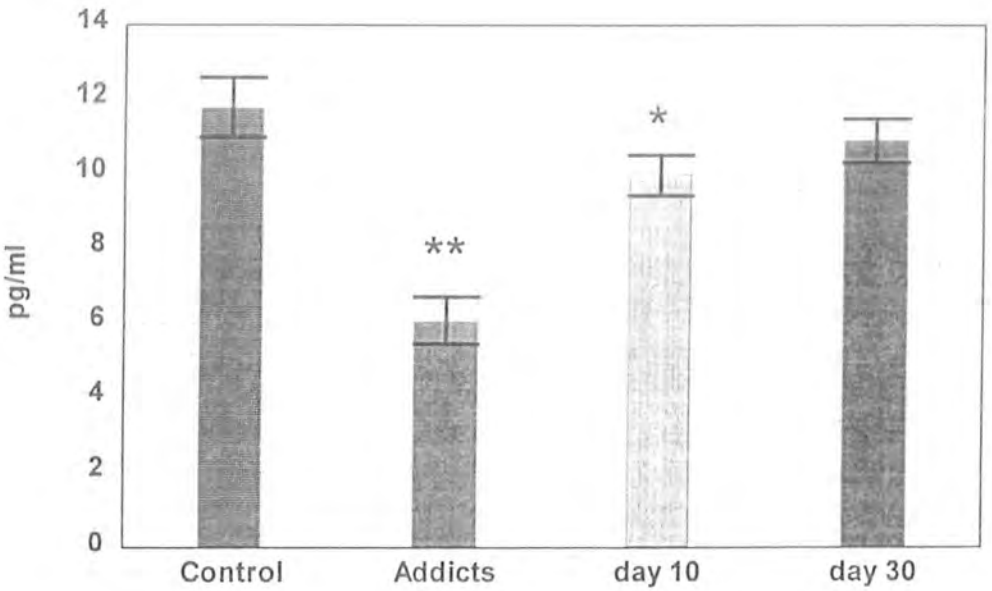

Fig. 9 Serum ACTH levels (mean ± SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex. ** (p<0.001) * (p<0.01) compared to control group.

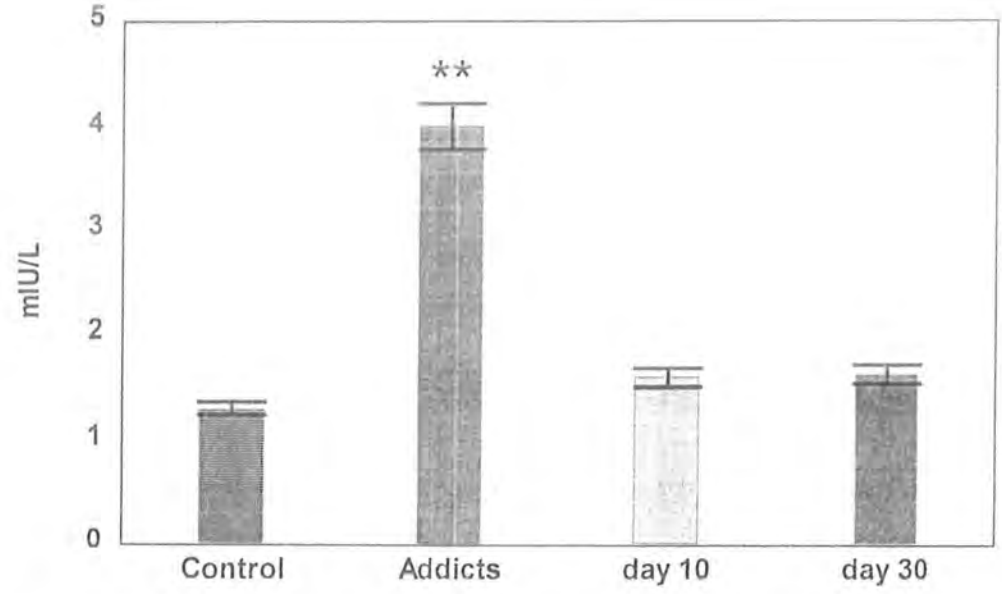

Fig. 10 Serum GH levels (mean ± SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex.
** ($p<0.001$) compared to control group.

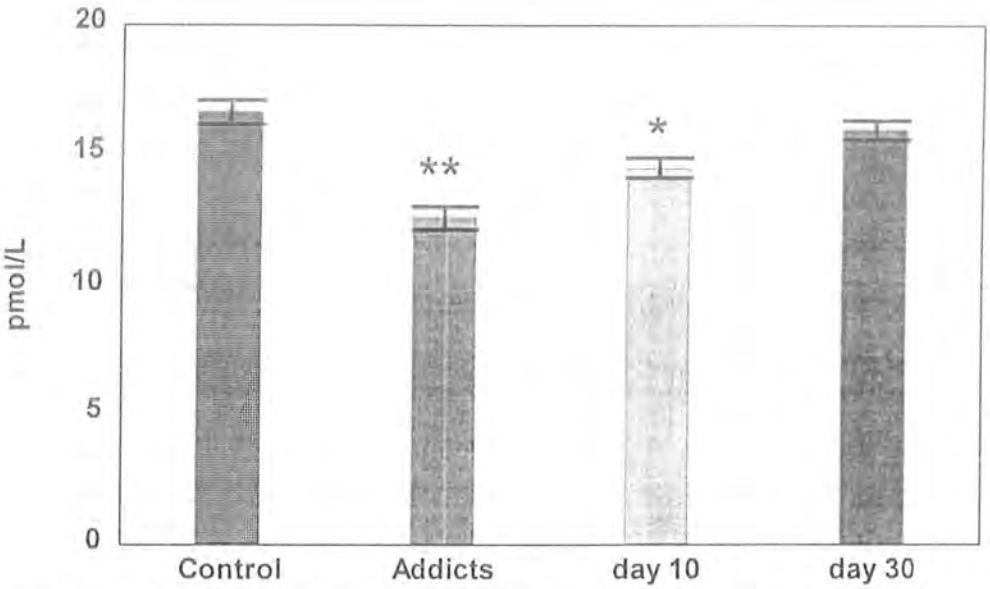

Fig. 11 Serum Free T4 levels (mean ± SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex.
** ($p<0.001$) * ($p<0.01$) compared to control group.

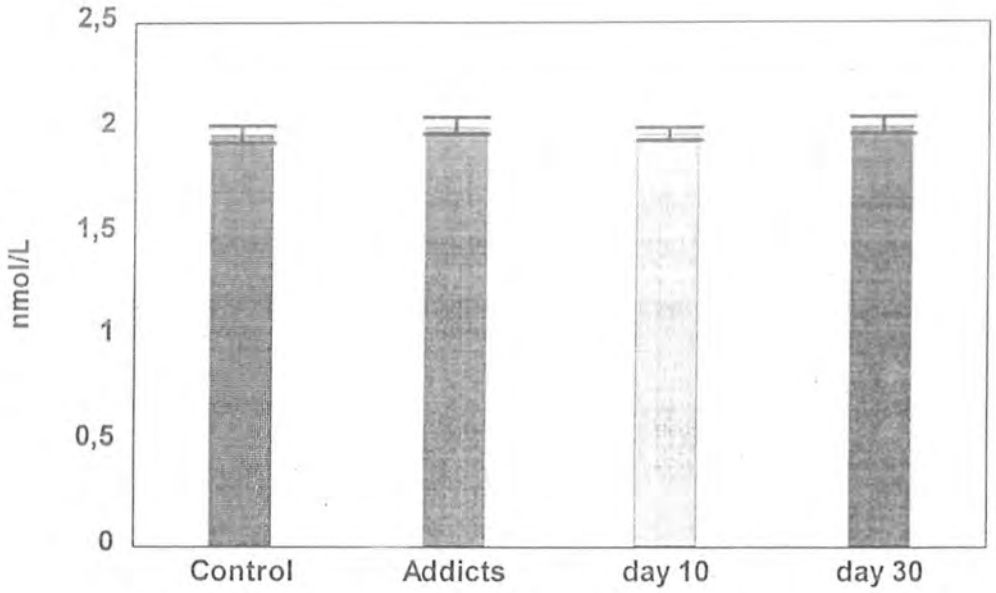

Fig. 12 Serum T3 levels (mean ± SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex.

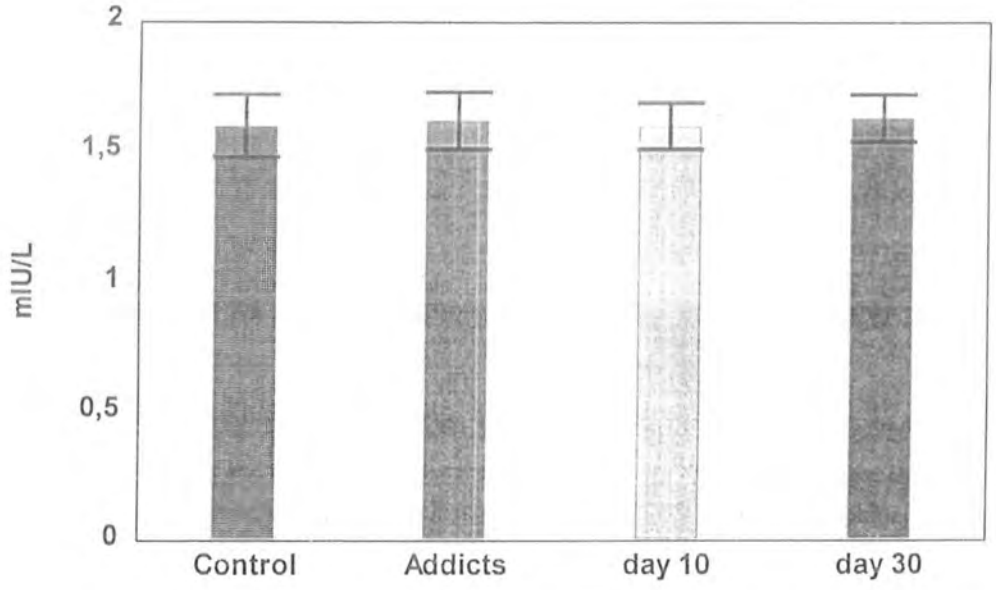

Fig. 13 Serum TSH levels (mean ± SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex.

Insulin and Glucose

Similar results were obtained for insulin and glucose as for GH. The mean insulin and glucose levels in the healthy controls were 10.3 ± 0.65 μIU/ml and 4.63 ± 0.07 mmol/l respectively. On the other hand, the levels of both were significantly elevated ($p < 0.001$) in the addicts prior to detoxification (Figs. 14, 15) suggesting an adverse influence of the drug. After detoxification, a sharp decline in the two metabolites below the level of the addicts occurred on the 10^{th} day. And their levels became similar to those of the controls (Fig. 14, 15) on the 10th day (11.4 ± 0.53 μIU/ml and 4.39 ± 0.06 mmol/l respectively) as well as the 30th day (10.62 ± 0.46 μIU/ml and 4.61 ± 0.06 mmol/l respectively). Evidently, withdrawal treatment caused early recovery of the addicts.

Gastrin

Serum gastrin was high in the addicts as opposed to the control subjects (75.6 ± 4.5 and 35.6 ± 2.3 pg/ml respectively). The difference was statistically significant ($p<0.001$). Heroin thus had an adverse effect on gastrin. The mean gastrin level decreased significantly below the level of the addicts following detoxification on the 10^{th} day which was maintained near this level on the 30^{th} day as well (34.78 ± 2.27 pg/ml and 32.7 ± 1.74 pg/ml respectively) (Fig. 16). These values remained statistically indistinguishable from the value for the control subjects which indicates early recovery from the effects of the drug.

Parathormone (PTH)

In the control subjects, the mean PTH level was 2.83 ± 0.19 pmol/l but dropped sharply and significantly in the addicts to a mean of 1.25 ± 0.13 pmol/l showing that heroin exerted a depressing effect on PTH. Withdrawal therapy significantly enhanced its level as early as the 10^{th} day of detoxification (Fig. 17) to a mean value of 2.66 ± 0.12 pmol/l. Thus early restoration to near control level occurred and the situation remained similar even on the 30^{th} day of detoxification.

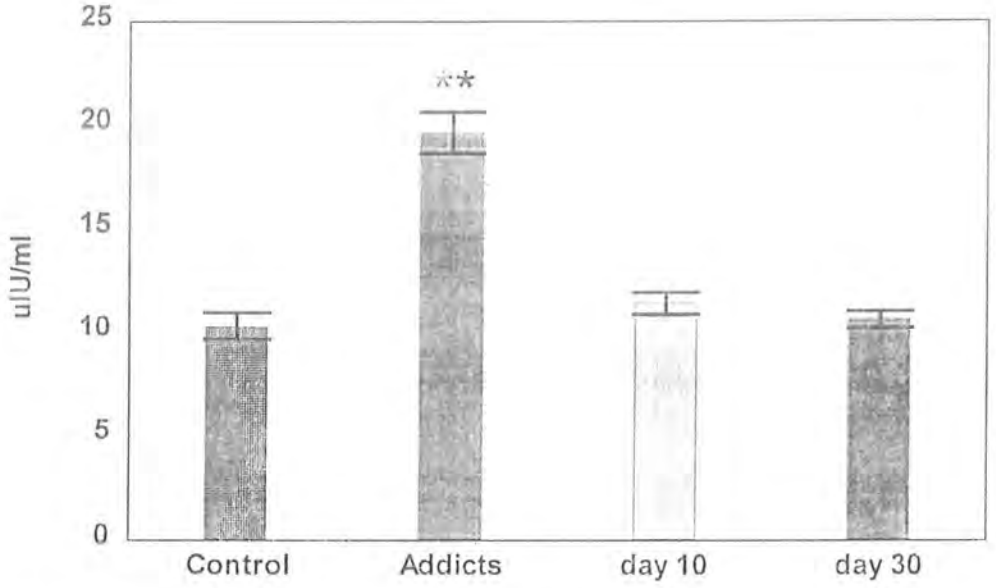

Fig. 14 Serum Insulin levels (mean ± SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex. ** ($p<0.001$) compared to control group.

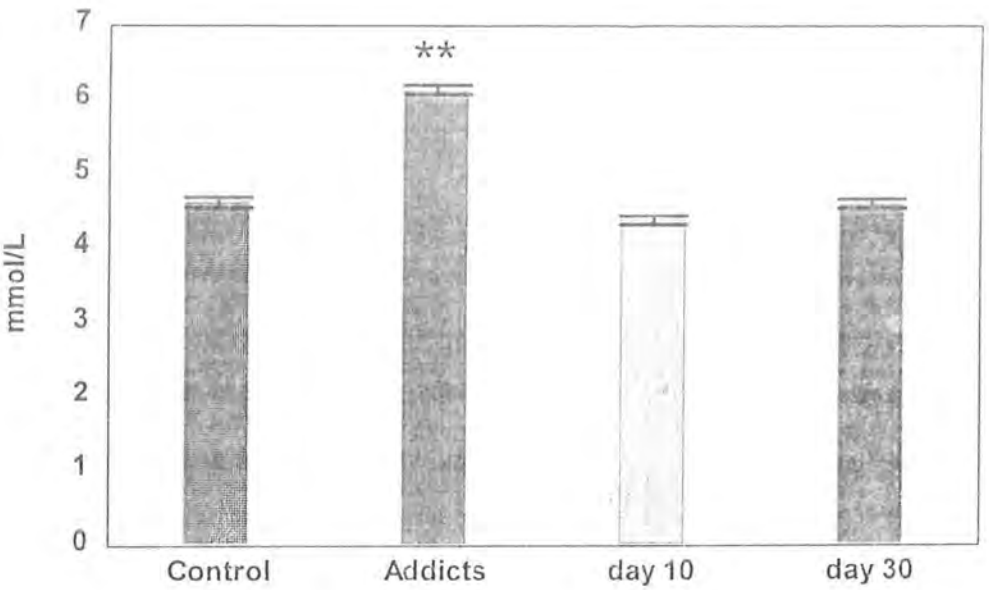

Fig. 15 Blood glucose levels (mean ± SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex. ** ($p<0.001$) compared to control group.

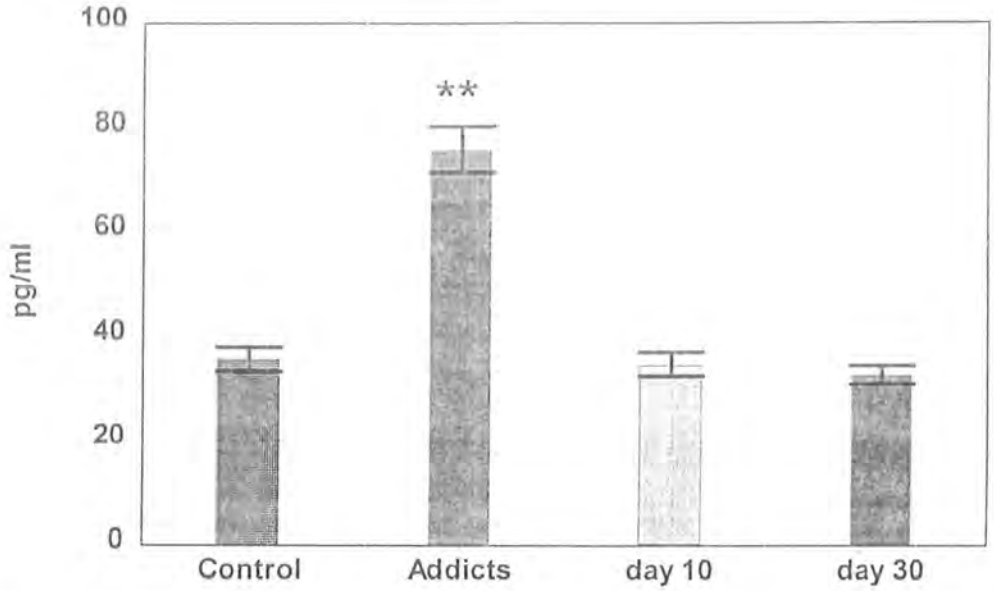

Fig.16 Serum Gastrin levels (mean ± SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex. ** ($p<0.001$) compared to control group.

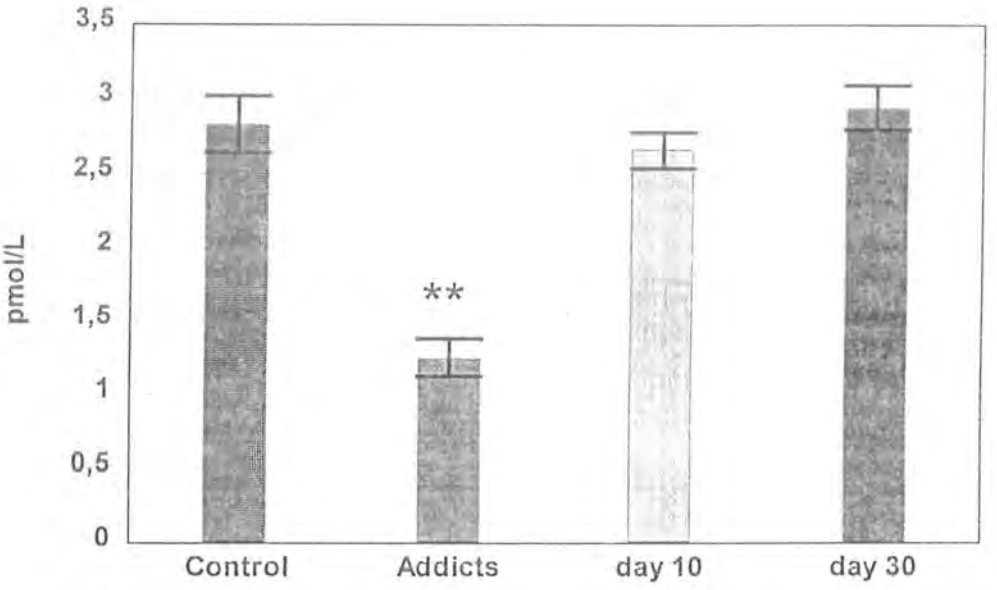

Fig. 17 Serum PTH levels (mean ± SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex. ** ($p<0.001$) compared to control group.

SEMEN ANALYSIS

In the control group the mean sperm count was 73.3 ± 2.19 million/ml, while in the addicts before detoxification it was 56.5 ± 1.96 million/ml. The sperm count remained unchanged from the addict-level and hence was significantly lower ($p < 0.001$) than in the controls even after the 10th and 30th day of detoxification (57.1 ± 1.88 and 58.6 ± 1.89 million/ml respectively) (Fig. 18). Thus heroin exerted a long lasting effect on the sperm count since no recovery was evident even on the 30th day of withdrawal.

Percentage of sperm motility in the addicts was also significantly lower ($p < 0.001$) than in the control subjects both before and after detoxification (Fig. 19). In the control subjects it was 64.83 ± 1.32 while in the addicts it dropped to 24.83 ± 1.47 before treatment and 26.9 ± 1.57 and 34.2 ± 1.97 on the 10th and the 30th days of detoxification respectively.

These results show that heroin brought about lasting effect on sperm motility as well. However, it is noteworthy that motility on the 30th day rose significantly ($p<0.01$) above the value for the 10th day suggesting a trend toward improvement following withdrawal therapy.

Semen fructose

Semen fructose was similarly adversely affected by heroin addiction (Fig. 20). In the control subjects the mean fructose was 15.8 ± 0.30 mmol/l while in the addicts it dropped to 8.1 ± 0.47 mmol/l, showing nearly 50% depression. On the 10th day of detoxification therapy, no significant change compared to the addicts was recorded (8.4 ± 0.49 mmol/l). The fructose value for the 30th day group, on the other hand, deviated significantly from the level of the addicts indicating delayed improvement, though complete restoration was still not achieved (compared with the control group).

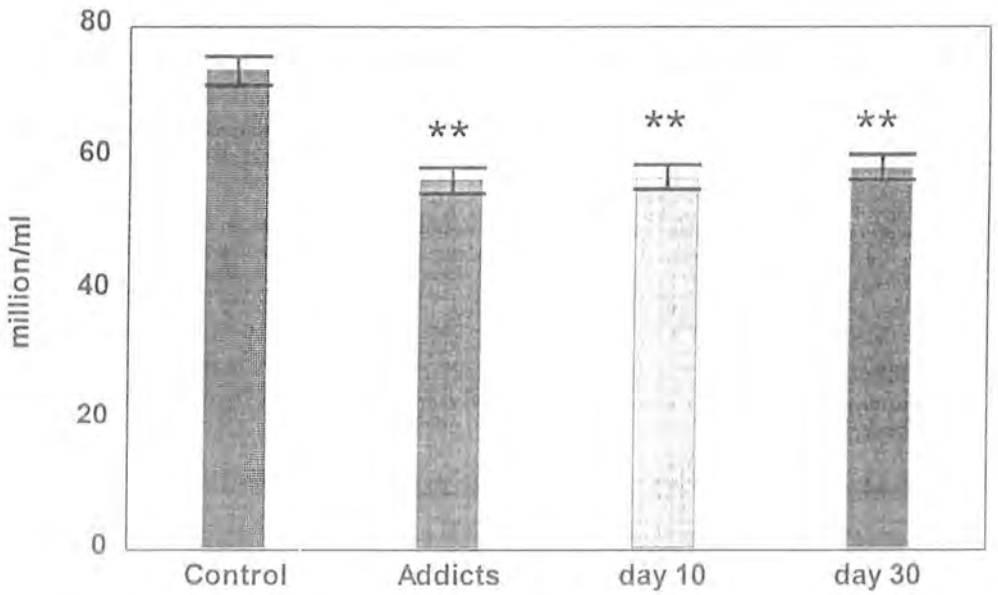

Fig. 18 Sperm concentration in semen samples (mean ± SEM) from heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex. ** ($p<0.001$) compared to control group.

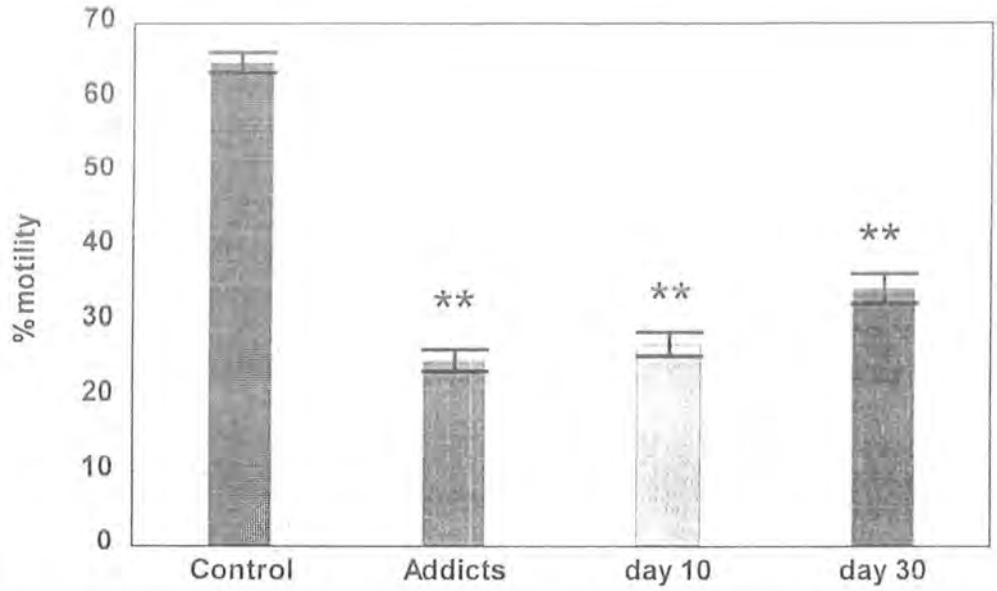

Fig. 19 Percentage forward motility of sperm in semen samples (mean ± SEM) from heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex. ** ($p<0.001$) compared to control group.

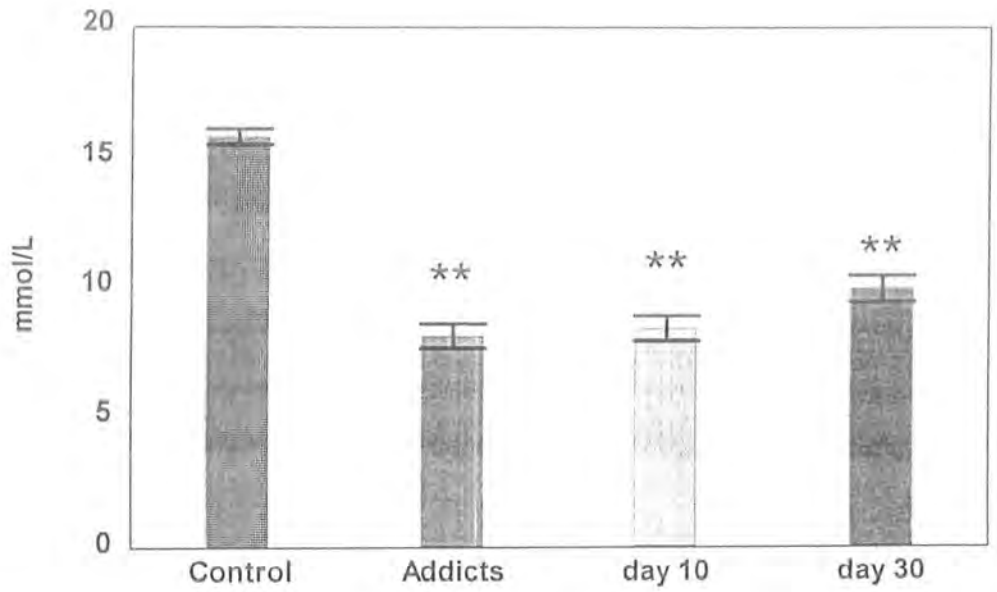

Fig. 20 Semen fructose levels (mean ± SEM) of heroin addicts before, 10 and 30 day of detoxification and in control subjects matched for age and sex. ** ($p<0.001$) compared to control group.

Na,K-ATPase in Erythrocytes

No significant difference was observed between the controls and the heroin addicts in Na,K-ATPase activity in the erythrocytes either before or 15 days after detoxification (Fig. 21). In the healthy controls, it was 8.86 ± 0.38 μmol Pi/g protein/hr given the optimal analytical conditions. The same mean value was recorded for the heroin addicts (8.86 ± 0.36 μmol Pi/g protein/hr) before detoxification. The mean activity 15 days after withdrawal showed only negligible change (8.9 ± 0.41 μmol Pi/g protein/hr).

Na,K-ATPase in Leucocytes

Na,K-ATPase activity in the leucocytes of the healthy controls, under the optimal conditions of the study, was 298 ± 12.2 μmol Pi/g protein/hr, while in the addicts it increased significantly (348 ± 11.6 μmol Pi/g protein/hr). On day 15th of withdrawal, the level reverted to the level of the controls (295 ± 12.7 μmol Pi/g protein/hr) indicating complete recovery (Fig. 22).

Intracellular sodium and potassium in erythrocytes

No significant difference was observed between controls and the addicts (either before or 15 days after detoxification) in the intracellular sodium and potassium levels (Figs. 23, 24). In the healthy controls, the levels were 5.1 ± 0.16 mmol/l cell water and 76 ± 2.9 mmol/l cell water respectively. In the heroin addicts, these were 5.07 ± 0.15 and 76.4 ± 3.1 mmol/l cell water respectively. Detoxification therapy did not bring about any change in these.

Intracellular sodium and potassium in leucocytes

Intracellular sodium and potassium in the leucocytes of the healthy controls, were 31.0 ± 1.75 mmol/l cell water and 140 ± 4.08 mmol/l cell water respectively. In the addicts these showed only negligible change (30 ± 1.66 mmol/l for sodium and 142 ± 4.51 mmol/l cell water for potassium). On day 15th of withdrawal, no distinguishable change was observed (Fig. 25).

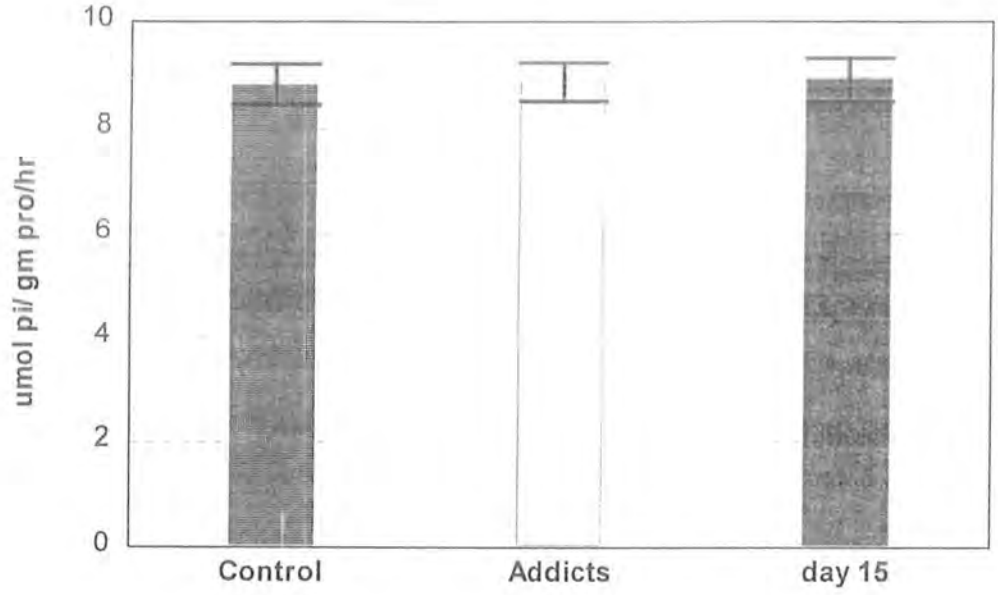

Fig. 21 Na,K-ATPase activity in erythrocytes (mean +SEM) of heroin addicts before, 15 day of detoxification and in control subjects matched for age and sex.

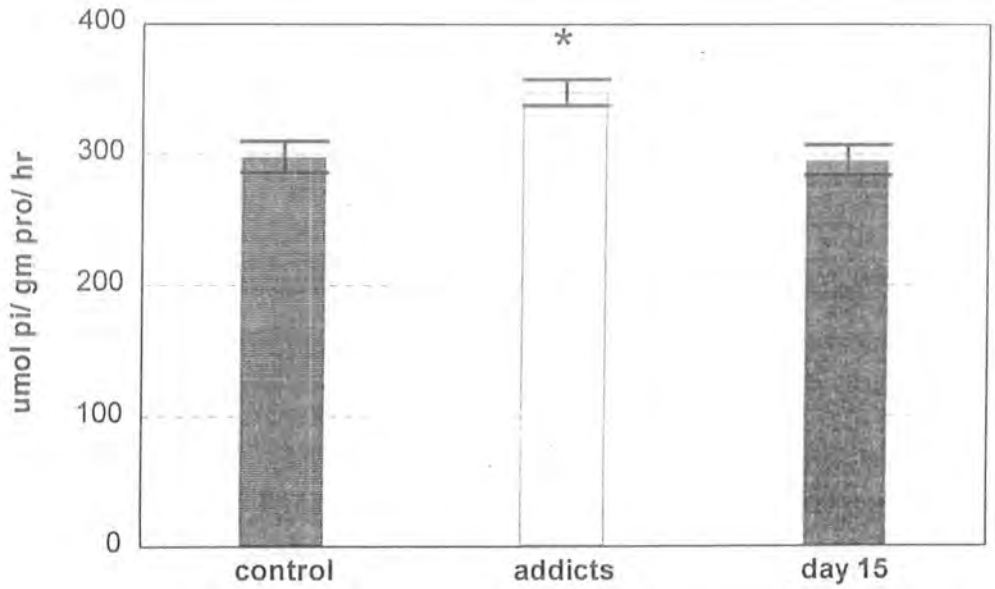

Fig. 22 Na,K-ATPase activity in leucocytes (Mean ± SEM) of heroin addicts before, 15 day after detoxification and control subjects matched for age and sex. * ($p<0.01$) compared to control group.

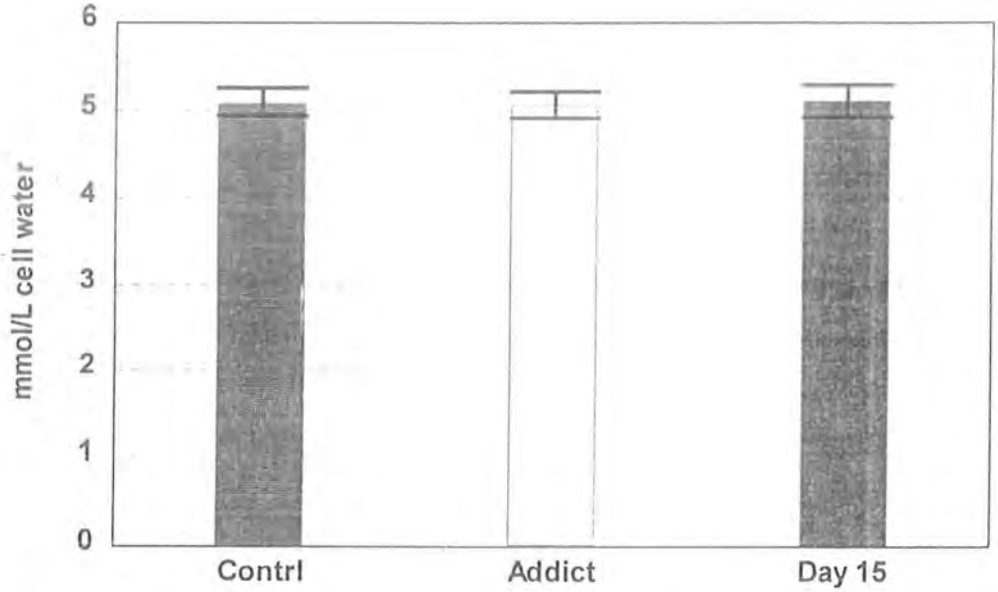

Fig. 23 Erythrocyte Intracellular sodium levels (mean ± SEM) of heroin addicts before, 15th day of detoxification and in control subjects matched for age and sex.

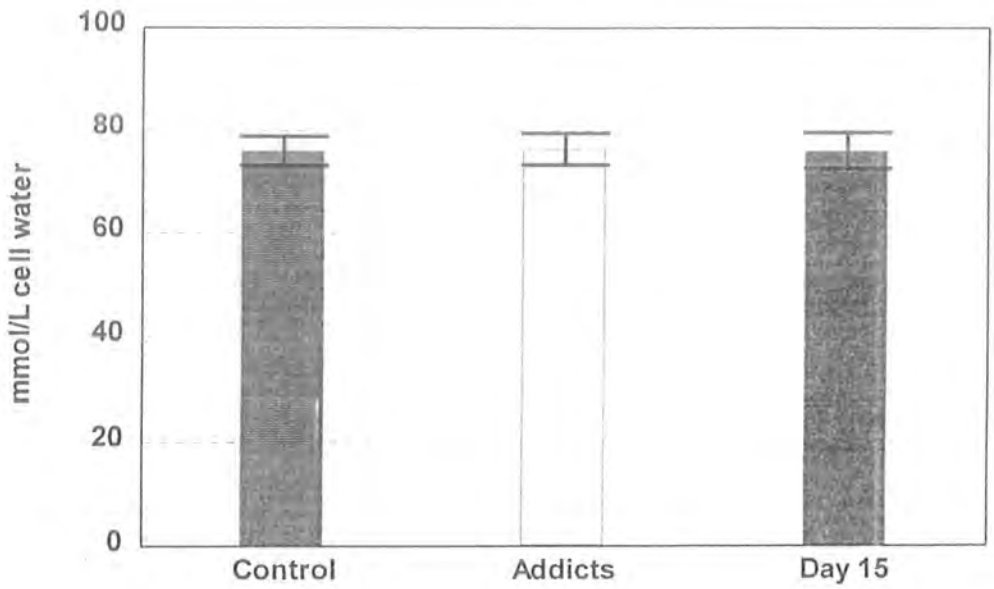

Fig. 24 Erythrocyte intracellular potassium levels (mean ± SEM) of heroin addicts before, 15th day of detoxification and in control subjects matched for age and sex.

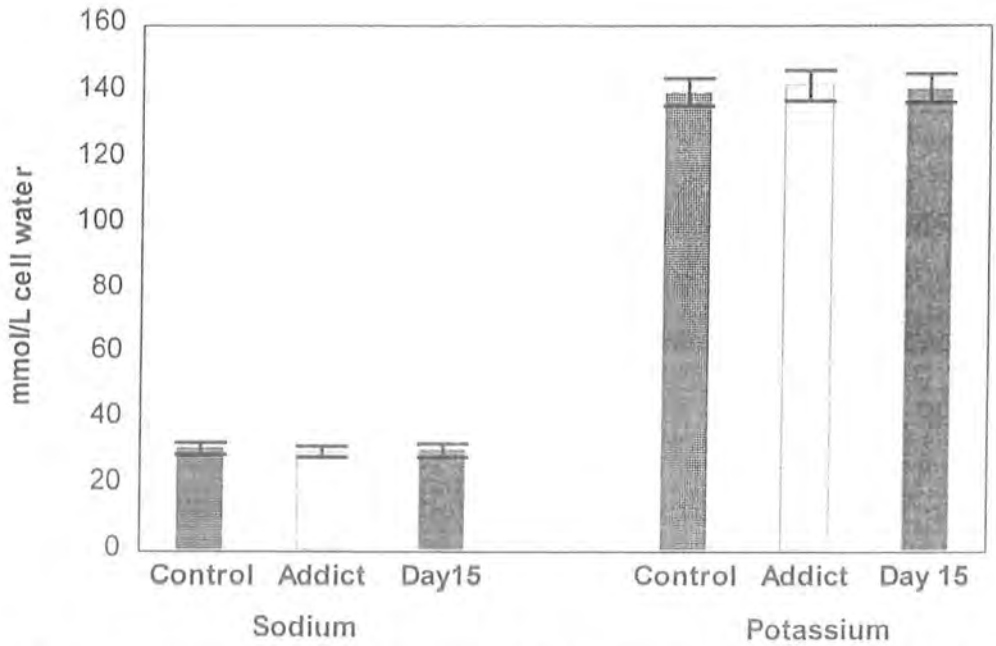

Fig. 25 Leucocyte intracellular sodium and potassium levels (mean ± SEM) of heroin addicts brfore, 15 days after detoxification and in control subjects matched for age and sex.

Comparison between Group I and Group II addicts

Table 9 describes the various hormonal analysis for the two classes of the addicts separated on the basis of length of addiction. It is evident from the data that with the exception of PRL, ACTH and cortisol, the basal levels of other hormones remained statistically indistinguishable in Group I and Group II addicts. The data for these parameters were also similar to those for the total duration subjects (2-16 yr). In contrast, the effect on PRL was very severe in the Group II addicts where it was drastically elevated (259 ± 17.1 mIU/ml). The difference with Group I (179 ± 10 mIU/ml) was highly significant ($p<0.001$). However, complete recovery from the effect of heroin occurred as early as 10^{th} day of drug withdrawal. Although the basal levels of T in the two groups were similar to those of the total duration group, recovery from the effect did not occur even on day 30 of detoxification either in Group I or Group II. The morning serum cortisol (basal, 10 and 30 day) levels in Group I addicts were similar to those of the total duration subjects. However, far great decrease in the basal levels occurred in the Group II addicts compared to Group I addicts. Also, the effect was irreversible even on day 30 of drug withdrawal. Although the evening basal level in the two groups were statistically similar and the 10 and 30 day levels deviated significantly from the within group basal level. Complete recovery from the heroin effect did not occur even on the 30^{th} day of detoxification in Group II subjects. The basal serum ACTH levels in Group I, Group II and total duration subjects remained similar. However, the Group I addicts recovered from the damage early (day 10). Such recovery was not evident in the Group II addicts either on day 10 or day 30 suggesting severe influence of duration of addiction on pituitary-adrenal axis (Table 9).

Regarding seminal parameters (sperm count, sperm motility and semen fructose level), significant difference was observed between the two groups studied (Table 10). In the chronic addicts, with longer addiction period (group II), no significant difference within group variation ($p>0.05$) in the three seminal parameters was observed at the two post detoxification intervals (10^{th} and 30^{th} day) revealing persistent impairment and lack of reversibility of the effect. In contrast, group I addicts showed a significant within group increase in the three seminal parameters over the baseline values (addicts) on day

30 of detoxification (Table 10). Since these values are statistically comparable to those of the corresponding controls (see Figs. 18, 19 and 20), they show that the impairment is reversible in group I addicts.

No appreciable change in Na,K-ATPase activity was found in the erythrocyte and the leucocytes of the two groups (Table 11).

Table 9: Comparison of various hormonal levels in Group I and Group II heroin addicts (B) and following 10 and 30 day detoxification. The values are means ± standard error of the mean.

HORMONE	CONTROL	TREATMENT	GROUP I 2-8 years (n = 44)	GROUP II 9-16 years (n = 16)
Free T_4 (pmol/l)	16.6 ± 0.47	B	12.9 ± 0.45	12.3 ± 0.39
		Day 10	14.4 ± 0.40	14.6 ± 0.42
		Day 30	16.3 ± 0.38	15.6 ± 0.41
Total T_3 (nmol/l)	1.97 ± 0.04	B	2.02 ± 0.04	2.00 ± 0.04
		Day 10	1.99 ± 0.03	1.97 ± 0.05
		Day 30	2.02 ± 0.04	2.00 ± 0.05
TSH (mIU/l)	1.60 ± 0.12	B	1.62 ± 0.12	1.62 ± 0.13
		Day 10	1.62 ± 0.10	1.59 ± 0.13
		Day 30	1.62 ± 0.08	1.64 ± 0.11
FSH (mIU/ml)	4.28 ± 0.24	B	9.31 ± 0.60	9.84 ± 0.71
		Day 10	7.30 ± 0.48	7.71 ± 0.59
		Day 30	5.02 ± 0.38	5.39 ± 0.42
LH (mIU/ml)	6.12 ± 0.38	B	3.71 ± 0.30	3.27 ± 0.35
		Day 10	4.08 ± 0.30	3.96 ± 0.33
		Day 30	6.23 ± 0.33	5.95 ± 0.40
Prolactin (mIU/ml)	99.6 ± 3.87	B	179 ± 10	259 ± 17.1 **
		Day 10	101 ± 6.05	95.0 ± 7.0
		Day 30	100 ± 6.0	99.0 ± 7.50
Testosterone (nmol/l)	19.4 ± 0.86	B	12.5 ± 0.59	12.1 ± 0.64
		Day 10	13.9 ± 0.55	13.0 ± 0.67
		Day 30	15.1 ± 0.60	15.3 ± 0.60
SHBG (pmol/l)	19.7 ± 1.28	B	41.7 ± 2.59	39.8 ± 2.87
		Day 10	30.8 ± 2.04	28.0 ± 2.30
		Day 30	24.2 ± 1.60	24.3 ± 1.95
DHEA-SO4 (µg/dl)	209 ± 4.32	B	212 ± 4.99	209 ± 5.80
		Day 10	207 ± 4.90	207 ± 5.10
		Day 30	209 ± 4.80	211 ± 5.40
Estradiol (pmol/l)	39.3 ± 1.71	B	40.1 ± 1.69	37.7 ± 1.74
		Day 10	36.5 ± 2.00	36.6 ± 3.75
		Day 30	38.5 ± 2.80	37.5 ± 3.33

** $p<0.001$ significant relative to Group I

Table 9: continued

HORMONE	CONTROL	TREATMENT	GROUP I 2-8 years (n = 44)	GROUP II 9-16 years (n = 16)
GH (mIU/l)	1.31 ± 0.06	B	4.00 ± 0.20	4.04 ± 0.25
		Day 10	1.66 ± 0.08	1.73 ± 0.11
		Day 30	1.59 ± 0.09	1.76 ± 0.12
Insulin (μIU/L)	10.3 ± 0.65	B	19.5 ± 0.77	19.8 ± 1.05
		Day 10	11.4 ± 0.46	11.4 ± 0.53
		Day 30	10.3 ± 0.40	10.8 ± 0.44
Glucose (mmol/l)	4.63 ± 0.07	B	6.03 ± 0.06	6.26 ± 0.08
		Day 10	4.41 ± 0.06	4.35 ± 0.06
		Day 30	4.55 ± 0.06	4.64 ± 0.07
Cortisol (μg/dl) Morning	17.8 ± 0.73	B	14.3 ± 0.40	10.9 ± 0.46 **
		Day 10	15.2 ± 0.46	11.0 ± 0.54 **
		Day 30	17.8 ± 0.59	12.4 ± 0.70 **
Cortisol (μg/dl) Evening	6.58 ± 0.38	B	5.03 ± 0.28	4.70 ± 0.31
		Day 10	5.25 ± 0.32	4.90 ± 0.36 *
		Day 30	6.64 ± 0.33	5.17 ± 0.37 **
ACTH (pg/ml)	11.78 ± 0.79	B	7.06 ± 0.60	5.17 ± 0.66
		Day 10	8.90 ± 0.54	6.15 ± 0.62 *
		Day 30	11.9 ± 0.55	9.23 ± 0.62 **
Gastrin (pg/ml)	35.6 ± 2.3	B	75.0 ± 4.80	76.1 ± 5.01
		Day 10	34.9 ± 2.20	34.6 ± 2.33
		Day 30	33.7 ± 1.70	32.1 ± 1.82
PTH (pmol/l)	2.83 ± 0.19	B	1.25 ± 0.12	1.25 ± 0.15
		Day 10	2.70 ± 0.12	2.63 ± 0.12
		Day 30	2.90 ± 0.16	2.98 ± 0.16

* p<0.01 ** p<0.001 significant relative to Group I

Table 10: Comparison of seminal parameters in Group I and Group II heroin addicts (B) and following 10 and 30 day detoxification. The values are means ± standard error of the mean.

SEMINAL PARAMETERS	CONTROL	TREATMENT	GROUP I 2-8 years (n = 44)	GROUP II 9-16 years (n = 16)
Sperm count (million/ml)	73.3 ±2.19	B	58.6 ± 1.80	54.3 ± 1.96
		Day 10	59.2 ± 1.85	54.8 ± 1.89 *
		Day 30	62.8 ± 1.88	54.4 ± 2.03 **
% Motility	64.8 ± 1.32	B	25.9 ± 1.40	23.1 ± 1.45
		Day 10	29.8 ± 1.55	23.2 ± 1.64 *
		Day 30	40.3 ± 1.68	27.0 ± 2.12 **
Semen Fructose (mmol/l)	15.8 ± 0.30	B	8.5 ± 0.44	7.8 ± 0.49
		Day 10	8.70 ± 0.46	8.0 ± 0.51 *
		Day 30	11.38 ± 0.50	8.42 ± 0.52 **

* p<0.01 ** p<0.001 significant relative to Group I

Table 11: Comparison of Na,K-ATPase activity in Group I and Group II heroin addicts (B) and following 10 and 30 day detoxification. The values are means ± standard error of the mean.

ENZYME	CONTROL	TREATMENT	GROUP I 2-8 years (n = 12)	GROUP II 9-16 years (n = 5)
Erythrocyte Na,K-ATPase (μmol pi/g protein/hr)	8.86 ± 0.38	B	8.86 ± 0.38	8.87 ± 0.38
		Day 15	8.85 ± 0.40	8.92 ± 0.41
Leucocyte Na,K-ATPase (μmol pi/g protein/hr)	298 ± 12.2	B	346 ± 9.0	350 ± 10.0
		Day 15	299 ± 11.0	290 ± 12.0

DISCUSSION

DISCUSSION

The present study reveals that regardless of the duration of chronic addiction the majority of physiological parameters examined are adversely affected by heroin addiction. Also, the damage done by heroin is not permanent and, in most cases the impairment is reversible. The function of the pituitary is clearly altered. Serum FSH, LH, PRL, GH and ACTH levels have been shown here to undergo significant modification in the heroin addicts. The only pituitary hormone which is unaffected by heroin turns out to be TSH. In addition, testosterone together with its binding protein SHBG as well as cortisol and thyroxin which are directly controlled by the pituitary also undergo change. Equally affected are parathormone, gastrin, insulin and glucose. Drastic and irreversible changes occur in respect to factors related to sperm function i.e. sperm count, sperm motility and seminal fructose. While Na,K-ATPase remains unaffected in the erythrocytes, its activity is reversibly altered in the leucocytes. When the data was analyzed on the basis of duration of chronic addiction by separating the addicts into two classes (2-8 yr, 9-16 yr), only PRL, to some extent T and particularly cortisol and ACTH are far more severely influenced in the group addicted for longer duration. The same holds true for the semen parameters (sperm count, sperm motility and semen fructose)

The harm of heroin addiction is known to extend to majority of physiological systems and a number of endocrine abnormalities have been recognized in the past in the heroin addicts. It is noteworthy that the receptors for the opiates are distributed throughout the CNS. The highest concentration of these exists in the limbic system (frontal and temporal cortex, amygdala and hippocampus), the striatum, midbrain, laminae I, II, IV and V of the dorsal horn in the spinal cord, the thalamus and the hypothalamus (Goodman and Pasternak, 1985). The median eminence of the hypothalamus contains high density of the opiate receptors (Atweh and Kuhar, 1983) where the anterior pituitary-regulating substances are released through the portal capillary system and modulation of pituitary hormone secretion by endogenous and exogenous opiates is known to take place (Clement-Jones and Besser, 1983). According to Pert and Synder (1973) and Yen *et al.* (1985), the opiates specifically affect the pituitary physiology. Increase in the volume of the pituitary gland of the addicts has been demonstrated by Teoh *et al.* (1993). These studies lend support to the present

observations showing significant changes in the serum levels of the pituitary hormones, GH, PRL, ACTH, LH and FSH.

Reproductive function in men and women is crucially dependent on a precisely coordinated cascade of hormonal signals from the hypothalamus, the pituitary and the gonads. Secretion of gonadotropins from the pituitary is initiated by pulsatile release of the gonadotropin releasing-hormone (GnRH) from the hypothalamus. The secreted gonadotropins functionally divide the gonads of both men and women into two components,: the gametogenic compartment mainly controlled by FSH and the steroidogenic compartment largely dependent on LH. Maintenance of normal reproductive function ultimately requires the collaboration of these two functionally distinct units within the gonads as well as their feedback via gonadal steroids and other peptides acting at the level of both the hypothalamus and the pituitary (Mirin et al., 1976).

One of the most consistently disturbed neuroendocrine systems under heroin addiction shown in the past (Martin *et al.*, 1973; Brakke *et al.*, 1974; Tolis *et al.*, 1975; Cicero *et al.*, 1977, Mendelson *et al.*, 1980; Smith and Gilbeau, 1985; Ragni *et al.*, 1988; Ahmed and Ahmed, 1993; Bonavera *et al.*, 1994) and the present work is the hypothalamo-pituitary-gonadal axis. Both FSH and LH have been shown positively to undergo alteration in the addicts. Interestingly, FSH levels in the addicts examined here are enhanced whereas those of LH are depressed. The current information in this area reveals considerable discordance in respect to relationship between heroin and these gonadotropins and the duration of change. The already existing observations, show enhancement of both serum FSH and LH (Tepperman , 1973; Cushman and Kreek, 1974; Afrasiabi *et al.*, 1979; Ragni *et al.*, 1988; Ahmed and Ahmed, 1993), depression of both FSH and LH (Martin *et al.*, 1973; Bakke *et al.*, 1973; Tolis *et al.*, 1975; Cicero *et al.*, 1976b, 1977; Mendelson *et al.*, 1980; Smith and Gilbeau, 1985; Singer *et al.*, 1986; Giri and Kaufman, 1994; Mena-Valdivia *et al.*, 1995) and no effect on either of the gonadotropins (Cushman *et al.*, 1972a; Azizi *et al.*, 1973; Wang *et al.*, 1978; Bolleli *et al.*, 1979). According to Giri and Kaufman (1994), decrease in FSH and LH results from low pulses of GnRH caused by the opiates. There is evidence that endogenous opiates too cause depression of serum FSH and LH levels and that the opiate antagonist, naloxone,

reverses this effect on serum LH (increases it) indicating that they are mediated by specific opiate receptors. The normal role of endogenous opiates, thus, appears to be inhibition of the gonadotropins (Hammer *et al.*, 1994; Kujjo *et al.*, 1995; Sannella and Petersen, 1997). In this context, the enhancement of FSH and LH reported by other workers in the past is difficult to explain. The conflict in results from different laboratories is not limited to what has been described above but also extends to observations which show that FSH and LH respond in opposite ways under heroin addiction and such a response of the two gonadotropins has been obtained in the present investigation. The level of FSH increases and that of LH decreases in the heroin addicts. There is abundant evidence to support such opposite effects of these gonadotropins. Cicero *et al.* (1976a,b) have described reduction in serum LH following injection of opiates. This has also been observed by other workers (Mendelson *et al.*, 1980; Sheridan and Buchanan, 1980; Mirin *et al.*, 1980; Smith and Gilbeau, 1985). A reduction in LH has been obtained not only in human addicts but also in rams given opiate injections (Fitzgerald and Perkins, 1994). It has been reported by different workers that the depression in serum LH is due to inhibition of GnRH by the opioids, both endogenous and exogenous, which activate opiate mu-receptors (Ferrer *et al.*, 1997; Giri *et al.*, 1996; Pedron *et al.*, 1996). Hyperprolactenemia (see below) is also inhibitory to GnRH secretion. Only a few studies are available which show a significant decrease in FSH and no change in LH in the heroin addicts (Lafisca *et al.*, 1981; Khan *et al.*, 1991). The fact that in the heroin addicts studied here FSH increases while LH decreases, needs to be looked at in relation to the observed changes in the levels of the androgens (see below).

Another of the consistent abnormalities accompanying heroin addiction is impotence, decreased libido, problems in the ejaculate and depression of serum testosterone (George, 1971; Azizi *et al.*, 1973; Cushman and kreek, 1974; Mendelson *et al.*, 1975; Cicero *et al.*, 1976a; Bolelli *et al.*, 1979; Mendelson and Mello, 1982, Ragni *et al.*, 1988; Ahmed and Ahmed, 1993; Pedrazzoni *et al.*, 1993). The situation regarding testosterone level is, however, confusing since conflicting data exist revealing either no change (Cushman, 1973; Lafisca *et al.*, 1981) or low levels in acute cases of intake and high levels under chronic conditions (Malik *et al.*, 1992). Perhaps, metabolic adaptation of the addicts occurs after prolonged use of heroin. According to the observations on the

subjects examined presently who were chronic drug users, the drastic decrease in testosterone level is correlated with enhanced FSH, depressed LH and no effect on DHEA-SO_4 and E_2. Evidently, heroin effects the pituitary-gonadal axis and that its influence seems to be not directly on the gonads, at least under the condition of the present study. Cicero *et al.* (1976b) have provided similar results regarding LH and testosterone in the addicts receiving opiates (Sheridan and Buchanan, 1980; Mirin *et al.*, 1976,1980). Further confirmation of a correlation between LH and testosterone comes from reversal of the levels of the two hormones when the opiate antagonist, naltrexone, is administered (Mendelson *et al.*, 1980; Smith and Gilbeau, 1985). Also, opiate infusion into rams not only reduces LH levels but also the number of ejaculations, an effect which is reversed by naloxone administration (Fitzgerald and Perkins, 1994). It may be noted in relation to the conflicting data available on effect of heroin (and other opiates) that the response of testosterone or other androgens (such as dehydroxytestosterone) may depend on the time of initiation of drug intake. Mendelson *et al.* (1984) have indicated that young men who begin heroin usage during puberty do not show long term effects of chronic addiction on testosterone levels. Relatively normal psychosexual growth and development have been obtained by these workers in adult men who began chronic use (8 years) of heroin at the age of 11-15 years. The observations of Malik *et al.* (1992) are also interesting, showing the least effect in short-time addicts, moderate increase in fair-time addicts and the largest increase in the long-time addicts. Little information is available on the effect of heroin on progesterone and estradiol levels in male addicts. The work of Azizi *et al.* (1973), Wang *et al.* (1978) and Lafisca *et al.* (1981) shows no effect of the drug on these hormones. In view of the biosynthetic scheme for estradiol, a reduction in testosterone level is expected to bring about decrease in E_2 level owing to unavailability of the latter in sufficient amount.

Sex hormone-binding globulin (SHBG) has a high affinity for testosterone and estradiol (Merceir-Bodard, 1970) and it protect the circulating steroids from degradation. Testosterone binds with it more tightly than estradiol. Only about 1-3% testosterone is in free form. Burke and Anderson (1972), Chopra and Tulchinsky (1974) and Kley *et al.* (1975) have presented evidence which indicates that the level of SHBG is dependent upon a balance between testosterone (T) and estradiol (E_2). An increase in estradiol raises

SHBG while increase in testosterone reduces it (Vermeulen *et al.*, 1969). FSH is also a potent stimulator of androgen-binding protein (Fritz *et al.*, 1976). Increased SHBG has a feminizing influence and its decrease a masculinizing effect (Burke and Anderson, 1972). Wang *et al.* (1978) have attributed high SHBG to low $T:E_2$ ratio. Accordingly, a significantly low testosterone level combined with high SHBG and FSH indicates abnormal testicular function in the heroin addicts. High SHBG in the heroin addicts has been considered to indicate failure of liver to metabolize it, which in turn results in low free testosterone levels (Kley *et al.*, 1975; Baker *et al.*, 1976). The results of the present study clearly reveal a correlation between high SHBG and depressed testosterone level. Whether heroin addiction caused any liver damage cannot be specified since tests for this could not be carried out but the above observation does correlate with the noted abnormalities of sperm count, sperm motility and the impaired male accessory gland physiology (see below).

The abnormalities of the accessory glands and sperm count as well as their forward motility reported here constitute lasting effects of heroin addiction. All seminal parameters, sperm count, forward motility of the spermatozoa and seminal fructose level underwent drastic drop in the heroin addicts examined. None of these effects turned out to be reversible at least within the time limits of the duration of abstinence therapy applied. Such marked alterations in these parameters have been recorded for heroin addicts in past works as well. It has been suggested by some workers that the semen abnormalities are almost always preceded by irreversible damage to the seminiferous epithelium (Katz *et al.*, 1982). Both seminal and prostatic secretions and sperm motility are known to drop under heroin addiction (Cicero *et al.*, 1975; Katz *et al.*, 1982; Ragni *et al.*, 1985; Singer *et al.*, 1986). About 70% of the human ejaculate is composed of seminal vesicle secretion and the prostate also makes its contribution to the ejaculate (Barak *et al.*, 1994). Fructose, a major constituent of the ejaculate, is known to control sperm motility. The drastic decrease in its level as reported presently has a direct bearing on the impaired motility of the spermatozoa. The spermatozoa depend on fructolysis augmented by respiration for the maintenance of their motility. Chronic morphine and methadone administration also produces pronounced reduction in the size and secretory activity of the male rat's accessory organs (seminal vesicle and prostate) (Cicero *et al.*, 1975). Ragni

et al. (1988) have demonstrated asthenozoospermia (low sperm motility) in all heroin addicts and suggested that seminal pathology involving reduced sperm motility constitutes an early indication of heroin toxicity even though the sex hormone levels may be normal. It is noteworthy that in the subjects of the present investigation, the impaired accessory organ function are accompanied by lowered serum testosterone. Testosterone has been thought to be necessary for normal secretory activity of the seminal vesicle and the prostate (Ragni *et al.*, 1985; Gonzales *et al.*, 1994). Hypofunction of these glands causes pathological semen in the form of low sperm motility as well as reduced seminal fructose in the heroin addicts (Ragni *et al.*, 1985). Singer *et al.* (1979) have further shown earlier that sperm motility decreases with decreased sperm count but the volume of the ejaculate does not have any correlation with sperm count. Hence the last factor may not be a decisive one in oligospermia (low sperm count). In several studies it has further been shown that an impaired reproductive function is not only accompanied by lowered testosterone (Cicero *et al.*, 1977; Bolleli *et al.*, 1979; Mendelson and Mello, 1982; Ragni *et al.*, 1988; Ahmed and Ahmed, 1993; Pedrazzoni *et al.*, 1993; Gonzales *et al.*, 1994) but also with high prolactin release (El-Gothamy and El-Samahy, 1992) and low thyroxin (Chandrasekher *et al.*, 1985; El-Gothamy ans El-Samahy, 1992), an observation which receives ample support from the data of the present investigation. Also, the present study reveals far more severe impairment of the seminal parametrs (sperm count, sperm motility and semen fructose) in the group addicted for longer period (9-16 yr). The depth of damage was more pronounced in this group with no evidence of reversibility of the ensuing impairment.

Interestingly, in most studies, serum prolactin has been shown to increase significantly in the adult heroin addicts (Pelosi *et al.*, 1974; Tolis *et al.*, 1975; River *et al.*, 1977; Dupont *et al.*, 1977; Chan *et al.*, 1979; Cushman, 1980; Lafisca *et al.*, 1981; Spagnoli *et al.*, 1987a, b; Ragni *et al.*, 1985, 1988; Ahmed and Ahmed, 1993; Priou *et al.*, 1995; Walsh and Clark, 1996). Thus the results of the present study showing decreased testosterone, enhanced PRL and depressed thyroxin are in full accord with the proposal that these parameters are associated with impaired seminal functions. According to Tolis *et al.* (1978), the increase in PRL is apparently mediated by pharmacologic blockade of receptor sites for dopamine (Loh *et al.*, 1976), which is thought to be the hypothalamic

prolactin inhibiting factor. This further shows that heroin and other opioids exert primary influence on the hypothalamic-pituitary axis. Walsh and Clark (1996) and Peccin-Thompson et al. (1996) have argued that it is the activation of the mu-opioid receptors which causes increase in prolactin secretion. In addition, the arduous environmental factors and the social conditions sustained by the addicts may also contribute to hyperprolactenemia (Ragni *et al.*, 1984). According to Tolis *et al.* (1975) hyperprolactenemia in the heroin addicts, may be the cause of low gonadotropins. Data analyzed for the two groups of addicts with different lengths of addiction are interesting. The difference in the serum prolactin has been shown here to be severe in the chronic addicts with longer period of addiction (9-16 yr) compared to group with the addiction period of 2-8 yr.

The other physiological parameters indicating disturbance of the hypothalamo-pituitary axis which have been shown here to be affected by heroin addiction are levels of ACTH, cortisol and GH. The observed reduction in ACTH and cortisol levels in the addicts receives support from several other studies (Hellman *et al.*, 1975; Ho *et al.*, 1980; Morley *et al.*, 1980; Glass, 1982; Vescovi *et al.*, 1990; Mutti *et al.*, 1992; Schurmeyer, 1995; Priou *et al.*, 1995; Windh *et al.*, 1995). Schoffelmeer *et al.* (1997) have demonstrated that exposure to drugs of abuse increases striatal dopamine release along with enhancement in plasma corticosterone. The suggestion is that an impairment of the adaptive response to stress may occur in the heroin addicts (Vescovi *et al.*, 1989). The discovery of a common precursor protein for both beta lipotropin (βLPH) and ACTH (Eipper and Main, 1980) and of their common hypothalamic control through corticotropic releasing factor (CRF) (Krieger and Martin, 1981) has led to the hypothesis that in the heroin addicts there may either be a suppressed synthesis and/or altered enzymatic cleavage of anterior pituitary proopiocortin. Morphine treatment reduces the *in vitro* biosynthesis of proopicortin and endorphin-like peptides in the neurointermediate lobe of the pituitary in the rat (Gianoulakis *et al.*, 1981). Regulation by morphine of the normal hypothalamic-pituitary-adrenal mechanism combating stress through increased ACTH and cortisol levels has also been suggested by Calogero *et al.* (1996). According to El-Daly *et al.* (1996), there is increased release of the peptides derived from proopiomelanocortin (POMC) after acute morphine treatment for a short time and

decreased release of these peptides after chronic treatment. Thus the opioid peptides and their receptors play important physiologic role in controlling secretion of CRF. In contrast to the observation of drug-induced ACTH and cortisol suppression is the demonstration of either increased levels of ACTH and cortisol by a few workers (O'Donohue and Dorsa, 1982; Gil-Ad *et al.*, 1985) or no change in their levels (Cushman *et al.*, 1970; Mendelson *et al.*, 1975; Afrasiabi *et al.*, 1979). Such results are difficult to rationalize in the light of above proposed mechanism governing the effect of heroin and other opioids on the CRF-ACTH axis. It is noteworthy that in contrast to several observations that heroin addiction causes disturbance of the diurnal rhythm of serum cortisol and ACTH (Cushman *et al.*, 1970; Facchinetti *et al.*, 1984; Keim *et al.*, 1995), the present study revealed no such alteration in the cortisol rhythm in the addicts. Although the morning and evening cortisol levels underwent significant decrease, the diurnal rhythm was still maintained. According to Facchinetti *et al.* (1984), the altered diurnal rhythm is due to certain degree of impairment in the function of serotoninergic fibers that regulate circadian rhythmicity of CRF. A substantial decrease in cortisol in the evening alone has been demonstrated in the addicts by some workers (Ghodse and Reed, 1977; Renault *et al.*, 1971; Kreek *et al.*, 1983; Facchinetti *et al.*, 1984; Keim *et al.*, 1995) and opposite effect by others (Eisenman *et al.*, 1958). The present study further reveals that duration of addiction has dire influence on the pituitary adrenal axis. Longer period of addiction has a more deleterious effect. It is shown here that restoration of the levels of two hormones (ACTH and cortisol) is blocked in the chronic heroin addicts with the longer period of addiction when compared to the addicts with the shorter addiction period. Apparently, the ability to meet stress of addiction is altered substantially.

The information regarding the effect of heroin addiction on serum GH is also fairly conflicting, largely owing to varying conditions under which observations have been made by various workers. In the present work, serum GH levels have been shown to increase significantly in the addicts. High and non-suppressing GH levels are known to occur in the addicts after glucose tolerance test (Ghodse and Reed, 1984), in cold-stimulated humans and rats (Idanpaan-Heikkila, 1996b) treated with morphine or even in situations where the only treatment has been intracerebroventricular injection of morphine sulphate (Hashiguchi *et al.*, 1996a,b). Opioid agonists are also known to be

potent stimulators of GH release in both rat and man (Cushman, 1972; Grossman and Rees, 1983; Priou *et al.*, 1995). The opiates stimulate GH secretion, probably, either by inhibiting dopaminergic tone and hence by reducing somatostatin release (Matcovish, 1981; Racagni *et al.*, 1982; Tuomisto and Mannisto, 1985) or by modulating adrenergic mechanism (Idanpaan-Heikkila *et al.*, 1996a). According to Dickson and Vaccarino (1994), the opiates affect growth hormone-releasing factor (GRF) in the hypothalamus which in turn induces the release of GH from the pituitary. Again mu and kappa receptors but not delta receptors are thought to be involved in regulating GH secretion (Eason *et al.*, 1996). Volpi *et al.* (1992) have observed that the control of GH secretion mediated by GABAergic beta receptors is impaired in the heroin addicts and have furthermore suggested that the neuroendocrine alterations may represent a trait marker of heroin addiction or that it is a consequence of long addiction to heroin. In contradistinction to the above works cited and the results of the present investigation, some studies have failed to record any change in serum GH level in the heroin addicts (George *et al.*, 1974; Brambila *et al.*, 1980; Delitala *et al.*, 1983; Volpi *et al.*, 1992) which merely indicates further the inconsistencies in the available informations.

While the responses of FSH, LH, PRL, GH and ACTH in the addicts suggest marked impairment of the hypothalamo-pituitary mechanism by heroin, TSH and T_3 remained unaffected in the addicts in the present study. Intrestingly FT_4 decreased significantly. Depressed FT_4 in the presence of normal TSH indicates impaired negative feedback mechanism along the pituitary-thyroid axis in the addicts. Low FT_4 might also be a cause of decreased appetite in the heroin addicts. The current status regarding the hypothalamic-pituitary-thyroid axis, under the influence of heroin and related opiates, seems to be quite uncertain. Some studies have provides ample evidence of increase in both T_4 and T_3 in the addicts and of borderline hyperthyroidism under acute heroin addiction (Azizi *et al.*, 1974; Ho *et al.*, 1977; Webster *et al.*, 1977; Glass, 1982). It is thought, in this context, that decreased peripheral turnover of T_4 and associated increase in the binding proteins for this hormone are responsible for this hyperthyroidism. Other workers have observed that the opiates act as depressors of thyroid function, probably due to inhibition of the hypothalamic-pituitary axis causing low TSH (George and Lomax, 1965; Redding *et al.*, 1966; Brakke *et al.*, 1974). There is one report available

which indicates lack of any effect of heroin on T_4, T_3 and TSH (Brambilla *et al.*, 1980). Detoxification in the present subjects studied was accompanied by several signs of withdrawal syndrome i.e irritability, tremors, sweating and elevation in blood pressure. These symptoms are also characteristic of hyperthyroidism suggesting sudden elevation in depressed thyroxin in the addicts. According to Gahn and Sevarino (1996) preprothyrotropin-releasing hormone (ppTRH) is highly expressed in the central gray in rats during withdrawal.

Both insulin and glucose increased drastically in the addicts studied here. It has been shown by Ipp *et al.* (1978, 1980), and Giugliano *et al.* (1987) that the opiates enhance insulin and glucagon release by suppressing endogenous somatostatin. High fasting levels of insulin with a delayed rise following glucose tolerance test have been obtained by Ghodse and Reed (1984). This resistance is thought to be due to high and non-suppressing growth hormone levels, a situation similar to acromegaly before the onset of pancreatic insufficiency. Elevated growth hormone and glucagon result in increased serum glucose (Reid and Yen, 1981; Passariello *et al.*, 1983, 1986). These workers suggested that negative interference exerted by heroin upon carbohydrate metabolism, either directly or through mediation by increased glucagon and GH levels, may condition the state of fasting hyperinsulinemia, which in turn tends to maintain fasting hyperglycemia. Hashiguchi *et al.* (1995) have shown that intracerebroventricular (ICV) injection of morphine-6-glucoronide (M6G) and morphine produces progressive hyperglycemia accompanied by high catecholamine and corticosterone levels in rats.

The present observation of a clear rise in gastrin in the addicts in spite of reduced appetite is in conflict with the work of the past workers. Jaffe and Martin (1975) has described that the opiates are inhibitors of gastric secretion, while Magee (1975) have reported increased gastric acid secretion with gastrin remaining normal. Thus in the light of the above study, the opiates seems to act directly on the oxyntic glands without the mediation of gastrin release. The present investigation agrees with Unvas (1969) and Yamaguchi (1974) who reported gastrin release from antrum under the influence of morphine. Yamaguchi (1974) observed that morphine increases gastrin release. Direct vagal excitation of oxyntic glands and endogenous gastrin play an important role in food-induced gastric secretions in innervated and denervated fundic pouches respectively.

Exogenous gastrin stimulates gastric secretions activating cholinergic post-ganglionic nerve fibers in conscious dogs. The author concluded that not only the central but also peripheral cholinergic stimulation of endogenous gastrin-release is responsible for the secretory enhancement by morphine in dogs and morphine-induced secretion cannot be attributed to histamine levels in blood as suggested by Thomson and Walton (1964). Studies using isolated perfused stomach have shown that cholinergic stimulation is mediated by the inhibition of antral somatostatin, which exerts direct inhibitory effects on G cells (Saffouri *et al.*, 1980).

Since very little work has been done on other hormones in the addicts, it is difficult to fully compare the observed decrease in PTH in the heroin addicts. The only available study carried out in the past is in agreement with the results presented here (Pedrazzoni *et al.*, 1993).

As mentioned earlier, the analysis of Na,K-ATPase was undertaken in view of some evidence that endogenous and exogenous opiates affect transmitter release through action on this enzyme system (Skou, 1965; Schwartz *et al.*, 1975; Sjolund *et al.*, 1977; North and Egan, 1983) as there is intimate relationship between neurotransmitter release and Na,K-ATPase activity (Visi, 1978). Examination of the enzyme activity in the erythrocytes and leucocytes revealed its activation only in the leucocytes. No effect occurred in the erythrocytes of the heroin addicts. The intracellular concentration of sodium and potassium was indistinguishable statistically, both in erythrocytes and leucocytes. Unchanged sodium and potassium values in leucocytes of the heroin addicts, in the presence of increased Na,K-ATPase activity, might be due to the enhanced Na:Na exchange, or increase in the rest of the passive sodium influx. The Na,K-ATPase system has been studied in the past under a variety of conditions (*in vitro, in vivo*) and in different tissues to test various opioids. Thus the result tends to vary from study to study. When morphine is applied *in vivo*, it stimulate the enzyme activity but has no effect under *in vitro* conditions (Jain *et al.*, 1974; Desaiah *et al.*, 1977). Lack of effect of morphine on Na,K-ATPase in the mouse brain has been shown by Desaiah *et al.* (1979). Similar results have been obtained by Maeda *et al.* (1988) who tested morphine and met-enkephalin on rat cardiac Na,K-ATPase. A stimulatory influence of morphine and met-enkephalin on the enzyme has been noted in the frog spinal cord (Hajeck *et al.*, 1985)

Lee and Sun (1984) observed that only a slight inhibition of the enzyme activity occurs in the synaptic plasma membrane in response to beta-endorphin but no effect occurs with met-enkephalin. Naloxone, an opiate antagonist, inhibits the activity of the sarcolemal Na,K-ATPase (Levin *et al.*, 1993). One of the reasons for discrepancies among the results of the present and the above studies may be the heterogeneity of Na,K-ATPase isozymes in different tissues (Fambrough *et al.*, 1983).

The present investigation shows that the withdrawal therapy partly or fully corrects the damage caused by chronic heroin addiction to most physiological parameters. However, as further demonstrated here, the duration of chronic addiction may be an important factor in determining the depth of effects and how soon the addicts may recover following withdrawal therapy. It appears that permanent damage occurred in respect to sperm count, sperm motility and seminal fructose which was severe in the addicts of 9-16 yr duration. The impairment turned out to be irreversible for as long as 30 days post treatment. The fact that sperm count was permanently depressed in the addicts affirms Katz's suggestion (1982) that spermatogenesis is irreversibly affected. It seems that chronic heroin addiction has profound effect on the endocrine mechanisms regulating reproductive functions since withdrawal therapy was followed by a delayed recovery of FSH, LH, T and SHBG. All other system which were examined and which experienced impairment in the addicts reverted to normal (control level) within 10 days. Reversibility of heroin and opiate-induced damage to various endocrine and other physiological systems has been a general observation in most studies where no particular assessment of effects of duration has been focussed upon (George *et al.*, 1974; Hellman *et al.*, 1975; Fachinetti *et al.*, 1984; Ghodse and Reed, 1984; Mendelson *et al.*, 1984; Giugliano *et al.*, 1987, Vescovi *et al.*, 1989; Mutti *et al.*, 1992; Pedrazzoni *et al.*, 1993; Gerra *et al.*, 1994; Keim *et al.*, 1995; Windh *et al.*, 1995;).

Post-detoxification study revealed that full reversibility occurs in respect to most of the endocrine functions governed by hypothalamic-hypophyeseal axis, even though it is delayed. The partial recovery of the seminal parameters from the effect of heroin, 30 days after discontinuation of the drug indicates a slow restoration of normalcy. Slightly reduced spermatogenesis and dysfunction occur in the secretory activity of accessory glands caused by deleterious effects of heroin, which might have required more time for

full recovery. So a longer period of post detoxification observation is required to see whether full recovery of these parameters occurs or not. In our study further investigations for testosterone and semen analysis beyond 30 days was abandoned because of poor patient co-operation as they refused to be detained at rehabilitation centers for reasons of their own. The fact that the damage done to most systems by heroin is reversible especially if addiction is not overly prolonged, this augers well for the addicts and provides sufficient incentive for timely remedial measures and rehabilitation steps.

Analysis of the data by separating into short duration and long duration chronic addiction revealed that PRL, ACTH, cortisol and all semen parameters undergo far greater impairment due to heroin use. The effect on PRL was very severe in the longer duration addicts (9-16 yr) where it was drastically elevated compared to group with the addiction period 2-8 yr. However, recovery occurred as early as 10^{th} day of the drug withdrawal in both groups. The present study further reveals that duration of addiction has dire influence on the pituitary-adrenal axis. Longer period of addiction has a more deleterious effect. It is shown here that restoration of the levels of two hormones (ACTH and cortisol) is blocked in the chronic addicts with the longer period of addiction when compared to the addicts with the shorter addiction period. Regarding seminal parameters (sperm counts, sperm motility and semen fructose) severe impairment was observed in the group addicted for longer period (9-16 yr). The depth of damage was more pronounced in this group with no evidence of reversibility of the ensuing impairment.

In conclusion the overall results obtained in the present study show that heroin acts on the hypothalamo-hypophyseal system by altering FSH, LH, ACTH, and GH levels. The concentration of testosterone and cortisol in the serum declines. The diurnal rhythm of cortisol secretion is preserved in the addicts in the present study. Heroin addiction increases the serum concentration of prolactin (far more severely in the long duration addicts), GH, gastrin and insulin along with fasting glucose level. The depressed thyroid activity (low FT_4) and elevated prolactin level in addicts may also have been a contributing factor in inhibiting catabolism of fructose and causing dysfunction of the testis respectively. As a consequence, both spermatogenesis and potency are impaired with reduced sperm count and markedly affected sperm motility may lead to subfertility.

The most striking finding of the present study is that the intensity of damaging effects of heroin can vary depending on the duration of addiction as is evident from comparison of group I and group II addicts. The hypothalamic-gonadal and hypothalamic-adrenal axes along with the seminal parameters are the ones most adversely influenced by heroin addiction in general. Furthermore, the duration of addiction appears to have selective influence on serum prolactin, ACTH and cortisol levels. It seems that the ability to meet the stress of addiction is far more deeply altered with increasing length of addiction to the drug. The study also indicates that rehabilitation of the addicts is possible if timely action is taken. With increasing time of addiction it may take longer to rehabilitate the addict.

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