FOR

MY DEAREST AMMI AND ABBA JEE

FOR THEIR IMMENSE STRUGGLE AND

SACRIFICES IN EDUCATING ME.

THE EFFECT OF ANABOLIC - ANDROGENIC STEROIDS ON THE GROWTH & METABOLISM OF CARP

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ERRATA

Due to a computational error the concentrations of the proteins, RNA/DNA and protein/DNA were calculated wrongly. For correct values please divide protein and RNA/DNA by 2 and protein/DAN by 4.

PROTEIN	RNA/DNA	PROTEIN/DNA
TABLE NO.	TABLE NO.	TABLE NO.
52-54	52-55	52-55
62-64	62-65	62-65
72-74	72-75	72-75
93-95	83-84	83-84
103-105	93-96	93-96
113	103-106	103-106
	112-115	112-115

SEE PAGE 157 ALSO

SUMMARY

The Effect of Anabolic-Androgenic Steroids on the Growth and Metabolism of Carp

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Groups of juvenile common carp *(Cyprinus carpio)* were fed diets supplemented with eight anabolic-androgenic steroids and one anti-androgenic steroid at doses of 1.0, 2.5, 5.0 and 10 mg/kg dry diet for sixty or ninety days, and their effect on growth, food conversion and body composition observed. Seven of these nine steroids when given at lower doses, significantly increased the growth rate and food conversion efficiency. The effect of these drugs on hepato-, reno-, cranio and viscero-somatic index was variable. In all the drugs the concentration of hormones which induced growth also brought concurrent changes in RNA, DNA and proteins of liver, kidney, brain and muscle. The uptake and disappearance of the ^{3}H-testosterone was also studied. Twenty days after the withdrawal of the labelled steroid, the concentration of radioactivity in the carp muscle was 2.79 ng/g of the fresh weight. It is concluded that anabolic-androgenic steroids can manifest pronounced anabolic activity and have great potential as growth promotants in aquaculture.

KEY WORDS : Carp - Growth - Anabolic - Androgenic Steroids - Nucleic Acids - Steroid Residues.

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CONTENTS

CHAPTER ONE

INTRODUCTION

Growth rate and efficiency of food utilisation are two of the most economically important characters in the production of meat animals. In these days of world shortage of both plant and animal protein, and with energy crises looming on the horizon, the necessity both to reduce maintenance costs by speeding up growth rate and by improving the efficiency of conversion of feed to animal protein has become more important and urgent than ever.

There has been considerable work on the nutritional requirements of fish for maximising growth responses (Cowey and Sargent, 1972, 1977, 1979; Cowey, 1975; Halver, 1972). However very little attention has been given to the modulation and/or regulation of growth by means of hormones (Weatherley, 1976), although recently the role of hormones in the biology and management of fish has been stressed (Schreck and Scanlon, 1977).

In mammals growth hormone, thyroxine and androgens have been implicated in the normal growth of the individual. In fish the role of growth hormone and thyroxine has also been fairly well established (Donaldson et al., 1979), but our knowledge about the role of sex steroids in fish growth is still elementary.

Studies on the effect of sex steroids in fish have been mainly restricted to sex reversal, inducement of sterility and the development of mono-sex culture (Schreck, 1974; Guerrero, 1975; Nakamura, 1975; Takahashi, 1974, 1975a, 1975b, 1975c, 1975d; Katz, et al., 1976; Simpson, 1976; Johnstone, et al., 1978, 1979; Shelton, et al., 1978; Tayamen and Shelton, 1978; Okada, et al., 1979).

Following the example of the livestock industry where production efficiencies have been made using sex steroids, estrogens and their derivatives were investigated for their effects on growth in fish. The results of these studies were confusing and contradictory (Ghittino, 197O; Bulkley, 1972; Cowey et al., 1973; Matty and Cheema, 1978; Yu et al., 1979).

During the last decade, anabolic-androgenic steroids have been used in growth studies. Hirose and Hibiya (1968a, 1968b) reported positive effects of intramuscular injections of 4-chlorotestosterone on the growth of goldfish and rainbow trout, where as stanozolol, when given to immature goldfish and channel catfish did not induce any significant growth (Bulkley and Swihart, 1973). 17α-methyltestosterone, a synthetic derivative of testosterone, which is active orally, has been studied in detail in different salmonids (McBride and Fagerlund, 1973, 1976; Fagerlund and McBride, 1975b, 1977; Simpson, 1976; Yamazaki, 1976; Higgs, et al., 1977; Saunders, et al., 1977; Yu, et al., 1979). Matty and Cheema (1978)

have reported the anabolic actions of dimethazine and norethandrolone in rainbow trout. Recently Donaldson, et al., (1979) have summarised the studies on the hormonal control of growth in fish.

Growth is largely protein accretion, and RNA and DNA levels may be used as a measure of growth rate (Bulow, 1970, 1971; Haines, 1973). However, RNA/DNA and protein/DNA ratios are considered a more accurate index of growth and cell size than the individual values of these cellular components (Winick and Noble, 1965; Winick, et al., 1972).

One very important aspect of the control of fish growth by androgens is the effect of withdrawal of the hormone before the fish is marketed for human consumption. This is an obligatory factor in aquacultural practice, because for the removal of hormone a suitable protocol has to be followed which insures that all the residues of the drug have been removed from the fish, when it leaves the farm. To date, only Fagerlund and McBride (1978) have reported the uptake and disappearance of ^{3}H-testosterone after oral administration in the blood and tissues of coho salmon. It was shown that after ten days of withdrawal of the labelled hormone, the concentration of radioactivity left in the body tissues was less than 1 ng/g.

No studies of sex steroids or anabolic-androgenic steroids on growth have been made in carp, nor has the effect on

growth and biochemistry been studied subsequent to steroid withdrawal. Therefore, the present studies were carried out to assess further the role of anabolic-androgenic steroids on the growth and major tissue biochemistry of the carp, *Cyprinus carpio*.

CHAPTER TWO

LITERATURE SURVEY

2.1. Structure of Tissues which Elaborate Androgens

The basic functions of the fish testis are two fold: the production of spermatozoa and secretion and release of steroid hormones. Testicular structure in teleosts is more variable than any other group of vertebrates. In most teleosts the testes are paired elongated structures attached to the dorsal body wall. The detailed account of the structure of testis and the process of spermatogenesis can be found in the excellent reviews by Dodd (1955, 196Oa, 1972, 1975), Dodd and Wiebe (1968), Lofts (1968), Lofts and Bern (1972), Hoar (1969) and de Vlaming (1974).

2.2. Cellular Site of Androgen Secretion

The site of androgen synthesis within the fish testis is debateable. Recent cytological, histochemical and ultrastructural studies on the testes of several species have shown the presence of steroidogenic tissue which demonstrates the cytological and histological features of well established steroid-secreting cells of mammalian testis and ovary. Detailed account of the fine structure of the cellular organelles related to steroidogenesis can be found in Christensen (1975), Christensen and Gillim (1969), Fawcett (1975), and Fawcett et al. (1969). Much of the earlier literature on fish have been summarised by Chieffi (1966), Lofts (1968), Lofts and Bern (1972), and Guraya (1976). A brief summary of the vast literature follows here.

Two distinct types of arrangement of sex steroid producing cells in the teleost testis have been described (Marshall and Lofts, 1956; Lofts, 1968; Lofts and Bern, 1972). This tissue is composed of interstitial Leydig cells, lobule boundary cells, or both. However, in some cases Sertoli cells (sustentacular cells) and semen have also been implicated in steroidogenesis. As most fishes are seasonal breeders, this steroidogenic tissue of the testis has also been shown to undergo conspicuous changes according to the cycle.

2.2.1. Interstitial Leydig Cells

Interstitial Leydig cells similar to those of amniotic vertebrates testes are distributed singly or in small groups in the interstices between the lobules of several elasmobranchs and teleosts (Chieffi, 1967; Bara, 1969; Hyder, 1970; Gresik et al., 1973; Hoar and Nagahama, 1978; Leatherland and Sonstegaard, 1978). Using the 3β-hydroxysteroid dehydrogenase (3β- HSDH) technique it has been suggested that in several species of teleosts the interstitial cells are the site of androgen production (Della Corte et al., 1961; Lupo and Chieffi, 1963; Stanley et al., 1965; Bara, 1966, 1969; Delrio et al., 1967). There is a direct correlation between histological signs of activity in interstitial tissue and androgens extractable from the testes of *Gasterosteus aculeatus* (Gottfried and Van Mullem, 1967). Ultrastructural examination of the interstitial cells of some teleosts have shown them to have the same characteristic

features of abundant agranular or smooth endoplasmic reticulum and tubular mitochondrial cristae which typify steroid producing cells (*Gasterosteus aculeatus* and *Poecilia reticulata*, Follenius and Porte, 1960; Follenius, 1964, 1968; rainbow trout, Oota and Yamamoto, 1966; *Trichogaster leeri*, Horstmann and Breucker, 1972; *Cyprinus carpio* and *Carassius auratus*, Leatherland and Sonstegaard, 1978; *Oncorhynchus kisutch* and *Oncorhynchus gorbuscha*, Hoar and Nagahama, 1978). Ultrastructural evidence suggests that the interstitial cells of *Oryzias latipes* are steroidogenic, but there is an absence of 3β-HSDH activity in these cells (Gresik et al., 1973). This later study indicates that caution must be taken in interpretation of histochemical results.

2.2.2. Lobule Boundary Cells and Sertoli Cells

In the second type of arrangement, the Leydig cells arise not in the interstices, but in the lobule walls and have been named lobule boundary cells. They were first designated as lobule boundary cells in testes of *Esox lucius* (Marshall and Lofts, 1956; Lofts and Marshall, 1957). Later on, the presence of these cells was reported in the testes of a variety of teleosts inhabiting both fresh and seawater (*Salvelinus fontinelis*, Henderson, 1962; lake chub, Ahsan, 1966; *Tilapia mossambica*, Yaron, 1966; *Salmo salar*, O'Halloran and Idler, 1970; *Cichlosoma nigrofasciatum*, Nicholls and Graham, 1972; *Belone belone* Upadhyay and Guraya, 1971). The typical interstitial distribution of endocrine cells is

generally absent from fishes that develop lobule boundary cells, however, some fish appear to have both of them (Yaron, 1966; Nicholls and Graham, 1972).

Sertoli cells are prominent feature of the testis in some teleosts (Lofts, 1968, 1972; Hoar, 1969). Functionally, the Sertoli cell may be nutritive, contractile, supportive and/or steroid producing endocrine cell (Lofts, 1968, 1972; Lofts and Bern, 1972). The Sertoli cells take on a glandular appearance as the testicular germinal content matures in *Fundulus heteroclitus* (Lofts et al., 1966; Bara, 1969). Sertoli cells also give strong 3β-HSDH reaction in *Cymatogaster aggregata* (Wiebe, 1969). Ultrastructural evidence also suggests that the Sertoli cells in *Poecilia reticulata* may be steroidogenic (Billard, 1970). Stanley et al. (1965) suggested that lobule boundary cells in *Gobius paganellus* may actually be Sertoli cell homologues. Some other workers also related the Sertoli cells with lobule boundary cells (Nicholls and Graham, 1972; Billard et al, 1972; Van den Hurk et al, 1974, 1978; Grier, 1976). Recently Hoar and Nagahama (1978) have shown that lobule boundary cells lack ultrastructural characteristics of Leydig cells and are clearly homologous with Sertoli cells.

2.2.3. Spermatozoa

Ozon and Collenot (1965) showed the presence of 3β- and 17β-HSDH activity in the spermatozoa of *Squalus acanthias*. Testosterone and 3β, 17α-dihydroxy-5-pregnen-20-one are

believed to be present in the sperm of *Scyliorhinus stellaris* (Gottfried and Chieffi, 1967). Considerable concentration of steroid hormones were reported by Simpson et al. (1963a, 1964) in the semen of *Squalus*. Apart from these steroids some enzymes which catalyze the synthesis of corticoids from cholesterol were also reported. These results seem to be the special cases, as no steroid was detected in the semen of four other elasmobranchs (Simpson et al., 1963b).

The results of the studies reported above indicate that interstitial Leydig cells, lobule boundary cells and/or Sertoli cells are the sites of steroidogenesis in the testes of fish. But most important sites are interstitial Leydig cells or lobule boundary cells or both, and these cells share many characteristic features of the typical steroid secreting cells of the mammals (Christensen, 1975; Fawcett, 1975; Connell and Connell, 1977).

2.3. Occurrence of Androgenic Steroids in Fish

In fish it has been established by experiments involving injections of testicular extracts and castration followed by replacement therapy that the testes are a source of androgenic sex hormones upon which the state of development and functional activity of the secondary sexual characters and sexual behaviour depend (Dodd, 1955, 1960a, 1972, 1975; Hoar, 1955, 1957, 1965, 1969; Pickford and Atz, 1957; Nandi, 1967; Baggerman, 1968; Lofts, 1968;

Lily, 1969, 1972; Chester Jones et al., 1972, 1974; de Vlaming, 1974).

The recent important advances in our knowledge of occurrence, biosynthesis and metabolism of steroid hormones in vertebrates can be attributed, to a large extent, to the development of new analytical techniques. Sandor and Idler (1972) has given a detailed account of different methods involved in extraction, estimation and biosynthesis of different steroid hormones in non-mammalian species.

During the last twenty years, steroid hormones have been isolated from different non-mammalian vertebrates (Gottfried, 1964; Ozon, 1966, 1969, 1972a, b; Nandi, 1967; Chieffi, 1966, 1972). However, main emphasis in this regard have been on fish and it is in this group that conclusive identification of C_{19} (Androgenic hormones) compounds have been achieved.

2.3.1. Biochemical Isolation and Identification of Androgenic Hormones

2.3.1.1. Elasmobranchs

The first attempt to isolate androgens from the testes of elasmobranch fish was by Chieffi and Lupo (1961), who isolated and identified testosterone, androstenedione and progesterone from the neutral fractions of the testicular extracts of *Scyliorhinus stellaris*. An analysis of 110 g

of testes yielded 25mg of neutral and 81mg of phenolic fractions. When the neutral fraction was analysed by paper chromatography, five spots were identified, three of which corresponded to testosterone, androstenedione and progesterone on the basis of the constant Rf values and absorption maxima at 240mμ. The spots corresponding to androstenedione and progesterone showed the characteristic blue colour with Zimmerman reagent. The spot corresponding to testosterone was oxidised to androstenedione with chromic oxide and showed the same Rf as the authentic androstenedione. According to semiquantitative estimation these steroids were present as 50μg/kg for testosterone, 70μg/kg for androstenedione, and 100μg/kg for progesterone. These authors also reported estradiol-17β in the phenolic fraction amounting to about 20μg/kg of the tissue. This was the first time that progesterone was reported in the testes of any vertebrate.

Investigations on the semen of the elasmobranch *Squalus acanthias* resulted in complete identification of 11-deoxycorticosterone (500μg/100 g), progesterone (8μg/100g), androstenedione (2μg/100g), dehydroepiadnrosterone (2μg/100g), pregnenolone (14μg/100g), androsterone (5μg/100g) and possibly aldosterone (<1μg/100g) (Simpson et al., 1963a). This situation is considered to be a special one as no steroid have been found in the sperm of four other species of elasmobranchs, *Lamna cornubica*, *Scyliorhinus caniculus*, *Galeus Vulgaris* and *Raja batis* (Simpson et al., 1963b). A short communication by Gottfried and Chieffi (1967)

reported the presence of testosterone (6µg/100g) and 3β, 17α-dihydroxy-5-pregnen-20-one (100µg/100g) in the sperm of *Scyliorhinus stellaris*.

Idler and Truscott (1966) identified testosterone in the plasma of two elasmobranchs, *Raja radiate* and *Raja ocellata*. The identification of the testosterone was based on extensive tests comprising paper, thin layer and gas chromatography, formation of derivatives (oxidation and acetylation), double isotope analysis and finally aromatization of testosterone to estradiol-17β. The hormone was found both in free and conjugated forms. The conjugated steroid was in the form of glucuronide. When testosterone was liberated after hydrolysis with β-glucuronidase, it was assumed to be in the form of sulphate. In *Raja radiata* free testosterone was present in concentrations of 2.8 - 10.2 (mean, 7.4) µg/100 ml and 2.2 - 20.8 (mean, 10.0) µg/100 ml of plasma in *R. ocellata*. Females also contained free testosterone (0.47 in *R. radiate* and 0.59 µg/100 ml of plasma in *R. ocellata*). Other species of *Raja* have not been examined extensively for testosterone in plasma but analysis of individual samples of blood from mature male fish gave testosterone levels as follows. *Raja laevis* (14.0 µg/100 ml) *R. erinacea* (0.6 µg/100 ml) and *R. clavata* (18.0 µg/100 ml). Six immature males of *R. clavata* averaged 5.0 µg/100 ml of plasma (Idler et al., 1968).

Concentration of dehydroepiandrosterone and androsterone

in plasma of *Torpedo marmorata* have been shown to vary with the sexual cycle (Buonanno et al., 1964). By thin layer and gas chromatography, Lupo di Prisco et al.,(1967) have demonstrated the presence of testosterone (free or conjugated) in plasma of female *Torpedo marmorata*. The steroid was present in mature (3.5µg/100 ml) animals in the period preceding gestation (1.56µg/100 ml) during gestation (0.80µg/100 ml) and post gestation period (2.36 µg/100 ml).

Fletcher et al.,(1969) studied the production and metabolic clearance rate of testosterone in sexually mature male and female *R. radiata*. These authors observed that the difference in the concentration of the steroid in the plasma was not due to the difference in production rate (0.38±0.07 µg/kg/hr for male and 0.33±0.05µg/kg/hr for female), it was in fact, due to the metabolic clearance rate, which was higher (10.4±0.57 ml/kg/hr) in females than in males (7.93±0.57 ml/kg/hr). A circadian rhythm in the concentration of testosterone was also noted in both male and female.

Darrow and Fletcher (1972) quantified the testosterone and testosterone glucuronide in testicular and peripheral plasma of mature *Raja radiata*. It was shown that free testosterone was greater in the testicular than in the peripheral plasma. In three of the five fish examined the testosterone glucuronide was higher in the testicular

effluent plasma than the peripheral plasma. The authors suggested that probably testosterone glucuronide was a normal secretory product of the testis.

2.3.1.2. Teleosts

Substances of the chromatographic mobility similar to that of testosterone have been extracted from the testes of *salmo irideus* and *Cyprinus carpio* (Galzigna, 1961). Grajacer and Idler (1963) revealed the presence of testosterone in the testes of *Oncorhynchus nerka*, which was conjugated with glucuronic acid. Enzymatic hydrolysis yielded a compound which was identified to be testosterone on the basis of rigorous chemical analysis. Hadd and Rhamy (1965) showed that linkage of testosterone with glucuronic acid is probably through the 17β- position. Traces of testosterone and large quantities of estrogens were recovered from the testis of *Morone labrex* (Lupo and Chieffi, 1963). Later on, Lupo di Prisco and Chieffi (1965) extracted testosterone, androstenedione and androsterone from the gonads of hermaphrodite fish, *Serranus scriba*. These steroids were also extracted and measured from the testis of *Gasterosteus aculeatus* using gas chromatography (Van Mullem and Gottfried 1966; Gottfried and Van Mullem, 1967).

Apart from the testes, testosterone has also been extracted and quantified from the peripheral plasma of different teleosts. Grajacer and Idler (1961, 1963) isolated testosterone from both male and female plasma of

Oncorhynchus nerka. In both sexes, testosterone existed in free and conjugated with glucuronic acid. The concentration of free testosterone after spawning in male and female was 1.7 and 7.8µg/100 ml of plasma. While conjugated steroid in male and female was 13.7 and 7.6µg/100 ml. Although the total concentration of testosterone was equal (about 15µg/100 ml of plasma), the conjugated fraction was higher in male than in female (Schmidt and Idler, 1962).

Androgens in teleosts may differ from those of other vertebrates, in that the testosterone may have a hydroxyl or keto (OXO) group at C-11 position in the steroid nucleus. Idler et al.,(1960, 1961a, 1961b, 1964) and Schmidt and Idler (1962), were the first to identify 11-ketotestosterone in the peripheral blood of *Oncorhynchus nerka* of both sexes. Atlantic salmon (*galmo salar*) also contain 11-ketotestosterone in its peripheral blood (Idler et al., 1964). During the spawning migration of sockeye salmon the concentrations of testosterone and 11-ketotestosterone changed interestingly in both male and female fish. Before migration the female contained less than 2.5µg/100 ml of 11-ketotestosterone but after the migration had begun 11-ketotestosterone rose to 7.1µg/100 ml and testosterone decreased to 3.2µg/100 ml. In males the situation was reversed. Testosterone levels rose and 11-ketotestosterone fell during migration. In male fish testosterone levels increased from 4.3 to 11µg/100 ml of

plasma and 11-ketotestosterone decreased from 7.9 to 4.8µg/100 ml. Apart from 11-ketotestosterone, adrenosterone has also been reported to be a plasmatic steroid in salmon (Idler et al., 1961c).

11-ketotestosterone is potent androgen both in chick, sockeye salmon and in *Oryzias latipes* (Arai, 1967; Idler et al, 1968) and is ten times more active than testosterone in promoting secondary male sex characteristics in a female teleost (Arai and Tamaoki, 1967a); 17α-hydroxyprogesterone and testosterone are both precursors to 11-ketotestosterone *in vivo* in pacific salmon (Idler and Truscott, 1963) and the data suggested that 17α-hydroxyprogesterone had a route to 11-ketotesterone in addition to one including testosterone (Idler et al, 1968). The details of pathways for synthesis of both testosterone and 11-ketotesterone in teleosts will be given in Section 2.5.

2.4. Levels of Androgen in Fish

Using a competitive protein binding assay, Schreck et al., (1972) described a relationship between the plasma androgen concentrations to gonadal development. Male and female of near maturity had 12.9±2.6 and 8.9±2.8 µg/ml of testosterone, while mature male of the same stock had testosterone levels twice as much (27.4±5.4µg/ml) as the less mature males. Orchidectomy reduced the plasma testosterone to 6.8µg/ml in twenty-one days.

Katz and Eckstein (1974) studied the steroid concentration in the blood of female *Tilapia aurea* during initiation of spawning. No estradiol-17β was detected, instead testosterone, 11-ketotestosterone, 11β-hydroxytestosterone and deoxycorticosterone were present. After the initiation of spawning there was 8.0, 2.5 and 38.0 fold increase in the concentration of testosterone, 11-ketotestosterone and deoxycorticosterone (DOC). 11β-hydroxytestosterone was only present after initiation of the spawning. The final concentration for these steroids were as follows: Testosterone, 4.83; 11-ketotestosterone, 17.05; 11β-hydroxytestosterone, 7.02; and DOC, 22.24 µg/ml of the blood.

In plaice (*Pleuronectes platessa L.*), Wingfield and Grimm (1976, 1977) studied the presence and levels of different steroids in the plasma of mature female, male and immature fish. It was shown that the plasma of mature female contain cortisol, testosterone, progesterone, estradiol-17β and estrone based on paper chromatography, colour reaction and by the formation of sequential derivatives. Later on, the concentration of these steroids was measured by competitive protein binding (CPB) and radio-immunoassay (RIA). The concentration of testosterone in female was highest (6.2±0.4µg/100ml) in February coinciding with the spawning while in males the concentration (12.2±1.5µg/100ml) was highest in December. Lowest values of testosterone in female and male were encountered in March (0.29±0.13µg/100ml)

and June (0.10±0.02µg/100 ml) respectively. In immature plaice, no seasonal change was seen in males or females and the concentration of testosterone was 1.0µg/100 ml throughout the year.

Campbell et al.,(1976) measured (by double isotope derivative assay (DIDA) the steroids in the plasma of winter flounder (*Pseudopleuronectes americanus* Walbaum) and the effect of season on the concentration of steroids. Among the eight steroids identified both in male and female, testosterone and 11-ketotestosterone were present in both sexes. There was a slight change in the concentration of testosterone in male fish while 11-ketotestosterone concentrations rose dramatically near the spawning. The value of pooled samples from prespawning fish was 17.9µg/100ml for 11-ketotestosterone. Testosterone levels were highest prior to spawning in plasma from female fish but concentrations of 11-ketotestosterone remained extremely low throughout the year.

In a study on the androgens in the blood of ambisexual and gonochoristic teleosts Idler et al.,(1976) measured (by DIDA) the levels of 11-ketotestosterone and 11β-hydroxytestosterone in *Serranus cabrilla* (a simultaneous hermaphrodite), *Pagellus acarne* (protandric), *Pagellus erythrinus* (protogynous?) *Diplodus sargus* (Protandric?), *Pseudopleuronectes americanus* (male) and *Salmo salar* (male). The sexual status of these fish was determined according to Reinboth (1962, 1970). All

the ambisexual species contained in their blood respectable amounts of 11β-hydroxytestosterone; 11-ketotestosterone was only present in gonochoristic species. In these later species, the pattern for androgens was typical of teleosts, that is, a predominance of 11-ketotestosterone and low concentration of 11β-hydroxytestosterone. The level of steroids present in the peripheral blood was as follows:

Species	Sex	Steroid	
		11-ketotest-osterone (µg/100ml)	11β-hydroxy-testosterone (µg/100ml)
Serranus cabrilla	?	Not detected	1427.00
Pagellus acarne	Male	Not detected	1884.00
Pagellus erythrinus	Male	Not detected	687.00
Diplodus sargus	Male	Not detected	1019.00
Pseudopleuronectes Americanus	Male	1137.00	11.00
Salmo salar	Male	221.00	12.00

The concentration in all the studies reported above were determined either by competetive protein binding or by double isotope derivative analysis. Both of these methods are tedious and require large amounts of samples, making multiple sampling from small fish impossible. In some cases the samples from several individuals were pooled. There are two serious flaws in the studies reporting the concentrations of steroids from pooled samples. Firstly,

the reading does not exhibit the individual variation within the sample and secondly, a number of samples are required. Another method of determining the steroids in different tissues of the body is gas chromotography. Although a small amount of sample is required in this technique, nevertheless, this technique is more time consuming. Simpson and Wright (1978) developed a method for testosterone and 11-ketotestosterone in fish plasma by a radio-gas chromatographic technique. Sangalang and Freeman (1977) and Sangalang et al.,(1978) have developed a radioimmunoassay (RIA) for testosterone and 11-ketotestosterone in the plasma of fish using antiserum to testosterone and 11-ketotestosterone. These methods require only about 200µl of plasma. Publication of these methods will certainly help in evaluating the exact amounts of the steroid present and during different stages of the sexual maturity of fish which were hitherto not possible.

2.5. Biosynthesis of Androgens in Fish

Radioactive testosterone and 11-ketotestosterone were isolated in a free state from the plasma of a sexually mature *Oncorhynchus nerka*, one hundred minutes after *in vivo* injection of 17α-hydroxyprogesterone-4-^{14}C (Idler and Truscott, 1963). These authors showed that the 11-hydroxycorticosteroids (Cortisol and Cortisone) were not transformed *in vivo* into C19 steroids substituted at C11. On the other hand testosterone-4-^{14}C and 17α-hydroxyprogesterone-4-^{14}C were precursors of 11-ketotesto-

sterone. Considering the percentage of the conversion, the authors, postulated a pathway to the synthesis of 11-ketotestosterone through androstenedione, 17β-hydroxyandrostenedione, and adrenosterone in addition to a pathway via testosterone. In conclusion a C21-C19 lyase (desmolase), 17β-HSDH, 11β-hydroxylase and 11β-HSDH have been confirmed in salmon (Idler and McNab, 1967).

Simpson et al. (1969) studied the biosynthetic capabilities of the testes of *Microstomus kitt*, a teleost. They recovered 17α-hydroxyprogesterone, androstenedione and testosterone after incubation with progesterone-4-^{14}C. Eckstein (1967) and Eckstein and Eylath (1968, 1970) studied the biosynthesis of steroids *in vitro* in ovaries and testes of *Mugil cephalus* and *Mugil capito*. In both tissues testosterone and 11-ketotestosterone were identified with predominant concentrations over other steroids. An interesting point to note in these studies is that, these species are euryhaline and do not reproduce in fresh water, but their gonads produced the required sex steroids in fresh water and in the later study the concentration of testosterone and 11-ketotestosterone were higher in ovaries from fresh water. Ovarian homogenates from fresh water *Tilapia aurea* when incubated with radioactive progesterone produced testosterone and 11-ketotestosterone (Eckstein, 1970).

Arai et al. (1964) showed that the testes of *Tribolodon*

hakonensis, a cyprinid, contain enzymes which *in vitro* catalyze the transformation of progesterone-4-^{14}C into 17α-hydroxyprogesterone and androstenedione. No testosterone was detected showing the absence of 17β-HSDH. But in a subsequent study Arai and Tamaoki (1967a and b) after incubating testicular homogenates of *Salmo gairdneri* with progesterone-4-^{14}C, obtained 17α-hydroxyprogesterone, 17α, 20β-dihydroxy-4-pregnen-3-one, androstenedione and testosterone. The metabolic pathway was similar to the previous study. Moreover, they also showed that androstenedione-4-^{14}C was metabolised by the testes to testosterone, 11β-hydroxytestosterone and 11-ketotestosterone.

Colombo et al.,(1970) studied the bioconversion of pregnenolone-4-^{14}C into testicular steroids of *Gobius paganellus*. On the basis of the different steroids identified it was concluded that free and conjugated steroids were formed and testosterone formation was identified both via 17β-hydroxyprogesterone and via dehydroepiandrosterone pathway. In subsequent papers (Columbo et al., 1972 a and b), the steroid biosynthesis was studied in a protandrous hermaphrodite (*Sparus auratus*) and comparisons were made with other species. The mature gonads of *Sparus auratus* when incubated with pregnenolone-4-^{14}C yielded predominantly progesterone and 17α-hydroxyprogesterone and these steroids were the only compounds found in the ovaries during the sex reversal. In testis androstenedione was identified both in mature

and during sex reversal period, but its conversion to testosterone was significant only in the maturing testes. Recently, Kime and Hews (1978) has studied the steroid formation in the testes of perch (*Perca fluviatilis*) and (*Esox lucius*) by using radioactive pregnenolone, progesterone and testosterone. In all the incubations involving tissues from pike, 11-ketotestosterone and 11β-hydroxytestosterone were the major end products. Testosterone being formed at a very low rate. The above two 11-oxygenated steroids were also isolated from perch testes, but only when testosterone was used as a precursor. Moreover, in all the incubations regarding perch, there were indications of the presence of testosterone glucuronide while no conjugates could be isolated from pike incubations.

On the basis of all the studies reviewed above, a pathway for the formation of testosterone and 11-ketotestosterone has evolved which is given in Figure 1. It must be kept in mind while looking into these studies that actual secretion of a steroid cannot be proven by merely studying *in vitro* conversions or by the demonstration of the presence of a steroid in question. In fact, studies should be carried out on the blood leaving the gonads. There are known technical difficulties for these type of studies in fish, but recently very sensitive methods have been reported for the estimation of testosterone and 11-ketotestosterone in the blood of fish up to picogram

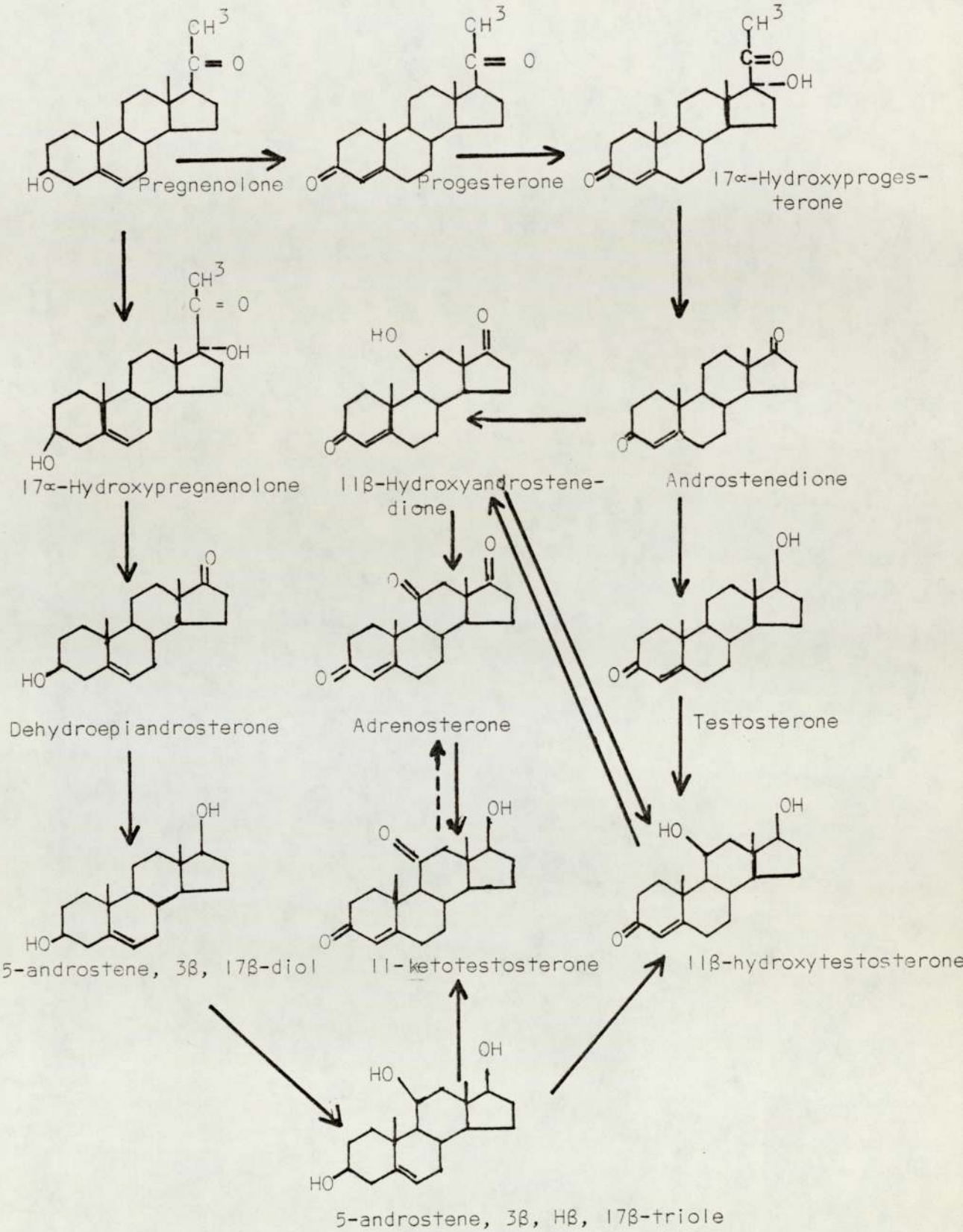


FIGURE 1 Formation of testosterone and 11-ketotestosterone in the testes of teleost fish. From in vitro and in vivo studies

levels and which require very little amount of plasma (Sangalang and Freeman, 1977; Sangalang et al., 1978; Simpson and Wright, 1978).

2.6. Anabolic-Androgenic Steroids

2.6.1. Introduction

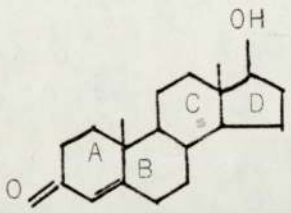
Androgens constitute a class of steroids characterised by their biological effects on the primary and secondary characteristics of various male animals. Naturally occurring androgens are steroids with 19 carbon atoms (C19 compounds) having an oxygen function (ketonic or hydroxyl) at C-3 and C-17, and partial unsaturation of the A-ring. The most potent naturally occurring androgen is testosterone. This definition must be variously modified to accommodate some analogous synthetic compounds (anabolic-androgenic steroids) possessing varied androgenic-anabolic properties. The term anabolic-androgenic steroids (sometimes referred as anabolic steroids) applies to those steroids that, *inter alia*, promote the synthesis and storage of cytoplasmic protein and stimulate the growth of the tissues in general. Sometimes the term "myotrophic steroids" is also used for these drugs, referring to the site of protein synthesis, i.e. muscle. Theoretically, for clinical use, anabolic steroids should possess the true anabolic activity of typical androgens, such as testosterone, but should lack all androgenic properties, such as virilizing effects.

The search for substances that possess a prepondrance of anabolic activity has been a concern of many investigators for the last thirty-five years. The compounds with total anabolic activity have not been reported as yet. Chemical modifications of testosterone or its derivatives have led to some synthetic compounds which show a satisfactory disassociation between anabolic and androgenic properties. Such drugs are generally referred as anabolic steroids but precisely speaking they are actually anabolic-androgenic steroids. Many attempts have also been made to find orally active compounds which have much greater clinical applications than intramuscularly administered drugs. Some of the commercially available anabolic steroids are shown in Figure 2.

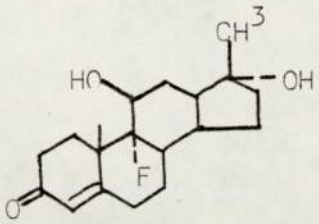
Excellent accounts of the biological, clinical and side effects of anabolic steroids are available (Kruskemper, 1968; Vida, 1969; Counsell and Klimstra, 1970; Camerino and Sciaky, 1975; Harvey, 1975; Kochakian, 1975, 1976; Murad and Gillman, 1976). In the following pages a summary of the action of these drugs will be presented.

2.6.2. Disassociation of anabolic-androgenic effects (Structure Activity Relationship)

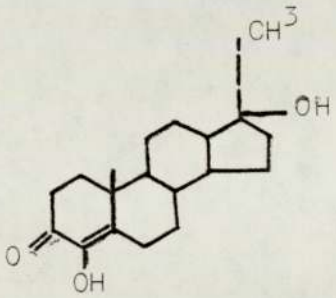
Much of the research efforts have been expanded over the last two decades in laboratories throughout the world in search of a molecule that possess all the anabolic properties and none of the androgenic ones. Although



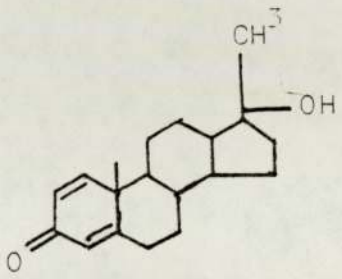
Testosterone



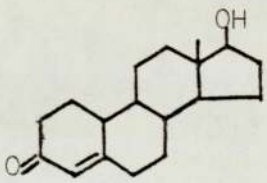
Fluoxymesterone



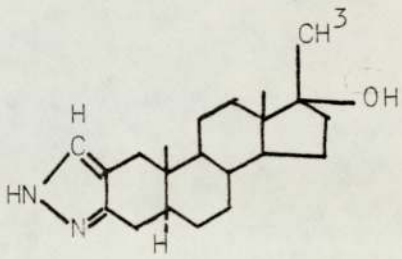
Oxymesterone



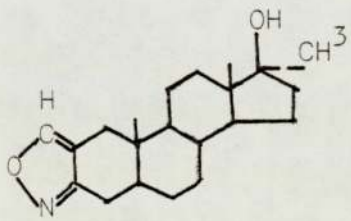
Methandienone



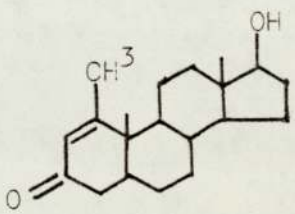
Nandrolone



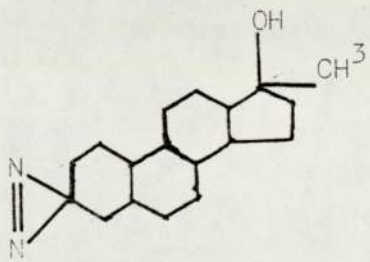
Stanozolol



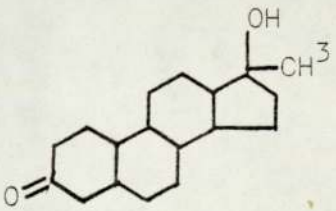
Androisoxazole



Methenolone



Methyldiazinol

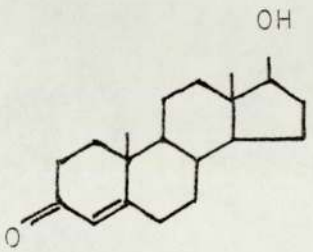


Mestanolone

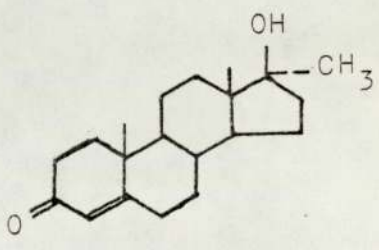
FIGURE 2 Some of the commercially available anabolic-androgenic steroids

absolute success has not been achieved but major strides in the desired direction have been made. The evaluation of the two properties of the steroids in question have been studied in laboratory mammals and in most cases testosterone or 17∝-methyltestosterone or both have been used as standard substances. Potts et al (1976) has discussed different methods available for the screening of the drug in question for its anabolic-androgenic properties. The data on these aspects can also be found in Kruskemper (1968), Vida (1969), Anstall (1974) and Camerino and Sciaky (1975). In this section the steroids used in the present study will be discussed.

2.6.2.1. Testosterone and 17∝-Methyltestosterone



Testosterone
(17β-hydroxyandrost-4-en-3-one)



17∝-methyltestosterone
(17∝-methyl-17β-hydroxyandrost-4-en-3-one)

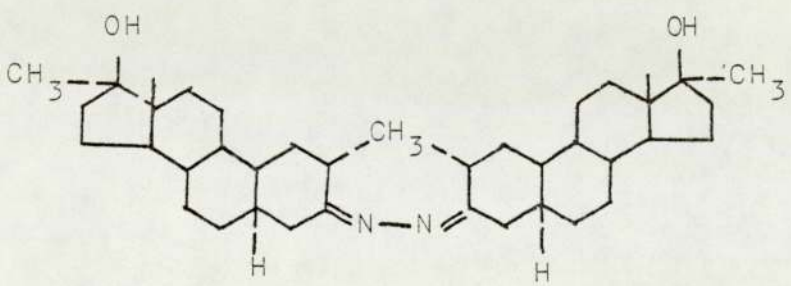
Testosterone and methyltestosterone differ only modestly in activity orally or parenterally, especially in comparison

with other protein anabolic steroids. No great case appears justifiable for meaningful differences in biological effects for selecting one from the other. Duration of activity may favour the methylated steroid when administered orally.

Parenterally, testosterone at 25 mg/kg increased the growth of reporductively mature female rats 100% and produced a 40% improvement in feed conversion. The same dose caused a 76% decrease in the number of vaginal estrous days, which is indicative of pituitary inhibition. Testosterone produces a progesterone-like stimulation of the endometrium in Claudberg assay (Klein and Parkes, 1937).

Methyltestosterone at 6mg/kg body weight increased the growth rate 35% in the same test system and improved 32% feed conversion when administered orally. A 48% decrease in number of vaginal estrus days was noted. Progestine like activity has also been reported for methyltestosterone (Klein and Parkes, 1937).

2.6.2.2. Dimethazine (Roxilone, Richter)



(2∝,17∝-Dimethyl-17β-hydroxy-5∝-androstan-3,3´-Azine)

Dimethazine was synthesized by De Ruggieri et al.,(1962) by condensation of 2α, 17α-dimethyl-5α-androst-17β-ol-3-one with its hydrazone derivative. The oral anabolic activity of dimethazine was studied in rats by Bianco et al. (1962) at a total dose of 6mg, using a modification of Metcalf and Broich (1961) radiochemical method with 17α-methyltestosterone as a standard. The specific activity of levator ani muscle when using dimethazine was 2.3 times that of untreated animals. No difference was noted between the untreated animals and those treated with 17α-methyltestosterone.

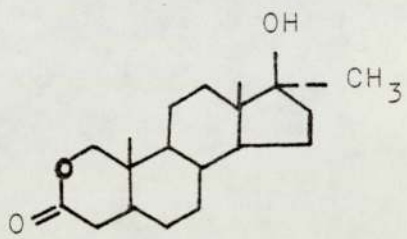
Matscher et al. (1962) reported the activity in protein anabolism of dimethazine compared with 17α-methyltestosterone, oxymetholone, stanozolol and testosterone propionate. Tests were carried out on rats in various experimental situations. Weight gain with resepct to controls, a myotropic effect and a nitrogen-retaining effect was always observed. The myotrophic-androgenic ratio was found to be (2.1:0.15) 14.0. Lupo et al. (1962) also concluded that at the given doses dimethazine showed no estrogenic, progestational or cortical activity in rats or rabbits.

Dorfman and Kincl (1963) found the anabolic activity to be 0.27, the androgenic activity, 0.12 (seminal vesicles, SV) and 0.06 (ventral prostate, VP) times the activity shown by 17α-methyltestosterone. Hence, dimethazine possess slight anabolic property but has almost no androgenic activity, this fact leads to the favourable

myotrophic-androgenic index.

Maggi (1964) reported the favourable results on treatment with dimethazine in a group of ninety patients of both sexes affected by various morbid syndromes. Recently, dimethazine capronate has been introduced into the market as a depot form having a long duration of action.

2.6.2.3. Oxandrolone (Anavar, Searle)



Oxandrolone
(17α-methyl-17β-hydroxy-2-oxa-5α-androstan-3-one)

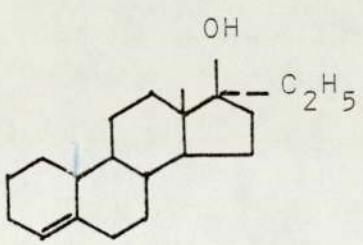
The synthesis of oxandrolone was reported in 1962 by Pappo and Jung. Oxandrolone was found by the oral administration, to be more active as an anabolic agent than 17α-methyl-17β-hydroxy-5α -androstan-3-one, based on the nitrogen retention test and was practically devoid of any androgenic properties.

The pharmacological properties were studied in castrated rats by Lennon and Saunders (1964). When compared by oral administration with 17α-methyltestosterone, it was 3.22 times more active as a myotopic agent and just under a quarter (0.24) as active as an androgenic agent. The myotropic-androgen index was thus 13.3.

Oxandrolone showed some activity in the inhibition of gonadotrophin secretion. In man, it proved to have an anabolic activity in oral administration equivalent to 6.3 times that of 17α-methyltestosterone (Fox et al., 1962). Nitrogen retention was evident at doses of 0.6mg/day.

Albanese et al. (1963) found oxandrolone to be twice as active as norethandrolone in stimulating a positive nitrogen balance in hospital patients and in mongoloid children. Ray et al. (1963) noticed an increase in height of the mongoloid children with daily doses of 0.25 to 0.5 mg/kg of body weight without significant enhancement in bone age, thus confirming its low androgenicity.

2.6.2.4. Ethylestrenol (Maxibolin, Organon)



(17α-ethyl-19-norandrost-4-en-17β-ol)

Ethylestrenol has been prepared, along with other analogues by De Winter et al. (1959) during a study of the relationship between structure and activity of 19-norsteroids. The biological properties of ethylestrenol have been studied in rat by Overbeeke et al. (1962). Arnold et al. (1963) investigated the relative oral and anabolic to androgenic activity ratios in comparison with androisoxazole and testosterone. The anabolic effect was evaluated by nitrogen balance studies and androgenic effect on the basis of the increase in weight of the ventral prostate gland in a ten day assay. Under these conditions, the ratios of their anabolic to androgenic activities with respect to 17α-methyltestosterone were: ethylestrenol (1.7 / 0.21) 8.1; androisoxazole (1.55 / 0.22) 7.0; and testosterone (0.38 / 0.57) 0.67.

Androgenically ethylestrenol has been shown to be as active as norethandrolone, but since it is four times more anabolic, this fact is not of great importance in therapeutic application (Junkmann and Suchowsky, 1962).

Overbeeke et al. (1961) reported the anticatabolic activity of ethylestrenol on rat body weight given hydrocortisone. It has also been shown that at therapeutic doses, it did not change the rate of excretion of 17-hydroxycorticosteroids (Van Varenbergh et al., 1961). The anticatabolic action of ethylestrenol on oral administration (8mg) has also been seen in patients treated with dexamethasone.
Ethylestrenol has been widely used in clinical practice,

net retention of calcium, phosphorus and nitrogen has also been reported (Nowakowski, 1962; Van Wayjen and Buyze, 1962).

2.7. Metabolism of Anabolic-Androgenic Steroids

Before considering the metabolic effects of anabolic-androgenic agents, one must take into account the factors affecting the biological activities of the steroid hormones. The question can be raised whether the steroids administered as drugs still possess the original structure of the drug at the receptor site or whether structural changes have taken place in the course of the delivery to the receptor site. This question becomes especially important in the view of finding (West et al, 1951) that the truly anabolic effect of testosterone begins only at a time when all but traces of the effective doses of the steroid have been metabolised and excreted by the body. Many excellent and detailed accounts of metabolism of anabolic-androgenic steroids have been published and the reader is referred to them. (Fotherby and James 1972; Gower, 1975; Wilson, 1975; Kochakian and Arimasa, 1976).

2.8. Metabolic Effects of Anabolic-Androgenic Steroids

2.8.1. Historical Perspective

The first clear demonstration of an effect of the product of the testis was not produced until 1935. Kochakian (1935) and Kochakian and Murlin (1935) reported a marked decrease in protein metabolism by nitrogen balance technique in

castrated dogs given an extract from human male urine. Shortly thereafter, testosterone was characterised (David et al., 1935) as the internal secretion of the testis and methods for its synthesis from cholesterol were developed (Butenandt and Hanish, 1935; Ruzicka and Wettstein, 1935). Kochakian and Murlin (1936) prepared androst-4-ene-3,17-dione, administered it to castrated dogs and reproduced the effect on nitrogen metabolism produced by the urinary extracts. Shortly afterwards, testosterone became available and also proved to be effective (Kochakian, 1937), thus establishing a protein anabolic action for androgens. Later on a battery of anabolic-androgenic steroids were synthesized and studied in detail, in laboratory animals, human beings and in livestock production. A brief summary of these studies will follow in the coming sections.

2.8.2. Studies in Mammals

Several review articles have appeared on different aspects of the anabolic actions of steroids (Kochakian, 1946, 1950, 1959, 1964b, 1965, 1969c, 1969d, 1975, 1976; Berczeller and Kupperman 1960; Camerino and Sala, 1960; Segaloff, 1966; Kruskemper, 1968; Vida, 1969). It appears from these studies that anabolic effects of these steroids are due to the positive nitrogen balance and an increase in body weight. This positive nitrogen balance is due to a decrease in urea production and excretion. Fecal nitrogen is not changed. It is suggested that this property of these steroids is direct and not mediated by any other endocrine

gland, as is evident, when various other endocrine organs are removed. The protein anabolism is due to better utilisation of the ingested protein.

Induction of nitrogen retention by the steroids is accompanied by a parallel change in Na^+, K^+, Cl^- and PO_4^{3-}, indicating the new formation of protoplasm. The urinary urea and blood non-protein nitrogen (NPN) and urea also change parallel with changes in urinary nitrogen.

Castration induces a transitory small post-operational increase in nitrogen excretion and possibly a small decrease in energy metabolism in rat. The effect of castration, testosterone and related steroids on body weight of several animal species has been studied at different ages, duration of castration, dose and duration of hormone treatment and during different endocrine and nutritional conditions. These factors have a variable modifying influences on body weight and organs weight. Of all the species studied, the mouse presents the widest spectrum of responsible tissues (For review see Kochakian, 1976).

The role of testosterone as growth stimulant in man has also been reviewed recently (Van der Werff Ten Bosch, 1977). It has also been shown that testosterone and 5α-androstan-17-one control the genetic and gold thioglucose induced obesity in mice (Kandutsch et al.,1972; Yen et al,1978).

2.8.3. Regulation of Protein Synthesis by Anabolic-Androgenic Steroids

In many studies on the changes in weight of the muscles and several organs with proportionate changes in protein content established that the positive nitrogen balance stimulated by anabolic-androgenic compounds was an expression of the synthesis of protein in many tissues other than the accessory sex organs. Availability of labelled amino acids gave the opportunity to study these processes directly both *in vivo* and *in vitro*.

2.8.3.1. Liver

Hauschildt and Grossman (1953) studied the incorporation of 1-^{14}C-glycine into protein by slices from the rat and human liver after addition of crystalline testosterone (30-300 µg/100mg of liver slices) and other steroids. The testosterone had a uniform depressing effect of glycine incorporation that was not altered by addition of 2.3mg of DPN to the incubation medium. Desoxycorticosterone (DOC) and estradiol-17β also had a depressing effect, while cholesterol and cortisone did not produce any effect.

Bernelli-Zazzera et al. (1958a, 1958b) castrated rats at 200-250 g body weight, five days later a group of rats was partially hepatectomized, and two hours later 2 mg of either testosterone propionate or 4-chlorotestosterone was injected daily. Seventy-two hours after the first

injection, liver from treated animals were removed and the liver slices were incubated with 1-^{14}C-glycine. After one hour of incubation, it was observed that treatment of the hormone did not affect the incorporation of radio-activity into the liver protein of the castrated rats, but further enhanced the already increased incorporation into that of partially hepatectomized-castrated rats. Similar results were obtained when 2-^{14}C-phenylalanine was used instead of glycine and kidney was substituted for liver.

Piceni-sereni et al. (1961) and Careddu et al. (1961) both utilised Bernelli-Zazzera et al. (1958a, 1958b) method to study the incorporation of labelled amino acids into liver or kidney proteins of albino rats under the effect of 4-chlorotestosterone (0.5mg/100g body weight). The rate of amino acid incorporation was not changed up to thirty days.

2.8.3.2. Kidney

Experiments *in vivo* indicate a small enhancement by testosterone phenylpropionate of the incorporation of ^{14}C-formate into the protein of the kidneys of immature castrated rats (Jakubovic and Cekan, 1966). Similar results were also obtained with testosterone treatment of adult male rats (Farabollini, 1969).

The regulation of growth of the mouse kidney by anabolic-androgens is well understood now (Kochakian, 1977). Studies

with kidney slices (Frieden et al., 1957) from male and female and homogenates from female mice (Frieden et al., 1961) demonstrated a small increase (30-35%) in the incorporation of 1-^{14}C-glycine into the trichloroacetic acid (TCA) precipitable material after androgen administration. By the use of Zamecnik and Keller (1954) cell free system Kochakian et al. (1963) demonstrated a marked decrease after castration and a very great enhancement by testosterone propionate in the incorporation of 1-^{14}C-leucine into acid precipitable material by the postmitochondrial fraction of the mouse kidney. Later on, it was shown that earliest effect of androgen was between 20-24 hours (Kochakian, 1969a, 1969b).

The protein biosynthesis consists of several parts. The first step, amino acid activation, changed exactly according to the changes in weight of the kidney (Kochakian et al., 1963). The site of protein synthesis is the polysomes and the concentration of these particles was decreased after castration and increased after androgen administration (Kochakian et al., 1969). Messenger ribonucleic acid (mRNA) apparently was not decisive factor. The addition of mRNA or poly uracil (Poly U) RNA enhanced the incorporation of U-^{14}C-phenylalanine into acid precipitable material by postmitochondrial fraction (Kochakian and Hama, 1969; Kochkian et al., 1974) or a polysome-cytosol system (Kochakian et al.,1974) prepared from the kidneys of normal, castrated or testosterone

propionate treated mice but did not restore the rate of incorporation in preparations from the castrated mouse kidneys to the level of androgen treated or normal mice. It was also shown that the cytosal contain factor (s) which is present probably in the transferases system and which participate in the effect of androgens (Kochakian and Hama, 1969; Kochakian et al.,1974; Kochakian, 1977).

The primary site of action of androgen appear to be genetic in nature. The lack of the effects of castration and androgen administration in guinea pig kidney of the incorporation of ^{14}C-leucine by the postmitochondrial fraction (Kochakian, 1964a) is probably due to the lack of necessary gene locus or else the gene is repressed.

All of the above studies were performed with post-mitochondrial fraction. But in mice there is some evidence that both mitochondrial and nuclear fractions can synthesize proteins and show some dependency on the androgens (Koth et al., 1972).

2.8.3.3. Skeletal Muscle

One of the sites of action of anabolic - androgenic hormones is skeletal muscle (expression of the protein anabolic activity). Effect of these steroids on the weight of individual muscles has been reviewed by Kochakian (1964b, 1966, 1975, 1976). Here, a brief account of the most recent studies is given.

The effect of methyltrienolone was studied in rat, mouse, cock and man (Dube et al., 1976) on the binding with specific receptors in skeletal as well as in perineal muscles. It was shown that skeletal muscle has the specific receptors for the anabolic-androgenic steroids binding, which are necessary for action of steroids on target organs. Recent studies by Kreig and Voigt (1976, 1977) have clearly demonstrated that skeletal muscle contain a cytosolic receptor for anabolic-androgenic steroids which is qualitatively similar to the receptors in bulbocavernosus/levator ani (BCLA) and prostate. Although the concentrations of these receptors are very low (seventy times lower than BCLA and about ten times lower than the prostate), nevertheless, they show a great affinity for anabolic steroids.

Grigsby et al. (1976) studied the effect of testosterone treatment on the incorporation of labelled amino acids in different skeletal muscle protein in rabbit. It was shown that testosterone affects the incorporation of ^{3}H-leucine into myofibrillar proteins but not in sarcoplasmic proteins.

It has been shown by perfusing the hindlimb (Stratman, 1978) and hemicorpus muscle (Dohm et al.,1979) of rat, that testosterone does not affect the protein synthesis in these muscles but an anabolic steroid (17β-hydroxy-17α-methyl-2-oxandrostan-3-one; SC 11585) was effective in

in stimulating the protein synthesis and the uptake/or exchange of several amino acids. In fact the capacity of SC 11585 in protein synthesis was equal to insulin in the same system and that insulin and testosterone acted synergistically while insulin and SC 11585 acted additively. The authors concluded from their results that insulin is necessary for testosterone to be anabolically active (Sireck and Best, 1953).

2.8.3.4. Anticatabolic Effects of Anabolic-Androgenic Steroids in Skeletal Muscle

Adrenal corticoids (mainly glucocorticoids) are known to act as catabolic hormones on the skeletal muscle, which is the main source of amino acid and proteins in the body. Glucocorticoid act by favouring hepatic gluconeogenesis on the expense of other peripheral tissues, the skeletal muscle providing the maximum precursors in the form of amino acid and proteins. The net result of the enhanced levels of glucocortiocoids in the body are negative nitrogen balance, myopathy and a marked reduction in muscle mass with muscle weakness (Eisenstein, 1967; Litwack and Singer, 1972; Leung and Munck, 1975; Mayer and Rosen, 1977; Shoji and Pennington, 1977).

Anabolic-androgenic steroids are known to antagonise the glucocorticoids in skeletal muscle. These studies have been reviewed elsewhere (Kruskemper, 1968; Kochakian, 1976; Mayer and Rosen, 1977).

2.8.3.5. Perineal Complex (Accessory Sex Organs)

The perineal complex (BCLA, seminal vesicles and prostate gland) were the first organs which were studied for anabolic action of anabolic-androgenic steroids. In fact, even now the biological evaluation of the new compounds is based on the responses of these organs. There are several reviews and recent research papers dealing with the aspects of protein synthesis in these organs (William-Ashman, 1965, 1975; William-Ashman and Reddi, 1971, 1972; William-Ashman et al., 1964; Villee et al., 1975; Heyns et al., 1976; Krieg and Voigt, 1976, 1977; Liao, 1977; Tremblay et al., 1977; Albert et al., 1978; Mulder et al., 1978).

2.8.4. Effect of Anabolic-Androgenic Steroids on the Nucleic Acid Metabolism

The pioneer studies by Brachet (1950) and Casperson (1950) demonstrated the necessity of nucleic acid in protein synthesis. The role of nucleic acid in protein metabolism has been reviewed many times, recent studies on skeletal muscle and other organs can be seen in (Medvedev, 1964; Bergen, 1974; Swick and Song, 1974; Thompson and Heywood, 1974; Young, 1974, 1976; Kochakian, 1976, 1977).

2.8.4.1. Liver

A number of studies utilising different radioactive precursors provide the evidence for change in nucleic

acid metabolism in the liver after castration or steroid administration. Cantarow et al. (1958) injected adult male rats every other day for twelve days with a large dose (10mg) of testosterone propionate and eighteen hours before autopsy 20mg of 2-^{14}C-uracil (500μCi/m mole) in normal saline. The specific activity of RNA of treated rats was increased six-fold. Increase in liver RNA has also been reported in mice and rats after administration of anabolic-androgenic steroid, and a parallel decrease in liver weight and RNA content was also observed after castration (Farabollini, 1969; Kochakian and Harrison, 1962).

There are some conflicting reports on the effect of steroids on the DNA content and weight of liver. No change in DNA content of liver was found after castration or treatment with testosterone or 17α-methyltestosterone, although the weight of the castrated mice came to normal level. But, 17β-hydroxy-5α-androstan-3-one increased the DNA after twenty-eight and forty-two days studied at the same time (Kochakian and Harrison, 1962). Cheeke et al. (1965) studied the growth of liver and reported no increase in DNA of the liver of rats castrated at three weeks of age, for subsequent five weeks in comparison with normal rats. Similarly, 17α-methyltestosterone was without any effect in normal rats as far as RNA and DNA were concerned (Krolikowska-prasal, 1970).

Tata (1966) treated castrated male rats with testosterone propionate and found increases in labelled nuclear RNA and RNA polymerase activities well before any rise in total nuclear protein was seen. Truckenbrodt (1969) reported that weight of liver of immature normal mice increased at a faster rate than mice castracted at eighteen days of age. The protein and DNA contents increased in proportion to weight, but RNA showed a greater increase (12%) compared with castrated mouse at sixty days of age.

2.8.4.2. Kidney

2.8.4.2.1. Total Concentrations

The failure of the initial studies to demonstrate a change in RNA after castration and small changes after androgen administration probably was due to the wide variation in the mice used (Rabinovitch, 1952). Subsequent studies clearly demonstrated a decrease in the concentration of RNA after castration and an increase after androgen stimulation to and above normal. Furthermore, DNA was slightly decreased after castration and restored to normal by androgen after a long period (Kochakian and Harrison, 1962; Kassenaar et al., 1962). The changes in DNA are not related to the nuclear volumes, which is said to be more dependent on the protein (non-histones) content. Further confirmation of the regulation of nucleic acids by androgens was provided by Truckenbrodt (1969) and Heim et al. (1969) who compared the increase in RNA and DNA in male

and female mice with age and the increase in nucleic acids after the injection of 3-oxo-1-methylandrost-1-en-17β-ol, and 17β-enanthate at different ages.

2.8.4.2.2. Subcellular Distribution

RNA exist in many different forms and in different parts of the cell. Subcellular fractionation demonstrated that the nuclear, mitochondrial and soluble RNA's changed in direct proportion with the changes in weight of the mouse kidney after castration and androgen administration (Kochakian 1969a). The microsomal RNA, however, decreased at a more rapid rate than the kidney weight after castration and increased at a more rapid rate after androgen administration. These changes were reflected both in polysomes and monosomes (Kochakian et al., 1969). The polysomes from the kidneys of testosterone propionate treated mice on gradient centrifugation exhibited a small and consistent shift towards the heavier polysomes with a concomitant decrease in monosomes. Electron microscopy revealed conformational changes in polysomes. Castration resulted in linear polysomes while the administration of testosterone propionate stimulated helical formation. Phenol extraction of post-mitocrondrial fraction, the microsomal fraction, the monosomes and the polysomes did not reveal any changes in the respective RNA gradient profile as a result of castration or androgen administration. The injection of 0.5mg/day of testosterone propionate into adult normal male and female mice for three, ten and

sixteen days gave only suggestive increases in the concentration of free monosomes and polysomes by electron microscopy (Tessman, 1968).

2.8.4.2.3. Biosynthesis

Studies *in vivo* with labelled precursors have provided further information on the influence of anabolic-androgenic steroids not only on the synthesis of RNA but also the nucleotides.

6-^{14}C-orotic acid as a precursor for RNA synthesis gave a marked increase of incorporation into the various subcellular RNA's (Kochakian and Hill, 1966; Kochakian et al., 1972). Yamanaka et al. (1969) using $^{32}P\text{-}PO_4^{3-}$, studied the effect of testosterone on the RNA synthesis in rat kidney. A maximal increase in RNA synthesis three hours after the injection of 5mg testosterone propionate with a return to normal levels twenty-four hours later was reported. Analysis of the newly formed RNA showed it consisted of ribosomal RNA and DNA like RNA.

Various RNA's are synthesized in nuclei (Davidson, 1976). Castration decreased the ability of isolated kidney nuclei to polymerise nucleotides and the injection of testosterone into mice rapidly restored the RNA polymerase activity (Avdalovic and Kochakian, 1969).

Recent papers on the effect of testosterone or its

analogues on the RNA metabolism clearly show that these steroids exerts a definite and powerful positive effect on the RNA synthesis which is due to the increase in activity of RNA polymerase and due to a decrease in RNA catabolism (Dubowsky and Kochakian, 1973; Bullock and Bardin, 1974; Avdalovic and Bates, 1975; Janne et al., 1976; Petrovic et al., 1977).

2.8.4.3. Skeletal Muscle

The effect of anabolic-androgenic steroids on the nucleic acid metabolism have not been studied in detail in skeletal muscle and only a few reports are avilable.

The skeletal muscle of the rat show only small changes after castration and androgen treatment (Kochakian et al., 1956). Saunders et al. (1962) found no change in RNA/DNA ratio of the rectus femoris muscle five weeks after castration at three weeks of age. The injection of testosterone (20mg/kg/day for seven days) after four weeks of castration produced a slight but non-significant increase in RNA/DNA ratio. No clear cut response of guinea pig muscle RNA or DNA was observed after treatment with testosterone (Kochakian et al., 1964). Cheek et al (1965) also observed no changes in the DNA content of the quadriceps muscles of rats castrated for five weeks.

Breuer and Florini (1965, 1966) and Florini (1970) demonstrated that anabolic-androgenic steroids increase

the RNA-polymerase, DNA priming efficiency and an activation of tempelate activity. Recently Marcais and Kochakian (1971) has reported synthesis of RNA and increase in polysomes of the temporal muscle of guinea pig after testosterone administration, which was decreased by castration.

2.8.4.4. Perineal Complex (Accessory Sex Organs)

In contrast to skeletal muscle, there are numerous studies on the effect of anabolic-androgenic steroids on the nucleic acid metabolism in these sex dependent organs (William-Ashman, 1965, 1975; William-Ashman and Reddi, 1971, 1972; William-Ashman et al, 1964; Liao and Fang, 1969; Liao, 1975, 1977; King and Mainwaring, 1974; Villee et al, 1975; Mainwaring, 1977; Parker and Scrace, 1978; Tunn et al, 1979; Wang and Loor, 1979).

2.9. Anabolic-Androgenic Steroids in Livestock Production

The large increases in the cost of feed stuffs and protein is a real challenge to the progress of animal production both in developed and developing countries. This can be counteracted by improving the efficiency of feed utilisation and by selecting those animals which have faster growth rates. Generally, the increase in feed efficiency is obtained by the addition of compounds in the feed with hormonal effects. In this connection, both estrogens and androgen derivatives have been employed. The compounds having estrogenic properties have been used more extensively

than the androgens as far as the livestock industry is concerned. The role of natural or synthetic estrogens used in livestock industry have been reviewed many times (Umberger, 1975; Trenkle, 1969, 1976; Trenkle and Burroughs, 1978; Abou-Akkada and El-Shazly, 1976; Bird, 1976; Nesheim, 1976; Velle, 1976, 1977; McMartin et al, 1978; Scott, 1978). In the following discussion only recent reports using androgens or their derivatives will be considered.

The derivative of testosterone which is commonly used in livestock industry to increase the growth rates of finishing cattle is trenbolone acetate. Trenbolone acetate is sold in Britain as "Finaplex", and is available in 300 mg pellets (one animal dose). This compound is not active orally in livestock and is heat labile. Androgenic side effects are also very low (Scott, 1978). All the available data show that trenbolone acetate is a potent anabolic steroid in cattle, steers, and heifers and increase growth rate than the controls (Scott, 1978; Roche et al, 1978; Heitzman, 1979). When used with other anabolic drugs like resorcyclic acid lactone or hexoestrol, trenbolone give additive effects (Roche et al, 1978; Galbraith and Watson, 1978).

Trenbolone acetate is also effective in increasing growth rate of intact female rats but was not effective in

castrated males. It also reduced the myofibrillar protein degradation as judged by methylhistidine excretion. It significantly increased total carcass nitrogen and decreased the total fat by 8.3%.(Vernon and Buttery, 1978a, 1978b; Buttery et al., 1978). Recently methods have been developed by which tissue concentrations of trenbolone and trenbolone acetate can be measured (Hoffmann, 1978; Ryan and Hoffmann, 1978; Holder et al., 1979) in slaughtered animals so that the residue levels in the muscle be kept in the permissive limits.

2.10. Epidemiological and Side Effects Related to the Use of Hormonal Agents in Animal Production

The principal types of hormone agents used in the production of meat for human consumption are estrogens, progestagens, androgens, and anabolic-androgenic steroids. As reported in Section 2.9 natural or synthetic estrogens (Diethylstilbestrol (DES), were the first compounds used for this purpose and their use was started in early 1950. But the use of DES in clinical practice was started earlier than this and even in 1947, Gusberg reported the occurrence in women, who were receiving daily doses of 1 mg DES for twenty-four days of each month, of uterine adenomatous hyperplasia. Gusberg and Hall (1961) suggested that cancer of the uterus may be related to the intake of potent estrogens. Recently it has been reported that administration of DES to pregnant women may result in an increased

incidence of cervical and vaginal cancer in their daughters, but there is no apparent incidence of cancer in their sons (Herbst, 1976; Herbst et al., 1972, 1975). Excellent recent reviews can be found on the induction of cancer or toxicology of the estrogens, and the present legal position regarding the use of these materials in the USA (Coulston and Wills, 1976; Kroes et al., 1976; Roe, 1976; Garcia et al., 1977; Horning et al., 1978; McMartin et al., 1978; Christopherson and Mays, 1979). The following paragraphs will be on the side effects of anabolic-androgenic steroids.

There is much evidence that androgens in general and testosterone in particular, favours the development of liver cell tumours in mice (Roe, 1976). Recently, Noble (1976, 1977a, 1977b and 1977c) reported that androgenisation of Nb rats is associated with increased incidence of skin, rectal, prostatic, mammary and bladder tumours. Some of these effects have been discussed by Bruchovsky et al. (1978). One very conspicuous effect of the anabolic steroid therapy is liver dysfunction, intrahepatic cholestasis and hepatocellular carcinoma (Klastin, 1974; Nishino, 1975; Sherlock, 1975; Wynn, 1975; Plaa and Priestly, 1977; O'Shea, 1978).

The incidence of jaundice and intrahepatic cholestasis has consistently been reported after the use of 17α-methylated steroids. The structure-activity relationship in steroids

inducing hepatic dysfunction in man has been studied by De Lorimier et al. (1965). The incidence of bromosulphophth-alien (BSP) retention was highest (100%) with normethandrone and norethindrone, moderate (43-85%) with methyltestosterone, fluoxymestrone and methandriol, and low (20%) with ethisterone. It was concluded that the 3-ketone group predisposed to a greater potential for BSP retention than the 3-hydroxyl group, and that the nature of the 17-alkyl group could modify the response. Moreover, BSP retention was found to be unrelated to the anabolic, androgenic or progestational activity of a given steroid (De Lorimier et al., 1965).

It appears from the above discussion that the residues of the drug used for the production of livestock will have some effects on their consumption by human beings. One aspect to monitor the level of residues in the tissues of experimental animals, is to estimate accurately the levels of the original drug or their metabolites in them. For this purpose very sensitive methods are needed. Recently some methods have appeared in the literature on the determination of these additives in food and tissue residues (Hoffmann and Karg, 1976; Hoffmann and Oettel, 1976; Hoffmann and Rattenberger, 1977; Hoffmann, 1978, 1979). Methods have also been reported for checking the levels of anabolic steroids in human beings (Brooks et al., 1979; Hample and Starka, 1979).

2.11. Antiandrogens

Dorfman (1970) has defined the term antiandrogen as follows:

"Antiandrogens are substances which prevent androgens from expressing their activity at target sites. The inhibitory effect of these substances, therefore, should be differentiated from compounds which decrease the sythesis and/or release of hypothalamic (releasing) factors, from the anterior pituitary hormone (gonadotrophins, particularly LH) and from material which acts directly on the gonads to inhibit biosynthesis and/or secretion of androgens".

Cyproterone acetate, a synthetic steroid, is a hydroxyprogesterone derivative and fullfils the criteria laid by Dorfman (1970). This steroid is antiandrogenic, progestational, and antigonadotropic (Neuman and Steinbeck, 1974). The antiandrogenic activity is a result of competitive androgen antagonism at target organ sites. Cyproterone has been shown to inhibit the formation of the nuclear-androgen receptor complex and retention of dihydroxytestosterone (DHT) is inhibited in the accessory sex organs of rodents (Fang and Liao, 1971; Fang et al., 1969; Neuman et al., 1970).

Cyproterone acetate has sex-specific and sex-unsepcific effects. Principally, cyproterone acetate influences all those organs which are functionally or morphologically androgen dependent. Recently, Vom Saal (1978) has

shown that cyproterone acetate exposure during the pregnancy retards the growth of the foetus and also increase the incidence of foetal deaths. Panesar et al. (1979) have shown that cyproterone reduces the weight of the adrenal, concentrations of corticosterone in plasma and inhibited the 3β-hydroxysteroid dehydrogenase-5-ene, 4-ene isomerase complex in *in vitro*. The detail effects of cyproterone acetate can be seen in the following reviews (Neuman and Steinbeck, 1974; Neuman and Schenck, 1976; Neuman et al., 1976; Neuman, 1977).

2.12. Effect of Anabolic-Androgenic Steroids on Growth and Food Conversion in Fish

The most important components of production changes in farmed fish are food, plant and capital expenditure. Major economy can be achieved if faster growth rate and higher food conversions are obtained. Such cost savings have been achieved in animal husbandry (for details see Section 2.9) by adding anabolic hormones to the diets. In these studies natural estrogens and their derivatives gave better results. Extrapolating the growth potential of estrogens in the ruminants and poultry, these compounds were the choice of certain workers and their effect on growth in fish were studied.

2.12.1. Estrogens

Ghittino (1970) fed rainbow trout on diets containing DES at levels of 5g and 500g/kg of food and at a level of 1%

of their body weight. The growth of trout receiving these levels of DES was lower than that of controls. Bulkley (1972) studied the effect of DES on the channel catfish (*Ictalurus punctatus*) growth. The DES was mixed in the food at the concentration of 6.7, 67 and 670 µg/kg body weight and this food was given at 3% of their body weight per day. After one week a drop in the appetite was observed in all experimental groups, which was more pronounced in animals on higher doses of the DES. At the end of the twenty-five days there was a decrease in the growth rate, massive fluid retention in the abdominal cavity and a strong renotrophic effect (increase in renosomatic index) observed in experimental fish.

Cowey et al. (1973) studied the effect of lower doses (o.6, 1.2, 2.4 mg/kg food) of DES in O-group plaice, *Pleuronectes platessa*. At the lowest treatment level, a marked growth promoting action of DES with no undesirable side effects was apparent during a ten week period. Highest dose caused a slight suppression of growth. There was also an increase in the food conversion efficiency of the experimental fish showing higher growth rate.

Fagerlund and McBride, (1975a) tested two estrogens, estradiol and DES on the growth of Coho salmon parr. Estradiol was ineffective but DES resulted in reduced

growth rate and increased mortality of the experimental fish.

Similarly in a study on the role of estradiol-17β in sex reversal of rainbow trout and atlantic salmon, Johnstone et al. (1978) noted a growth suppressing effect of this steroid during feeding, but after the withdrawal of the drug the growth rate of the treated fish increased and after one hundred and fifty days there was no difference in control and experimental fish. Matty and Cheema (1978) has shown that DES (1.2 mg/kg food) had an adverse effect on growth of rainbow trout fingerlings.

Recently, the effect of estradiol-17β on bone calcification in goldfish was studied by Mugiya (1978). Calcium deposition on vertebrae and ribs was significantly inhibited (43% and 38%) with an increase in plasma Ca^{++} level.

From the studies reported above, it is clear that the role of estrogens in fish growth is confusing. Work with other compounds and fish species can throw some light on this aspect of fish biology.

2.12.2. Anabolic-Androgenic Steroids

The role of anabolic-androgenic steroids in growth of mammals have been discussed in Section 2.9. Hutchinson

and Campbell (1964) were first to report the effect of ethylestrenol on the growth of *Tilapia melanopleura*. Although, these authors could not achieve any growth response with this compound, however, later on, many workers used different other and some time the same (ethylestrenol) steroid with positive effects on growth of fish.

Hirose and Hibiya (1968a, 1968b) noted a significant increase in growth rate of goldfish (*Carassius auratus*) and rainbow trout (*Salmo gairdneri*) from 4-chlorotestosterone. When given intramuscularly, this steroid induced maximum growth at dosage levels of 0.5mg/fish/week in goldfish and 2.5mg/fish/week in rainbow trout and both in sexually active and inactive seasons. The administration of higher doses (1.2 mg/fish/week goldfish) resulted in diminished growth, probably due to androgenic side effects, such as hypertrophy of the liver and kidney and suppression of gonads. The increase in growth rate was mainly due to the increase in appetite and to some extent on food conversion. Chemical composition of the muscle remained unchanged. Two other androgenic steroids, methylandrostenediole and testosterone propionate were also used as reference purposes. Both hormones caused undesirable renotropic changes.

The anabolic activity of stanozolol (17β-hydroxy-17α-methylandrostan-3,2-C-pyrazole) was evaluated by Bulkley and

Swihart (1973) in Channel Catfish and goldfish. The hormone was mixed in the food and given in doses of O, 0.25, 2.5 and 25 mg/kg body weight/day. The hormone at the given doses caused only slight improvements in weight gain. The slight weight gain was noticed immediately after feeding the drug but was not apparent after twenty-eight days of feeding of the drug. It was concluded by the authors that stanozolol given in the food is not efficacious in promoting growth in immature cat fish and goldfish. This steroid also increased the renosomatic index (RSI) of goldfish at the highest dose level. No effect on hepatosomatic index (HSI) was observed.

The effect of 17∝-methyltestosterone (MT), a synthetic derivative of testosterone, which is active orally has gained maximum attention, as far as the growth experiments in fish is concerned, and several studies have been reported on the potential of this steroid as a growth stimulant in different species of pacific and atlantic salmon and rainbow trout. Majority of these studies have been reported by the Vancouver Group (McBride and Fagerlund, 1973, 1976; Fagerlund and McBride, 1975b, 1977; Saunders et al, 1977).

McBride and Fagerlund (1973) and Fagerlund and McBride (1975b) studied the effect of 17∝-MT on the growth of Coho and Chinook salmon. In their first experiment (1973)

the feeding was continued for forty-two days. At the termination of the experiment the fish (coho) receiving 10 mg/kg (10ppm) of the steroid were 29% and fish (chinook) receiving 1ppm exhibited a 17% net weight gain over the respective controls. A marked thickening of the skin and degenerative changes in the testes were noted in coho, ovary was not affected.

The experiments with coho salmon reported above were extended (Fagerlund and McBride, 1975b) up to seventy-two weeks, fifty-seven of which the fish remained in freshwater but after this period they were slowly transferred to the sea water. At the end of the freshwater residency, the fish receiving 10 and 1 ppm of the steroid showed a net increase in weight of 125% and 71% respectively over the controls. Both test groups gained in length too, but 10 ppm group gained less resulting into fish with higher condition factor. After transfer to salt water at the normal time of smolting, there was a decrease in rate of growth in weight and length of both the treated groups. The decrease was more pronounced in 10 ppm group. Testes of these fish were degenerated, but no effect on ovaries was observed. The lipid content of the flesh increased from 2.4 to 3.5% and there was also a decrease in flesh from 35% to 28.5%. No change in the moisture content of the flesh was noted.

The same group (Fagerlund and McBride, 1977) further

reported the effect of temperature and transfer to sea water on growth of coho salmon. When the fish were raised in fresh water at 11.5 or 16.5°C and fed MT in doses of 0.2 and 1.0 ppm, the growth rates were higher at 16.5°C than at 11.5°C. After two hundred and sixty nine days of treatment, the group receiving 0.2 mg/kg at 16.5°C weighed 231% more than the group at 11.5°C and there were minor defects in the testes.

In another experiment, coho was raised at 11.6-12.2°C. One group received MT at a dose of 1 mg/kg food. After eighty-four days these fish were 55.1% heavier than the controls. These fish were slowly acclimated to salt water and feeding of MT continued. After two hundred and sixty eight days in sea water the experimental group weighed 63.1% more than the control group and testes of the majority of treated fish were either normal or affected very little. Similar results were reported for *Salmo gairdneri*, *Oncorhynchus gorbuscha* and *Salmo salar* (Fagerlund and McBride, 1977; Saunders et al., 1977).

Apart from MT, Vancouver Group also studied the effect of other anabolic-androgenic steroids in coho salmon (McBride and Fagerlund, 1976). In addition to MT, testosterone, 11-ketotestosterone, 4-chlorotestosterone acetate, oxymetholone and progesterone were incorporated into the diet and were fed to juvenile coho. The drugs were administered for thirty-two or thirty-four weeks at

concentrations of 1 and 10 mg/kg food. Significant increases in both body weight and length at both concentration were recorded for MT, testosterone, 11-ketotestosterone and oxymetholone. Progesterone and 4-chlorotestosterone acetate failed to induce any response. The failure of 4-chlorotestosterone and progesterone in producing growth is surprising in that these results are contrary to Hirose and Hibiya (1968a, 1968b) and to Ashby (1957) who reported the anabolic action of these steroids in *Salmo gairdneri* and *Salmo trutta* respectively. Route of administration seems to play an important part in eliciting the anabolic action, because in earlier studies the hormones were either injected or dispensed in water, but in the later study they were given *per os*.

Fagerlund et al (1978) studied the effect of MT (1 ppm) and testosterone (5 ppm) on the growth, appetite and food conversion efficiency (FCE) of under yearlings of coho salmon for one hundred and three days. At the end of the experiment, fish on satiation ration were 41.8 (MT) and 13.2% (testosterone) heavier than the controls, but lower increases were noted when the fish were placed on the restricted ration. No change in the condition factor was observed. Both hormones increase the appetite (g food/ g wet weight) by 16.8 and 6.2% for MT and testosterone respectively. Food conversion efficiencies were also higher in experimental fish, but differences from controls were more pronounced in restricted ration groups than in

animals fed to satiation. FCE for MT on satiation and restricted ration was 10.0 and 22.1% respectively. Corresponding values for testosterone were 2.6 and 8.8%. Different effects on moisture, protein, fat and ash contents were also reported.

Effect of MT in the diet on growth and appetite was also studied in goldfish, *Salmo gairdneri* and *Oncorhynchus nerka* by Yamazaki (1976). Positive growth responses and effect on appetite were noted. While lower doses of MT increased the growth, higher doses inhibited it and androgenic side effects were also noted.

When fertilised eggs of *Tilapia nilotica* were incubated in water containing adrenosterone (an oxidation product of 11-ketotestosterone; 5.0 mg/litre), a significant enhancement of body growth was noted, while gonads were degenerated. The growth rate of the treated fish were higher ($P<0.05$) even after five months of withdrawal of the steroid treatment (Katz et al., 1976).

Simpson et al. (1976) studied the effect of 17α-MT and ethylestrenol on the growth and food conversion in rainbow trout and salmon parr. In all the experiments with rainbow trout both steroids were effective in promoting growth rates and food conversion efficiencies. MT, however, was not effective in salmon parr while ethylestrenol was. There was an increase in the carcass

lipid content with concomitant decrease in visceral fat and weight. There was no effect on HSI, but control fish had livers and heart with fatty infiltrations and some lesions were also found in the kidneys. The treated fish on the other hand exhibited liver without any fat in them and no lesion in the kidneys were found, also, the musculature of the control fish was loosly packed while ethylestrenol treated fish had densly packed myofibrils and looked more likely of the wild fish.

The effect of two anabolic-androgenic (dimethazine and norethandrolone) steroids on the growth and protein metabolism was studied by Matty and Cheema (1978) in rainbow trout. When given in the diet at the level of 2.5 and 5.0 mg/kg food for sixty days both steroids significantly increased weight gain. This increase in weight gain was due to both the increase in protein synthesis and improved food conversion efficiency. Higher doses of dimethazine induced an initial growth response which was subsided after forty-eight days. No change in the HSI was noted, but RSI in dimethazine treated fish was higher than the controls. Recently Yu et al,(1979) have reported the growth stimulant effects of MT in Coho salmon.

In a study on the combinations of different hormones on the growth of Coho salmon, MT was studied in combination with growth hormone (GH) or thyroxine (T4) or with both (Higgs et al, 1977). All the hormones when given to fish

(GH and T4 by intramuscular injections and MT *per os*) singly or in combination with other hormone at 10°C increased growth rate. The sequence noted for growth was as follows: (GH+MT+T4) > (GH+MT) > (GH+T4) > (GH) > (MT+T4) > (MT) > (T4) > Controls. (GH+MT+T4); (GH+MT); (MT+T4) acted additively. Significant differences in the condition factor, water (GH), lipid (T4; T4+MT; GH+MT) were also recorded. Other changes in thyroid, pancreas, ovaries, testes and interrenal tissues were also noted. Donaldson et al. (1979) have recently summarised the studies on the hormonal control of growth in fish.

It then appears from the above literature that anabolic-androgenic steroids have a future in aquaculture for increased growth rates, food conversion efficiency and other carcass changes which are beneficial for both the fish farmer (increased production capacity) and consumer (competetive prices and probably better meat quality).

2.13. The Role of Anabolic-Androgenic Steroids in Sex-Reversal and Mono-Sex Culture of Fish

Two very obvious important benefits of the role of sex steroids in sex reversal (sex inversion is a better term; Reinboth, 1970; Mittwoch, 1975) in aquacultural management can be seen in the culture practice of tilapia and salmonids, trout and salmon). To maximise the yield of harvestable sized fish, tilapia culturists have laboriously hand-sexed

and stocked monosex genetic males instead of bisexual or all female groups, because of higher growth rates of males and that female starts spawning when they are still small, resulting into overcrowded ponds and stunted growth.

On the other hand, the processes of sexual maturation in salmonids are associated with losses in food conversion, a deterioration of flesh quality and an increased susceptibility to bacterial and fungal invasion. In later stages of sexual maturation, salmonids suffer changes in skin and muscle pigmentation which reduce their market acceptability. In rainbow trout, the male mature in second year while female in third year of their lives. A change of sex ratio of the population in favour of female (delayed maturity) will be more economical for the farmer because food energy will be channelised more towards somatic growth rather than the sexual products.

The process by which sex is determined has a genetic basis in all vertebrate groups, which ultimately depends upon the chromosomal arrangements. In some fishes, distinct sex chromosomes are not present, instead some part of chromosomes (autosomes) are linked with sexual differentiation and colour pattern (Mittwoch, 1975). The details of the sex determination mechanisms and natural sex reversal can be found in earlier reviews (Dodd, 1960b; Atz, 1964; Yamamoto, 1969; Chan, 1970; Chan et al, 1975; Reinboth, 1970; Vanyakina, 1972; Harrington, 1971, 1974,

1975; Schreck, 1974; Schreibman and Kallman, 1978).

There are two basic approaches to have a mono-sex culture of a given species. Firstly, by inverting the genetic sex of the individual and then mating it with normal fish of opposite sex to get a mono sex population of the desired sex depending upon the sex determination mechanism of the particular species. Secondly, by direct treatment of the population with the appropriate steroid to manipulate the sex and to get a fast growing standing crop for marketing. Both of these techniques have been tried recently involving tilapia, salmonids and cyprinodonts using either estrogens (Nakamura and Takahashi, 1973; Takahashi, 1975a; Johnstone et al, 1978; Shelton et al, 1978; Jensen and Shelton, 1979) or androgens (Clemens et al., 1966; Clemens and Islee, 1968; Takahashi, 1974, 1975b, 1975c, 1975d; Guerrero, 1975; Nakamura, 1975; Anon., 1976; Yamazaki, 1976; Anderson and Smitherman, 1978; Johnstone et al., 1978; Shelton et al., 1978; Tayaman and Shelton, 1978; Okada et al, 1979). These references should be consulted for details of the different processes involved in these studies.

2.14. Side-Effects Induced by Anabolic-Androgenic Steroids in Fish

A lot of reports have been published on the effect of sex steroids on the different tissues of the fish. These reports have evolved essentially from two types of studies. Firstly,

the observations made on the samples while studying the growth of the gonads and the role of the steroids in it. Secondly, there are observations made mainly on gonads, liver, kidney and skin in order to study the side effects of growth promoting steroids. In the short summary that follows, only those studies will be reported which report the side effects of the doses of steroids employed to study the growth phenomenon with some reference to the key studies from gonadal development.

2.14.1. Liver

In rainbow trout, 4-chlorotestosterone showed an increasing tendency in HSI. This tendency seemed to be related to hypertrophy of the liver cells which were laden with glycogen (Hirose and Hibiya, 1968a, 1968b). McBride and Van Overbeeke (1971) showed that 11-ketotestosterone and 17α-MT induced degenerative changes in livers of treated fish (*Oncorhynchus nerka*), which according to authors resembled changes in sexually maturing pacific salmon, reported by Robertson and Wexler (1960).

Anabolic-androgenic steroids (Stanozolol and ethylesternol) did not bring any changes in the livers of goldfish and salmon (Bulkley and Swihart, 1973; Simpson, 1976). Similarly, no changes in HSI have been reported in rainbow trout fed dimethazine and norethandrolone for sixty days (Matty and Cheema, 1978).

2.14.2. Kidney

In mammals, apart from the secondary sexual organs, the kidney is also considered to be a "target" organ for anabolic-androgenic steroids (Kochakian, 1976, 1977). In fish, the most conspicuous effects of 11-ketotestosterone and 17α-MT were glomerular dilation accompanied by sclerosis of glomerular capilleries and a thickening of Bowman's capsule (McBride and Van Overbeeke, 1971). Hirose and Hibiya (1968a, 1968b) also reported hypertrophied and degenerated kidneys after treatment with 4-chlorotestosterone, the effect was more marked in higher doses of the steroid. Stanozolol also induced renal hypertrophy in goldfish (Bulkley and Swihart, 1973). The hypertrophy of the kidney has also been reported in *Salmo trutta* (testosterone treatment) and *Cottus hangiongensis* during spawning period in Male (Ashby, 1957; Goto, 1974). Mourier (1970, 1972, 1976a, 1976b) studied the effect of androgens and antiandrogen (Cyproterone acetate) on the kidney of three spined stickleback. By studying the ultrastructure he showed that kidney hypertrophied after treatment with sex hormones and this hypertrophy is related with nest building behaviour of this species. Cyproterone acetate inhibited this response within fourteeen days while castration inhibited the kidney in seven days. Recently, Matty and Cheema (1978) has described the renotrophic responses to dimethazine in rainbow trout.

2.14.3. Skin

Changes in skin colouration and texture with gonadal maturation in teleosts have been observed quite frequently and associated with the variations in the levels of endogenous gonadal hormones. Fagerlund and Donaldson (1969) observed changes in external colouration of castrated sockeye salmon, treated with 11-ketotestosterone or 17α-MT. Effect of these steroids was studied by McBride and Van Overbeeke (1971) and marked changes in the epidermal region of the skin of gonadectomized sockeye salmon were noted. The effect of these hormones on the skin appear to be a direct one rather than mediated by any other organ of the body. Similar results have been reported with other androgenic hormones by Yamazaki (1972, 1976).

2.14.4. Gonads

It is not possible to review the voluminous literature concerned with the effect of sex steroids on the testes and ovaries in fish. Detailed and excellent reviews are available on the subject for consultation (Dodd, 1955, 196Oa, 1972, 1975; Dodd and Wiebe, 1968; Hoar, 1955, 1957, 1965, 1969; Barr, 1968; Lofts, 1968; Lofts and Bern, 1972; Chester-Jones et al., 1972; Donaldson, 1973; DeVlaming, 1974).

CHAPTER THREE

3.1. Materials and Methods

3.1.1. Fish and Quarantine Procedure

The fish used in the present study were common carp (mirror carp variety of *Cyprinus carpio L*) and were obtained from Cleaves Farm, Yorkshire (used in experiments with methyltestosterone) and Cotswald Carp Farm, Burton-on-the-Water, Gloucestershire (all other drugs except methyltestosterone). The fish from the farms were brought directly to a stocking tank in the quarantine area, which was sterilised beforehand and situated in the main fish culture unit of the department. All the equipment used in this room was also sterilised.

On arrival into the quarantine area, the fish were fed for the first seven days on a food containing antibiotic to eliminate the possibility of any disease. After this time the fish were fed a normal diet.

To eliminate any external parasites, the fish were bathed in one hundred and fifty parts per million (ppm) formalin for one hour, after a week of their arrival into the facility. Three days after, they were further treated twice on successive days in 2ppm of malachite green. Fish were kept in quarantine for three weeks, during which time they were checked for any external parasites or any other signs of abnormality.

When the fish came out of quarantine, they were transferred to a hot room, where all the experiments were carried out. In the hot room the temperature was raised 3°C per day, while the fish were in a stocking tank with recirculating water. They were kept there for a fortnight (at least one week after the required temperature of the experiment was reached). After this time the fish were transferred to experimental tanks.

3.1.2. Experimental Tanks

The tanks used in the study were all glass aquaria of forty litres capacity with twenty-five fish in them. Tap water (chemical analysis given in Table 1) was used throughout the study period. The fish were kept at a constant temperature of 25±2.0°C and photoperiod was fixed at twelve hours day and twelve hours night. For light fluorescent tubes were used.

In order to avoid the accumulation of the steroid metabolites, which might have had an effect (deleterious or beneficial) on growth, and to avoid any possible breakdown of the steroid given, due to the bacterial action (Ashby, 1957), the tanks were cleaned daily and filled up to the required mark with water of the same temperature from a header tank.

TABLE 1 ANALYSIS OF TAP WATER USED IN THE PRESENT STUDY

pH	7.0	
O_2	8	ppm
Na^+	6	ppm
K^+	1	ppm
Ca^{++}	8	ppm
Mg^{++}	1	ppm
Mn^{++}	<0.1	ppm
Fe^{+++}	<0.1	ppm
Cl^-	10	ppm
NO_3^{2-}	2	ppm
SO_4^{2-}	4	ppm
PO_4^{3-}	0.5	ppm
$(NH_4)^+$	0.05	ppm

3.1.3. Stock Diet

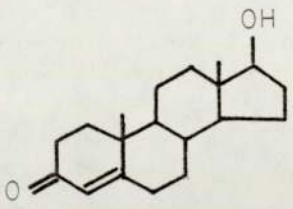
Omega trout pellets (No.3; Edward Baker Limited, Suffolk) were used as stock diet. Huisman (1976) has shown that carp can easily adapt the trout diets with no apparent effect on their growth. In fact the protein content of this diet was higher than the optimal requirements of carp for protein (Ogino and Saito, 1970; Ogino et al., 1976; Sin, 1973a, 1973b). The proximate composition of the diet was as follows:

Protein	=	50.0%
Lipid	=	8.5%
Moisture	=	8.0%
Fiber	=	2.5%
Ash	=	10.0%
Nitrogen Free Extracts	=	20.5%

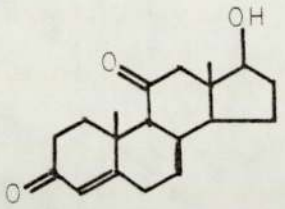
3.1.4. The Drugs

Eight different anabolic-androgenic and an antiandrogen (Cyproterone acetate) were used in growth evaluation experiments either alone or in combinations (one experiment with three drugs). The formulae of these drugs are shown in Figure 3. These include:

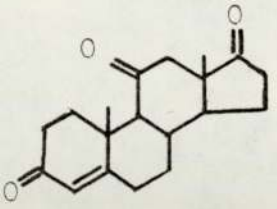
1. TESTOSTERONE: 17β-hydroxyandrost-4-en-3-one
2. 11-KETOTESTOSTERONE: 17β-hydroxyandrost-4-en-3,11-dione
3. ADRENOSTERONE: 4-androsten-3,11,17-trione
4. METHYLTESTOSTERONE: 17α-methyl-17β-hydroxyandrost-4-en-3-one



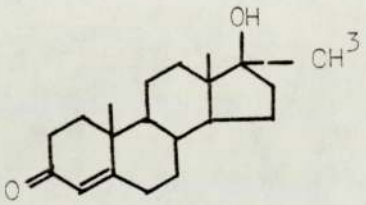
Testosterone
(Sigma)



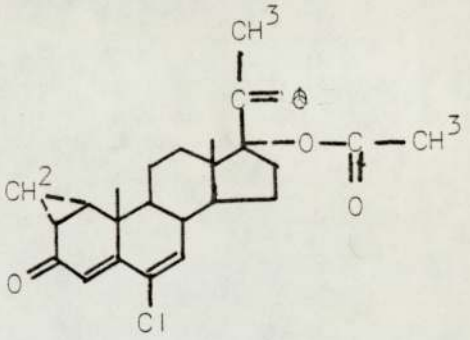
11-Ketotestosterone
(Sigma, USA)



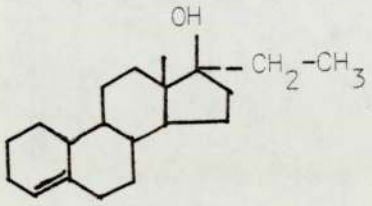
Adrenosterone
(Sigma)



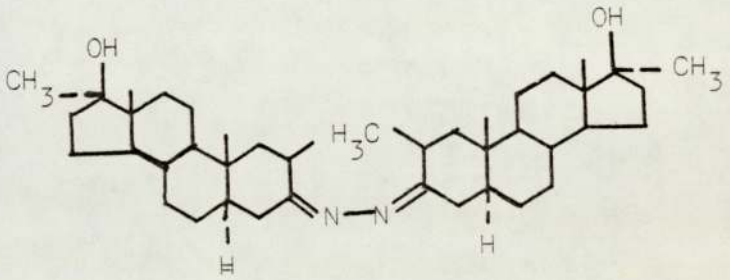
17α-methyltestosterone
(Sigma)



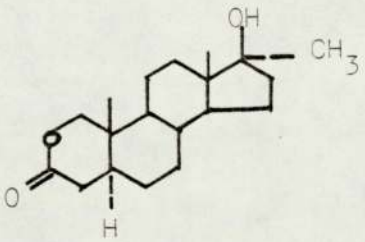
Cyproterone Acetate
(Schering, West Germany)



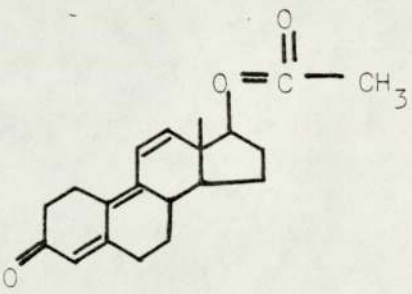
Ethylestrenol
(Maxibolin)
(Organon, USA)



Dimethazine
(Roxilon) (Richter, Italy)



Anavar
(Oxandrolone)
(Searle, USA)



Trenbolone Acetate
(Finaplix)
(Roussel Uclaf)

FIGURE 3 Formulae of the drugs used in the present study

5. DIMETHAZINE: 2α,17α-dimethyl-17β-hydroxy-5α-androsten-3,3'-azine
6. OXANDROLONE: 17α-methyl-17β-hydroxy-2-oxa-5α-androstan-3-one
7. ETHYLESTRENOL: 17α-ethyl-19-norandrost-4-en-17β-ol
8. TRENBOLONE ACETATE: 17β-acetoxy-3-oxoestra-4,9,11-triene
9. CYPROTERONE ACETATE: 1α,2α-methylene-6-Chloro-Δ^4-6-pregnadiene-17α-ol-3,20-dione-17α-acetate

There were two major reasons for selecting these drugs. Firstly, the steroids used in these studies are either natural steroids, normally present in the body of the fish or synthetic steroids, actively used in clinical practice or livestock production. Secondly, these steroids have been used in other fish (mainly salmonids) for growth experiments, except in case of oxandrolone, trenbolone acetate, and cyproterone acetate, so that some data is available for comparison purposes.

Testosterone, 11-ketotestosterone, adrenosterone and methyltestosterone were purchased from Sigma, St. Louis. While dimethazine, oxandrolone, ethylestrenol, trenbolone acetate and cyproterone acetate were gifts from Ormonoterapia Richter, Italy; Searle, USA; Organon, USA; Roussel Uclaf, France; and Schering, Germany, respectively.

3.1.5 Experimental Diets

All hormones given in Section 3.1.4. were administered orally with the food. The steroids were incorporated in the diet as follows: 50mg of the hormone was dissolved in 50ml of absolute ethanol. The concentration of this stock solution was so adjusted that the required amount was contained in 10ml of absolute ethanol. This solution was then evenly sprayed with a chromatographic sprayer over 500g of the food pellets. The pellets were thoroughly mixed during and after the spray and left at room temperature for one or two hours for ethanol evaporation. Except for the exclusion of the hormone, an identical procedure was followed for the control diets. The diets were stored at 4°C, required amounts were removed daily and after they came to room temperature were fed to the fish.

3.1.6. Concentration of the Drugs and Feeding Rates

Throughout the course of this study the concentration of the drugs given to the fish were kept constant for all the drugs. The fish received 0.00, 1.0, 2.5, 5.0, and 10.0 mg/kg (ppm) of the drug in the food.

Fish were fed at a rate of either 8% (only for methyltestosterone) or 5% (rest of the drugs) of their body weights daily. The 5% ration level has also been recommended by Jauncey (1979) for mirror carp at temperatures between 25-28°C. This 5% or 8% ration was divided into three equal parts and was fed three times a day between 0900

hours and 1900 hours by hand over a period of fifteen to twenty minutes to ensure the maximum utilisation of the food and that hormone may not be dissolved in the water.

Fish were fed six days a week and no food was given on the day of weighing. The hormone supplemented drugs were administered for sixty days (all drugs except methyltestosterone) or ninety days (only for methyltestosterone) to all experimental groups and after that period they were placed on normal diets for a further thirty days (all drugs except methyltestosterone) or sixty days (only for methyltestosterone) to examine the effect of hormone withdrawal. All other conditions remained the same.

3.1.7. Weighing and Measuring Procedures

After every fifteen days of the drug fed, the fish were anaesthetised with either MS-222 (tricaine methane-sulphonate, Sandoz, 40mg/l) or Benzocaine (ethyl-4-aminobenzoate, BDH, 40mg/l) and were weighed individually to the nearest 0.01g on a DSG-200 (International Electronics Limited, Lancashire) digital balance, after removal of excess water by an absorbant paper towel. Total lengths were also measured to the nearest 0.1cm. After every weighing the ration levels were adjusted according to the new mean weight of each tank of fish.

3.1.8. Biochemical Procedures

After sixty or ninety days of the hormone feeding when the

experimental groups were returned to the normal diet, to observe the hormone withdrawal phenomenon, samples of fish were taken from each group for the determination of total proteins and nucleic acids (RNA, DNA) in the brain, kidney, liver and muscle. Cholesterol in these tissues was determined only in methyltestosterone treated fish. In addition, muscle water, muscle fat, crude proteins (total nitrogen x 6.25) and ash content were also determined. A second sample was also taken when the experiments were terminated and analysed for the contents outlined above. In addition free (only in methyltestosterone treated fish) and protein bound amino acids in muscle tissue from ethylestrenol treated fish were also determined. In one experiment (one hundred and twenty days) total plasma proteins, glucose and free amino acids were also determined. Different methods employed for these determinations are given below.

3.1.8.1. Nucleic Acids

The extraction of nucleic acids from different tissues of animal is a difficult task, and differs from animal to animal. Munro and Fleck (1966) have summarised these problems encountered in determination of total nucleic acids in different tissues of the body.

In the present study the methods proposed by Wannemacher et al. (1965), Shibko et al. (1967) and Munro and Fleck

(1969) were tried using different tissues of carp. Significantly different values were obtained for the same tissue when analysed at the same time by the above methods, for example, very high values for DNA were observed using ultraviolet (UV) technique, but when the same fraction was determined by colour methods of Ceriotti (1952) using 0.04% indole or by Burton's (1956) diphenylamine method, less than 25% of UV values were observed, showing RNA and probably proteins were interfering in UV determinations.

Due to the above problems, a method was evolved from the existing methods by compromising or altering certain conditions or concentrations of the reagents to suit the needs of the present study. Details of this method are given below.

A suitable amount of tissue was homogenised in 5ml of ice cold distilled water using an all glass homogeniser. To 1ml of this homogenate were added 8ml of 10% (v/v) perchloric acid (PCA). The precipitate (ppt) formed was separated from the supernatant by centrifugation at 5000 revolutions per minute (rpm). The ppt was subsequently washed twice with 5ml portions of 10% PCA and centrifuged. The supernatants were discarded and ppt was drained by inverting the test tubes on a tissue paper for about 15-30 seconds. While the ppt was still moist 4ml of 0.3N NaOH were added and the samples were incubated for sixty

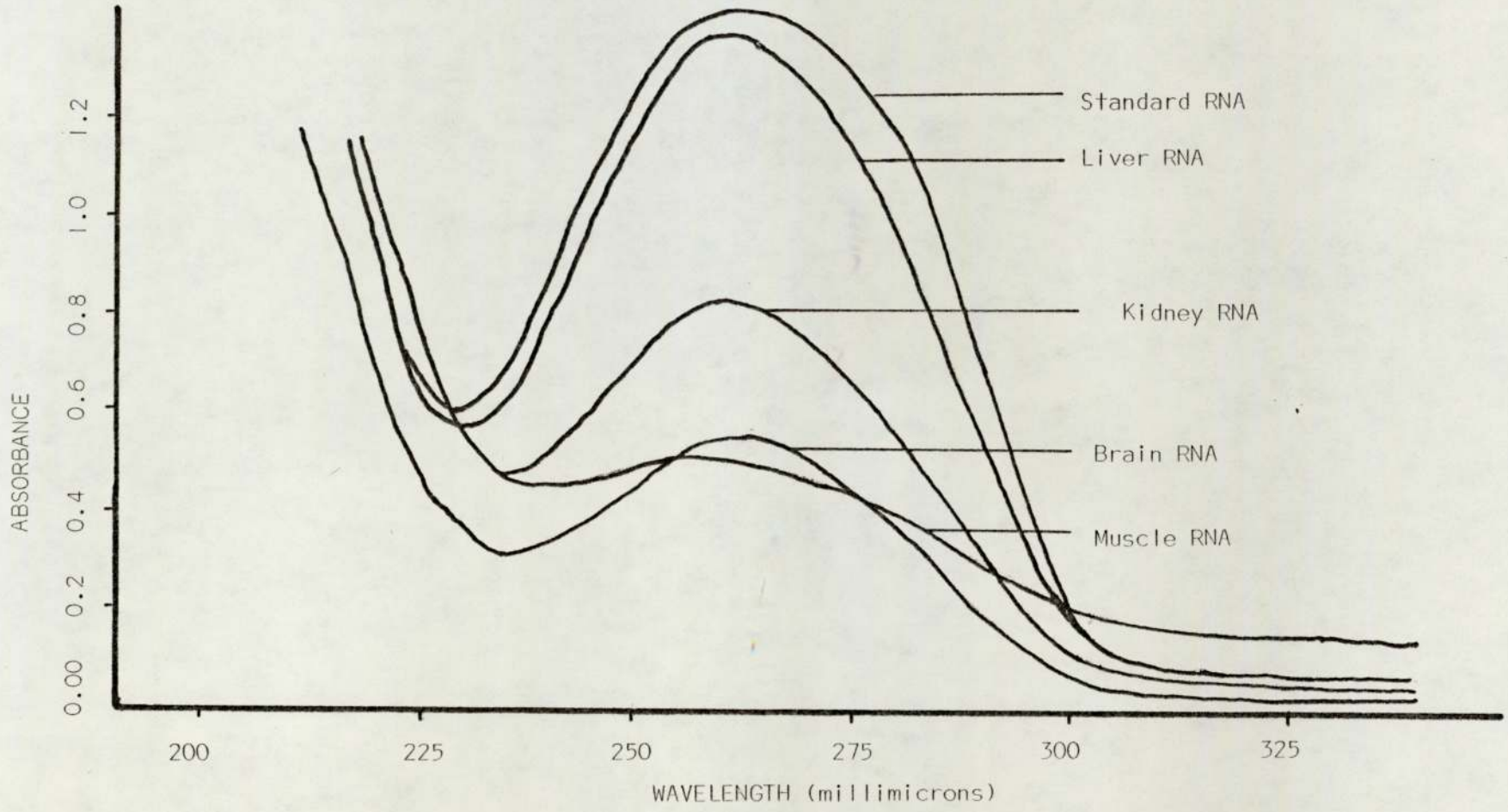


FIGURE 4 Absorption spectrum of RNA extracted from different tissues of carp with standard RNA (40 µg/ml)

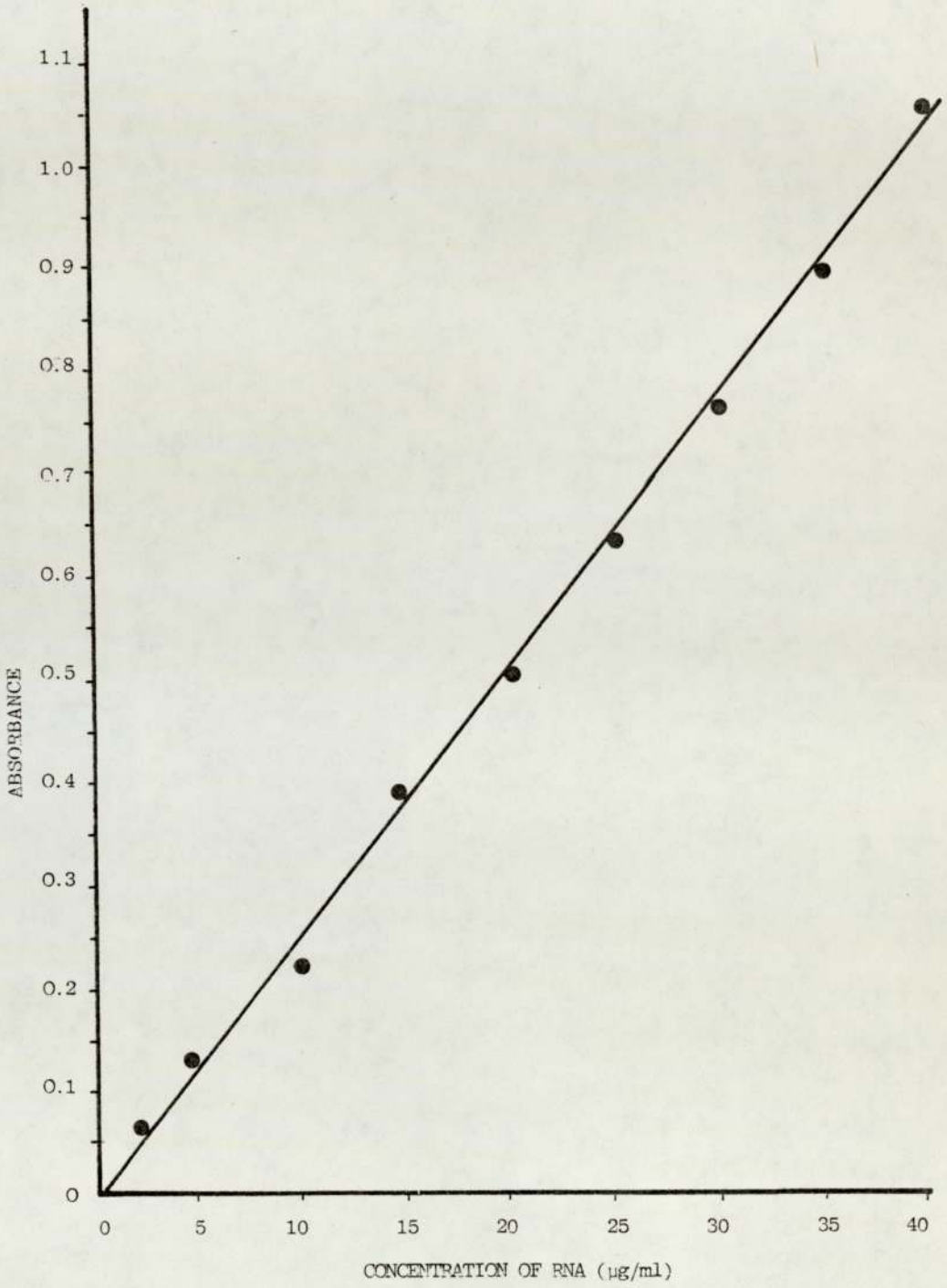


FIGURE 5 STANDARD CURVE OF RNA

minutes at 37±1°C in a constant temperature water bath, with occasional shaking of the contents with a glass rod. The NaOH-hydrolyzate was cooled in ice and divided into two parts of 2ml each. These portions were used for protein as well as for RNA and DNA estimations.

RNA and DNA were separated from 2ml of the hydrolyzate by adding 1ml of 60% (w/v) solution of PCA. All the samples were ice-cooled for fifteen minutes and then centrifuged for fifteen minutes at 4°C. The supernatant and two washings of the ppt with 5% PCA were pooled and used for RNA determinations, while the ppt was saved for DNA determinations.

RNA concentrations were measured by appropriate diluting the supernatant and washings with 5% PCA in Beckman-DB-Spectrophotometer at 260nm. Every time when the RNA was extracted from different tissues its absorption spectrum was noted in a Pye Unicam SP-800 Spectrophotometer equipped with SP-825 programme controller to check the authenticity of the extraction. A typical spectral analysis of RNA extracted from brain, kidney, liver and muscle of carp with standard RNA (calf liver RNA, Sigma, St. Louis) is given in Figure 4. To estimate the total RNA in the sample, these readings were compared with a standard curve (Figure 5), obtained from a highly pure sample of calf liver RNA in the range of 3-30 µg RNA/ml.

The DNA was determined from the ppt by the method of

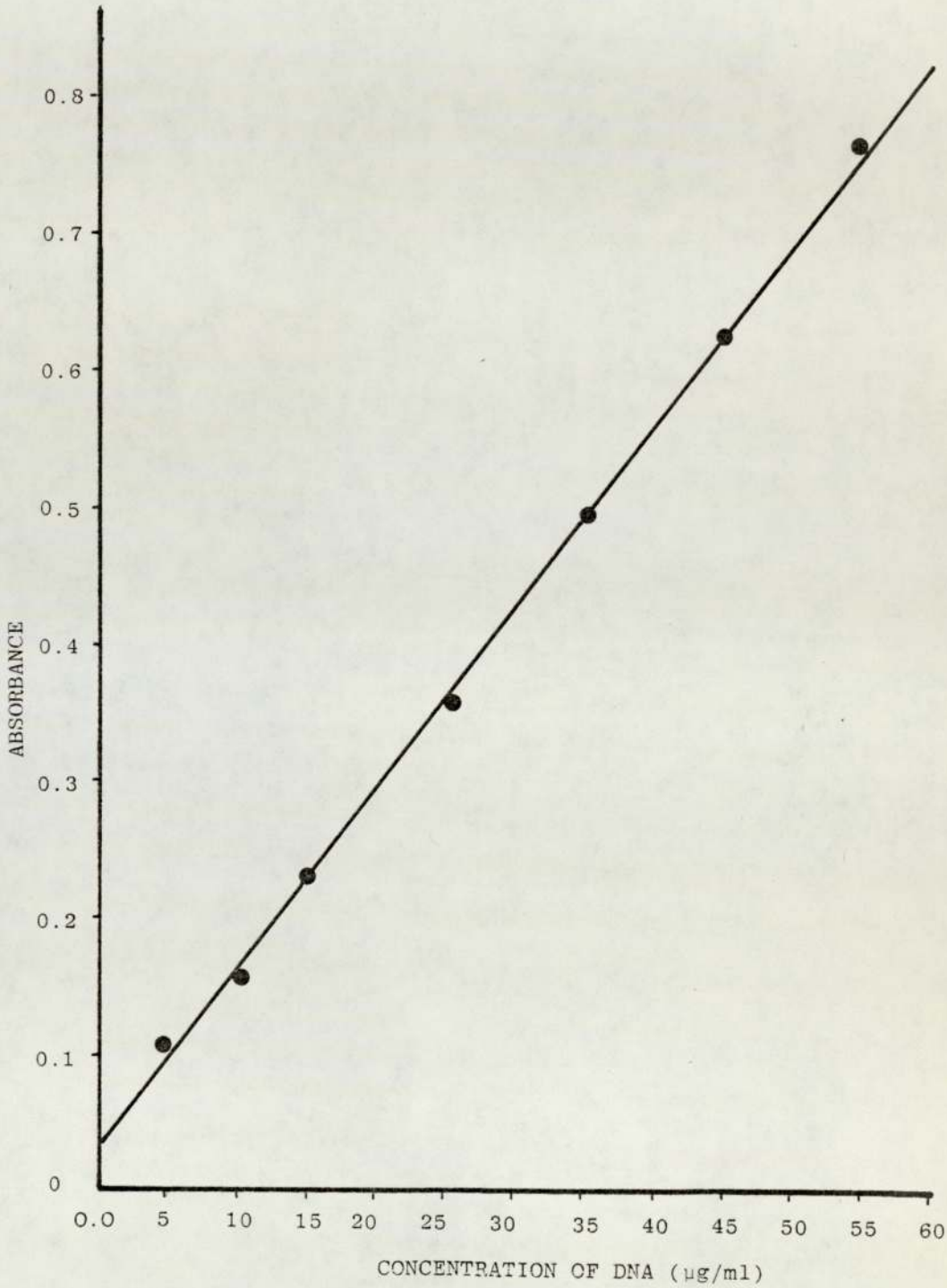
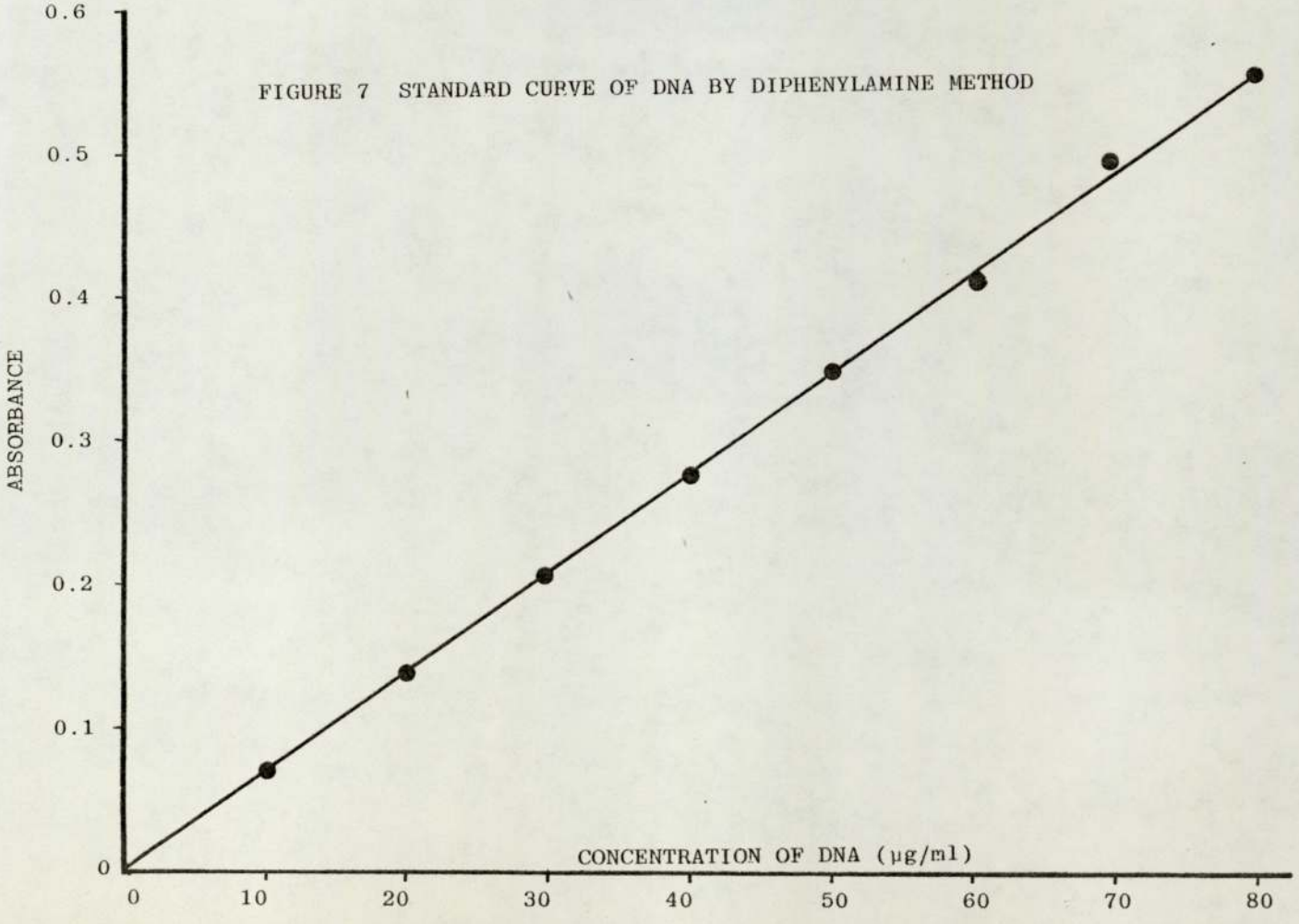


FIGURE 6 STANDARD CURVE OF DNA BY CERIOTTI METHOD

FIGURE 7 STANDARD CURVE OF DNA BY DIPHENYLAMINE METHOD



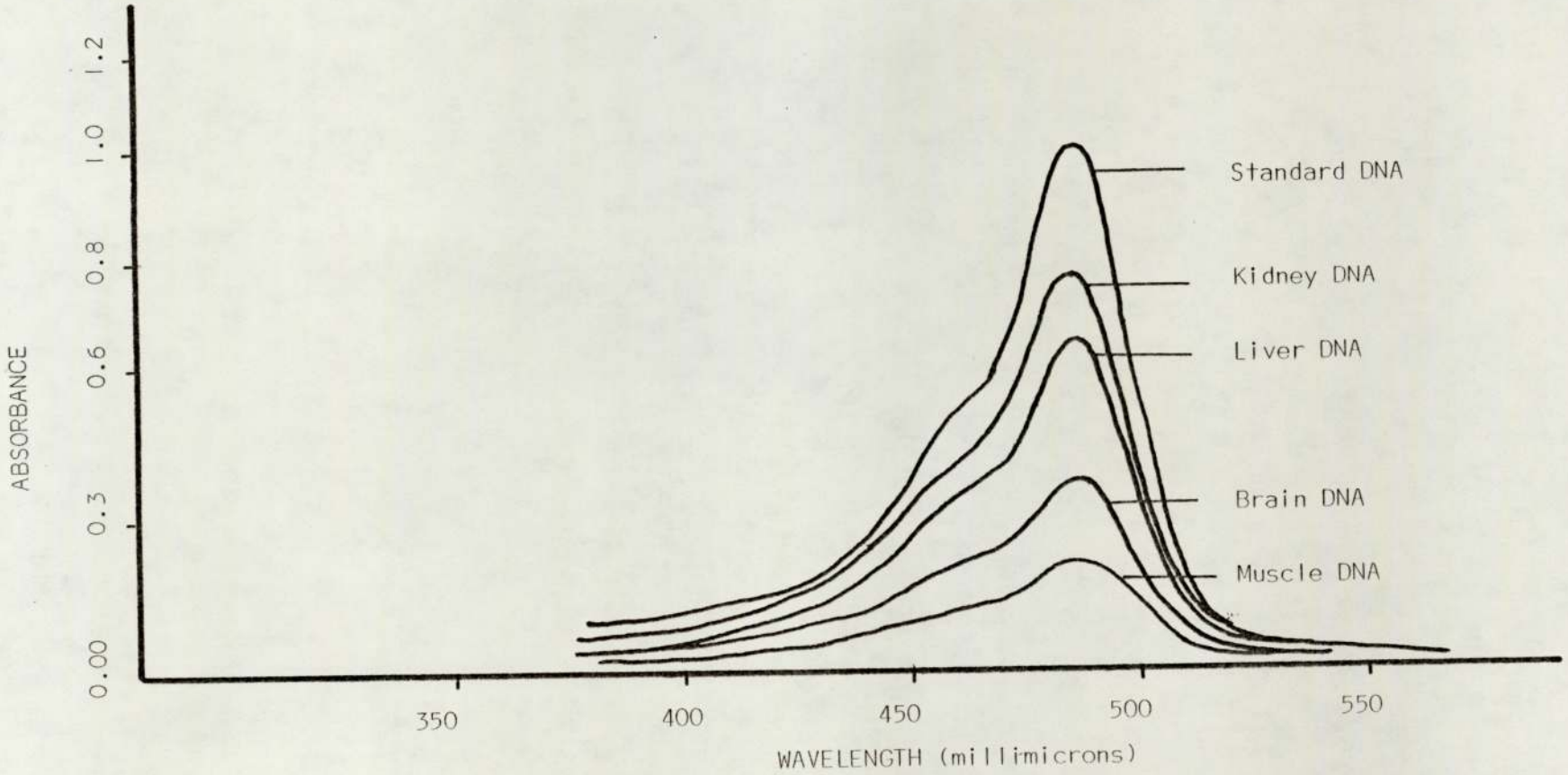


FIGURE 8 Absorption spectrum of DNA extracted from different tissues of carp with standard DNA

Ceriotti (1952) as described by Munro and Fleck (1969) and sometimes double checked by the diphenylamine method (Burton, 1956). The standard curve for these methods and a spectral analysis for the colour developed by DNA with O.O4% indole are given in Figures 6, 7, and 8.

3.1.8.2. Total Proteins

Total proteins were determined in tissues on one ml of the hydrolyzate obtained during nucleic acid determination (Section 3.1.8.1). The hydrolyzate was diluted twenty-five to fifty times with distilled water and proteins determined by the method of Lowry et al. (1951) as modified by Miller (1959) and Schacterle and Pollack (1973). Bovine serum albumin (Sigma, St Louis) was used as standard (Standard Curve given in Figure 9).

3.1.8.3. Amino Acids

In some experiments, free as well as protein bound amino acids were also determined in the muscle. For determination of the amino acids, the homogenate prepared in the Section 3.1.8.1. for nucleic acids and protein determinations were used. For free amino acids, to the 100 µl of the homogenate 1.1 ml of 3% sulphosalicylic acid was added. After some time, the ppt was separated by centrifuging the contents at 1500 g for fifteen minutes. 500 µl of the supernatant was loaded on the column of a Locarate amino acid analyser for ion exchange chromatography.

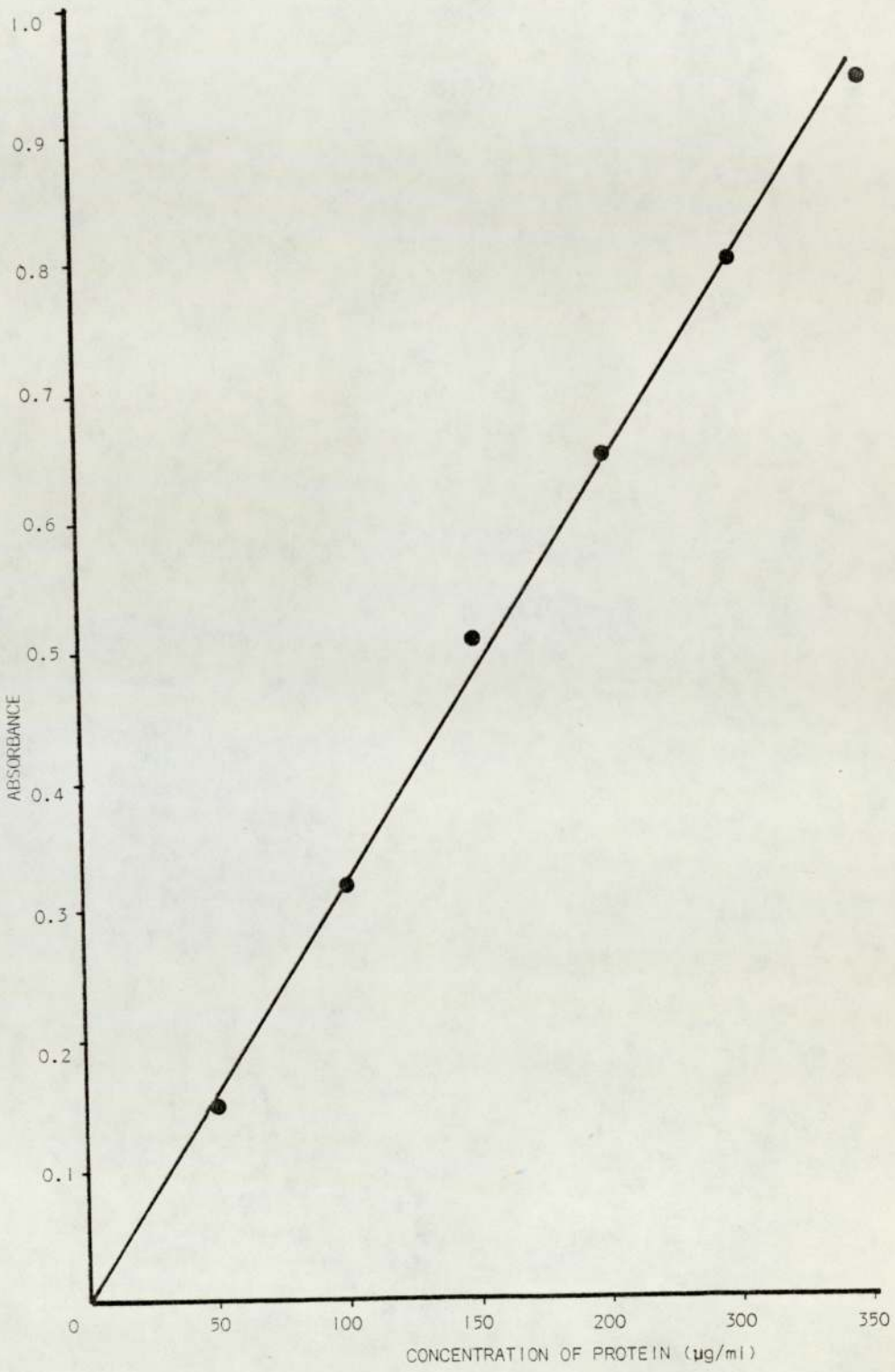


FIGURE 9 Standard Curve of Protein

For amino acids bound in the proteins of the muscle, to 1ml of the hydrolyzate 1ml of 6N HCl was added. The tubes were sealed under the atmosphere of nitrogen and placed in an oven at 110°C for twenty-four hours. After this time the tubes were removed from the oven, cooled and the contents were neutralised with 5.3ml of 2N NaOH. This neutralised hydrolyzate was then diluted with double distilled water to 50ml and centrifuged. 300µl of the supernatant was loaded on the column as in free amino acids.

The concentration of different amino acids were calculated from the chromatograms by the area method. For standards, twenty-five n moles each of the twenty amino acids were chromatographed exactly in the same way as the experimental samples.

A typical chromatograph for standard, free amino acids, protein bound amino acids and plasma free amino acids is given in Figures 10, 11, 12 and 13.

3.1.8.4. Determination of Proteases Activity of The Alimentary Canal

In the growth experiments with testosterone, 11-ketotestosterone and adrenosterone after sixty and ninety days the proteases activity of the alimentary canal was also determined. Due to the smaller size of the fish, the alimentary canals of four fish from sixty days group

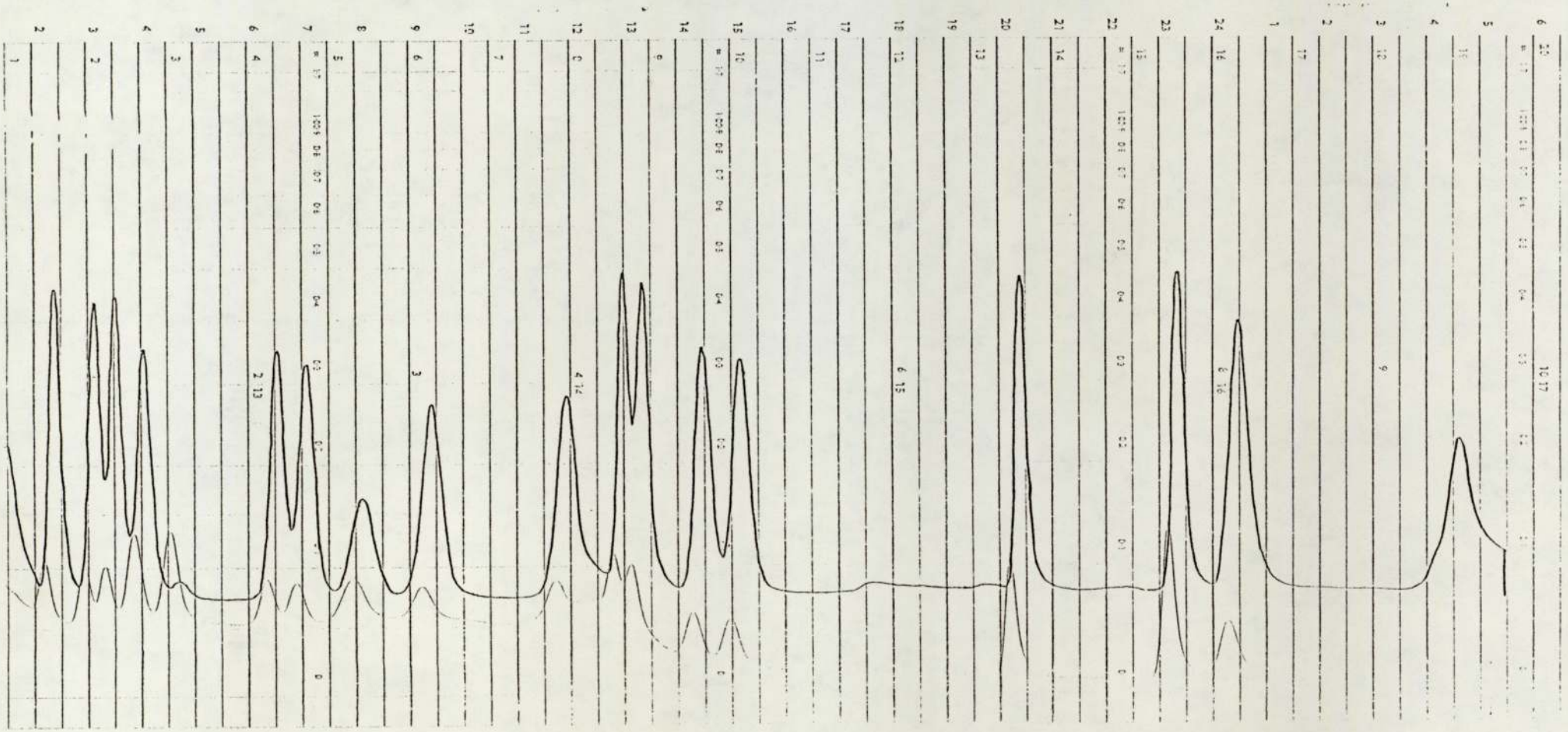


FIGURE 10 Chromatograph of standard amino acids

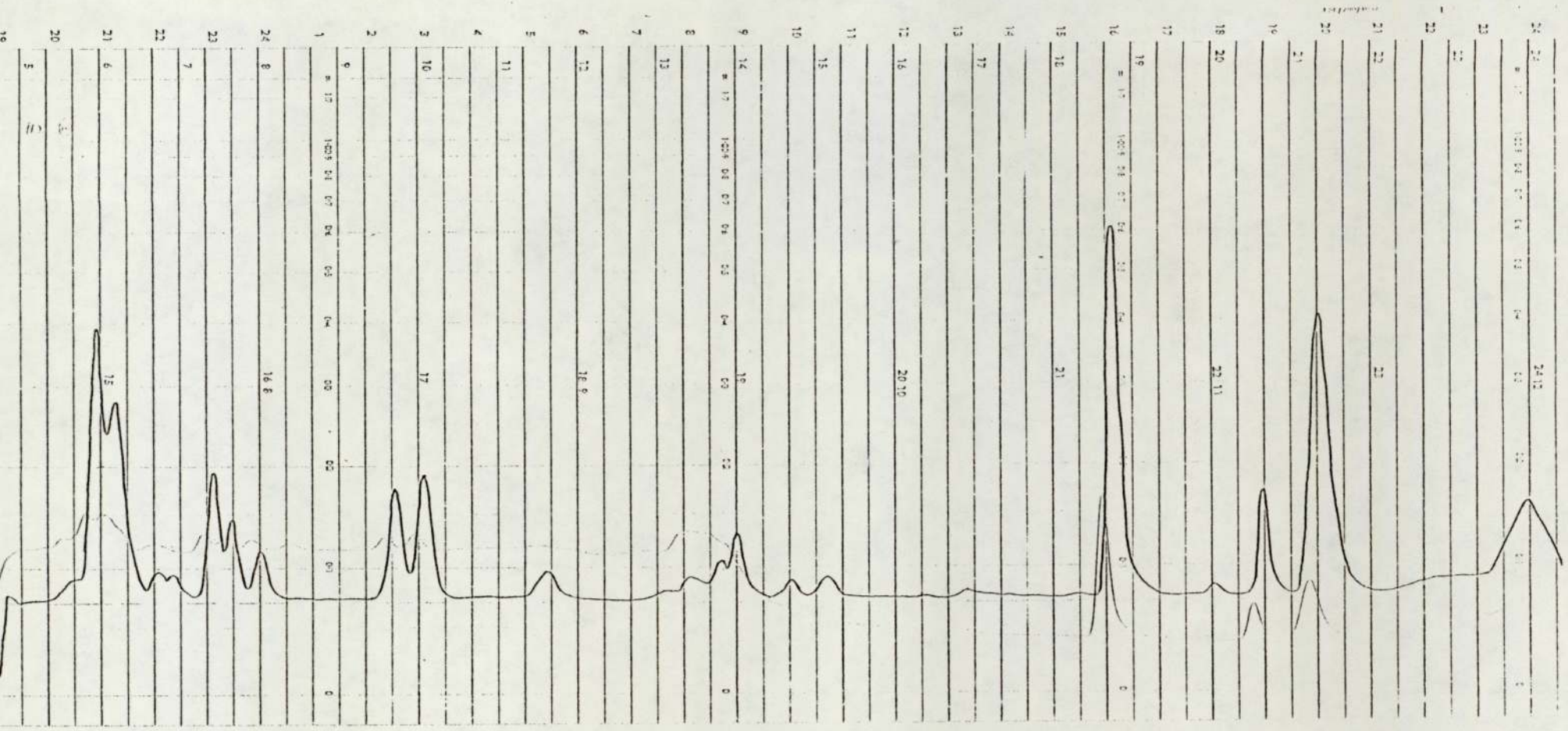


FIGURE 11 A typical chromatograph of free amino acids of muscle of carp.

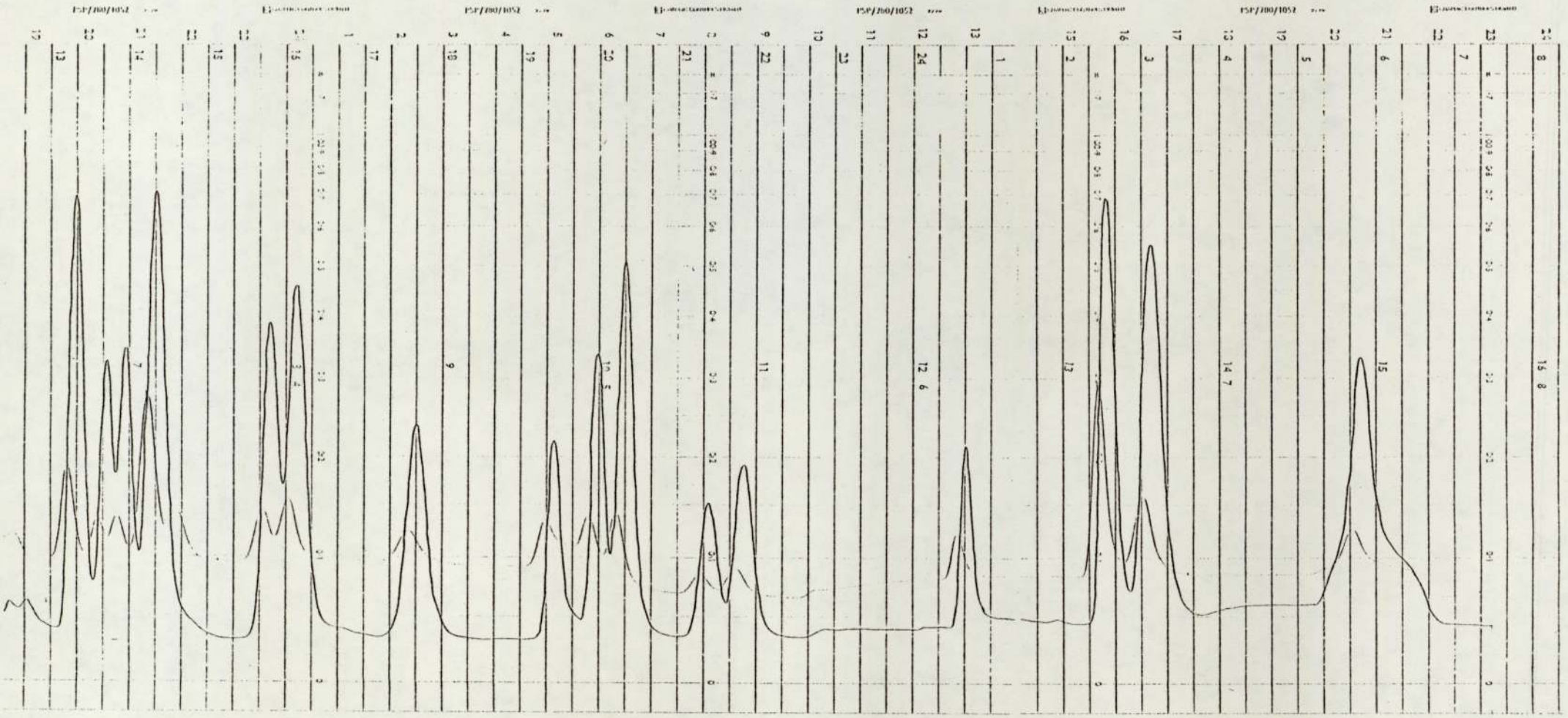


FIGURE 12 Protein Bound Amino Acids of Muscle of Carp

A typical chromatograph

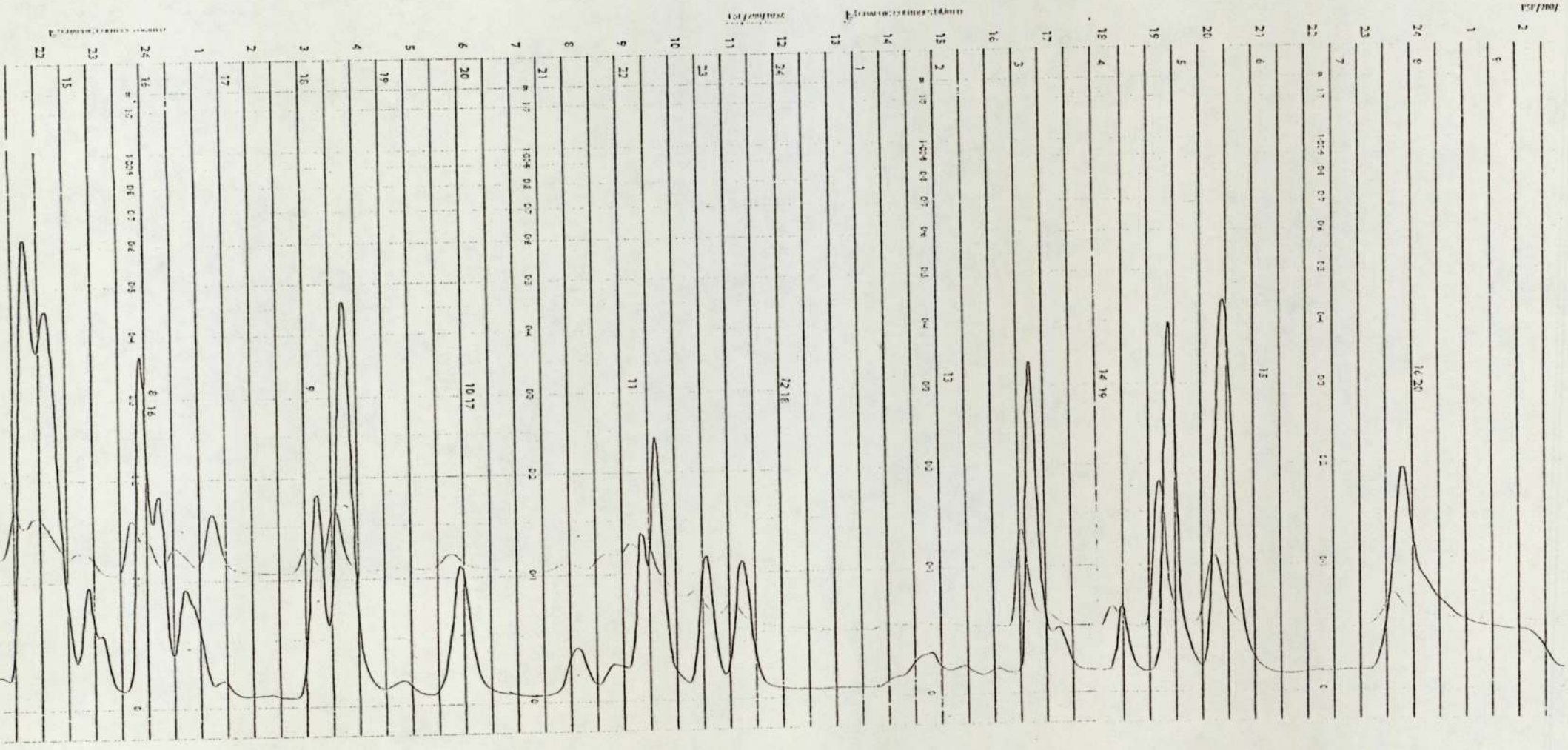


FIGURE 13 Plasma free amino acids of carp.
A typical chromatograph

were pooled for determination of proteases activity. While at ninety days alimentary canal from each fish was treated separately. The alimentary canals were washed free of their contents with phosphate buffer (pH 7.5; 0.1M) and homogenised with five volumes of the same buffer and then centrifuged for twenty minutes at 10,000 rpm. The proteolytic activity of the supernatants was determined by incubating a reaction mixture as follows:

2% Casein (Vitamin free, Sigma) solution	0.3 ml
0.1M Phosphate Buffer (pH 7.5)	0.6 ml
Enzyme Preparation (10,000 rpm supernatant)	0.1 ml

The incubations were performed at 30°C from zero to thirty minutes on three replicates from the same enzyme preparations. The reaction was stopped after every ten minutes by addition of 10% PCA. The amount of hydrolytic products in supernatant were measured by the method described in Section 3.1.8.2. Only those incubations were included in the data which gave a linear plot on three consecutive determinations. Total protein content of the enzyme preparation was also determined by the method described in Section 3.1.8.2.

3.1.8.5. Cholesterol and Glucose

Cholesterol was determined in tissues of methyltestosterone treated fish only and was estimated by the method of Bowman and Wolf (1962).

Plasma glucose was determined in one experiment only and was estimated on Beckman autoglucose analyser by the glucose oxidase method.

3.1.8.6. Proximate Muscle Composition

3.1.8.6.1. Moisture

Moisture content was determined by drying the samples in an oven at 105°C for forty-eight hours.

3.1.8.6.2. Crude Lipid

Total lipid content of the muscle was determined by the method of Bligh and Dyer (1959) as modified by Higgs et al. (1975).

3.1.8.6.3. Crude Proteins

Crude protein was determined by micro-Kjeldahl method for the determination of total nitrogen (A.O.A.C. 1975) and multiplying it with 6.25 to convert total nitrogen to crude protein.

3.1.8.6.4. Ash

Ash content was determined by heating samples in a muffle furnace for twenty-four hours at a temperature of 500°C.

3.1.9. Distribution and Disappearance of Radioactive Testosterone from the Blood and Tissues of Carp after Oral Administration

The fish used in this experiment were mirror carps of

59.22±3.80g weight. They were fed 10 ppm of testosterone incorporated in the diet for two months, three times a day. After this time the fish were fed diet containing ^{3}H-testosterone, 2 millicuries of 1,2,6,7-^{3}H-testosterone (The Radiochemical Centre, Amersham; Specific activity 88.5 Ci/m mole; 3.27 T Bq/m mole) and 5mg of cold testosterone (Sigma, St. Louis) were mixed in 5ml of absolute ethanol and this mixture was then sprayed over the 500g of Omega trout pellets (Edward Baker Limited, Suffolk; No. 4) as described in Section 3.1.5. The specific activity of the testosterone in solution was 867.6 decompositions per minute/ng (dpm/ng). The radioactive diet was given to the fish three times a day until fish rejected the diet. These diets were fed for twelve days. On the thirteenth day and thereafter the fish were fed normal diets without any hormone.

3.1.9.1. Fish Sampling

Four fish were taken at 0900 hours before the first meal of the day as follows: 1,2,3,4, and twelve days after the feeding of the radioactive diet had commenced and 1,3,5,7, 13 and twenty days after the withdrawal of the radioactive drug. The fish were deeply anaesthetised in a strong solution of Benzocaine (80 mg/l), weighed and slaughtered. 0.1ml of the blood was taken in a scintillation vial by directly puncturing the heart. After all the blood was drained from the fish, tissue samples were excised and weighed. Following tissues were taken in the amounts

described:

Liver	=	40-70 mg
Kidney	=	20-40 mg
Head Kidney	=	20-30 mg
Brain (mid-brain plus some parts of fore and hind brain)	=	40-70 mg
Spleen	=	25-40 mg
Heart (ventricle only)	=	25-40 mg
Gill (gill filaments and gill rackers)	=	60-120 mg
Skin (just above the lateral line and below the dorsal fin and without any fat)	=	60-120 mg
Testis	=	40-70 mg
Muscle (above the lateral line and just behind the head region)	=	100-150 mg
Blood	=	0.1 ml
Anterior Intestine (anterior most part of the alimentary canal)	=	40-70 mg
Posterior Intestine (posterior most part of the intestine, just before the cloaca including faeces, if any)	=	40-70 mg
Gall Bladder		as whole

All the tissues were sampled within thirty minutes of sacrificing the fish.

The tissues and blood samples were digested in the

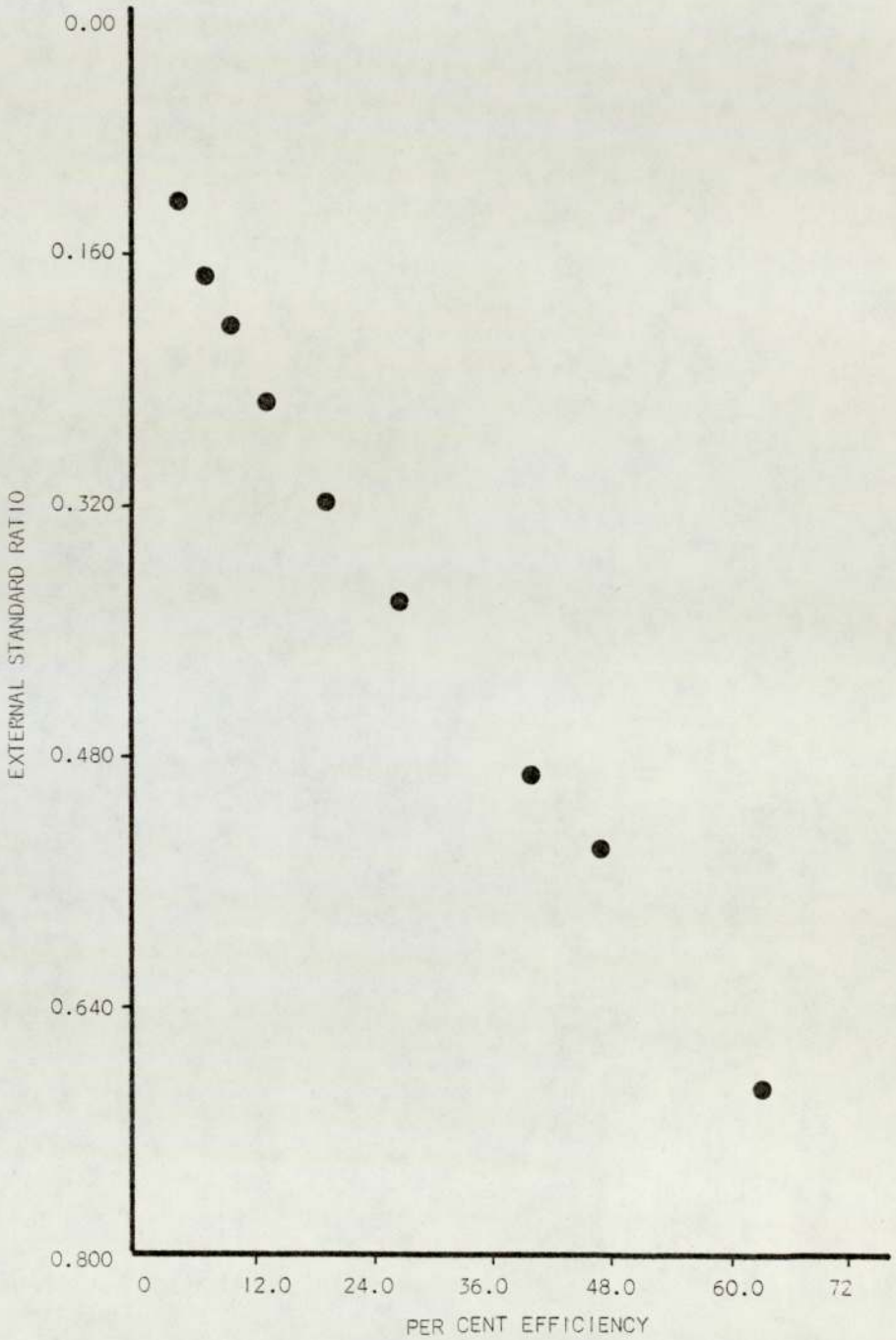


FIGURE 14 QUENCH STANDARDS FOR RADIOACTIVITY

scintillation vial with 1ml of Protosol* (Tissue solubiliser; NEN, Massachusetts) at 40±2°C for twenty-four hours in a water bath. After removing from the water bath, the tissue digests were cooled and 0.1ml of glacial acetic acid was added followed by 10ml of the toluene based premixed scintillation solution (ECONOFLUOR*;NEN Massechusetts). The vials were capped tightly and were placed in a refri-gerator overnight to supress the chemoluminescence and background counts. For blood 5ml of the above solution (11.2ml) was diluted to 10ml with scintillation cocktail. Radioactivity was determined in a Packard tricab 2660 Scintillation Counter. Quenching was determined from a quench curve (Figure 14) drawn from a set of quench standards treated exactly in the same manner as the experimental samples.

Regression line for radioactivity of tissue (dpm/g) versus time were calculated by the method of least squares. From the slopes the half life of the hormone was determined by the method of Fagerlund and McBride (1978).

3.1.10. Analysis of Experimental Data

3.1.10.1. Condition Factor

The index of fatness or leanness, the condition factor "k" was computed from the formula:

$$k = \frac{W(g)}{L(cm)^3} \times 100$$

---

* Registered Trade Mark

3.1.10.2. Specific Growth Rate

Specific growth rate for weight and length data was calculated according to the formula of Huisman (1976). The formula is given below:

$$Wt = Wo\ (1+ \alpha/100)^{t}$$ where

Wt = Weight or length at day t

Wo = Weight or length at day o

t = Number of days between t and o

α = Specific Growth Rate

3.1.10.3. Food Conversion Efficiency

Food conversion efficiency was computed from weight gain (g) to food given (g).

3.1.10.4. Tissue-Body Indices

Different tissue body indices were calculated from the formula:

$$\frac{\text{Weight of the tissue (g)}}{\text{Total Weight of the Fish (g)}} \times 100$$

3.1.10.5. Statistical Analysis

All the data were analysed by the single factor analysis of variance (ANOVA) and its priori and/or posteriori test according to Sokal and Rohlf (1969).

For all the biochemical analysis samples were taken from at least four different fish and in the case of methyltestosterone all analysis were performed on duplicate samples. In selecting the fish for analysis, care was taken

to choose the fish from each group which did not deviate in weight more than 15% from the mean of the sample.

CHAPTER FOUR

4.1. Results

4.1.1. Testosterone

4.1.1.1. Weight and Length Data

The weight and length data accumulated over a period of ninety days (sixty days of drug feeding (Phase 1) and thirty days of drug withdrawal (Phase 2) is presented in Figures 15 and 16 and Tables 2 and 3. The faster growth rate of experimental groups compared with the controls is apparent but the groups receiving 1.0 and 10.0 mg/kg (ppm) of testosterone achieved maximum weight and length after sixty days. The same pattern was seen after thirty days of the withdrawal of the drug. At sixty days the net percentage increase in weight over the controls was 54.44, 26.39, 29.72 and 52.78 respectively for 1.0, 2.5, 5.0 and 10.0 ppm groups. Similarly, these groups were 59.66, 47.06, 47.06, 41.18% longer than the controls.

The data concerning specific growth rate (SGR) is given in Table 4. Although all the experimental groups had higher growth rates than the controls during the phase 1, the SGR for weight dropped for 1.0, 2.5 and 5.0 ppm groups, while the 10.0 ppm group was still higher in SGR. The SGR for length was not changed and was higher in experimental groups both in phase 1 and phase 2 of the experiment (Table 4).

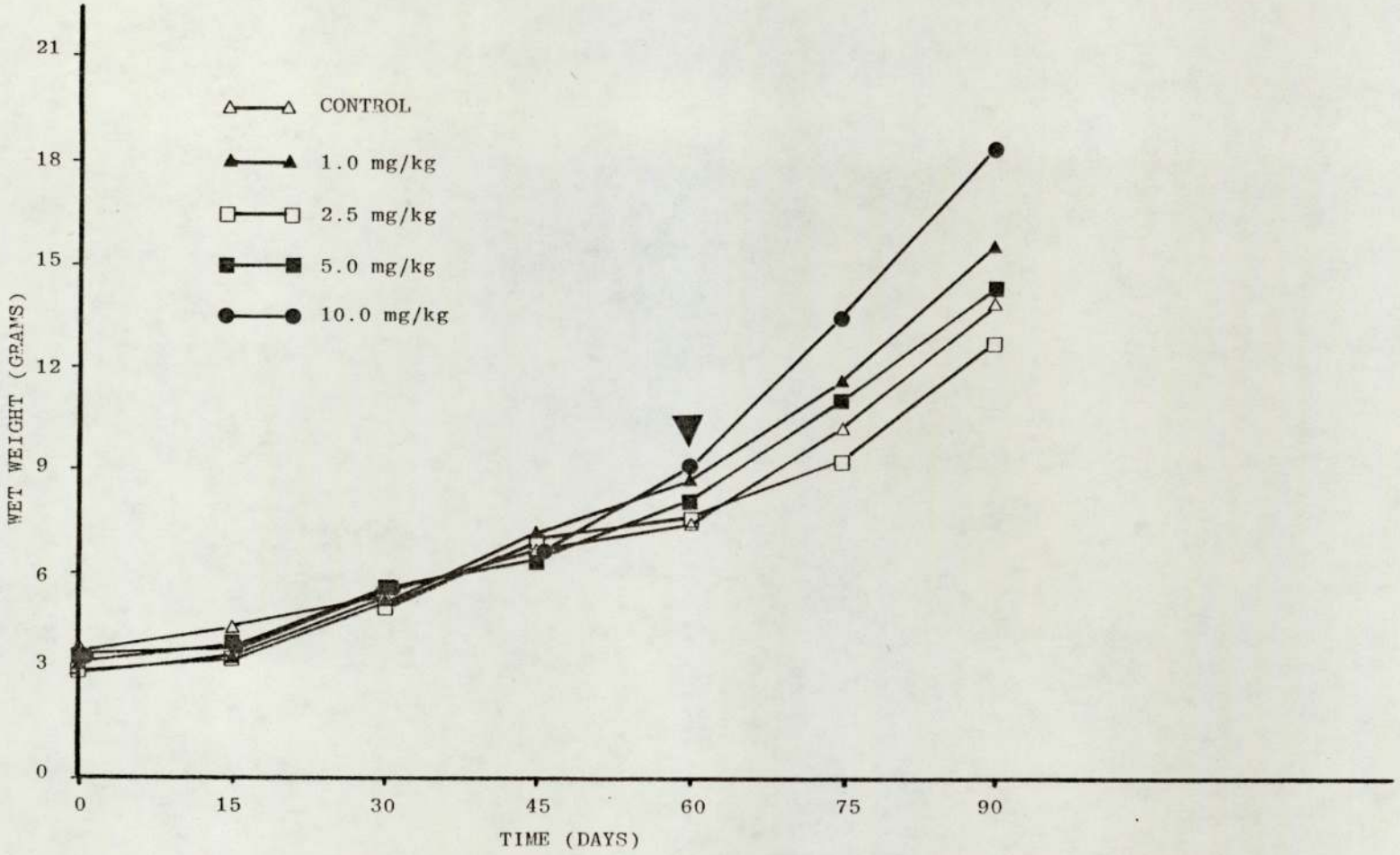


FIGURE 15 MEAN WEIGHTS OF DIFFERENT GROUPS OF CARP RECEIVING TESTOSTERONE SUPPLEMENTED DIETS. DRUG WAS WITHDRAWN AFTER 60 DAYS (ARROW).

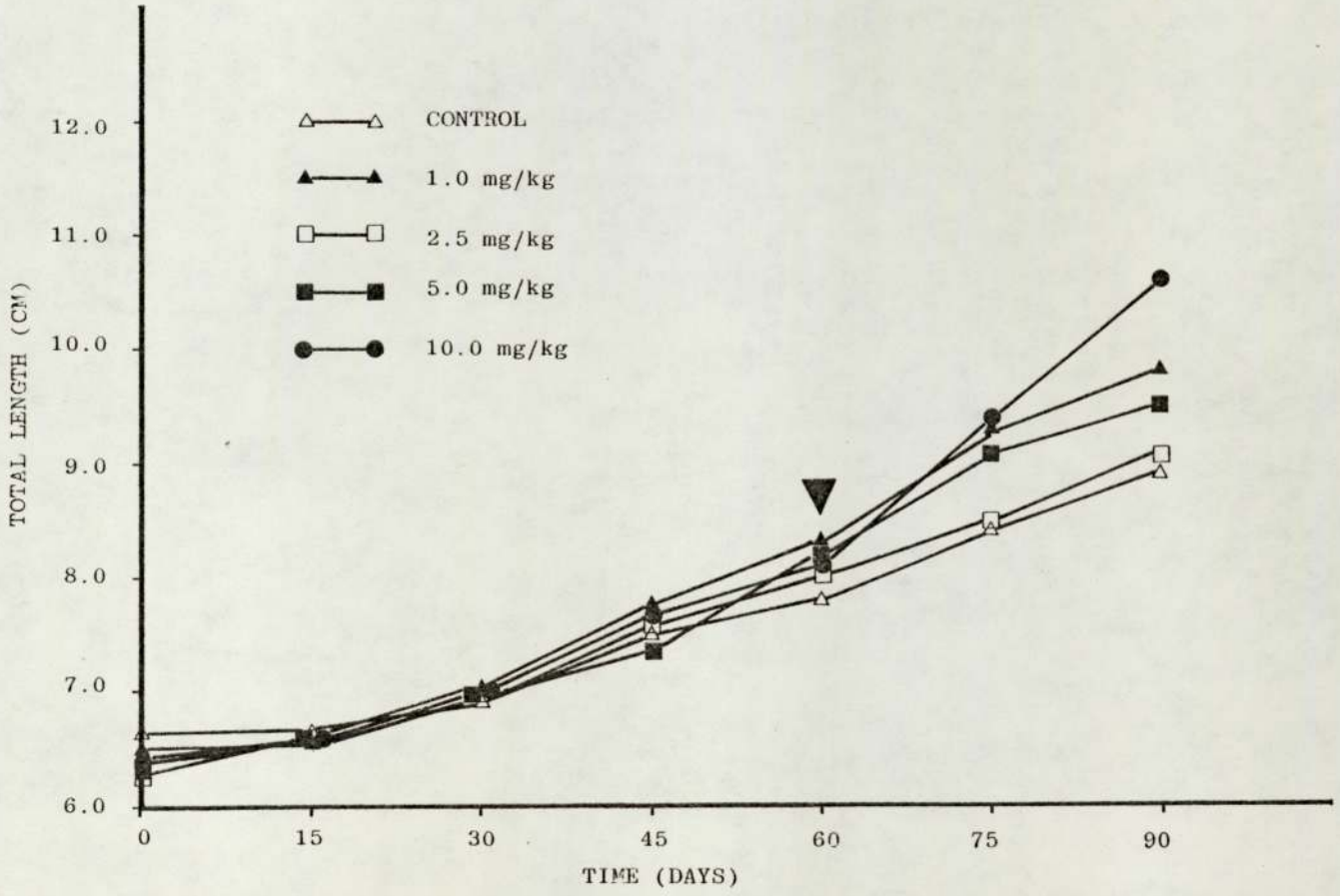


FIGURE 16 MEAN LENGTHS OF CARP GIVEN TESTOSTERONE *PER OS*. DRUG WITHDRAWN AFTER 60 DAYS (ARROW).

The effect of testosterone feeding on the condition factor and food conversion efficiency (FCE) is presented in Table 5. All the experimental animals were converting food into flesh more efficiently than the control group during the phase 1. A slight depression in FCE was seen in phase 2, when the drug was withdrawn, but the overall values for FCE (0-90 days) were still higher in experimental groups as compared with the control fish.

4.1.1.2. Tissue - Body Indices

A significant decrease in cranio-somatic index (CSI) was observed in 5.0 ($P<0.01$) and 10.0 ($P<0.05$) ppm after sixty days of the drug feeding. In phase 2, no change was observed in CSI. A statistically significant decrease was also seen in hepato-somatic (HSI) and reno-somatic (RSI) index in phase 1. In phase 2, an increase was detected in HSI and no change was seen in RSI. A decrease in viscero-somatic index (VSI) was also observed. The data concerning these changes is given in Table 6 and Figures 17 and 18.

4.1.1.3. Biochemical Analysis

4.1.1.3.1. Liver (Table 7)

A significant increase in total proteins, RNA/DNA, protein/RNA and protein/DNA was observed in those groups which showed maximum growth (1.0 and 10.0 ppm) after sixty days of drug feeding. At ninety days, a significant increase in total proteins ($P<0.001$) and RNA/DNA ($P<0.05$) was observed in only 1.0 ppm group. Protein/RNA was

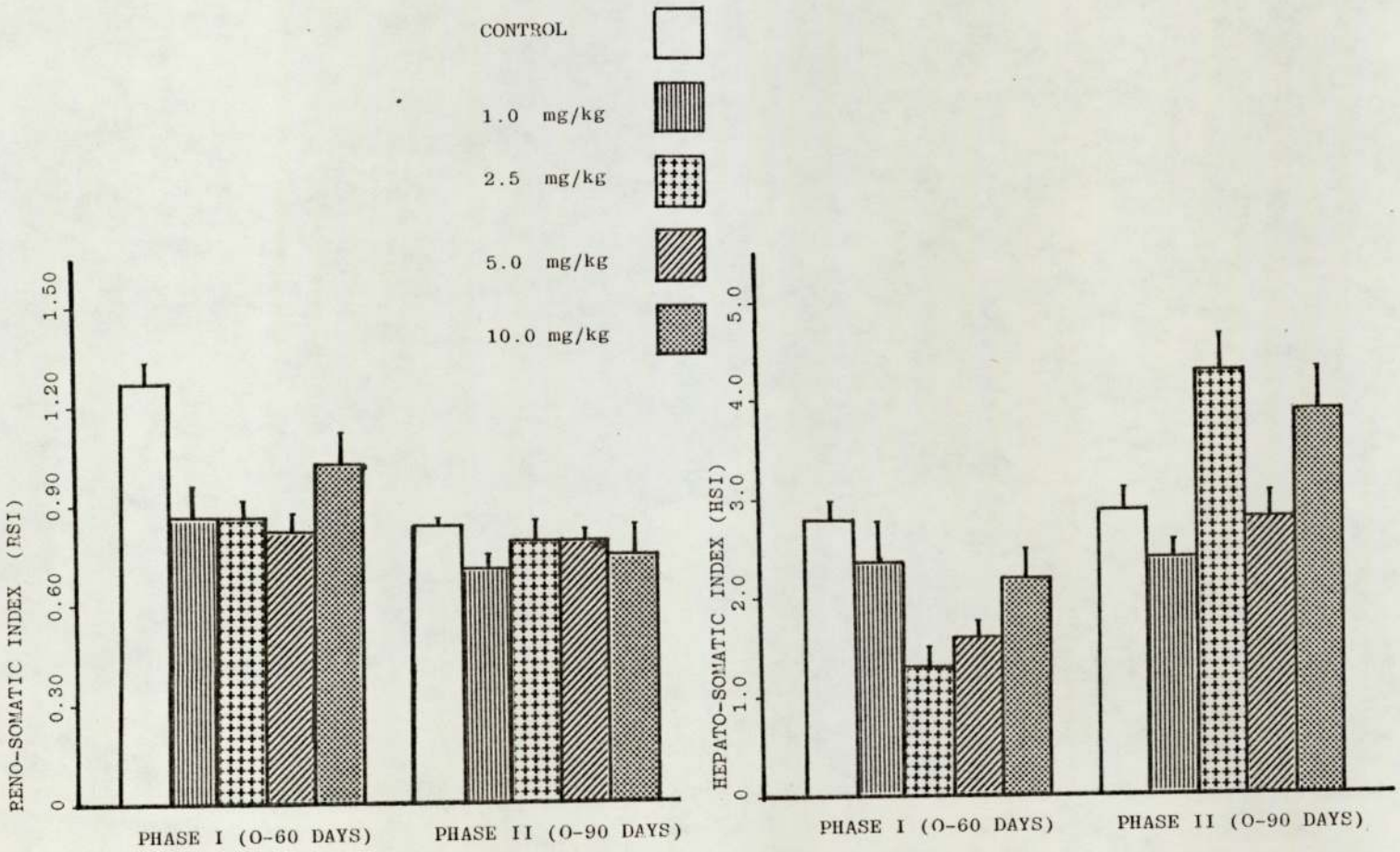


FIGURE 17 EFFECT OF TESTOSTERONE ON THE RSI AND HSI OF CARP.
DRUG WAS WITHDRAWN AFTER 60 DAYS.

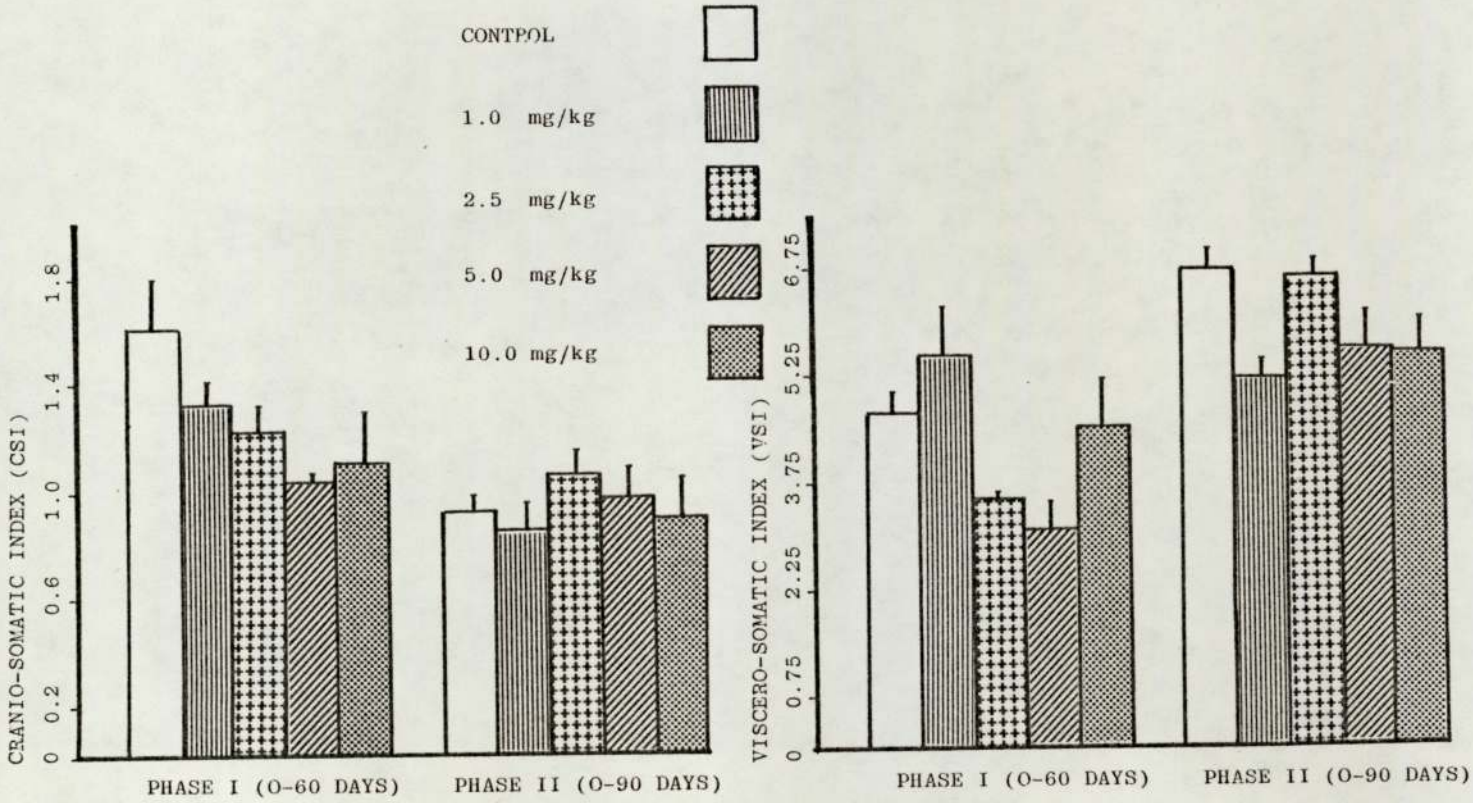


FIGURE 18 EFFECT OF TESTOSTERONE GIVEN *PER OS* ON THE CSI AND VSI OF CARP. DRUG WAS WITHDRAWN AFTER 60 DAYS.

significant in 2.5 ($P<0.05$) and 10 ppm ($P<0.001$) group. No change was observed in protein/DNA.

4.1.1.3.2. Kidney (Table 8)

There was a significant increase in proteins ($P<0.001$), RNA/DNA ($P<0.001$) and protein/DNA ($P<0.001$) ratios at sixty days in 10.0 ppm group. RNA/DNA and protein/DNA were significant in other groups also after sixty days. At ninety days, no change was observed in proteins, RNA/DNA and protein/DNA, but protein/RNA was significantly higher in 10.0 ppm groups.

4.1.1.3.3. Brain (Table 9)

Total proteins showed an increase ($P<0.001$) in all the experimental groups, so was the case for RNA/DNA ($P<0.01$) except in 5.0 ppm groups. Protein/RNA ($P<0.01$) was significant only in 1.0 and 5.0 ppm groups. Protein/DNA was significant ($P<0.01$) in all except 1.0 ppm group at the end of phase 1. In phase 2, no difference was seen in protein and protein/DNA. RNA/DNA was higher ($P<0.05$) in 10.0 ppm group, while protein/RNA was elevated ($P<0.05$) in 2.5 and 5.0 ppm groups.

4.1.1.3.4. Muscle (Tables 10 and 11)

No effect was seen as far as total proteins and protein/DNA is concerned both at sixty and ninety days. RNA/DNA ($P<0.001$) was significant only in 2.5, 5.0 and 10.0 ppm and protein/RNA in 2.5 and 10.0 ppm at sixty days. At

ninety days, a significant increase ($P<0.001$) in RNA/DNA and decrease in protein/RNA ($P<0.001$) was exhibited by 1.0 and 2.5 ppm groups only.

As far as the proximate composition is concerned, an increase in moisture content was seen in 1.0 and 2.5 ppm groups at sixty and ninety days. No effect on the crude protein was seen. Total lipids were increased ($P<0.05$) in 1.0 ppm group only but a decrease in muscle ash ($P<0.001$) was observed in all the experimental groups.

TABLE 2 CHANGES IN BODY WEIGHT OF CARP FED TESTOSTERONE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEAN (grams) ± S.E. of 25 FISH. PER CENT WEIGHT GAIN IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF TESTOSTERONE mg/kg FOOD				
	0.00	1.00	2.50	5.00	10.00
0	3.76±0.14	3.09±0.21	3.02±0.17	3.41±0.22	3.44±0.23
15	4.37±0.19 (16.22)	3.51±0.19 (13.59)	3.46±0.17 (14.57)	3.90±0.23 (14.37)	3.89±0.25 (13.08)
30	5.40±0.30 (43.62)	5.13±0.26 (66.02)	5.06±0.28 (67.55)	5.45±0.32 (59.82)	5.42±0.34 (57.56)
45	6.72±0.53 (78.72)	7.19±0.33 (132.69)	6.83±0.37 (126.16)	6.49±0.51 (90.32)	6.50±0.41 (88.95)
60*	7.36±0.61 (95.74)	8.65±0.46 (179.94)	7.57±0.45 (150.66)	8.08±0.47 (136.95)	8.94±1.35 (159.88)
75	10.28±1.26 (173.40)	11.48±0.77 (271.52)	9.27±0.79 (206.95)	11.05±0.86 (224.05)	14.61±2.27 (324.71)
90	13.98±2.16 (271.81)	15.64±1.08 (406.15)	12.73±1.32 (321.52)	14.38±1.21 (321.70)	18.40±1.71 (434.88)

* DRUG WITHDRAWN AFTER 60 DAYS.

TABLE 3 CHANGES IN TOTAL BODY LENGTH OF CARP FED TESTOSTERONE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEANS (cm) ± S.E. of 25 FISH. PER CENT INCREASE IN LENGTH IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF TESTOSTERONE mg/kg FOOD				
	0.00	1.00	2.50	5.00	10.00
0	6.63±0.08	6.42±0.13	6.23±0.11	6.30±0.14	6.35±0.15
15	6.66±0.09 (0.45)	6.44±0.12 (0.31)	6.47±0.10 (3.85)	6.54±0.12 (3.81)	6.48±0.14 (2.05)
30	6.91±0.12 (4.22)	7.0±0.12 (9.03)	6.97±0.13 (11.88)	6.93±0.14 (10.00)	7.03±0.15 (10.71)
45	7.48±0.18 (12.82)	7.74±0.12 (20.56)	7.56±0.14 (21.35)	7.35±0.18 (16.66)	7.60±0.17 (19.69)
60*	7.82±0.21 (17.95)	8.32±0.15 (29.60)	7.98±0.17 (28.09)	8.23±0.16 (30.63)	8.03±0.40 (26.46)
75	8.42±0.34 (27.00)	9.30±0.21 (44.86)	8.46±0.24 (35.79)	9.06±0.23 (43.81)	9.34±0.51 (47.09)
90	8.90±0.46 (34.24)	9.79±0.27 (52.49)	9.04±0.28 (45.10)	9.43±0.27 (49.68)	10.60±0.31 (66.93)

* DRUG WITHDRAWN AFTER 60 DAYS

TABLE 4 EFFECT OF FEEDING AND WITHDRAWAL OF TESTOSTERONE ON THE SPECIFIC GROWTH RATE (SGR) OF CARP. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg FOOD	SPECIFIC GROWTH RATE (WEIGHT)			SPECIFIC GROWTH RATE (LEGNTH)		
	0-60 DAYS	60-90 DAYS	0-90 DAYS	0-60 DAYS	60-90 DAYS	0-90 DAYS
0.00	1.15±0.22	2.16±0.13	1.49±0.25	0.29±0.11	0.43±0.04	0.34±0.08
1.00	1.77±0.43	1.99±0.13	1.85±0.28	0.45±0.15	0.55±0.14	0.48±0.11
2.50	1.59±0.46	1.75±0.28	1.64±0.31	0.42±0.07	0.42±0.02	0.42±0.06
5.00	1.47±0.29	1.94±0.12	1.63±0.21	0.46±0.11	0.46±0.13	0.46±0.08
10.00	1.63±0.34	2.44±0.64	1.90±0.36	0.41±0.10	0.93±0.06	0.59±0.13

TABLE 5 CHANGES IN THE CONDITION FACTOR AND FOOD CONVERSION EFFICIENCY OF CARP ADMINISTERED TESTOSTERONE IN THE DIET FOR 60 DAYS.

CONCEN-TRATION OF DRUG mg/kg FOOD	CONDITION FACTOR			FOOD CONVERSION EFFICIENCY		
	0 DAYS	60 DAYS	90 DAYS	0-60 DAYS	60-90 DAYS	0-90 DAYS
0.00	1.29± 0.21	1.54± 0.32	1.98± 0.26	0.26	0.58	0.40
1.00	1.17± 0.17	1.50 ±0.34	1.67 ±0.29	0.40	0.52	0.46
2.50	1.25 ±0.15	1.49 ±0.31	1.72 ±0.30	0.34	0.47	0.39
5.00	1.36 ±0.21	1.45 ±0.37	1.71 ±0.28	0.33	0.53	0.42
10.00	1.34 ±0.21	1.73 ±0.67	1.79 ±0.32	0.39	0.55	0.48

TABLE 6 EFFECT OF FEEDING TESTOSTERONE ON THE CRANIO-SOMATIC (CSI), HEPATO-SOMATIC (HSI), RENO-SOMATIC (RSI) & VISCERO-SOMATIC (VSI) INDEX OF CARP. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg FOOD	CSI		HSI		RSI		VSI	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	1.61± 0.19	0.94± 0.05	2.79± 0.16	2.91± 0.19	1.28± 0.06	0.84± 0.01	4.79± 0.21	6.72± 0.23
1.00	1.32± 0.11	0.84± 0.07	2.37± 0.42	2.42± 0.19	*** 0.87± 0.08	0.71± 0.04	5.56± 0.59	** 5.14± 0.25
2.50	1.22± 0.11	1.05± 0.09	*** 1.29± 0.19	** 4.29± 0.35	*** 0.87± 0.04	0.80± 0.06	3.52± 0.10	6.59± 0.24
5.00	** 1.03± 0.03	0.96± 0.08	** 1.63± 0.18	2.80± 0.27	*** 0.82± 0.05	0.80± 0.03	3.06± 0.36	* 5.55± 0.48
10.00	* 1.10± 0.2	0.87± 0.09	2.21± 0.29	* 3.91± 0.40	* 1.04± 0.09	0.75± 0.09	4.51± 0.68	* 5.58± 0.37

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL AND ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 7 EFFECT OF FEEDING TESTOSTERONE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN LIVER OF *Cyprinus carpio*. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg / kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	25.14± 1.83	19.91± 0.93	3.11± 0.16	6.33± 0.53	18.46± 0.94	15.46± 0.76	57.36± 4.22	97.62± 8.85
1.00	* 30.38± 2.83	*** 31.57± 2.12	** 4.72± 0.61	* 8.10± 0.94	** 22.34± 1.12	14.84± 0.38	*** 103.44± 8.46	119.94± 13.32
2.50	25.15± 1.54	19.54± 2.97	* 4.11± 0.15	7.31± 0.45	* 20.81± 0.59	* 12.09± 1.12	*** 85.41± 2.20	89.65± 12.91
5.00	28.49± 1.09	19.68± 1.13	3.96± 0.03	6.31± 0.55	17.62± 0.34	16.88± 0.68	69.87± 1.70	106.34± 10.23
10.00	** 34.61± 1.54	18.12± 1.45	* 4.17± 0.14	5.03± 0.27	** 21.94± 0.10	*** 22.03± 1.32	*** 91.33± 2.83	110.61± 6.41

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 8 EFFECT OF FEEDING TESTOSTERONE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN KIDNEY OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg / kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	31.07± 2.06	47.35± 2.13	1.96± 0.06	3.22± 0.60	17.67± 0.64	12.85± 0.46	34.65± 1.27	42.14± 9.75
1.00	35.47± 3.36	56.06± 4.81	3.50*** ± 0.27	4.18± 0.45	19.62± 1.09	12.21± 0.27	67.85*** ± 2 00	50.87± 5.10
2.50	36.38± 3.70	42.30± 2.97	2.98** ± 0.19	4.40± 0.87	21.71* ± 0.62	11.82* ± 0.07	64.40*** ± 3.03	52.18± 9.90
5.00	37.89± 4.04	52.50± 3.87	3.08** ± 0.22	3.46± 0.17	23.69*** ± 1.43	13.61± 0.25	72.19*** ± 4.27	46.87± 1.62
10.00	52.14*** ± 4.11	47.68± 4.32	3.73*** ± 0.19	4.83± 0.17	19.87± 0.91	15.38*** ± 0.33	74.03*** ± 5.04	73.72± 3.62

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 9 EFFECT OF FEEDING TESTOSTERONE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN BRAIN OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN/RNA		PROTEIN/DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	23.19± 1.16	34.07± 1.14	1.89± 0.08	2.84± 0.13	18.90± 0.88	16.19± 0.39	35.54± 0.47	45.87± 2.09
1.00	29.35± 1.01 *	42.81± 2.61	2.54± 0.05 ***	3.00± 0.06	14.97± 0.24 **	16.89± 0.19	37.98± 0.82	50.66± 1.32
2.50	36.76± 1.92 ***	23.22± 7.55	2.33± 0.05 **	2.94± 0.23	19.17± 0.71	19.09± 0.55 *	44.68± 0.84 *	56.46± 5.8
5.00	35.67± 2.95 ***	23.08± 3.01	2.09± 0.17	2.60± 0.17	25.00± 1.00 ***	18.79± 1.35 *	52.15± 4.66 **	48.30± 2.42
10.00	44.65± 1.28 ***	23.08± 0.84	2.30± 0.01 **	3.68± 0.40 *	20.23± 0.63	16.21± 0.45	46.53± 1.56 **	59.74± 7.26

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 10 EFFECT OF FEEDING TESTOSTERONE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN MUSCLE OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	15.28± 0.37	16.33± 0.73	5.69± 0.30	7.16± 0.14	63.58± 3.23	62.62± 3.01	363.49± 34.95	447.48± 18.39
1.00	16.56± 1.83	17.84± 0.84	5.61± 0.41	*** 14.64± 1.19	73.62± 4.50	*** 35.15± 2.70	408.75± 15.07	505.21± 9.53
2.50	15.21± 0.52	20.18± 1.52	** 8.91± 0.61	*** 17.26± 1.40	* 52.74± 3.49	*** 34.23± 2.73	471.81± 48.73	579.51± 18.24
5.00	16.58± 1.67	18.21± 0.40	*** 9.64± 1.21	8.27± 0.43	54.51± 3.31	67.69± 1.28	522.95± 69.02	558.21± 22.55
10.00	15.47± 0.68	19.86± 1.08	*** 10.01± 0.25	8.21± 1.10	*** 38.62± 2.21	67.95± 5.08	387.29± 27.05	549.09± 59.48

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL AND ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 11 EFFECT OF FEEDING TESTOSTERONE ON THE PROXIMATE COMPOSITION OF MUSCLE TISSUE FROM *Cyprinus carpio*. DRUG WAS FED FOR 60 DAYS ONLY.

CONCENTRATION OF DRUG mg/kg	MOISTURE mg/100 mg		CRUDE PROTEIN N x 6.25 (mg/100 mg)				TOTAL LIPIDS (mg/100 mg)				ASH (mg/100 mg)			
			ON WET BASIS		ON DRY BASIS		ON WET BASIS		ON DRY BASIS		ON WET BASIS		ON DRY BASIS	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	72.57± 0.70	72.43± 0.48	17.44± 1.00	18.13± 0.63	63.50± 2.63	65.88± 2,69	3.18± 0.04	5,11± 0.47	11.58± 0.10	18.51± 1.47	2.98± 0.18	2,14± 0.13	10.80± 0.53	7.78± 0.48
1.00	73.45± 0.38	* 74.32± 0.39	18.63± 0.38	17.75± 0.19	** 70.00± 0.69	68.94± 0.81	* 4.45± 0.32	4.54± 0.36	* 16.79± 1.41	17.73± 1.57	** 2.21± 0.02	1.85± 0.01	* 8.30± 0.37	7.20± 0.08
2.50	*** 75.35± 0.41	72.13± 0.58	17.25± 0.50	18.69± 0.75	** 70.06± 1.44	66.88± 1.69	3.45± 0.33	3,94± 0.10	13.96± 1.16	14.15± 0.38	*** 1.90± 0.13	2.70± 0.68	** 7.7± 0.49	9.85± 2.72
5.00	73.24± 0.32	73.28± 0.76	18.81± 0.44	18.06± 0.88	** 70.25± 0.81	67.56± 1.69	3.68± 0.10	4.55± 0.21	13.75± 0.53	17.05± 0.90	* 2.24± 0.05	2.34± 0.40	* 8.40± 0.11	8.83± 1.66
10.00	73.70± 0.57	71.85± 0.44	17.50± 0.50	19.00± 1.06	66.56± 0.94	67.44± 3.13	3.74± 0.31	5.35± 0.74	14,16± 0.95	18.98± 2.59	*** 1.96± 0.02	1.75± 0.10	*** 7.50± 0.07	6.23± 0.41

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

4.1.2. 11-Ketotestosterone

4.1.2.1. Weight and Length Data

The weight and length data for phase 1 and phase 2 is presented in Tables 12 and 13 and Figures 19 and 20. The experimental groups on this drug were growing faster than the controls and at sixty days they were 59.17, 74.72, 7.22 and 61.67% heavier and 91.60, 74.79, -4.20 and 59.66% longer than the controls for 1.0, 2.5, 5.0 and 10.0 ppm groups respectively. Unlike testosterone, the optimum dose for this natural steroid appears to be 2.5 ppm.

The specific growth rates for weight and length are given in Table 14 and show a clear increase over the controls at least in phase 1. The condition factor and food conversion efficiency is given in Table 15.

4.1.2.2. Tissue - Body Indices

A significant decrease ($P<0.05$) in 1.0 ppm for CSI and 1.0 ($P<0.05$) and 2.5 ($P<0.01$) ppm groups for HSI was observed at sixty days. The RSI of the treated fish was significantly decreased ($P<0.001$) in all the experimental groups (Table 16). At ninety days no change was observed in CSI, HSI and RSI, while VSI was lowered in all experimental groups, but was significant only in 2.5 ($P<0.05$) and 10.0 ($P<0.01$) ppm groups. Figures 21 and 22 give the details about these changes.

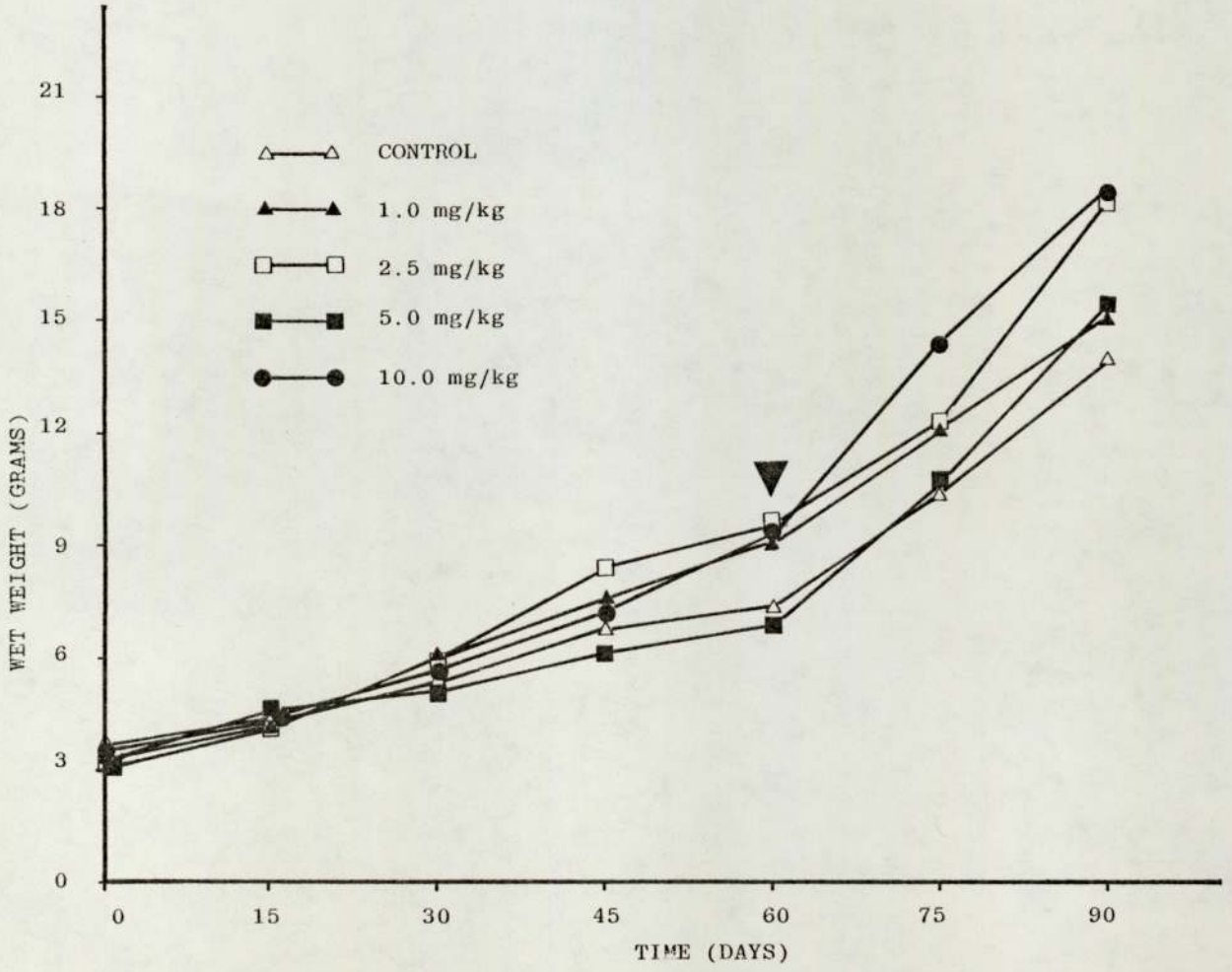


FIGURE 19 MEAN WEIGHTS OF DIFFERENT GROUPS OF CARP RECEIVING 11-KETOTESTOSTERONE SUPPLEMENTED DIETS. DRUG WAS WITHDRAWN AFTER 60 DAYS (ARROW).

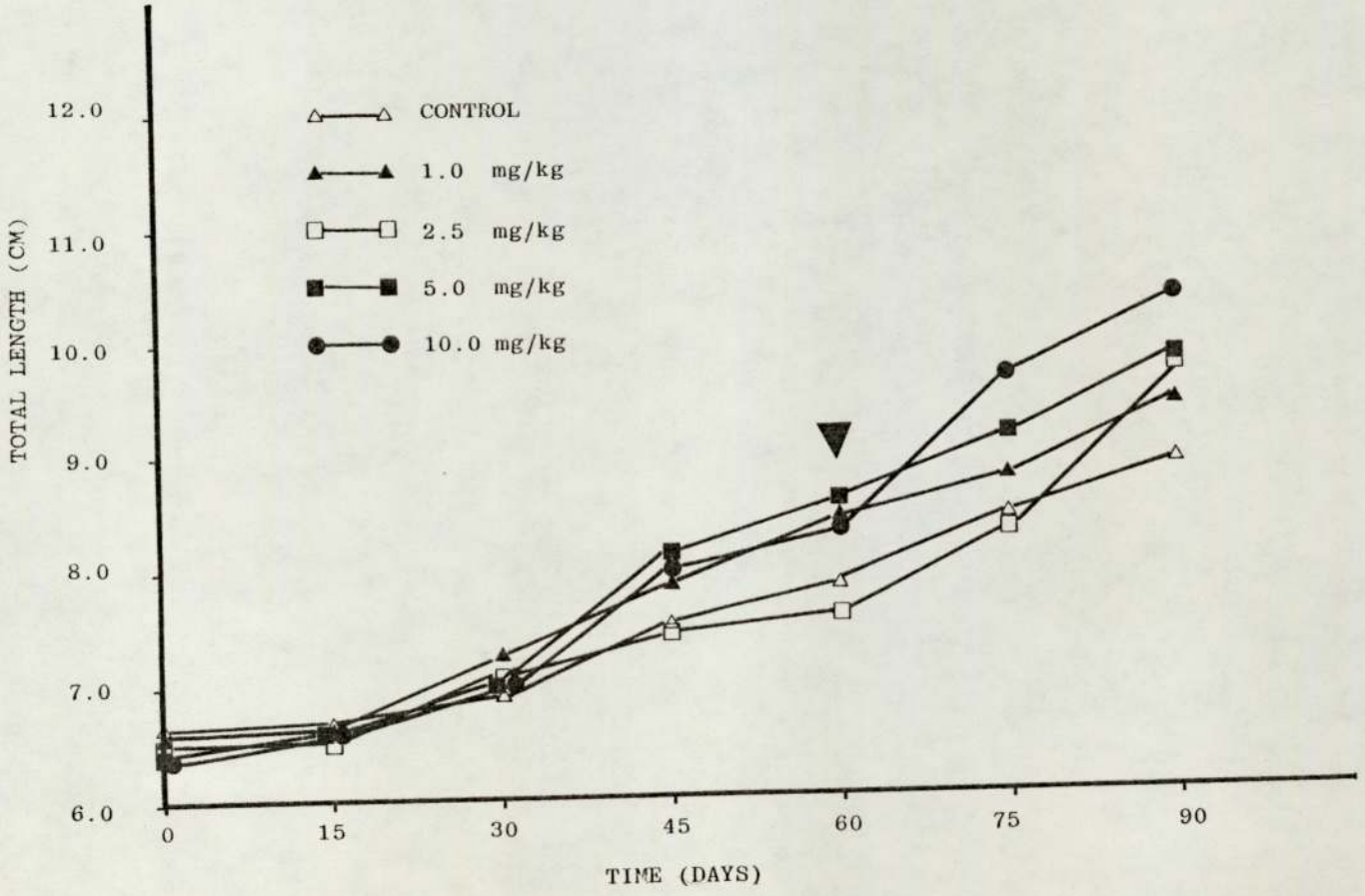


FIGURE 20 EFFECT OF 11-KETOTESTOSTERONE ON THE TOTAL LENGTH OF CARP. DRUG WAS WITHDRAWN AFTER 60 DAYS (ARROW).

4.1.2.3. Biochemical Changes

4.1.2.3.1. Liver (Table 17)

At sixty days, protein was significantly elevated ($P<0.05$) only in 5 ppm group, while RNA/DNA ($P<0.01$) and protein/DNA ($P<0.05$) was higher in all the treated groups. No changes were observed in protein/RNA ratios. After ninety days in phase 2, protein was higher ($P<0.01$) in 1.0, 5.0 and 10.0 ppm, while protein/RNA was higher in 2.5 and 10.0 ppm ($P<0.05$) groups. No change was observed in RNA/DNA and protein/DNA.

4.1.2.3.2. Kidney (Table 18)

A consistent increase in proteins ($P<0.001$), RNA/DNA ($P<0.001$) and protein/DNA (1.0 and 5.0 ppm) was noted. Protein/RNA was higher ($P<0.05$) in 1.0 but lower ($P<0.05$) in 5.0 and 10.0 ppm groups after sixty days of the feeding of the drug. In phase 2 (ninety days), although the protein exhibited no change from controls, the RNA/DNA was still higher ($P<0.05$) in experimental groups. Protein/RNA (2.5 ppm) and protein/DNA (2.5 and 10.0 ppm) were significantly higher ($P<0.05$).

4.1.2.3.3. Brain (Table 19)

Total proteins and RNA/DNA ratio was significantly raised ($P<0.001$) in 5.0 and 10.0 ppm groups. Protein/RNA was elevated only in 2.5 ($P<0.01$) and protein/DNA was increased ($P<0.001$) in all except 1.0 ppm group at the end of phase 1. In phase 2, a decrease ($P<0.05$) was observed in total

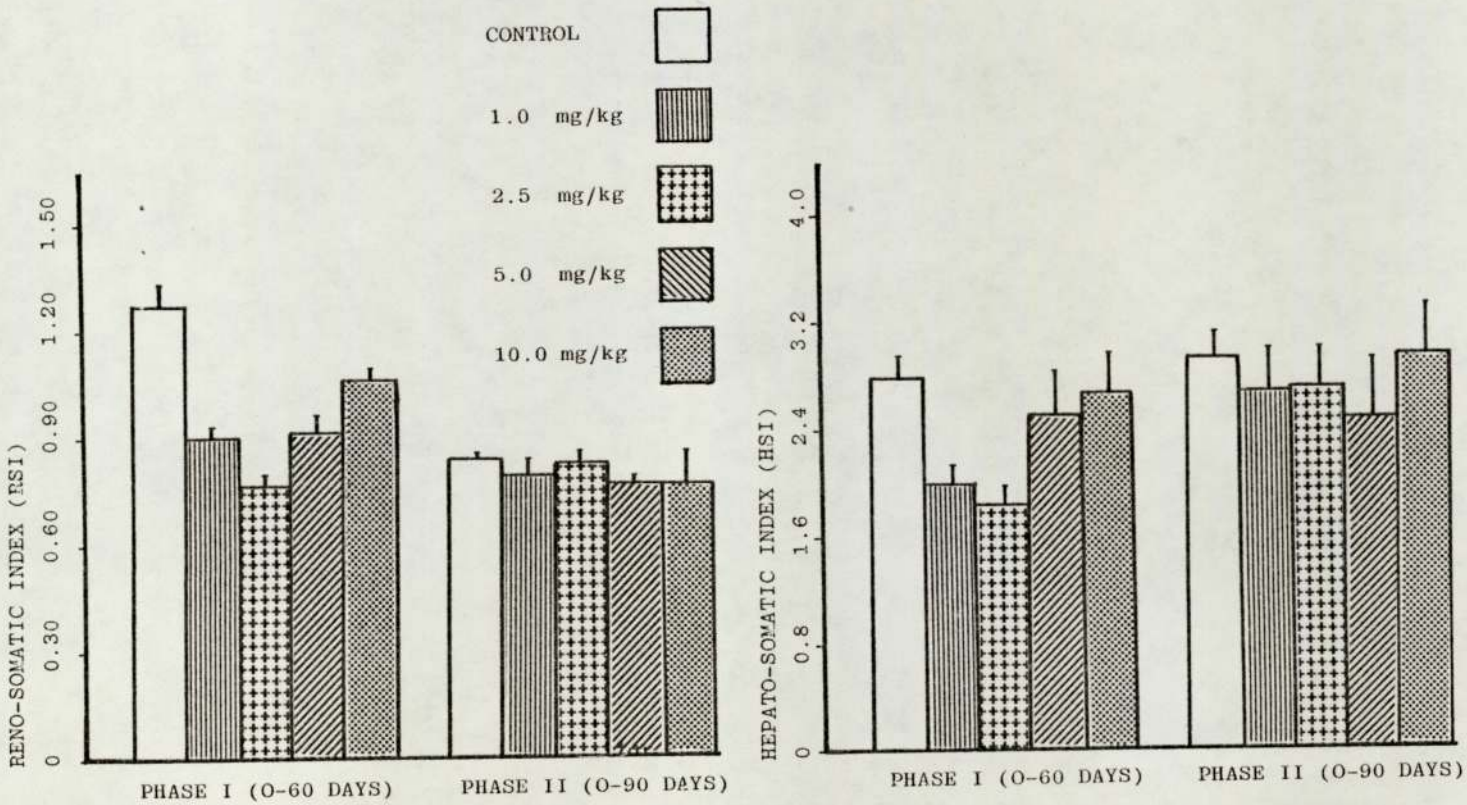


FIGURE 21 EFFECT OF FEEDING 11-KETOTESTOSTERONE ON THE RSI AND HSI OF CARP. DRUG WAS WITHDRAWN AFTER 60 DAYS.

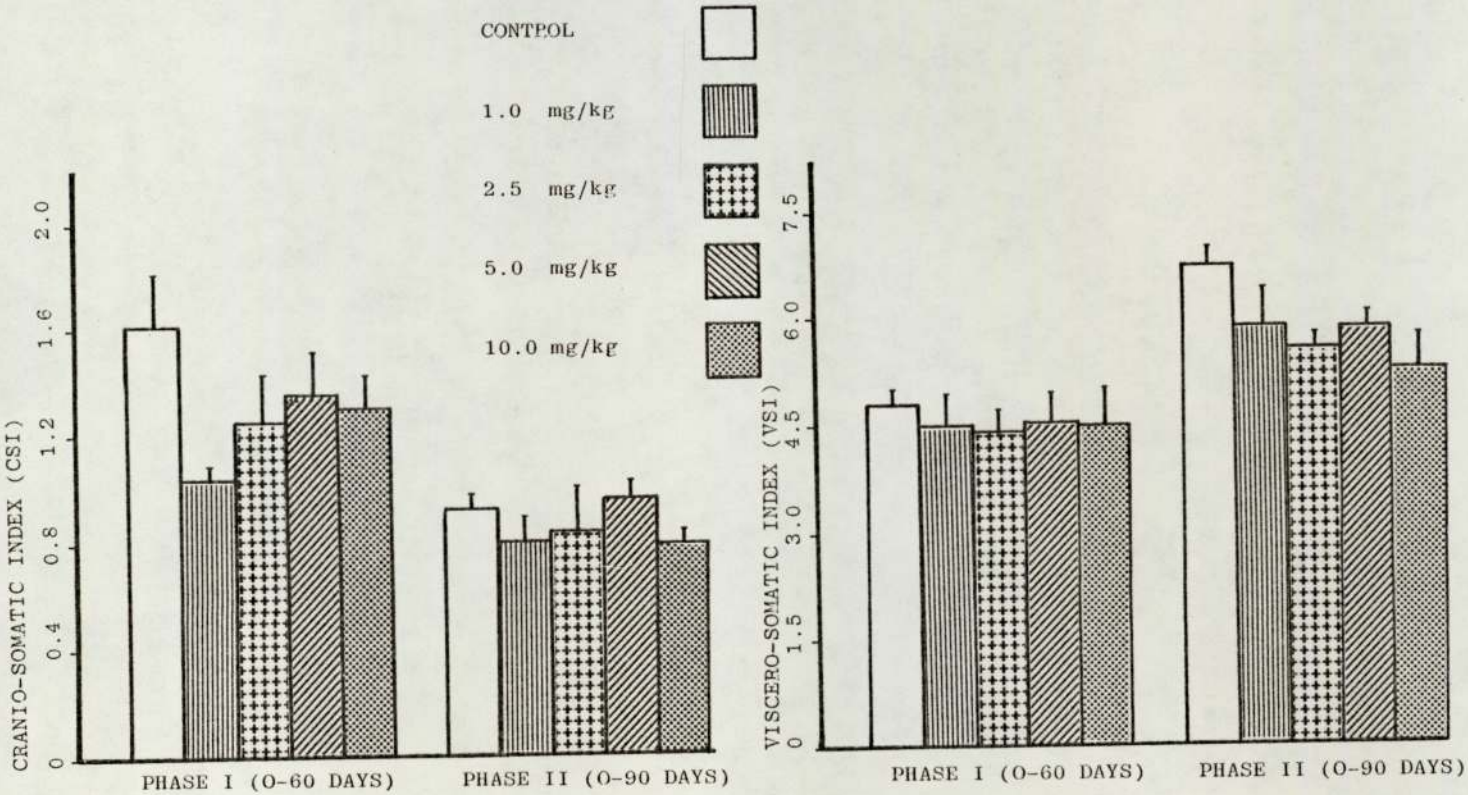


FIGURE 22 EFFECT OF FEEDING 11-KETOTESTOSTERONE ON THE CSI & VSI OF CARP.
DRUG WAS WITHDRAWN AFTER 60 DAYS.

proteins in 2.5 ppm group while RNA/DNA was higher ($P<0.05$) in 2.5 and 5.0 ppm. Protein/DNA was higher ($P<0.01$) in all but 10.0 ppm group.

4.1.2.3.4. Muscle (Tables 20 and 21)

An increase ($P<0.001$) was seen only in 10 ppm group in RNA/DNA and protein/DNA in phase 1. No effect on protein and protein/RNA was observed. In phase 2, RNA/DNA and protein/DNA was significantly higher ($P<0.01$) in all the groups. No change was observed in protein/RNA both in phase 1 and 2.

Proximate composition of the muscle is given in Table 21. In phase 1, moisture was increased only in 2.5 and 5.0 ppm ($P<0.01$). Although, no change in crude proteins was observed on wet weight bases, it was significantly higher ($P<0.01$) than the controls in 1.0 and 2.5 ppm groups on dry weight bases. No change in the total lipid and ash content (dry and wet bases) was observed.

At ninety days, moisture was higher ($P<0.05$) in 1.0 and 5.0 ppm groups only. No change in crude protein was observed. The total lipids ($P<0.001$) and ash ($P<0.01$) contents were decreased both on wet and dry bases.

TABLE 12 CHANGES IN BODY WEIGHT OF CARP FED 11-KETOTESTOSTERONE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEAN (g) ± S.E. OF 25 FISH. PERCENT WEIGHT GAIN IS GIVEN IN PARENTHESES UNDER ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF 11-KETOTESTOSTERONE mg/kg FOOD				
	0.00	1.00	2.50	5.00	10.00
0	3.76± 0.14	3.25± 0.18	3.17± 0.19	3.08± 0.18	3.62± 0.21
15	4.37± 0.19 (16.22)	4.16± 0.26 (28.00)	4.16± 0.26 (31.23)	4.21± 0.25 (36.39)	4.13± 0.25 (14.09)
30	5.40± 0.30 (43.62)	6.06± 0.38 (86.46)	5.84± 0.35 (84.23)	5.16± 0.36 (67.53)	5.71± 0.39 (57.73)
45	6.72± 0.53 (78.72)	7.62± 0.57 (134.46)	8.39± 0.54 (164.67)	6.08± 0.42 (97.40)	7.38± 0.59 (103.87)
60*	7.36± 0.61 (95.74)	8.98± 0.84 (176.31)	9.46± 0.59 (198.42)	6.94± 1.15 (125.32)	9.44± 0.94 (160.77)
75	10.28± 1.26 (173.40)	12.15± 2.08 (273.85)	12.20± 1.70 (284.86)	10.63± 3.31 (245.13)	14.34± 0.95 (296.13)
90	13.98± 2.16 (271.81)	15.07± 3.30 (363.69)	18.14± 3.38 (472.24)	15.39± 4.50 (399.68)	18.49± 1.17 (410.77)

* DRUG WITHDRAWN AFTER 60 DAYS.

TABLE 13 CHANGES IN THE TOTAL BODY LENGTH OF CARP FED 11-KETOTESTOSTERONE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEAN (cm) ± S.E. OF 25 FISH. PERCENT INCREASE IN LENGTH IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF 11-KETOTESTOSTERONE mg/kg FOOD				
	0.00	1.00	2.50	5.00	10.00
0	6.63± 0.08	6.47± 0.11	6.46± 0.13	6.43± 0.13	6.45± 0.13
15	6.66± 0.09 (0.45)	6.63± 0.13 (2.47)	6.60± 0.14 (2.17)	6.57± 0.15 (2.18)	6.60± 0.14 (2.33)
30	6.91± 0.12 (4.22)	7.24± 0.15 (11.90)	7.12± 0.15 (10.22)	6.89± 0.17 (7.15)	7.04± 0.16 (9.15)
45	7.48± 0.18 (12.82)	7.84± 0.20 (21.17)	8.13± 0.18 (25.85)	7.39± 0.17 (14.93)	7.91± 0.20 (22.64)
60*	7.82± 0.21 (17.95)	8.40± 0.26 (29.83)	8.54± 0.18 (32.20)	7.57± 0.36 (17.73)	8.35± 0.38 (29.46)
75	8.42± 0.34 (27.00)	8.75± 0.46 (35.24)	9.16± 0.42 (41.80)	8.28± 0.87 (28.77)	9.65± 0.21 (49.61)
90	8.90± 0.46 (34.24)	9.37± 0.64 (44.82)	9.76± 0.62 (51.08)	9.74± 0.76 (51.48)	10.32± 0.27 (60.00)

* DRUG WITHDRAWN AFTER 60 DAYS.

TABLE 14 EFFECT OF FEEDING & WITHDRAWAL OF 11-KETOTESTOSTERONE ON THE SPECIFIC GROWTH RATE (SGR) OF CARP. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg FOOD	SPECIFIC GROWTH RATE (WEIGHT)			SPECIFIC GROWTH RATE (LENGTH)		
	0-60 DAYS	60-90 DAYS	0-90 DAYS	0-60 DAYS	60-90 DAYS	0-90 DAYS
0.00	1.15± 0.22	2.16± 0.06	1.49± 0.25	0.29± 0.11	0.43± 0.04	0.34± 0.08
1.00	1.74± 0.30	1.75± 0.21	1.74± 0.20	0.45± 0.10	0.37± 0.07	0.42± 0.07
2.50	1.88± 0.40	2.20± 0.34	1.99± 0.29	0.48± 0.17	0.45± 0.02	0.47± 0.11
5.00	1.39± 0.26	2.69± 0.13	1.82± 0.32	0.28± 0.08	0.85± 0.17	0.47± 0.14
10.00	1.64± 0.28	2.27± 0.40	1.85± 0.26	0.45± 0.14	0.71± 0.18	0.53± 0.13

TABLE 15 CHANGES IN THE CONDITION FACTOR AND FOOD CONVERSION EFFICIENCY OF CARP ADMINISTERED 11-KETOTESTOSTERONE IN THE DIET FOR 60 DAYS.

CONCEN-TRATION OF DRUG mg/kg FOOD	CONDITION FACTOR			FOOD CONVERSION EFFICIENCY		
	0 DAYS	60 DAYS	90 DAYS	0-60 DAYS	60-90 DAYS	0-90 DAYS
0.00	1.29± 0.21	1.54± 0.32	1.98± 0.26	0.26	0.58	0.40
1.00	1.20± 0.16	1.52± 0.32	1.83± 0.38	0.37	0.38	0.38
2.50	1.18± 0.16	1.52± 0.33	1.95± 0.33	0.40	0.73	0.54
5.00	1.16± 0.13	1.60± 0.35	1.67± 0.35	0.28	0.88	0.53
10.00	1.35± 0.23	1.62± 0.39	1.68± 0.27	0.38	0.49	0.44

TABLE 16 EFFECT OF FEEDING 11-KETOTESTOSTERONE ON THE CRANIO-SOMATIC (CSI), HEPATO-SOMATIC (HSI), RENO-SOMATIC (RSI), & VISCERO-SOMATIC (VSI) INDEX OF CARP. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg FOOD	CSI		HSI		RSI		VSI	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	1.61± 0.19	0.91± 0.05	2.79± 0.16	2.91± 0.19	1.28± 0.06	0.84± 0.01	4.79± 0.21	6.72± 0.23
1.00	* 1.04± 0.04	0.80± 0.09	* 1.99± 0.14	2.68± 0.31	*** 0.90± 0.02	0.80± 0.05	4.49± 0.36	5.84± 0.49
2.50	1.25± 0.17	0.84± 0.18	** 1.83± 0.15	2.71± 0.28	*** 0.76± 0.02	0.83± 0.03	4.46± 0.29	* 5.52± 0.19
5.00	1.35± 0.16	0.95± 0.06	2.15± 0.31	2.47± 0.45	*** 0.92± 0.05	0.77± 0.10	4.58± 0.38	5.87± 0.18
10.00	1.30± 0.12	0.79± 0.06	2.69± 0.27	2.95± 0.37	*** 1.06± 0.02	0.77± 0.08	4.51± 0.48	** 5.23± 0.41

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 17 EFFECT OF FEEDING 11-KETOTESTOSTERONE ON THE CONCENCENTRATIONS OF PROTEIN, RNA & DNA IN LIVER OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	25.14± 1.83	19.91± 0.93	3.11± 0.16	6.33± 0.53	18.46± 0.94	15.46± 0.76	57.36± 4.22	97.62± 8.85
1.00	33.63± 3.43	** 27.39± 1.20	** 4.45± 0.27	7.02± 0.84	18.52± 1.13	16.80± 0.89	* 81.68± 3.64	120.04± 20.08
2.50	32.74± 4.17	21.04± 0.47	* 4.07± 0.44	6.00± 1.06	19.12± 0.46	* 18.78± 0.45	* 78.10± 9.54	113.36± 21.29
5.00	* 41.69± 4.23	** 27.34± 2.13	** 4.29± 0.19	6.40± 0.97	15.16± 1.32	14.18± 1.33	64.34± 3.95	90.15± 15.81
10.00	28.42± 1.73	*** 32.99± 2.16	*** 4.86± 0.12	7.53± 0.94	19.12± 2.17	** 18.87± 0.12	** 92.26± 8.46	141.62± 16.88

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALAYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 18 EFFECT OF FEEDING 11-KETOTESTOSTERONE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN KIDNEY OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	31.07± 2.06	47.35± 2.13	1.96± 0.06	3.22± 0.60	17.67± 0.64	12.85± 0.46	34.65± 1.27	42.14± 9.75
1.00	*** 52.47± 1.63	44.12± 1.29	*** 2.79± 0.09	* 4.92± 0.71	* 20.86± 2.19	12.66± 0.70	*** 57.75± 4.66	63.67± 13.28
2.50	** 40.70± 1.19	41.88± 0.95	2.13± 0.02	* 4.72± 0.25	16.26± 0.24	** 15.70± 0.53	34.68± 0.71	* 74.47± 5.97
5.00	** 39.76± 0.45	42.06± 7.55	*** 3.13± 0.14	* 4.94± 0.28	* 13.58± 0.31	13.75± 0.19	* 42.41± 0.92	* 67.81± 3.04
10.00	*** 49.76± 3.63	44.47± 2.26	*** 2.92± 0.13	* 4.68± 0.29	* 13.96± 0.53	14.04± 0.63	40.52± 0.69	65.22± 1.83

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 19 EFFECT OF FEEDING 11-KETOTESTOSTERONE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN BRAIN OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	23.19± 1.16	34.07± 1.14	1.89± 0.08	2.84± 0.13	18.90± 0.88	16.19± 0.39	35.54± 0.47	45.87± 2.09
1.00	24.43± 1.04	36.48± 0.65	1.86± 0.07	3.48± 0.41	21.21± 1.61	* 18.78± 0.94	39.14± 2.23	* 65.13± 8.22
2.50	24.30± 0.64	* 21.45± 0.50	1.87± 0.03	* 3.57± 0.20	** 24.80± 0.75	17.87± 0.38	*** 46.40± 1.57	* 63.71± 3.04
5.00	*** 34.83± 1.10	31.71± 4.85	*** 2.85± 0.12	** 4.19± 0.08	16.67± 0.75	18.31± 1.24	*** 47.31± 1.87	*** 76.48± 3.85
10.00	*** 40.41± 0.55	35.92± 0.85	*** 2.66± 0.26	2.50± 0.24	18.89± 1.66	** 19.89± 0.70	*** 48.95± 1.69	49.52± 4.34

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 20 EFFECT OF FEEDING 11-KETOTESTOSTERONE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN MUSCLE OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	15.28± 0.37	16.33± 0.73	5.69± 0.30	7.16± 0.14	63.58± 3.23	62.62± 3.01	363.49± 34.95	447.48± 18.39
1.00	16.01± 1.00	15.43± 0.29	8.89± 0.99	9.81± * 0.75	47.84± 5.61	61.28± 5.77	411.27± 25.22	596.31± 58.49
2.50	16.00± 0.58	17.34± 0.55	5.66± 0.41	12.99± *** 0.80	71.55± 1.19	67.29± 4.27	403.89± 27.46	870.88± *** 68.86
5.00	14.15± 0.43	19.49± *** 0.50	7.95± 0.54	11.30± ** 0.52	53.08± 2.85	62.65± 4.21	418.31± 14.59	705.79± ** 46.13
10.00	16.29± 0.41	17.31± 0.47	13.28± ** 3.09	10.66± ** 1.15	65.08± 3.20	63.24± 6.11	753.07± *** 86.48	663.13± * 60.91

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 21 EFFECT OF FEEDING 11-KETOTESTOSTERONE ON THE PROXIMATE COMPOSITION OF MUSCLE TISSUE FROM *Cyprinus carpio*. DRUG WAS FED FOR 60 DAYS ONLY.

CONCENTRATION OF DRUG mg/kg	MOISTURE mg/100 mg		CRUDE PROTEIN N x 6.25 (mg/100 mg)				TOTAL LIPIDS (mg/100 mg)				ASH (mg/100 mg)			
			ON WET BASIS		ON DRY BASIS		ON WET BASIS		ON DRY BASIS		ON WET BASIS		ON DRY BASIS	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	72.57± 0.70	72.43± 0.48	17.44± 1.00	18.13± 0.63	63.50± 2.63	65.88± 2.69	3.18± 0.04	5.11± 0.47	11.58± 0.10	18.51± 1.47	2.98± 0.18	2.14± 0.13	10.80± 0.53	7.78± 0.48
1.00	73.70± 0.82	* 74.68± 0.64	18.88± 0.44	17.63± 0.50	** 72.56± 0.88	69.63± 1.0	2.53± 0.38	*** 3.03± 0.24	9.57± 1.37	** 11.98± 0.94	1.91± 0.11	* 1.80± 0.13	7.25± 0.25	7.13± 0.51
2.50	** 75.04± 0.50	73.91± 0.48	18.13± 0.19	17.81± 0.13	** 72.50± 1.31	68.50± 1.69	2.11± 0.14	*** 3.07± 0.48	8.42± 0.44	** 11.82± 1.97	2.01± 0.06	** 1.55± 0.09	8.05± 0.32	** 5.93± 0.28
5.00	** 75.22± 0.53	* 74.68± 0.99	15.88± 0.44	17.38± 0.81	64.00± 0.69	68.63± 1.88	3.17± 0.31	*** 2.24± 0.25	12.72± 1.02	*** 8.86± 1.01	—	1.91± 0.09	—	7.60± 0.47
10.00	73.75± 0.37	72.07± 0.16	17.88± 0.38	19.44± 0.25	67.56± 0.75	69.56± 0.69	3.19± 0.70	*** 2.99± 0.12	12.24± 2.86	*** 10.69± 0.45	2.44± 0.30	** 1.64± 0.09	9.30± 1.13	** 5.85± 0.30

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

4.1.3. Adrenosterone

4.1.3.1. Weight and Length Data

Detailed statistics of feeding this drug relating to the growth of carp are given in Figures 23 and 24. A significant difference was noted both at sixty ($P < 0.01$) and ninety ($P < 0.05$) days for weight and length. Detailed statistical analysis is given in Tables 22 and 23. At sixty days, the groups on 1.0 and 10.0 ppm dose were growing faster than the rest of the groups. These were 89.72 and 132.78% heavier and 64.71 and 85.71% longer than the controls. At the termination of the experiment (ninety days) the experimental groups were still heavier and longer than the controls. At this time, the groups on 1.0, 2.5, 5.0 and 10.0 ppm gained 64.09, 30.14, 1.08 and 78.67 in weight and 81.06, 39.64, 18.94 and 94.27% in length (Tables 22 and 23).

As far as the SGR for weight is concerned, it was higher than the control group in the phase 1 and was equal in 1.0 and 10.0 ppm groups. After withdrawal of the drug, the SGR for weight declined in all the experimental groups except in 1.0 ppm group. SGR for length was higher in both the phases and there was no effect of the withdrawal of the steroid (Table 24).

Changes in the condition factor and FCE were parallel with changes in the weight and length and details can be seen in Table 25.

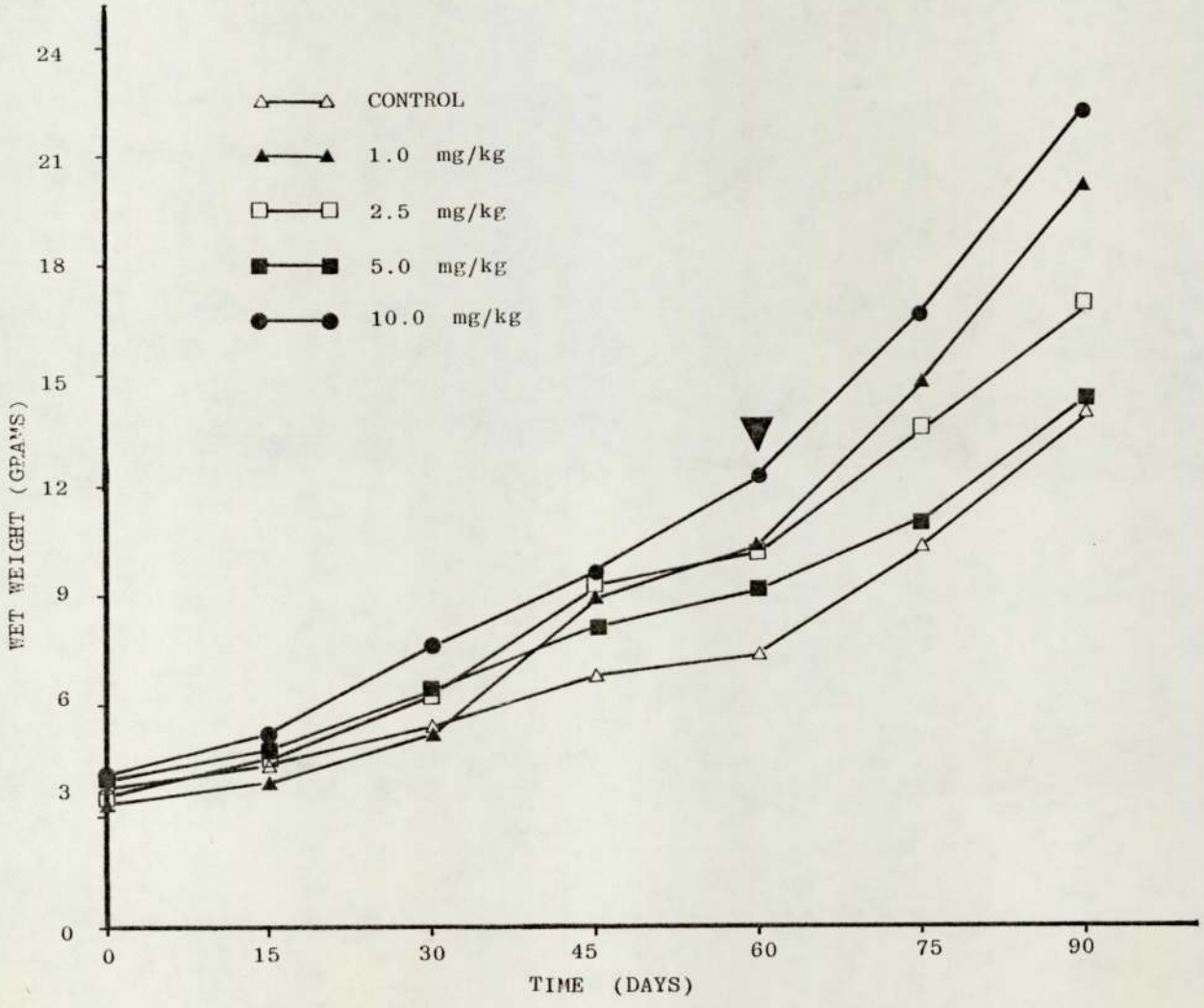


FIGURE 23 MEAN WEIGHTS OF DIFFERENT GROUPS OF CARP RECEIVING ADRENOSTERONE-SUPPLEMENTED DIETS. DRUG WAS WITHDRAWN AFTER 60 DAYS (ARROW).

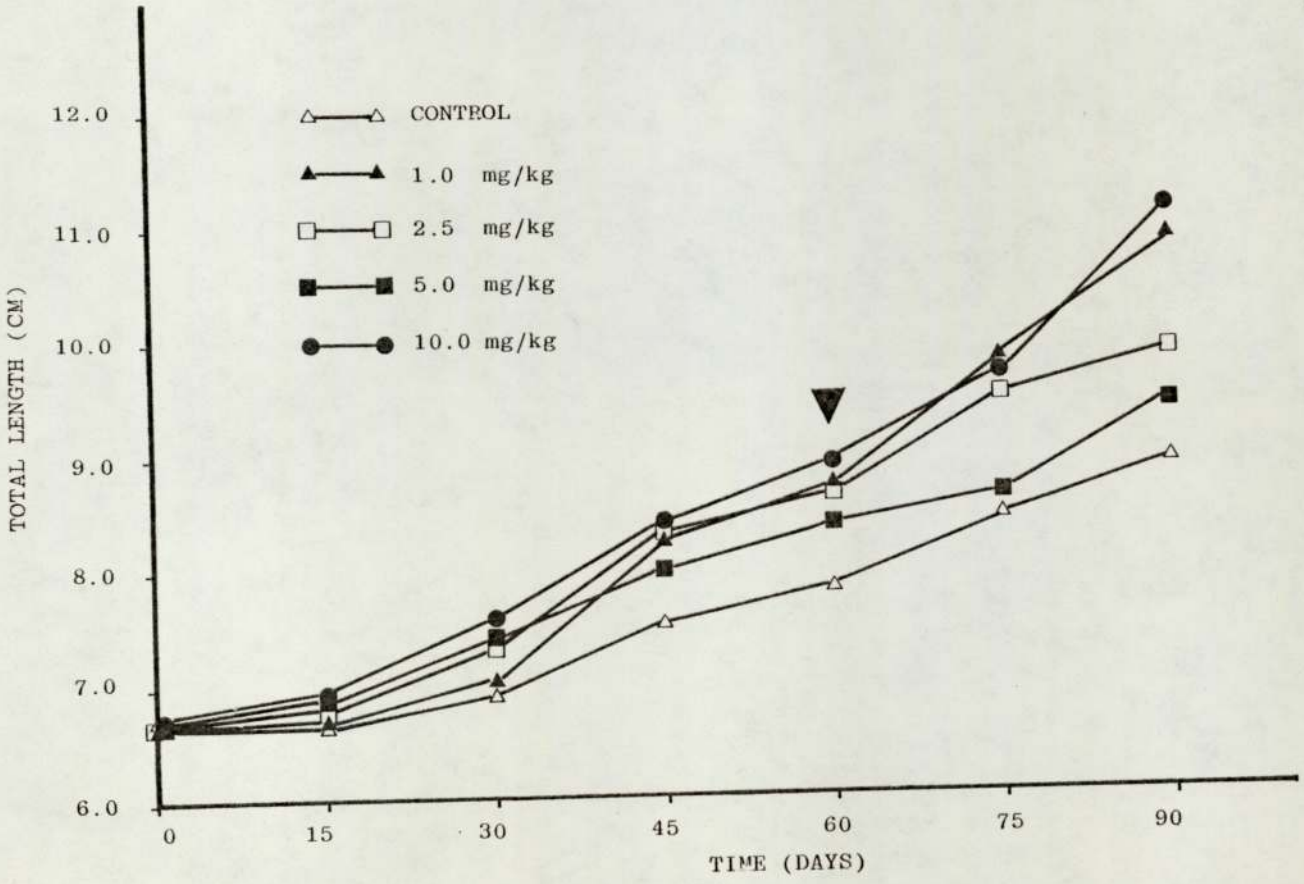


FIGURE 24 MEAN LENGTHS OF DIFFERENT GROUPS OF CARP RECEIVING ADRENOSTERONE-SUPPLEMENTED DIETS. DRUG WAS WITHDRAWN AFTER 60 DAYS (ARROW).

4.1.3.2. Tissue - Body Indices

Tissue-body indices are given in Table 26 and Figures 25 and 26. There were no differences in HSI and VSI after sixty days of the drug feeding. A decrease in CSI ($P<0.05$) and RSI ($P<0.01$) was observed. After ninety days, one month after the withdrawal, no change was noted in CSI HSI and RSI, while a decrease in VSI ($P<0.05$) was noted. The fact that CSI and RSI became equivalent to control values, show that the decrease in these indices, was the direct manifestation of the drug feeding.

4.1.3.3. Biochemical Changes

4.1.3.3.1. Liver (Table 27)

Total proteins were significantly higher only in 5.0 and 2.5 ppm groups ($P<0.05$) at sixty and ninety days respectively. RNA/DNA ratio although increased in all experimental groups, but was significant only in 5.0 and 10.0 ppm ($P<0.001$) groups at sixty days and at ninety days, a significant ($P<0.01$) decrease in RNA/DNA was noted in 5.0 ppm group. In phase 1, protein/RNA was signficant ($P<0.01$) only in 2.5 ppm group, while a steady increase was seen in all the experimental groups in protein/DNA ($P<0.05$). After ninety days, protein/RNA was higher ($P<0.05$) in 1.0 and 5.0 ppm groups, while no change was recorded in protein/DNA.

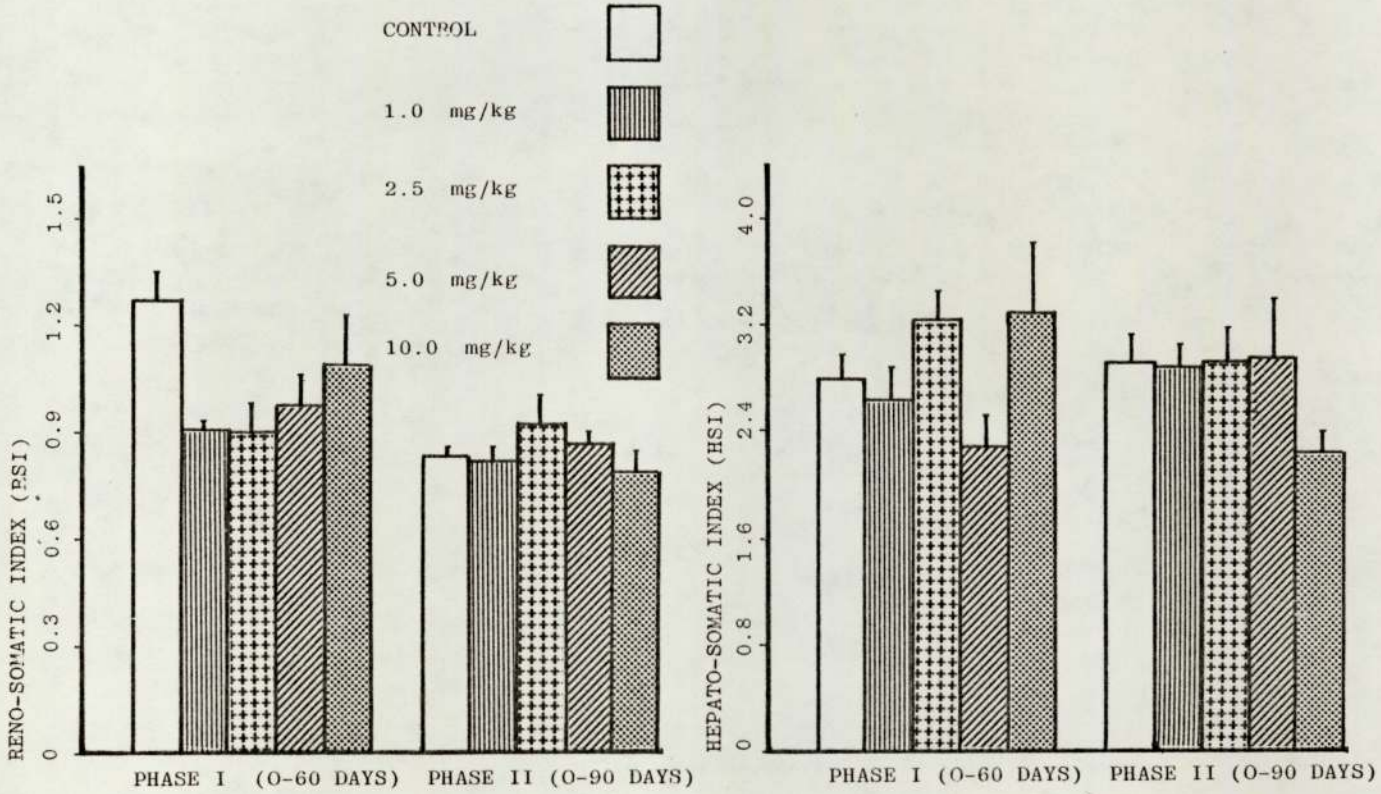


FIGURE 25 EFFECT OF ADRENOSTERONE GIVEN *PER OS* ON THE RSI AND HSI OF CARP. DRUG WAS WITHDRAWN AFTER 60 DAYS.

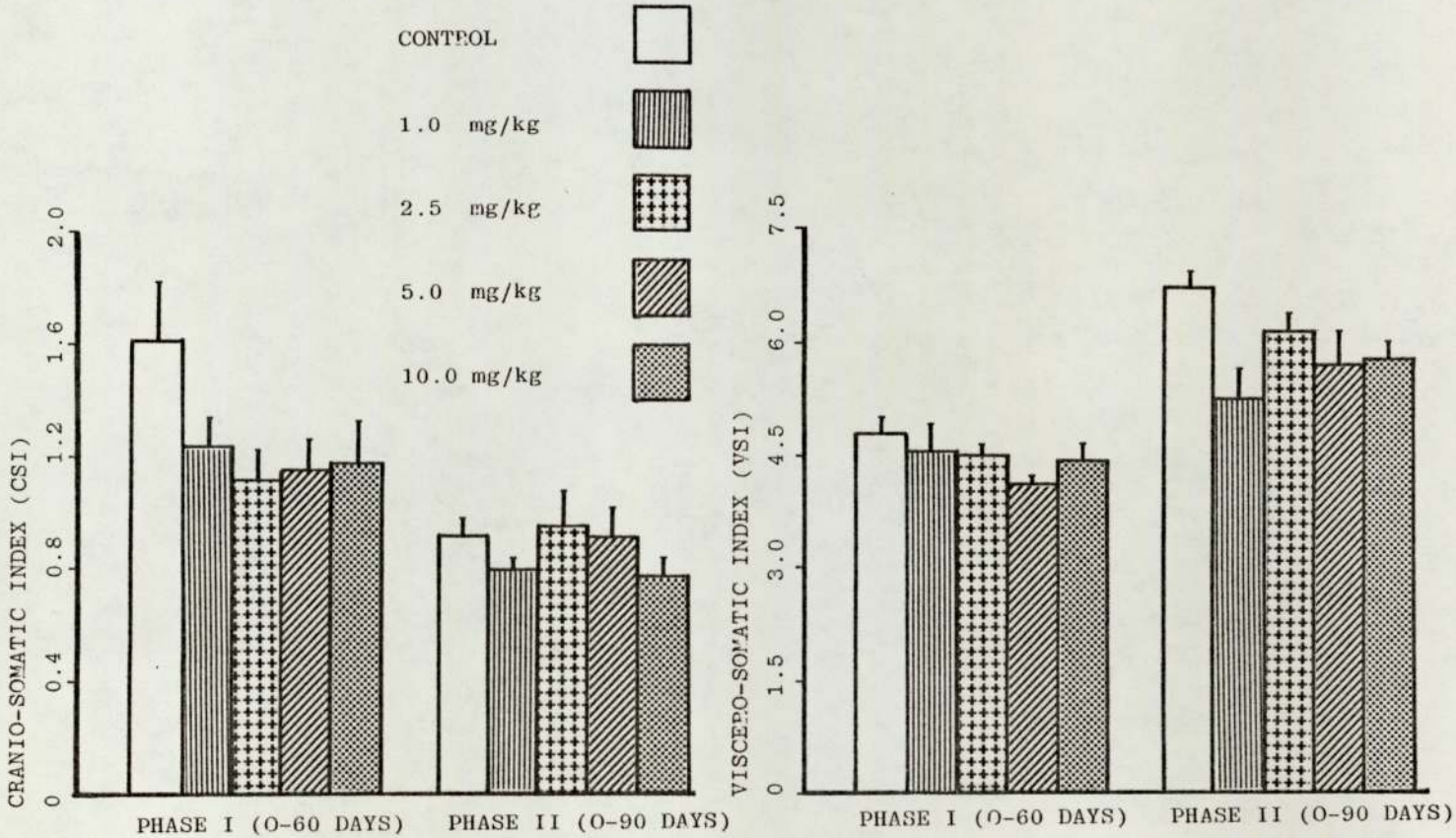


FIGURE 26 EFFECT OF ADRENOSTERONE GIVEN *PER OS* ON THE CSI AND VSI OF CARP. DRUG WAS WITHDRAWN AFTER 60 DAYS.

4.1.3.3.2. Kidney (Table 28)

At the end of phase 1, the total proteins in the kidney were higher in all the drug fed groups but were significant ($P<0.01$) only in 2.5 and 10.0 ppm group. RNA/DNA ratio was significant ($P<0.001$) in 1.0, 5.0 and 10.0 ppm groups. A significant ($P<0.05$) decrease was seen in protein/RNA ratio while protein/DNA was significantly ($P<0.05$) higher only in 10.0 ppm group. After ninety days, a decrease in proteins (10 ppm; $P<0.001$), increase in RNA/DNA (1.0 and 10.0 ppm; $P<0.05$), protein/RNA (2.5 and 5.0 ppm; $P<0.05$), and protein/DNA (1.0 and 10.0 ppm; $P<0.05$) was observed.

4.1.3.3.3. Brain (Table 29)

Total proteins were significantly higher ($P<0.001$) in all the phase 1 experimental groups. So was the case in RNA/DNA ($P<0.05$), but protein/RNA was significant only in 10.0 ppm group and protein/DNA in 2.5 and 10.0 ppm ($P<0.01$) groups. In phase 2, proteins (10.0 ppm; $P<0.001$) and RNA/DNA (2.5 and 5.0 ppm; $P<0.01$) were decreased, while protein/RNA ($P<0.001$) and protein/DNA ($P<0.05$) were increased.

4.1.3.3.4. Muscle (Tables 30 and 31)

Muscle exhibited significant increase in protein (10.0 ppm; $P<0.05$), RNA/DNA (1.0 and 2.5 ppm; $P<0.05$), protein/RNA (all groups; $P<0.01$) and protein/DNA (all groups; $P<0.001$) after sixty days of drug feeding. After the withdrawal period no change in protein and protein/RNA was observed. Increase was only seen in 1.0 and 2.5 ppm groups in RNA/

DNA ($P<0.01$) and protein/DNA ($P<0.05$).

The moisture content was increased in 2.5, 5.0 and 10.0 ppm groups ($P<0.01$) in phase 1, but no change was seen in phase 2 of the experiment. No change was observed in crude protein and total lipids both on wet and dry bases after sixty days of the drug feeding. After ninety days, no change was recorded in crude proteins, but total lipids were significantly ($P<0.001$) lowered both on wet and dry bases. Although, on wet weight basis no change was observed, ash content of the muscle on dry weight basis were lower than the controls ($P<0.05$).

TABLE 22 CHANGES IN BODY WEIGHT OF CARP FED ADRENOSTERONE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEAN (g) ± S.E. OF 25 FISH. PERCENT WEIGHT GAIN IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF ADRENOSTERONE mg/kg FOOD				
	0.00	1.00	2.50	5.00	10.00
0	3.76± 0.14	3.35± 0.19	3.70± 0.21	3.95± 0.29	3.96± 0.25
15	4.37± 0.19 (16.22)	3.91± 0.19 (16.72)	4.43± 0.26 (19.73)	4.76± 0.29 (20.51)	5.28± 0.47 (33.33)
30	5.40± 0.30 (43.62)	5.22± 0.30 (55.82)	6.25± 0.36 (68.92)	6.51± 0.44 (64.81)	7.72± 0.72 (94.95)
45	6.72± 0.53 (78.72)	8.80± 0.63 (162.69)	9.10± 0.63 (145.95)	8.10± 0.59 (105.06)	9.66± 0.87 (143.94)
60*	7.36± 0.61 (95.74)	10.18± 0.81 (203.88)	9.98± 0.76 (169.73)	9.06± 0.80 (129.37)	12.34± 1.56 (211.62)
75	10.28± 1.26 (173.40)	14.68± 1.70 (338.21)	13.53± 1.30 (265.68)	10.96± 1.36 (177.47)	16.64± 2.14 (320.20)
90	13.98± 2.16 (271.81)	20.12± 2.93 (500.60)	17.00± 2.90 (359.46)	14.28± 2.33 (261.52)	22.22± 2.83 (461.11)

* DRUG WITHDRAWN AFTER 60 DAYS.

STATISTICALLY SIGNIFICANT DIFFERENCES AMONG THE DOSES.
(SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF 1969)

60 DAYS

CONTROL VERSUS 1.00 = $P<0.01$

CONTROL VERSUS 2.50 = $P<0.05$

CONTROL VERSUS 10.00 = $P<0.001$

90 DAYS

CONTROL VERSUS 1.0 = $P<0.05$

CONTROL VERSUS 10.0 = $P<0.05$

TABLE 23 CHANGES IN TOTAL BODY LENGTH OF CARP FED ADRENOSTERONE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEAN (cm) ± S.E. OF 25 FISH. PERCENT INCREASE IN LENGTH IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF ADRENOSTERONE mg/kg FOOD				
	0.00	1.00	2.50	5.00	10.00
0	6.63± 0.08	6.67± 0.13	6.70± 0.12	6.70± 0.13	6.69± 0.19
15	6.66± 0.09 (0.45)	6.69± 0.11 (0.30)	6.80± 0.14 (1.49)	6.85± 0.14 (2.24)	6.92± 0.19 (3.44)
30	6.91± 0.12 (4.22)	7.03± 0.13 (5.40)	7.37± 0.14 (10.00)	7.39± 0.17 (10.30)	7.61± 0.22 (13.75)
45	7.48± 0.18 (12.82)	8.24± 0.20 (23.54)	8.29± 0.19 (23.73)	7.97± 0.20 (18.96)	8.34± 0.23 (24.66)
60*	7.82± 0.21 (17.95)	8.63± 0.24 (29.39)	8.62± 0.22 (28.66)	8.33± 0.26 (24.33)	8.90± 0.38 (33.03)
75	8.42± 0.34 (27.00)	9.73± 0.43 (45.88)	9.45± 0.30 (41.04)	8.58± 0.38 (28.06)	9.68± 0.42 (44.69)
90	8.90± 0.46 (34.24)	10.78± 0.49 (61.62)	9.87± 0.38 (47.31)	9.40± 0.53 (40.30)	11.10± 0.52 (65.92)

* DRUG WITHDRAWN AFTER 60 DAYS.

STATISTICALLY SIGNIFICANT DIFFERENCE AMONG DOSES.
(SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF 1969)

60 DAYS

CONTROL VERSUS 1.0 = $P<0.05$

CONTROL VERSUS 2.5 = $P<0.05$

CONTROL VERSUS 10.0 = $P<0.05$

90 DAYS

CONTROL VERSUS 1.0 = $P<0.05$

CONTROL VERSUS 10.0 = $P<0.01$

TABLE 24 EFFECT OF FEEDING AND WITHDRAWAL OF ADRENOSTERONE ON THE SPECIFIC GROWTH RATE (SGR) OF CARP. DRUG WAS FED FOR 60 DAYS ONLY.

CONCENTRATION OF DRUG mg/kg FOOD	SPECIFIC GROWTH RATE (WEIGHT)			SPECIFIC GROWTH RATE (LENGTH)		
	0-60 DAYS	60-90 DAYS	0-90 DAYS	0-60 DAYS	60-90 DAYS	0-90 DAYS
0.00	1.15± 0.22	2.16± 0.13	1.49± 0.25	0.29± 0.11	0.43± 0.04	0.34± 0.08
1.00	1.94± 0.66	2.30± 0.12	2.06± 0.42	0.45± 0.24	0.75± 0.04	0.55± 0.16
2.50	1.72± 0.49	1.79± 0.18	1.74± 0.31	0.44± 0.16	0.45± 0.11	0.44± 0.11
5.00	1.42± 0.28	1.53± 0.17	1.46± 0.19	0.37± 0.09	0.40± 0.15	0.38± 0.10
10.00	1.95± 0.22	1.98± 0.02	1.96± 0.14	0.49± 0.10	0.74± 0.13	0.57± 0.09

TABLE 25 CHANGES IN THE CONDITION FACTOR AND FOOD CONVERSION EFFICIENCY OF CARP ADMINISTERED ADRENOSTERONE IN THE DIET FOR 60 DAYS.

CONCENTRATION OF DRUG mg/kg FOOD	CONDITION FACTOR			FOOD CONVERSION EFFICIENCY		
	0 DAYS	60 DAYS	90 DAYS	0–60 DAYS	60–90 DAYS	0–90 DAYS
0.00	1.29± 0.21	1.54± 0.32	1.98± 0.26	0.26	0.58	0.40
1.00	1.19± 0.14	1.58± 0.31	1.61± 0.23	0.45	0.67	0.56
2.50	1.23± 0.21	1.56± 0.26	1.77± 0.29	0.37	0.50	0.43
5.00	1.31± 0.26	1.57± 0.29	1.72± 0.31	0.30	0.45	0.36
10.00	1.31± 0.32	1.75± 0.43	1.62± 0.32	0.44	0.52	0.48

TABLE 26 EFFECT OF FEEDING ADRENOSTERONE ON THE CRANIO-SOMATIC (CSI), HEPATO-SOMATIC (HSI), RENO-SOMATIC (RSI), AND VISCERO-SOMATIC (VSI) INDEX OF CARP. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg FOOD	CSI		HSI		RSI		VSI	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	1.61± 0.19	0.91± 0.05	2.79± 0.16	2.91± 0.19	1.28± 0.06	0.84± 0.01	4.79± 0.21	6.72± 0.23
1.00	1.24± 0.11	0.80± 0.03	2.64± 0.25	2.87± 0.21	0.92± 0.01 **	0.82± 0.03	4.59± 0.29	5.23± 0.40 **
2.50	1.11± 0.10 *	0.96± 0.12	3,24± 0.22	2.93± 0.22	0.91± 0.08 **	0.93± 0.07	4.48± 0.15	6.12± 0.24
5.00	1.16± 0.09 *	0.92± 0.10	2.27± 0.21	2.96± 0.39	0.97± 0.09 *	0.87± 0.03	4.16± 0.07	5.73± 0.42 *
10.00	1.18± 0.13 *	0.77± 0.05	3.26± 0.51	2.22± 0.17	1.09± 0.13	0.79± 0.05	4.40± 0.21	5.80± 0.29

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 27 EFFECT OF FEEDING ADRENOSTERONE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN LIVER OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA/ DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	25.14± 1.83	19.91± 0.93	3.11± 0.16	6.33± 0.53	18.46± 0.94	15.46± 0.76	57.36± 4.22	97.62± 8.85
1.00	23.79± 2.32	28.05± 1.91	3.77± 0.28	6.46± 0.62	16.56± 1.72	* 19.33± 0.47	62.09± 6.42	124.43± 10.64
2.50	22.42± 2.18	* 30.83± 5.39	3.16± 0.07	5.23± 0.29	** 25.79± 2.18	18.14± 1.01	* 81.18± 5.90	94.63± 6.31
5.00	* 38.60± 4.47	17.40± 1.77	*** 4.53± 0.25	** 4.16± 0.23	17.77± 1.07	* 19.24± 2.19	* 80.83± 8.20	81.03± 12.57
10.00	29.20± 4.42	24.60± 1.77	*** 4.58± 0.29	6.48± 0.52	17.69± 1.19	17.10± 0.77	* 81.26± 8.79	111.35± 12.11

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 28 EFFECT OF FEEDING ADRENOSTERONE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN KIDNEY OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	31.07± 2.06	47.35± 2.13	1.96± 0.06	3.22± 0.60	17.67± 0.64	12.85± 0.46	34.65± 1.27	42.14± 9.75
1.00	31.91± 4.40	46.62± 1.09	** 3.03± 0.15	* 5.08± 0.64	* 14.62± 0.56	13.97± 0.38	44.00± 1.17	* 70.27± 6.86
2.50	** 48.00± 6.72	43.86± 3.34	2.28± 0.11	3.94± 0.35	18.32± 1.08	* 14.81± 0.36	42.50± 4.23	58.43± 5.30
5.00	37.01± 1.81	43.81± 3.03	** 3.12± 0.24	3.03± 0.30	** 12.67± 1.67	* 15.74± 0.57	38.96± 5.19	47.75± 5.37
10.00	* 44.87± 1.46	*** 24.05± 2.50	*** 3.24± 0.38	* 4.99± 0.62	14.80± 0.52	13.66± 0.64	* 48.04± 6.44	* 69.30± 11.27

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL AND ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 29 EFFECT OF FEEDING ADRENOSTERONE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN BRAIN OF *Cyprinus carpio*. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	23.19± 1.16	34.07± 1.14	1.89± 0.08	2.84± 0.13	18.90± 0.88	16.19± 0.39	35.54± 0.47	45.87± 2.09
1.00	** 34.27± 1.32	36.15± 2.21	** 2.36± 0.06	2.86± 0.11	15.89± 0.70	** 20.32± 1.67	35.92± 0.41	* 58.69± 6.94
2.50	*** 49.22± 3.43	36.35± 0.41	2.12± 0.03	*** 1.93± 0.09	22.15± 1.65	*** 28.11± 0.66	** 46.80± 3.55	54.30± 3.41
5.00	** 36.60± 2.23	30.30± 3.58	* 2.31± 0.16	** 2.07± 0.11	17.98± 0.84	*** 25.15± 0.39	41.66± 3.83	52.21± 3.28
10.00	*** 48.95± 3.47	*** 20.82± 1.87	* 2.27± 0.13	2.78± 0.20	* 23.13± 1.43	*** 23.22± 0.89	*** 52.18± 2.71	** 64.26± 3.75

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 30 EFFECT OF FEEDING ADRENOSTERONE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN MUSCLE OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	15.28± 0.37	16.33± 0.73	5.69± 0.30	7.16± 0.14	63.58± 3.23	62.62± 3.01	363.49± 34.95	447.48± 18.39
1.00	18.93± 2.28	13.42± 1.46	* 7.44± 0.57	** 12.19± 1.52	** 87.96± 4.43	58.79± 2.58	*** 697.44± 27.72	* 710.58± 77.75
2.50	17.35± 1.12	15.83± 0.65	* 7.20± 0.46	*** 14.41± 1.27	* 81.32± 5.49	50.80± 2.32	*** 578.95± 23.49	* 739.90± 97.73
5.00	18.48± 0.39	15.10± 0.43	5.80± 0.55	8.06± 1.22	* 81.64± 7.70	76.54± 8.06	* 460.11± 3.94	622.46± 126.07
10.00	* 19.70± 1.70	15.50± 0.47	5.74± 0.30	8.92± 0.86	*** 105.38± 5.33	73.23± 4.57	*** 604.81± 40.67	644.53± 41.81

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 31 EFFECT OF FEEDING ADRENOSTERONE ON THE PROXIMATE COMPOSITION OF MUSCLE TISSUE FROM Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCENTRATION OF DRUG mg/kg	MOISTURE mg/100 mg		CRUDE PROTEIN N x 6.25 (mg/100 mg)				TOTAL LIPIDS (mg/100 mg)				ASH (mg/100 mg)			
			ON WET BASIS		ON DRY BASIS		ON WET BASIS		ON DRY BASIS		ON WET BASIS		ON DRY BASIS	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	72.57± 0.70	72.43± 0.48	17.44± 1.00	18.13± 0.63	63.50± 2.63	65.88± 2.69	3.18± 0.04	5.11± 0.47	11.58± 0.10	18.51± 1.47	2.98± 0.18	2.14± 0.13	10.80± 0.53	7.78± 0.48
1.00	74.15± 0.84	71.02± 1.49	17.63± 0.56	18.75± 1.31	68.13± 0.56	64,38± 1.50	2.82± 0.56	* 3.75± 0.14	9.63± 1.57	* 14,55± 0.54	2.22± 0.23	1.93± 0.14	8.60± 0.99	* 6.63± 0.15
2.50	** 76.08± 0.75	72.40± 1.23	17.31± 0.63	18.88± 0.88	72.19± 2.56	68.56± 2.00	3.14± 0.28	* 3.76± 0.64	11.31± 0.65	* 15.49± 2.44	* 1.79± 0.07	1.82± 0.05	7.45± 0.39	* 6.60± 0.22
5.00	* 75.19± 0.91	73.44± 2.60	17.56± 0.50	17.69± 1.69	70.88± 0.88	66.63± 0.44	2.23± 0.25	*** 2.73± 0.32	8.46± 0.70	*** 9.71± 1.69	2.14± 0.06	1.75± 0.22	8.65± 0.39	* 6.55± 0.32
10.00	*** 76.96± 0.95	72.91± 1.06	15.44± 1.19	18.50± 0.50	66.88± 3.06	68.44± 1.63	2.96± 0.37	*** 2.82± 0.14	10.84± 1.02	** 12.20± 0.28	* 1.80± 0.06	1.94± 0.10	7.80± 0.14	7.15± 0.18

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

4.1.4. Effect of Feeding Testosterone, 11-ketotestosterone and Adrenosterone on the Total Proteases Activity of the Alimentary Canal

In the experiments with the "natural" steroids (testosterone, 11-ketotestosterone and adrenosterone) total proteases activity of the gut was determined after sixty days of feeding of the drug and again after thirty days of drug withdrawal. The results of these experiments are shown in Tables 32, 33 and 34. After sixty days of the drug feeding, both total protein content and proteases activity was higher in experimental groups of all the three drugs. After withdrawal of the drug, except in the case of 1.0 ppm (testosterone) whose protein and proteases activity was still higher than the controls, all other groups of experimental fish exhibited a decline in both protein ($P<0.001$) and proteases activity ($P<0.001$ for testosterone and 11-ketotestosterone). In the case of 11-ketotestosterone, the proteases activity has a direct correlation ($r = 0.7482$) with the total mean weight and food conversion efficiency in all the experimental animals after sixty days of drug feeding.

TABLE 32 EFFECT OF FEEDING TESTOSTERONE ON THE TOTAL PROTEIN AND PROTEASE ACTIVITY OF THE INTESTINE OF CARP. DRUG WAS FED FOR 60 DAYS ONLY. PROTEASE ACTIVITY IS GIVEN AS O.D. UNITS PER 100 mg OF THE FRESH WEIGHT OF THE INTESTINE.

CONCEN-TRATION OF THE DRUG mg/kg FOOD	60 DAYS			90 DAYS		
	TOTAL PROTEIN mg/100 mg	O.D. UNITS AT 650	% DIFFERENCE O.D./ CONTROL	TOTAL PROTEIN mg/100 mg	O.D. UNITS AT 650	% DIFFERENCE O.D. / CONTROL
0.00	27.56	0.403		36.05± 0.60	0.937± 0.90	
1.00	35.10	0.483	19.85	40.81± 0.79	1.516± 1.79	51.12
2.50	37.68	0.612	51.86	22.80± 1.80	0.615± 0.59	-34.36
5.00	38.59	0.926	129.78	27.42± 2.10	0.783± 1.75	-16.44
10.00	37.60	0.752	86.60	27.43± 2.34	0.480± 0.38	-48.77

TABLE 33 EFFECT OF FEEDING 11-KETOTESTOSTERONE ON THE TOTAL PROTEIN AND PROTEASE ACTIVITY OF THE INTESTINE OF CARP. DRUG WAS FED FOR 60 DAYS ONLY. PROTEASE ACTIVITY IS GIVEN AS O.D. UNITS PER 100 mg OF THE FRESH WEIGHT OF THE INTESTINE.

CONCEN-TRATION OF DRUG mg/kg FOOD	60 DAYS			90 DAYS		
	TOTAL PROTEIN mg/100 mg	O.D. UNITS AT 650	% DIFFERENCE O.D./CONTROL	TOTAL PROTEIN mg/100 mg	O.D. UNITS AT 650	% DIFFERENCE O.D./CONTROL
0.00	27.56	0.403		36.05± 0.60	0.937± 0.90	
1.00	33.02	0.860	113.40	29.72± 1.30	0.501± 0.11	-46.53
2.50	34.72	1.340	232.51	30.00± 0.76	0.495± 0.45	-47.17
5.00	26.69	0.160	-60.30	34.01± 0.74	0.486± 0.40	-48.13
10.00	27.02	0.484	20.10	27.91± 1.52	0.586± 0.83	-37.46

TABLE 34 EFFECT OF FEEDING ADRENOSTERONE ON THE TOTAL PROTEIN AND PROTEASE ACTIVITY OF THE INTESTINE OF CARP. DRUG WAS FED FOR 60 DAYS ONLY. PROTEASE ACTIVITY IS GIVEN AS O.D. UNITS PER 100 mg OF THE FRESH WEIGHT OF THE INTESTINE.

CONCEN-TRATION OF THE DRUG mg / kg FOOD	60 DAYS			90 DAYS		
	TOTAL PROTEIN mg/100 mg	O.D. UNITS AT 650	% DIFFERENCE O.D./CONTROL	TOTAL PROTEIN mg/100 mg	O.D. UNITS AT 650	% DIFFERENCE O.D./CONTROL
0.00	27.56	0.403		36.05± 0.60	0.937± 0.90	
1.00	34.29	0.888	120.35	34.35± 3.00	0.775± 1.73	-17.29
2.50	30.09	0.541	34.24	24.25± 0.22	0.641± 1.04	-31.59
5.00	31.49	0.577	43.18	27.43± 1.38	0.755± 1.10	-19.42
10.00	34.16	0.953	136.48	28.66± 0.83	0.835± 0.64	-10.89

4.1.5. Methyltestosterone

4.1.5.1. Weight and Length Data

The weight and length data accumulated over a period of one hundred and fifty days (ninety days of drug feeding and sixty days of drug withdrawal) is presented in Tables 35 and 36 and Figures 27 and 28. It is evident that the experimental groups which received 1.0, 2.5 and 5.0 ppm of methyltestosterone were growing faster in weight than the controls throughout the study, despite the fact that the drug was withdrawn after ninety days ($P<0.01$ and $P<0.001$ for ninety and one hundred and fifty days respectively). The fourth group which received 10.0 ppm of the drug grew nearly parallel to the control group. At the time when experimental groups were transferred to control diets the percentage weight gain by each of them over the controls was 40.41, 39.00 and 18.25 for 1.0, 2.5 and 5.0 mg/kg groups respectively, while the 10.0 mg/kg group showed a decrease of 6.08% from the controls. At the same time, the length of these groups were 37.70, 49.48, 37.96 and 2.36% greater than the controls.

The specific growth rate for weight and length are given in Table 37. As is seen in this table, in phase 2 of the experiment there was no retardation in growth of the experimental groups compared with the control group. Although the SGR both for weight and length decreased for this period (ninety to one hundred and fifty days) for all the experimental groups compared with the phase 1

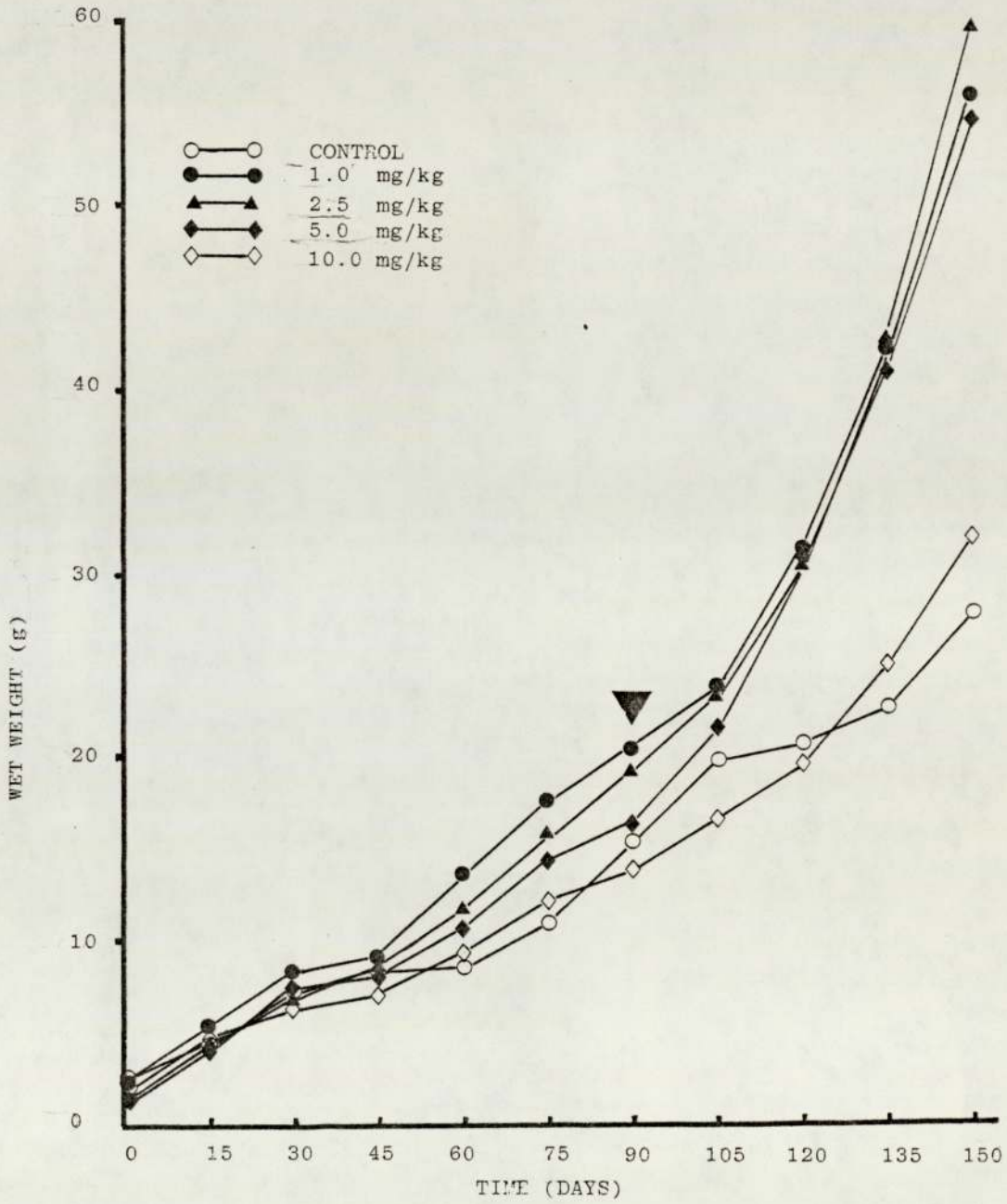


FIGURE 27 MEAN WEIGHTS OF CARP GIVEN METHYLTESTOSTERONE *PER OS*. DRUG WAS WITHDRAWN AFTER 90 DAYS (ARROW).

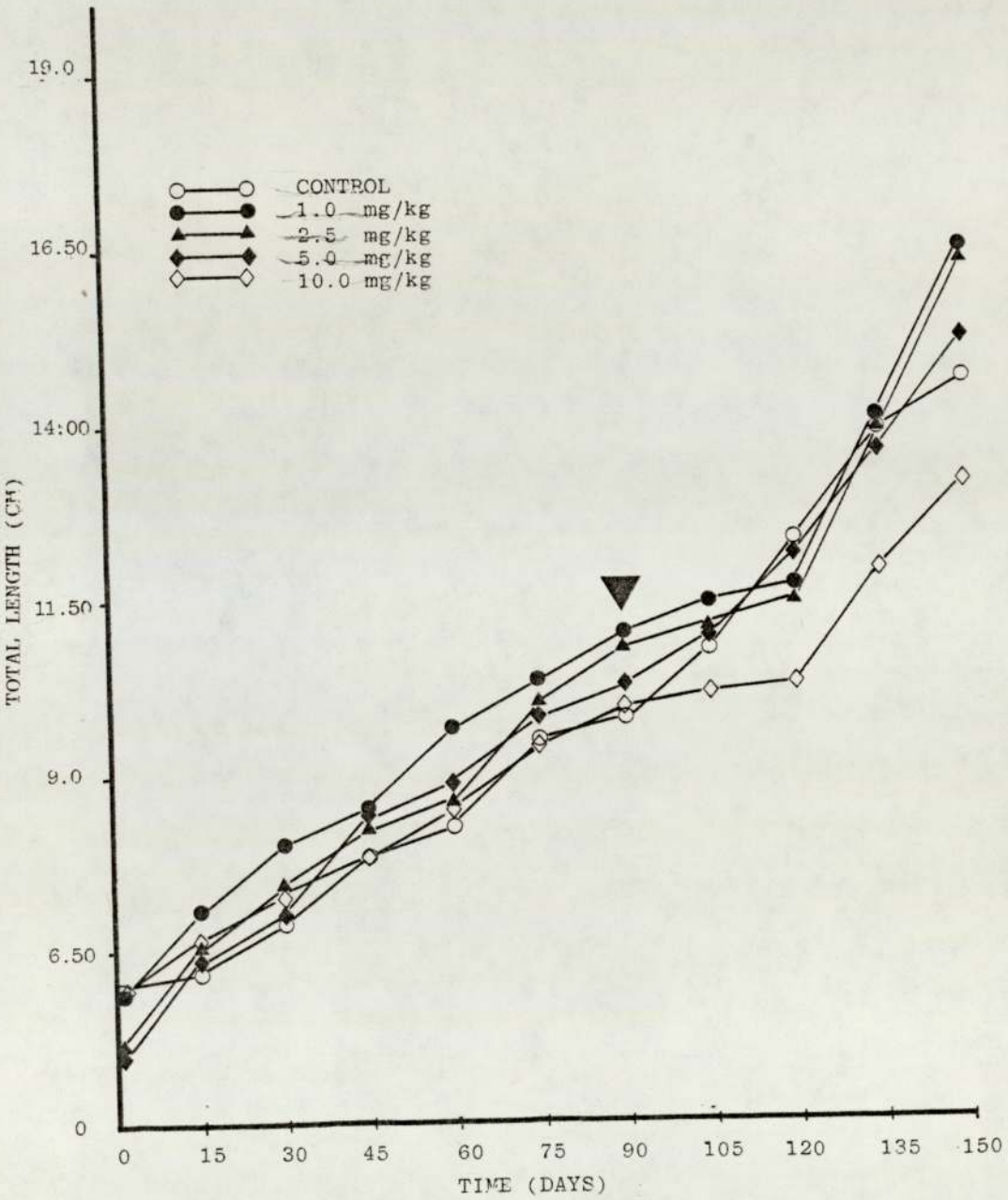


FIGURE 28 MEAN LENGTHS OF CARP GIVEN METHYLTESTOSTERONE *PER OS*. DRUG WAS WITHDRAWN AFTER 90 DAYS (ARROW).

values, the SGR for weight in these groups was still higher than the controls.

The regression equations for weight and length data in phases 1 and 2 of the experiment were also computed. The analysis of co-variance (Zar, 1974) showed that the slopes of the regression lines for weight at ninety and one hundred and fifty days were significantly different ($P<0.001$), but the slopes for length were not, demonstrating that methyltestosterone is more potent in increasing weight than length.

The condition factor and FCE is given in Table 38. The experimental groups were converting food into flesh more efficientyly both at ninety and one hundred and fifty days than the control group.

4.1.5.2. Tissue - Body Indices

The effect of feeding methyltestosterone on CSI, HSI, RSI and VSI was quite variable and is given in Table 39 and Figures 29 and 30.

4.1.5.3. Biochemical Changes

4.1.5.3.1. Liver (Table 40)

A significant increase in total proteins ($P<0.001$), RNA/DNA ($P<0.001$), protein/RNA ($P<0.05$) and protein/DNA was observed after ninety days in all the experimental groups. Cholesterol was significantly decreased ($P<0.001$)

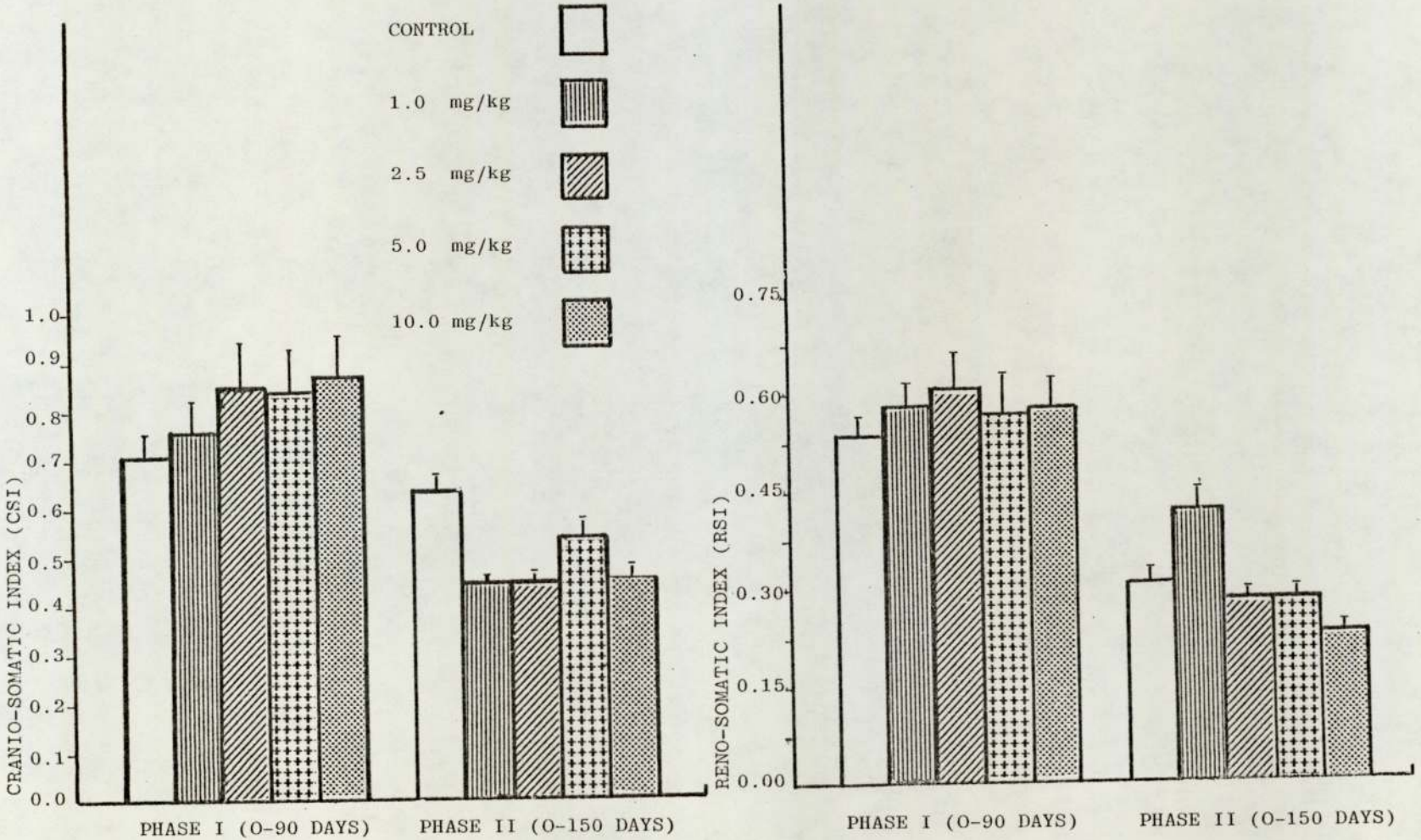


FIGURE 29 EFFECT OF METHYLTESTOSTERONE GIVEN *PER OS* ON THE CSI AND RSI OF CARP. DRUG WAS WITHDRAWN AFTER 90 DAYS.

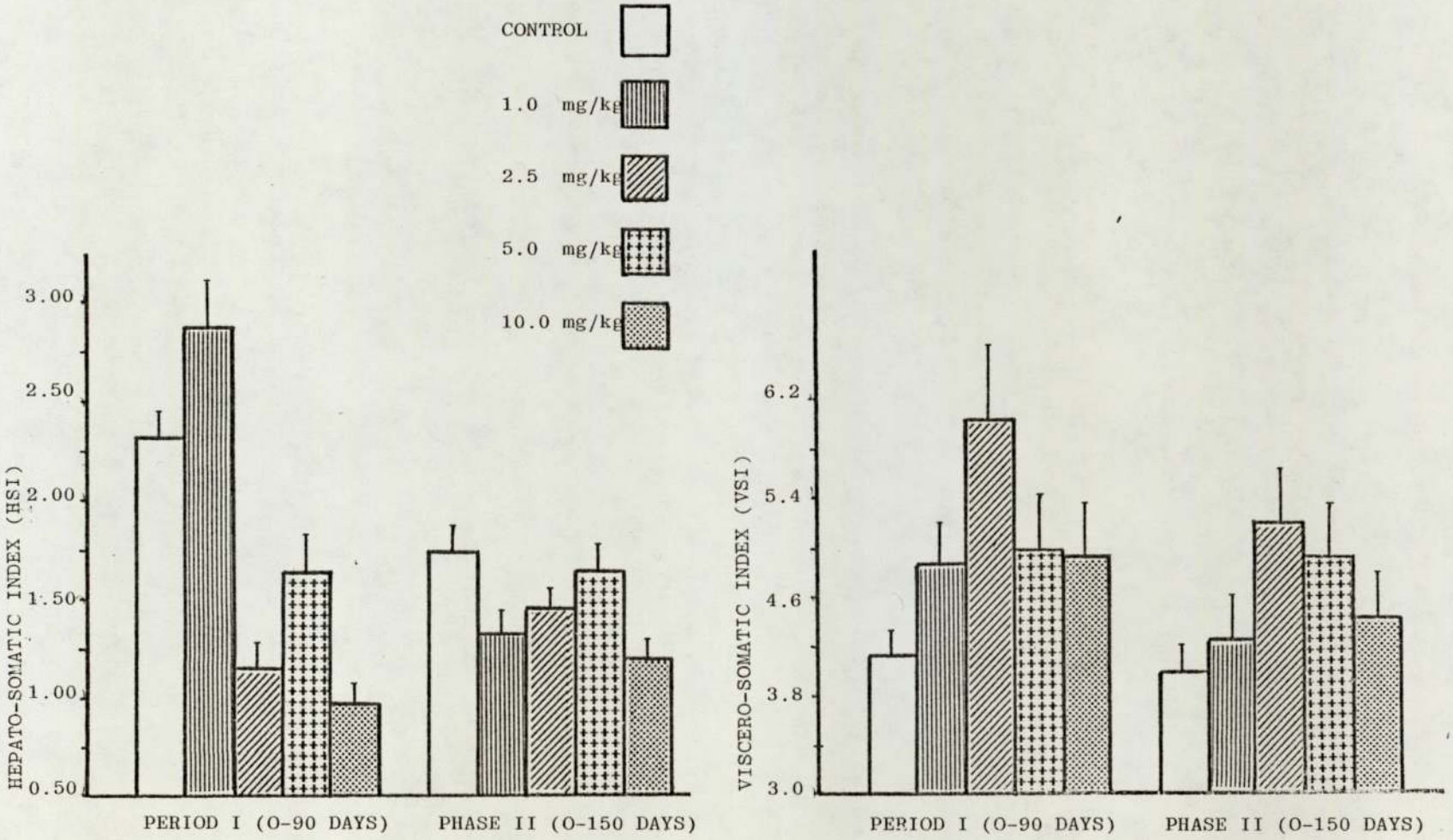


FIGURE 30 EFFECT OF METHYLTESTOSTERONE GIVEN *PER OS* ON THE HSI AND VSI OF CARP. DRUG WAS WITHDRAWN AFTER 90 DAYS.

in all experimental groups. While no change from control values was evident in total liver proteins and protein/RNA, the RNA/DNA and protein/DNA ratios were still higher in experimental groups at one hundred and fifty days. Cholesterol was significantly ($P<0.05$) less in 5.0 and 10.0 ppm groups.

4.1.5.3.2. Kidney (Table 41)

After ninety days, a significant increase in protein, protein/RNA and protein/DNA was observed. Except for 10.0 ppm group, where RNA/DNA increased ($P<0.001$), no change was seen in other groups. A significant decrease ($P<0.01$) in cholesterol was noted.

After one hundred and fifty days, a decrease in protein content in 1.0 and 2.5 ppm and an increase in RNA/DNA and protein/DNA ($P<0.001$) was observed. A significant ($P<0.001$) decrease in protein/RNA and cholesterol was observed.

4.1.5.3.3. Brain (Table 42)

Total proteins were increased ($P<0.01$) after ninety days. There was no difference in RNA/DNA and protein/RNA, while protein/DNA ($P<0.01$) and cholesterol ($P<0.001$) were decreased. In phase 2, the proteins were significantly higher in 10.0 ppm groups only. RNA/DNA and protein/DNA were reduced in experimental groups. No change was recorded in protein/RNA, and cholesterol did not show any defineable pattern.

4.1.5.3.4. Muscle (Tables 43, 44, 45 and 46)

A significant elevation from controls was noted in total proteins ($P<0.01$), RNA/DNA ($P<0.001$), protein/RNA ($P<0.05$), protein/DNA ($P<0.001$) and total cholesterol ($P<0.001$) after ninety days of drug feeding. After one hundred and fifty days, an increase in 1.0 ppm and decrease ($P<0.05$) in other groups was noted for total proteins. RNA/DNA and protein/DNA were still higher but protein/RNA and cholesterol were lowered.

The moisture content of the experimental groups was lower than the controls both in phase 1 and phase 2 of the experiment. A significant increase in total lipids and a non significant decrease in ash was noted in phase 1. In phase 2, the lipid content of the experimental decreased ($P<0.01$), while no difference was observed in ash content.

The effect of methyltestosterone feeding for ninety days and sixty days of drug withdrawal on the muscle free amino acids is given in Tables 45 and 46. It is clear from these tables that the experimental groups has less free amino acids in the muscle which is probably related to the active protein synthetic activity of this tissue in these fish.

TABLE 35 CHANGES IN BODY WEIGHT OF CARP FED METHYLTESTOSTERONE SUPPLEMENTED DIETS FOR 90 DAYS. VALUES GIVEN ARE MEANS (g) ± S.E. OF 30 FISH. PERCENT WEIGHT GAIN IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF METHYLTESTOSTERONE mg/kg DRY FOOD				
	0.00	1.00	2.50	5.00	10.00
0	2.60± 0.23	2.36± 0.13	1.50± 0.11	1.24± 0.06	2.29± 0.10
15	4.32± 0.20 (66.15)	5.29± 0.24 (124.15)	4.48± 0.34 (198.67)	4.00± 0.20 (222.58)	4.39± 0.22 (91.70)
30	7.25± 0.25 (178.85)	8.26± 0.54 (249.85)	6.75± 0.50 (350.00)	6.33± 0.31 (410.58)	6.34± 0.84 (176.86)
45	8.32± 0.66 (220.00)	9.58± 0.52 (305.93)	8.76± 0.61 (484.00)	8.66± 0.48 (588.39)	7.12± 0.31 (210.92)
60	8.57± 0.59 (229.62)	13.54± 0.98 (473.73)	11.76± 1.14 (684.00)	10.76± 0.75 (767.75)	9.35± 0.70 (308.30)
75	11.93± 0.67 (358.58)	17.50± 1.24 (641.53)	15.96± 1.32 (964.00)	14.41± 1.40 (1062.10)	12.26± 1.19 (435.38)
90*	15.42± 0.74 (493.08)	20.36± 1.07 (762.71)	19.32± 1.61 (1137.86)	16.40± 1.73 (1172.44)	14.33± 1.46 (475.62)
105	19.93± 1.23 (666.54)	23.69± 1.43 (903.81)	23.39± 2.77 (1459.33)	21.67± 2.10 (1647.58)	16.75± 1.90 (631.44)
120	20.84± 1.31 (701.54)	31.21± 2.72 (1222.46)	30.60± 4.83 (1940.00)	30.77± 2.32 (2381.45)	19.77± 2.67 (763.32)
135	22.85± 1.59 (778.85)	42.08± 3.01 (1683.05)	42.75± 5.09 (2750.00)	40.97± 4.61 (3204.03)	25.19± 2.13 (1000.00)
150	27.96± 1.93 (975.38)	55.87± 5.4 (2267.37)	59.72± 5.93 (3881.33)	54.59± 5.70 (4302.42)	32.10± 3.51 (1301.75)

* DRUG WITHDRAWN AFTER 90 DAYS.

STATISTICALLY SIGNIFICANT DIFFERENCES AMONG THE DOSES. (SINGLE FACTOR ANALYSIS OF VARIANCE ACCORDING TO SOKAL & ROHLF 1969).

90 DAYS		150 DAYS	
CONTROLS VERSUS 1.0	$P < 0.01$	CONTROLS VERSUS 1.0;2.5;5.0	$P < 0.001$
1.0 VERSUS 10.0	$P < 0.01$	1.0 VERSUS 10.0	$P < 0.001$
2.5 VERSUS 10.0	$P < 0.05$	2.5 VERSUS 10.0	$P < 0.001$
		5.0 VERSUS 10.0	$P < 0.001$

TABLE 36 CHANGES IN TOTAL BODY LENGTH OF CARP FED METHYLTESTOSTERONE SUPPLEMENTED DIETS FOR 90 DAYS. VALUES GIVEN ARE MEAN (cm) ± S.E. OF 30 FISH. PERCENT INCREASE IN LENGTH IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF METHYLTESTOSTERONE (mg/kg DRY FOOD)				
	0.00	1.00	2.50	5.00	10.00
0	5.97± 0.20	5.75± 0.12	5.11± 0.14	4.98± 0.08	5.95± 0.10
15	6.18± 0.28 (3.52)	7.07± 0.08 (22.96)	6.58± 0.11 (28.77)	6.38± 0.07 (28.11)	6.64± 0.07 (11.60)
30	6.88± 0.11 (15.24)	8.03± 0.12 (39.65)	7.42± 0.12 (45.20)	7.01± 0.08 (40.76)	7.28± 0.13 (22.35)
45	7.86± 0.14 (31.66)	8.54± 0.12 (48.53)	8.27± 0.14 (61.84)	8.46± 0.13 (69.88)	7.84± 0.09 (31.77)
60	8.26± 0.14 (38.66)	9.69± 0.15 (68.52)	8.63± 0.17 (68.88)	8.90± 0.14 (78.41)	8.56± 0.13 (43.57)
75	9.49± 0.20 (58.96)	10.34± 0.19 (79.83)	10.05± 0.20 (96.67)	9.83± 0.17 (97.39)	9.42± 0.20 (58.32)
90*	9.79± 0.17 (63.99)	11.01± 0.23 (91.48)	10.82± 0.33 (111.74)	10.25± 0.22 (105.82)	9.86± 0.20 (65.71)
105	10.77± 0.17 (80.40)	11.45± 0.27 (99.13)	11.09± 0.53 (117.03)	10.98± 0.24 (120.48)	10.16± 0.37 (70.75)
120	12.33± 0.32 (106.53)	11.70± 0.39 (103.48)	11.47± 0.59 (124.46)	12.10± 0.27 (142.97)	10.29± 0.41 (72.94)
135	13.88± 0.39 (132.50)	14.05± 0.64 (144.35)	13.93± 0.74 (172.60)	13.54± 0.53 (171.81)	11.89± 0.49 (99.83)
150	14.57± 0.72 (144.05)	16.43± 0.92 (185.74)	16.31± 1.12 (219.18)	15.15± 0.61 (204.22)	13.12± 0.63 (120.50)

* DRUG WITHDRAWN AFTER 90 DAYS.

STATISTICALLY SIGNIFICANT DIFFERENCES AMONG THE DOSES. (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF 1969)

90 DAYS	150 DAYS
CONTROLS VERSUS 1.0 = P< 0.01	CONTROLS VERSUS 1.0 = P<0.05
CONTROLS VERSUS 2.5 = P< 0.05	CONTROLS VERSUS 2.5 = P<0.05
1.0 VERSUS 5.0 = P< 0.05	1.0 VERSUS 10.0 = P<0.001
1.0 VERSUS 10.0 = P< 0.01	2.5 VERSUS 10.0 = P<0.001
2.5 VERSUS 10.0 = P< 0.01	5.0 VERSUS 10.0 = P<0.05

TABLE 37 EFFECT OF FEEDING AND WITHDRAWAL OF METHYLTESTOSTERONE ON THE SPECIFIC GROWTH RATE (SGR) OF CARP. DRUG WAS FED FOR 90 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg FOOD	SPECIFIC GROWTH RATE (WEIGHT)			SPECIFIC GROWTH RATE (LENGTH)		
	0-90 DAYS	90-150 DAYS	0-150 DAYS	0-90 DAYS	90-150 DAYS	0-150 DAYS
0.00	2.00± 0.55	1.00± 0.32	1.60± 0.37	0.60± 0.12	0.67± 0.13	0.62± 0.08
1.00	2.44± 0.69	1.70± 0.23	2.14± 0.43	0.73± 0.16	0.67± 0.27	0.70± 0.14
2.50	2.90± 0.95	1.90± 0.23	2.50± 0.58	0.84± 0.20	0.69± 0.29	0.78± 0.16
5.00	2.94± 1.08	2.03± 0.11	2.58± 0.64	0.81± 0.22	0.65± 0.07	0.75± 0.12
10.00	2.07± 0.53	1.35± 0.16	1.78± 0.33	0.56± 0.06	0.48± 0.21	0.53± 0.08

TABLE 38 CHANGES IN THE CONDITION FACTOR AND FOOD CONVERSION EFFICIENCY OF CARP ADMINISTERED METHYLTESTOSTERONE IN THE DIET FOR 90 DAYS.

CONCENTRATION OF DRUG mg/kg FOOD	CONDITION FACTOR			FOOD CONVERSION EFFICIENCY		
	0 DAYS	90 DAYS	150 DAYS	0–90 DAYS	90–150 DAYS	0–150 DAYS
0.00	1.22± 0.22	1.64± 0.23	0.90± 0.25	0.31	0.16	0.25
1.00	1.24± 0.15	1.53± 0.41	1.26± 0.46	0.39	0.24	0.33
2.50	1.12± 0.20	1.53± 0.33	1.38± 0.34	0.52	0.28	0.42
5.00	1.00± 0.25	1.52± 0.35	1.57± 0.51	0.55	0.30	0.45
10.00	1.09± 0.35	1.50± 0.31	1.42± 0.43	0.32	0.19	0.27

TABLE 39 EFFECT OF FEEDING METHYLTESTOSTERONE ON THE CRANIO-SOMATIC (CSI), HEPATO-SOMATIC (HSI) RENO-SOMATIC (RSI) & VISCERO-SOMATIC (VSI) INDEX OF CARP. DRUG WAS FED FOR 90 DAYS ONLY

CONCEN-TRATION OF DRUG mg/kg FOOD	CSI		HSI		RSI		VSI	
	90 DAYS	150 DAYS	90 DAYS	150 DAYS	90 DAYS	150 DAYS	90 DAYS	150 DAYS
0.00	0.71± 0.04	0.64± 0.03	2.31± 0.18	1.75± 0.12	0.54± 0.03	0.31± 0.03	4.12± 0.21	3.97± 0.16
1.00	0.76± 0.06	*** 0.44± 0.02	*** 2.87± 0.23	*** 1.32± 0.11	0.58± 0.04	* 0.42± 0.04	* 4.87± 0.39	* 4.23± 0.04
2.50	* 0.85± 0.08	*** 0.44± 0.03	*** 1.13± 0.12	*** 1.44± 0.09	0.61± 0.06	0.28± 0.02	*** 6.06± 0.62	** 5.21± 0.04
5.00	* 0.84± 0.08	*** 0.54± 0.04	*** 1.63± 0.18	1.65± 0.13	0.57± 0.06	0.28± 0.02	4.16± 0.45	3.92± 0.04
10.00	** 0.87± 0.08	*** 0.45± 0.03	*** 0.96± 0.09	*** 1.19± 0.10	0.58± 0.06	* 0.23± 0.01	* 4.92± 0.45	* 4.41± 0.09

SIGNIFICANTLY DIFFERENT FROM CONTROLS * = $P<0.05$

(SINGLE FACTOR ANALAYSIS OF VARIANCE ** = $P<0.01$

ACCORDING TO SOKAL AND ROHLF 1969) *** = $P<0.001$

TABLE 40 EFFECT OF FEEDING METHYLTESTOSTERONE ON THE BIOCHEMICAL COMPOSITION OF LIVER. DRUG WAS WITHDRAWN AFTER 90 DAYS.

CONCEN-TRATION OF STEROID mg/kg FOOD	PROTEINS mg / 100 mg		RNA / DNA		PROTEINS / RNA		PROTEINS / DNA		CHOLESTEROL mg / g	
	90 DAYS	150 DAYS	90 DAYS	150 DAYS	90 DAYS	150 DAYS	90 DAYS	150 DAYS	90 DAYS	150 DAYS
0.00	10.00± 0.45	17.69± 0.36	1.59± 0.13	4.93± 0.32	40.43± 9.94	35.54± 4.29	63.08± 13.23	173.06± 16.38	19.53± 0.72	10.60± 0.53
1.00	*** 20.74± 1.92	19.61± 1.56	*** 3.52± 0.26	4.97± 0.01	* 61.81± 10.24	47.24± 13.41	*** 214.72± 19.88	234.49± 57.59	*** 11.03± 0.88	11.11± 0.23
2.50	*** 28.93± 3.19	23.16± 2.12	*** 2.48± 0.48	*** 7.70± 0.10	* 77.47± 16.58	47.02± 4.29	*** 188.76± 20.78	** 388.35± 30.05	*** 9.69± 1.29	10.99± 0.17
5.00	*** 29.19± 0.23	19.72± 2.35	*** 3.31± 0.20	*** 15.46± 2.21	* 66.76± 3.94	30.19± 3.94	*** 220.27± 20.56	*** 464.81± 54.96	*** 12.16± 0.44	* 7.47± 0.17
10.00	*** 40.18± 1.68	22.45± 0.45	*** 4.25± 0.65	*** 14.26± 1.31	43.17± 13.26	26.38± 2.62	*** 181.12± 14.21	** 376.24± 37.32	*** 10.28± 0.28	* 7.34± 0.21

SIGNIFICANTLY DIFFERENT FROM CONTROLS * = $P < 0.05$

(SINGLE FACTOR ANALYSIS OF VARIANCE ** = $P < 0.01$

ACCORDING TO SOKAL AND ROHLF 1969) *** 1 $P < 0.001$

TABLE 41 EFFECT OF FEEDING METHYLTESTOSTERONE ON THE BIOCHEMICAL COMPOSITION OF THE KIDNEY. DRUG WAS WITHDRAWN AFTER 90 DAYS.

CONCEN-TRATION OF STEROID mg/kg FOOD	PROTEINS mg / 100 mg		RNA / DNA		PROTEINS / RNA		PROTEINS / DNA		CHOLESTEROL mg / g	
	90 DAYS	150 DAYS	90 DAYS	150 DAYS	90 DAYS	150 DAYS	90 DAYS	150 DAYS	90 DAYS	150 DAYS
0.00	18.02± 2.32	47.14± 7.38	3.17± 0.82	1.09± 0.07	25.55± 2.88	62.90± 1.76	77.71± 12.94	68.60± 1.92	29.45± 1.67	42.11± 2.31
1.00	** 31.25± 0.36	*** 22.02± 2.62	2.28± 0.43	*** 4.10± 0.10	*** 51.57± 6.94	*** 36.90± 2.10	* 115.17± 7.12	*** 151.37± 8.62	** 23.39± 1.41	*** 5.56± 0.12
2.50	** 27.32± 0.32	*** 39.61± 0.61	4.49± 0.06	*** 3.84± 0.04	** 47.27± 1.87	*** 35.78± 0.73	*** 211.90± 3.92	*** 137.35± 3.26	** 20.82± 1.27	*** 7.78± 0.37
5.00	** 30.31± 0.22	49.32± 0.30	4.73± 0.89	*** 4.94± 0.17	** 41.87± 5.54	*** 34.30± 2.01	*** 192.97± 11.54	*** 169.60± 9.96	** 21.30± 2.78	*** 11.70± 0.51
10.00	*** 51.29± 3.06	54.22± 10.02	*** 11.41± 1.69	*** 4.48± 0.12	34.96± 5.23	*** 39.58± 5.56	*** 389.88± 1.07	*** 177.44± 24.95	** 20.06± 0.77	*** 13.45± 0.16

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 42 EFFECT OF FEEDING METHYLTESTOSTERONE ON THE BIOCHEMICAL COMPOSITION OF BRAIN. DRUG WAS WITHDRAWN AFTER 90 DAYS.

CONCEN-TRATION OF STEROID mg/kg FOOD	PROTEINS mg / 100 mg		RNA / DNA		PROTEINS / RNA		PROTEINS / DNA		CHOLESTEROL mg / g	
	90 DAYS	150 DAYS	90 DAYS	150 DAYS	90 DAYS	150 DAYS	90 DAYS	150 DAYS	90 DAYS	150 DAYS
0.00	8.33± 0.34	22.27± 1.73	3.92± 0.78	9.39± 1.07	52.72± 2.49	69.61± 24.98	205.03± 27.71	653.54± 202.85	22.20± 0.13	15.80± 1.39
1.00	*** 13.41± 1.69	22.06± 1.60	3.39± 0.76	7.56± 1.23	47.42± 5.96	48.61± 4.88	155.84± 16.06	* 364.89± 44.87	*** 16.25± 0.24	*** 12.35± 1.10
2.50	10.70± 0.16	24.89± 0.31	1.82± 0.26	8.38± 0.39	60.49± 13.19	51.44± 2.92	** 106.10± 7.80	* 431.06± 17.89	*** 16.28± 0.65	*** 27.26± 1.23
5.00	** 12.84± 1.25	22.48± 0.63	2.43± 0.02	8.16± 0.67	52.91± 7.59	64.26± 1.99	* 128.74± 19.33	* 519.51± 24.41	*** 15.59± 1.36	*** 17.09± 1.52
10.00	*** 14.60± 0.81	*** 37.23± 0.84	2.78± 0.23	*** 4.85± 0.73	36.24± 5.13	56.18± 6.56	*** 99.49± 5.55	*** 262.42± 35.21	20.13± 0.60	*** 7.87± 1.11

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 43 EFFECT OF FEEDING METHYLTESTOSTERONE ON THE BIOCHEMICAL COMPOSITION OF THE MUSCLE. DRUG WAS WITHDRAWN AFTER 90 DAYS.

CONCENTRATION OF STEROID mg/kg FOOD	PROTEINS mg / 100 mg		RNA / DNA		PROTEINS / RNA		PROTEINS / DNA		CHOLESTEROL mg / g	
	90 DAYS	150 DAYS	90 DAYS	150 DAYS	90 DAYS	150 DAYS	90 DAYS	150 DAYS	90 DAYS	150 DAYS
0.00	10.78± 0.96	16.45± 1.13	4.41± 0.11	4.38± 0.47	73.79± 2.01	89.95± 3.59	324.68± 16.45	391.84± 9.15	1.51± 0.27	2.42± 0.19
1.00	11.96± 2.01	* 18.14± 1.07	5.59± 0.73	4.55± 0.61	74.91± 4.51	111.49± 20.84	* 422.25± 80.08	506.98± 94.73	1.78± 0.12	*** 4.42± 0.39
2.50	*** 16.94± 0.09	* 14.11± 0.87	** 7.06± 0.98	*** 10.88± 0.56	90.07± 4.60	* 68.81± 3.86	** 633.80± 11.96	*** 748.81± 41.99	** 2.78± 0.11	*** 0.52± 0.04
5.00	** 15.16± 0.39	* 14.19± 0.98	*** 7.43± 0.08	*** 10.48± 0.50	* 100.41± 4.05	* 62.49± 3.55	*** 739.35± 45.76	** 681.66± 26,78	*** 4.46± 0.08	*** 0.44± 0.08
10.00	** 16.70± 0.15	* 15.70± 0.55	*** 16.67± 0.71	*** 18.96± 2.18	70.91± 10.61	* 63.14± 6.83	*** 1189.35± 227.59	*** 1197.43± 129.57	*** 4.63± 0.66	*** 0.56± 0.06

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 44 EFFECT OF FEEDING METHYLTESTOSTERONE ON THE PROXIMATE COMPOSITION OF MUSCLE TISSUE FROM *Cyprinus carpio*. DRUG WAS FED FOR 90 DAYS ONLY.

CONCENTRATION OF DRUG mg/kg FOOD	MOISTURE mg/100 mg		TOTAL LIPIDS (mg/100 mg)				ASH (mg / 100 mg)			
			ON WET BASIS		ON DRY BASIS		ON WET BASIS		ON DRY BASIS	
	90 DAYS	150 DAYS	90 DAYS	150 DAYS	90 DAYS	150 DAYS	90 DAYS	150 DAYS	90 DAYS	150 DAYS
0.00	81.09± 2.04	76.68± 0.07	2.62± 1.28	3.37± 0.78	13.86± 6.77	14.45± 0.30	3.09± 0.57	2.33± 0.76	16.34± 3.01	9.99± 3.26
1.00	81.27± 0.22	*** 71.50± 2.54	2.72± 0.80	4.07± 0.55	14.52± 4.27	14.28± 1.93	2.78± 0.49	2.02± 0.08	14.84± 2.62	7.09± 0.28
2.50	*** 73.03± 0.20	* 73.87± 0.99	*** 6.31± 0.05	* 2.48± 0.19	23.40± 0.18	9.49± 0.73	2.49± 0.79	* 1.69± 0.05	9.23± 2.93	6.47± 0.19
5.00	** 76.84± 0.40	77.98± 0.42	* 4.31± 1.87	** 2.19± 0.22	18.61± 8.07	9.95± 1.00	2.41± 0.81	1.80± 0.12	10.41± 3.49	8.17± 0.54
10.00	** 75.23± 0.36	73.40± 0.92	* 4.18± 2.08	** 2.21± 0.20	16.88± 8.39	8.31± 0.76	2.92± 0.49	1.95± 0.17	11.79± 1.98	7.33± 0.64

SIGNIFICANTLY DIFFERENT FROM CONTROLS * = $P<0.05$

(SINGLE FACTOR ANALYSIS OF VARIANCE ** = $P<0.01$

ACCORDING TO SOKAL & ROHLF 1969) *** = $P<0.001$

TABLE 45 EFFECT OF FEEDING METHYLTESTOSTERONE FOR 90 DAYS ON THE FREE AMINO ACIDS (n mole/mg) OF THE MUSCLE OF THE CARP *Cyprinus carpio*.

a = where actual value does not follow with standard error, this means that other samples in that group had traces of the substance.

AMINO ACID	CONCENTRATION OF THE DRUG (mg/kg) FOOD				
	0.00	1.0	2.5	5.0	10.0
Taurine	18.97± 5.47	12.15± 1.06	16.52± 2.66	14.17± 0.32	15.41± 0.13
Aspartic	Traces	Traces	Traces	Traces	Traces
Threnonine	6.46± 2.24	3.36± 0.02	3.03± 0.56	3.64± 0.29	4.41± 0.04
Serine	2.34± 0.12	Traces	1.77	2.20	2.69± 0.08
Glutamic	1.57[a]	1.42	1.0± 0.18	1.32± 0.28	2.14± 0.02
Proline	Traces	Traces	Traces	Traces	Traces
Glycine	4.98± 0.02	3.87± 0.58	1.82± 0.03	2.94± 0.23	2.41± 0.17
Alanine	4.87± 0.01	4.70± 0.07	2.80± 0.30	4.23± 0.44	4.78± 0.02
Cysteine	Traces	Traces	-	Traces	Traces
Valine	-	-	-	-	-
Methionine	Traces	Traces	1.91	Traces	1.75± 0.10
Isoleucine	Traces	-	-	Traces	Traces
Leucine	Traces	Traces	Traces	Traces	Traces
Tyrosine	-	-	-	-	-
Phenylalanine	-	-	-	-	-
Histidine	12.5± 2.86	14.01± 1.26	9.46± 0.56	11.15± 0.93	14.42± 0.55
Ornithine	Traces	Traces	Traces	Traces	Traces
Lysine	8.16± 0.40	7.07± 2.79	3.53± 0.48	3.88± 0.32	9.05± 0.82
Ammonia	18.28± 0.10	12.65± 0.03	7.14± 0.59	10.05± 1.44	11.59± 0.18
Arginine	9.18± 0.19	4.99± 1.60	3.37± 0.13	7.64± 2.80	12.43± 0.12

TABLE 46 EFFECT OF WITHDRAWAL (60 DAYS) OF METHYLTESTOSTERONE ADMINISTERED *per os* FOR 90 DAYS ON THE FREE AMINO ACIDS (n mole/mg) OF THE MUSCLE OF *Cyprinus carpio*.

AMINO ACID	CONCENTRATION OF THE DRUG (mg/kg FOOD)				
	0.00	1.00	2.50	5.00	10.0
Taurine	24.71± 5.84	17.56± 0.56	14.67± 0.29	16.66± 0.95	24.73± 0.34
Aspartic	Traces	Traces	Traces	Traces	Traces
Threonine	6.24± 0.64	3.38± 0.02	3.49± 0.25	3.61± 0.14	5.87± 0.63
Serine	4.16± 0.31	3.39± 0.16	2.85± 0.14	3.47± 0.11	5.14± 0.24
Glutamic	4.30± 0.75	2.66± 0.26	1.70± 0.07	1.72± 0.04	2.98± 0.19
Proline	Traces	Traces	-	Traces	Traces
Glycine	8.96± 1.30	5.71± 0.15	5.96± 0.10	4.41± 0.17	5.75± 0.12
Alanine	8.41± 1.02	6.20± 0.89	4.50± 0.02	5.81± 0.15	8.69± 0.17
Cystine	-	-	-	-	-
Valine	3.24± 0.09	2.03± 0.32	1.45± 0.37	1.73± 0.16	2.29± 0.08
Methionine	Traces	-	5.84± 0.30	Traces	5.58
Isoleucine	2.65± 0.11	2.40± 0.07	3.47	1.76± 0.01	2.25± 0.05
Leucine	6.19± 1.61	2.74± 0.38	2.40± 0.14	2.79± 0.56	2.74± 0.05
Tyrosine	1.88± 0.23	1.31± 0.16	1.90	1.24± 0.06	1.49± 0.17
Phenylalanine	2.89± 0.09	1.62± 0.20	2.58	2.16± 0.13	2.51± 0.03
Histidine	21.95± 4.86	16.82± 0.21	16.15± 0.85	15.25± 0.14	20.50± 2.62
Ornithine	Traces	Traces	Traces	Traces	Traces
Lysine	8.33± 1.30	9.90± 4.00	4.42± 0.07	4.53± 0.05	4.52± 0.82
Ammonia	12.31± 2.56	8.94± 0.67	7.24± 0.61	5.67± 0.04	7.95± 0.40
Arginine	17.14± 0.52	10.98± 2.91	10.62± 0.37	10.55± 0.37	11.37± 0.14

4.1.6. Dimethazine

4.1.6.1. Weight and Length Data

The weight and length data for this drug is presented in Tables 47 and 48 and Figures 31 and 32. A significant increase in weight ($P<0.001$) and length ($P<0.001$) was observed after sixty days of feeding of this synthetic anabolic-androgenic drug. Although, the weight difference was not significant after thirty days of drug withdrawal, the length ($P<0.01$) was. At the end of the sixty days, the experimental groups gained net weight of 146.09, 182.40, 82.12, and 140.22% for 1.0, 2.5, 5.0 and 10.0 ppm groups, while difference in length was of the order of 135.14, 149.55, 113.51 and 141.44% greater than the controls for the four experimental groups.

Table 49 gives the SGR for this drug. It is clear that, although the SGR both for weight and length was higher during the drug feeding period in all the experimental groups, the SGR decreased in both weight and length after withdrawal of the drug, which ultimately resulted in retardation of the growth in phase 2. The overall values for SGR for ninety days both for weight and length were higher than the controls. Food conversion values (Table 50) followed the changes in SGR, after ninety days the FCE was equal in control and experimental groups.

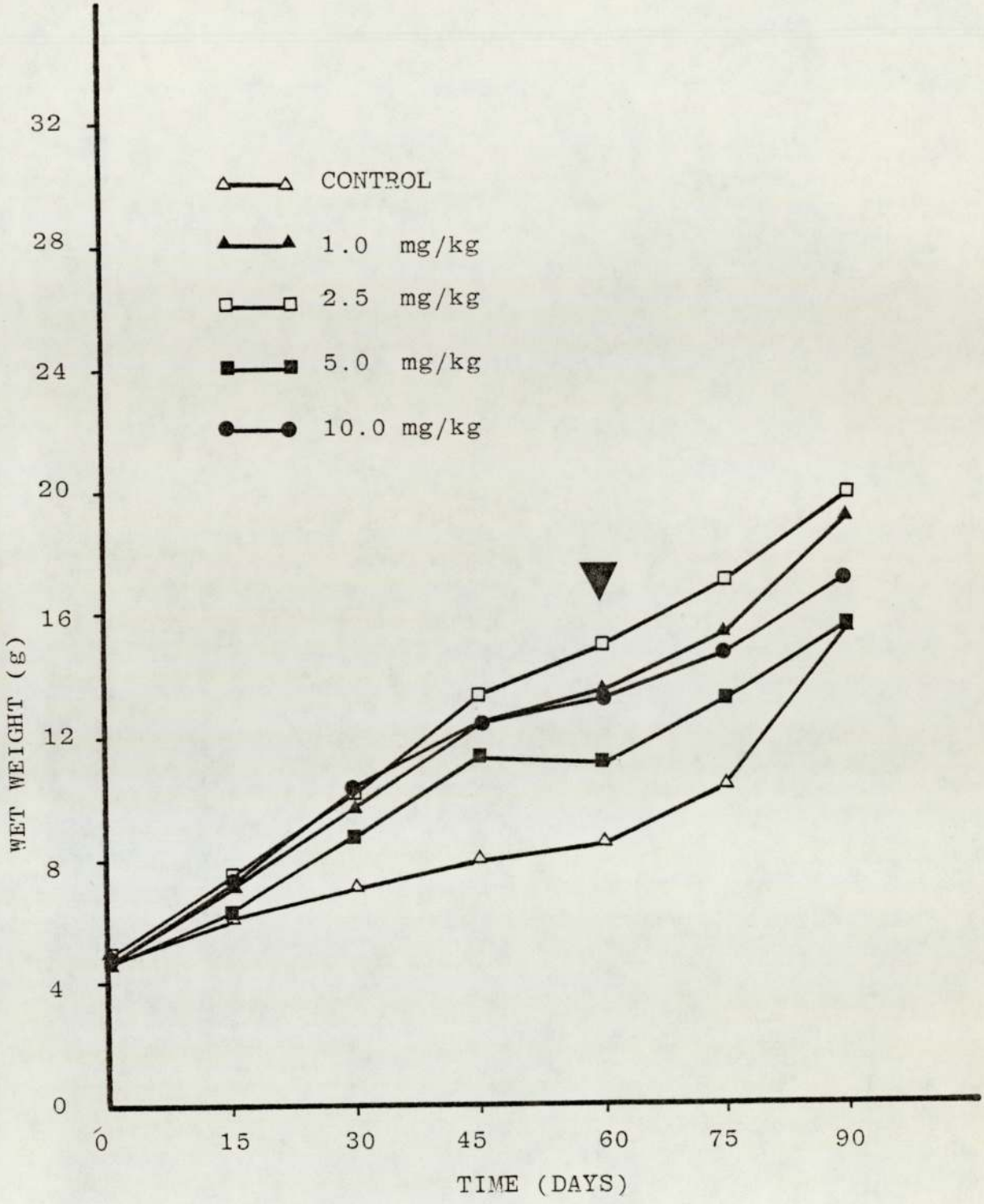


FIGURE 31 MEAN WEIGHTS OF CARP GIVEN DIMETHAZINE *PER OS*. DRUG WAS WITHDRAWN AFTER 60 DAYS (ARROW).

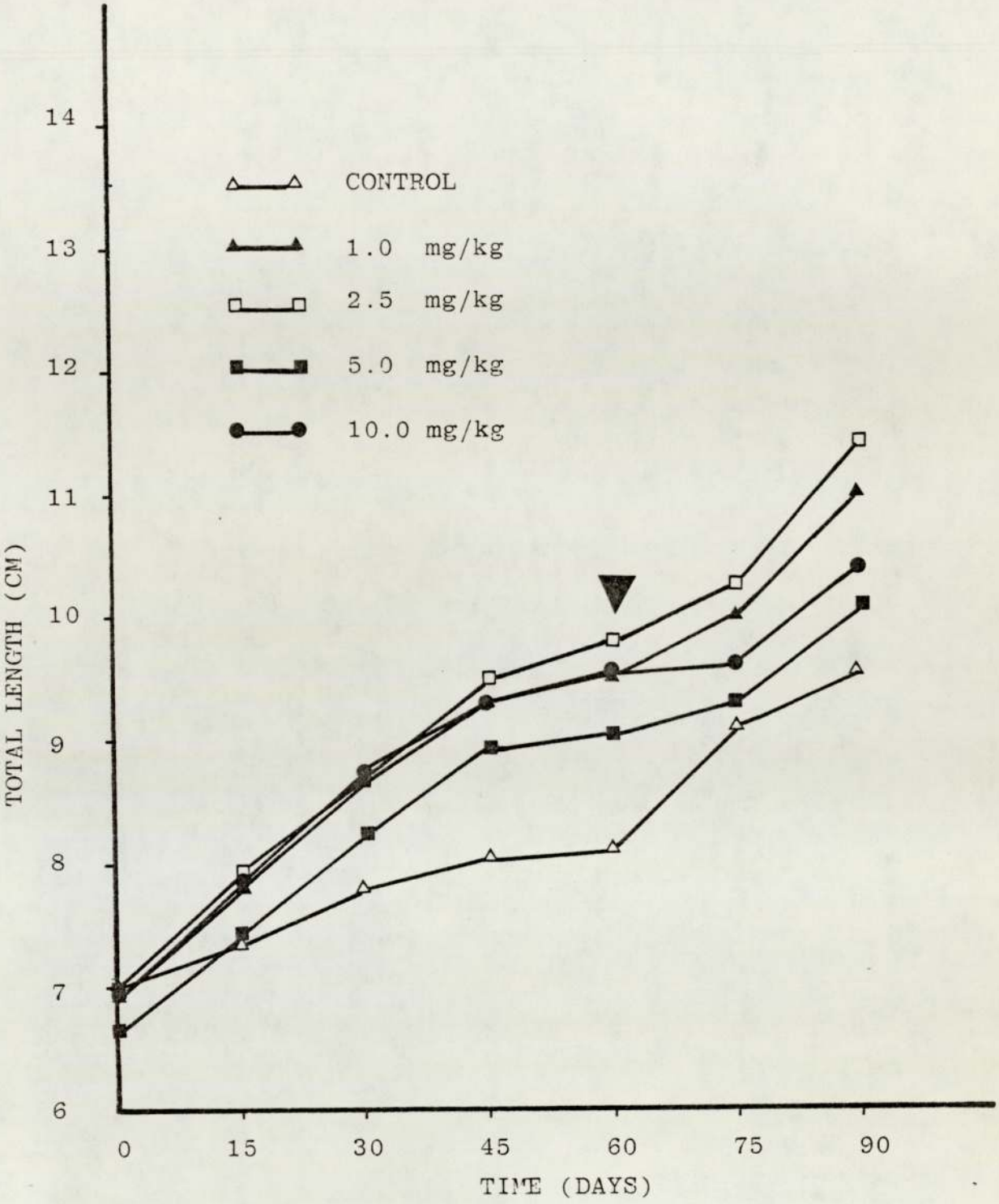


FIGURE 32 MEAN LENGTHS OF CARP GIVEN DIMETHAZINE *PER OS*. DRUG WAS WITHDRAWN AFTER 60 DAYS (ARROW)

4.1.6.2. Tissue-Body Indices

A significant decrease ($P<0.05$) was noted in CSI and HSI after sixty days. While RSI exhibited an increase ($P<0.05$), no effect was observed in VSI. After ninety days, there was no effect on HSI and RSI, while a decrease (2.5 ppm; $P<0.05$) in CSI and increase (1.0 and 2.5 ppm; $P<0.05$) in VSI was observed. The details are given in Table 51 and Figures 33 and 34.

4.1.6.3. Biochemical Changes

4.1.6.3.1. Liver (Table 52)

A significant elevation of total proteins, RNA/DNA and protein/DNA was observed after sixty days. No change was noted in protein/RNA. After ninety days, nearly all the parameters were comparable to the control values, which show a correlation with HSI at ninety days.

4.1.6.3.2. Kidney (Table 53)

Although no effect on total proteins and protein/RNA was noted, nevertheless, RNA/DNA ($P<0.05$) and protein/DNA ($P<0.01$) were higher at sixty days. After ninety days, the proteins were higher ($P<0.05$) in 2.5 and 5.0 ppm groups and RNA/DNA ($P<0.05$) in 2.5 ppm group only. A diminishing of the ratio of protein/RNA ($P<0.01$) and protein/DNA ($P<0.05$) was noted in 10 ppm group only.

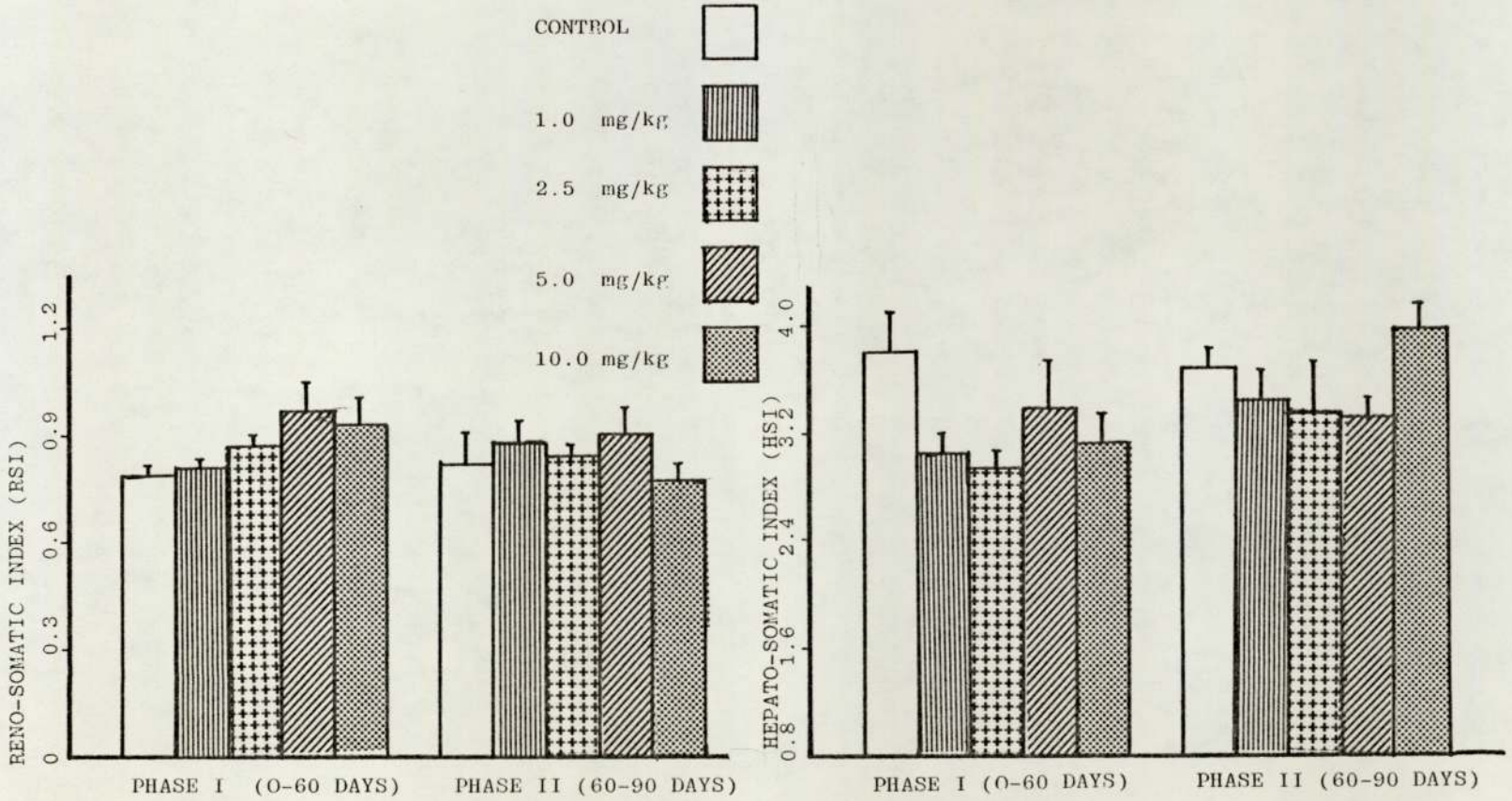


FIGURE 33 EFFECT OF DIMETHAZINE GIVEN *PER OS* ON THE RSI AND HSI OF CARP. DRUG WAS WITHDRAWN AFTER 60 DAYS.

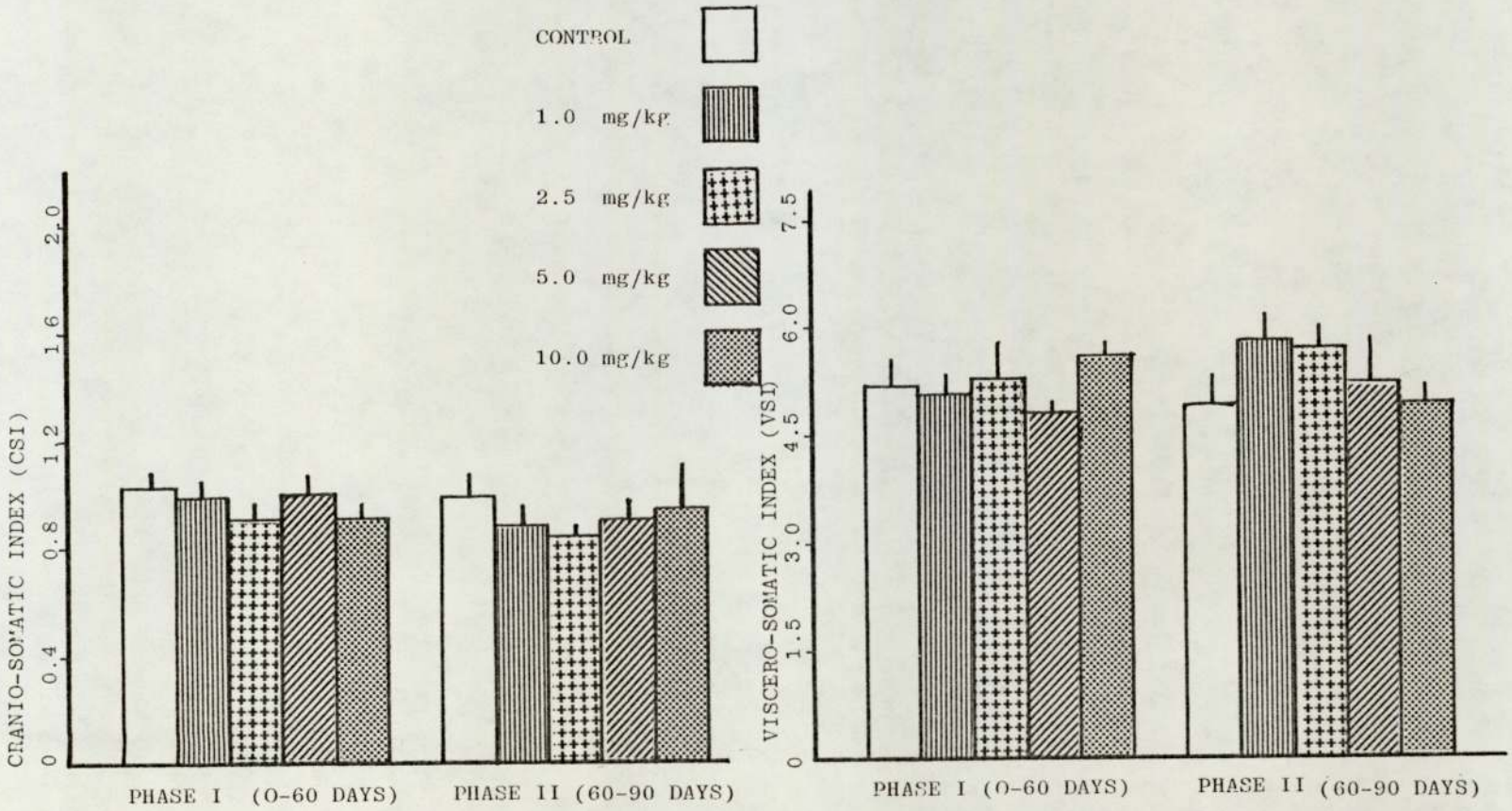


FIGURE 34 EFFECT OF DIMETHAZINE GIVEN *PER OS* ON THE CSI AND VSI OF CARP. DRUG WAS WITHDRAWN AFTER 60 DAYS.

4.1.6.3.3. Brain (Table 54)

After sixty and ninety days, a decrease in total proteins ($P<0.001$) and protein/RNA ($P<0.05$ and $P<0.001$) was noted. RNA/DNA increased after sixty days. Protein/DNA was significant only in 5.0 ppm group after sixty days.

4.1.6.3.4. Muscle (Tables 55 and 56)

After sixty days, protein was only significant in 2.5 ppm group. RNA/DNA, Protein/RNA and protein/DNA were significantly higher. After ninety days, no effect was noted in protein content. RNA/DNA was lower ($P<0.05$) in 1.0 ppm group. Protein/RNA was still higher than the controls and protein/DNA was higher only in 2.5 ppm group.

Moisture content was lower ($P<0.05$) only in 1.0 group after sixty days. No effect was seen in crude proteins and ash content of the muscle both at sixty and ninety days. Crude lipids were significantly lower only in 10.0 ppm ($P<0.05$) and 5.0 and 10.0 ppm ($P<0.05$) at sixty and ninety days respectively.

TABLE 47 CHANGES IN BODY WEIGHT OF CARP FED DIMETHAZINE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEAN (g) ± S.E. OF 25 FISH. PER CENT WEIGHT GAIN IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF DIMETHAZINE mg/kg FOOD				
	0.00	1.00	2.50	5.00	10.00
0	4.92± 0.35	4.63± 0.20	4.79± 0.20	4.60± 0.26	4.62± 0.20
15	6.10± 0.41 (24.06)	7.11± 0.33 (53.72)	7.49± 0.31 (56.25)	6.33± 0.23 (37.80)	7.28± 0.35 (57.72)
30	7.06± 0.50 (43.50)	9.70± 0.44 (109.74)	10.23± 0.49 (113.35)	8.76± 0.48 (90.56)	10.30± 0.53 (123.07)
45	7.99± 0.59 (62.35)	12.37± 0.56 (167.47)	13.34± 0.57 (178.31)	11.31± 0.66 (146.07)	12.36± 0.65 (167.85)
60*	8.50± 0.75 (72.72)	13.44± 0.73 (190.51)	14.90± 0.70 (210.70)	11.12± 0.74 (141.88)	13.22± 0.70 (186.51)
75	10.38± 0.77 (110.98)	15.28± 1.00 (230.47)	16.99± 0.81 (254.46)	13.23± 1.27 (187.78)	14.73± 0.98 (195.78)
90	15.50± 2.89 (215.11)	19.07± 1.49 (312.22)	19.85± 1.01 (314.13)	15.58± 1.92 (239.05)	17.18± 1.32 (272.14)

* DRUG WITHDRAWN AFTER 60 DAYS.

STATISTICALLY SIGNIFICANT DIFFERENCES AMONG THE DOSES, ACCORDING TO SINGLE FACTOR ANALYSIS OF VARIANCE (SOKAL & ROHLF 1969).

60 DAYS

CONTROL VERSUS 1.0 = $P < 0.001$

CONTROL VERSUS 2.5 = $P < 0.001$

CONTROL VERSUS 5.0 = $P < 0.05$

CONTROL VERSUS 10.0 = $P < 0.01$

1.0 VERSUS 5.0 = $P < 0.05$

2.5 VERSUS 5.0 = $P < 0.05$

5.0 VERSUS 10.0 = $P < 0.05$

TABLE 48 CHANGES IN TOTAL BODY LENGTH OF CARP FED DIMETHAZINE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEANS (cm) ± S.E. OF 25 FISH. PERCENT INCREASE IN LENGTH IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF DIMETHAZINE mg/kg FOOD				
	0.00	1.00	2.50	5.00	10.00
0	7.00± 0.15	6.91± 0.10	7.00± 0.10	6.66± 0.13	6.88± 0.10
15	7.36± 0.16 (5.16)	7.78± 0.12 (12.57)	7.93± 0.11 (13.30)	7.44± 0.12 (11.67)	7.83± 0.12 (13.87)
30	7.80± 0.18 (11.43)	8.66± 0.13 (25.30)	8.69± 0.16 (24.12)	8.22± 0.15 (23.27)	8.74± 0.15 (27.03)
45	8.03± 0.20 (14.66)	9.28± 0.14 (34.27)	9.48± 0.15 (35.44)	8.95± 0.17 (34.32)	9.28± 0.16 (34.94)
60*	8.11± 0.24 (15.91)	9.52± 0.17 (37.78)	9.77± 0.17 (39.60)	9.03± 0.19 (35.58)	9.56± 0.17 (39.02)
75	9.09± 0.22 (29.90)	10.01± 0.22 (44.89)	10.26± 0.17 (46.57)	9.28± 0.26 (39.30)	9.61± 0.22 (35.80)
90	9.56± 0.61 (36.51)	10.99± 0.29 (59.02)	11.42± 0.20 (63.79)	10.04± 0.35 (50.82)	10.39± 0.29 (51.02)

* DRUG WITHDRAWN AFTER 60 DAYS

STATISTICALLY SIGNIFICANT DIFFERENCES AMONG DOSES, ACCORDING TO SINGLE FACTOR ANALYSIS OF VARIANCE (SOKAL & ROHLF 1969)

60 DAYS		90 DAYS	
CONTROL VERSUS 1.0	$P < 0.001$	CONTROL VERSUS 1.0	$P < 0.01$
CONTROL VERSUS 2.5	$P < 0.001$	CONTROL VERSUS 2.5	$P < 0.001$
CONTROL VERSUS 5.0	$P < 0.001$	1.0 VERSUS 5.0	$P < 0.05$
CONTROL VERSUS 10.0	$P < 0.001$	2.5 VERSUS 5.0	$P < 0.01$
2.5 VERSUS 5.0	$P < 0.01$	2.5 VERSUS 10.0	$P < 0.05$

TABLE 49 EFFECT OF FEEDING AND WITHDRAWAL OF DIMETHAZINE ON THE SPECIFIC GROWTH RATE (SGR) OF CARP. DRUG WAS FED FOR 60 DAYS ONLY.

CONCENTRATION OF DRUG mg/kg FOOD	SPECIFIC GROWTH RATE (WEIGHT)			SPECIFIC GROWTH RATE (LENGTH)		
	0-60 DAYS	60-90 DAYS	0-90 DAYS	0-60 DAYS	60-90 DAYS	0-90 DAYS
0.00	0.92± 0.21	2.04± 0.48	1.29± 0.29	0.25± 0.07	0.55± 0.16	0.35± 0.10
1.00	1.81± 0.49	1.18± 0.22	1.60± 0.35	0.54± 0.14	0.49± 0.10	0.52± 0.10
2.50	1.92± 0.47	0.97± 0.06	1.61± 0.36	0.56± 0.13	0.54± 0.15	0.55± 0.10
5.00	1.50± 0.55	1.14± 0.02	1.38± 0.36	0.51± 0.15	0.36± 0.12	0.46± 0.11
10.00	1.78± 0.59	0.88± 0.11	1.48± 0.42	0.56± 0.15	0.28± 0.17	0.46± 0.13

TABLE 50 CHANGES IN THE CONDITION FACTOR AND FOOD CONVERSION EFFICIENCY OF CARP ADMINISTERED DIMETHAZINE IN THE DIET FOR 60 DAYS.

CONCENTRATION OF DRUG mg/kg FOOD	CONDITION FACTOR			FOOD CONVERSION EFFICIENCY		
	0 DAYS	60 DAYS	90 DAYS	0–60 DAYS	60–90 DAYS	0–90 DAYS
0.00	1.43± 0.24	1.59± 0.27	1.78± 0.44	0.18	0.49	0.31
1.00	1.40± 0.23	1.56± 0.25	1.44± 0.26	0.35	0.26	0.31
2.50	1.40± 0.23	1.60± 0.25	1.32± 0.26	0.38	0.21	0.30
5.00	1.56± 0.25	1.51± 0.25	1.54± 0.29	0.28	0.24	0.26
10.00	1.42± 0.23	1.51± 0.25	1.53± 0.27	0.33	0.19	0.27

TABLE 51 EFFECT OF FEEDING DIMETHAZINE ON THE CRANIO-SOMATIC (CSI), HEPATO-SOMATIC (HSI), RENO-SOMATIC (RSI) & VISCERO-SOMATIC (VSI) INDEX OF CARP. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg FOOD	CSI		HSI		RSI		VSI	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	1.03± 0.04	0.99± 0.05	3.07± 0.32	2.89± 0.13	0.79± 0.03	0.82± 0.08	5.19± 0.36	4.91± 0.25
1.00	0.99± 0.03	0.88± 0.06	* 2.23± 0.18	2.64± 0.20	0.81± 0.01	0.88± 0.06	5.05± 0.25	* 5.78± 0.08
2.50	* 0.91± 0.04	* 0.84± 0.02	* 2.13± 0.11	2.55± 0.36	0.87± 0.03	0.84± 0.02	5.26± 0.24	* 5.69± 0.47
5.00	1.00± 0.06	0.90± 0.07	2.57± 0.34	2.53± 0.11	* 0.97± 0.06	0.90± 0.07	4.82± 0.58	5.24± 0.14
10.00	* 0.91± 0.04	0.94± 0.05	* 2.32± 0.20	3.18± 0.13	* 0.93± 0.07	0.77± 0.05	5.57± 0.19	4.86± 0.15

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = P 0.05

** = P 0.01

*** = P 0.001

ERRATA

Due to a computational error the concentrations of the proteins, RNA/DNA and protein/DNA were calculated wrongly. For correct values please divide protein and RNA/DNA by 2 and protein/DAN by 4.

PROTEIN	RNA/DNA	PROTEIN/DNA
TABLE NO.	TABLE NO.	TABLE NO.
52-54	52-55	52-55
62-64	62-65	62-65
72-74	72-75	72-75
93-95	83-84	83-84
103-105	93-96	93-96
113	103-106	103-106
	112-115	112-115

TABLE 52 EFFECT OF FEEDING DIMETHAZINE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN LIVER OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	26.28± 2.41	31.28± 3.49	5.53± 0.48	8.88± 1.15	17.37± 1.26	16.37± 1.88	95.33± 9.69	142.11± 16.58
1.00	31.38± 3.41	26.75± 1.39	** 8.55± 0.23	10.78± 1.10	15.00± 1.08	* 11.99± 0.92	* 128.57± 11.32	127.14± 9.72
2.50	31.55± 1.02	* 19.91± 2.57	** 8.93± 0.67	9.02± 0.85	14.88± 0.72	16.10± 0.93	* 131.40± 3.44	144.44± 13.21
5.00	** 38.90± 4.48	25.82± 6.07	*** 11.34± 1.15	8.45± 0.56	14.86± 0.89	15.45± 1.51	*** 167.27± 15.34	129.17± 9.59
10.00	*** 41.97± 2.98	28.28± 0.80	*** 11.63± 1.22	8.27± 0.85	15.96± 1.28	14.41± 1.28	*** 175.92± 7.45	115.95± 2.71

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 53 EFFECT OF FEEDING DIMETHAZINE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN KIDNEY OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	40.81± 3.50	26.19± 2.92	3.52± 0.26	4.14± 0.26	14.41± 0.72	13.16± 1.34	49.58± 2.34	54.34± 6.78
1.00	37.89± 3.11	41.15± 2.89	3.44± 0.04	4.74± 0.53	13.56± 0.83	13.75± 0.43	46.66± 2.81	64.45± 4.79
2.50	47.59± 3.98	* 43.75± 7.93	* 4.35± 0.31	* 5.30± 0.46	15.03± 1.46	12.79± 0.76	* 64.58± 5.04	67.05± 4.41
5.00	41.38± 3.66	** 53.18± 1.06	* 4.39± 0.25	4.43± 0.27	14.65± 0.81	11.41± 1.25	* 64.44± 5.07	49.72± 2.77
10.00	33.11± 4.09	37.23± 7.10	* 4.50± 0.26	4.43± 0.23	16.93± 0.99	** 8.68± 0.70	*** 76.70± 8.36	* 38.02± 1.85

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 54 EFFECT OF FEEDING DIMETHAZINE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN BRAIN OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	41.36± 3.10	37.24± 1.27	2.73± 0.04	2.24± 0.04	18.81± 1.39	28.66± 0.57	51.56± 4.21	64.14± 1.09
1.00	*** 20.39± 3.16	*** 22.73± 2.54	2.78± 0.15	2.40± 0.28	17.12± 1.19	*** 20.53± 1.44	47.53± 4.05	49.93± 8.43
2.50	*** 17.84± 1.64	*** 25.92± 0.92	*** 3.14± 0.08	* 3.41± 0.43	* 14.73± 0.43	* 23.45± 1.61	46.05± 1.05	79.04± 9.60
5.00	47.78± 3.61	*** 24.86± 1.12	*** 3.13± 0.08	2.73± 0.22	22.29± 1.06	*** 20.78± 1.43	** 69.70± 3.81	55.79± 1.72
10.00	36.71± 4.02	*** 24.99± 1.93	*** 3.23± 0.06	2.78± 0.28	18.48± 1.55	*** 22.72± 1.02	59.84± 5.99	62.22± 3.39

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 55 EFFECT OF FEEDING DIMETHAZINE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN MUSCLE OF *Cyprinus carpio*. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	13.60± 0.75	14.86± 0.59	10.51± 1.41	12.71± 1.87	36.94± 3.28	49.23± 3.15	375.67± 23.41	614.76± 69.43
1.00	15.29± 1.24	13.33± 2.37	* 13.86± 0.66	* 8.19± 0.63	** 50.23± 1.71	** 83.69± 12.06	*** 693.38± 19.44	663.08± 41.17
2.50	* 18.24± 2.95	12.89± 0.61	** 15.72± 0.37	10.39± 0.27	* 48.61± 4.76	* 71.41± 4.93	*** 765.84± 84.32	* 738.95± 40.19
5.00	16.94± 0.90	14.19± 0.38	12.13± 0.04	9.77± 1.13	* 48.20± 2.65	* 71.53± 4.96	** 582.61± 34.62	682.92± 43.42
10.00	16.83± 1.87	13.09± 0.83	12.46± 1.45	13.16± 1.23	* 47.97± 2.96	53.49± 4.42	** 587.06± 46.61	690.26± 34.07

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 56 EFFECT OF FEEDING DIMETHAZINE ON THE PROXIMATE COMPOSITION OF MUSCLE TISSUE FROM Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCENTRATION OF DRUG mg/kg	MOISTURE mg/100 mg		CRUDE PROTEIN N x 6.25 (mg/100 mg)				TOTAL LIPIDS (mg / 100 mg)				ASH (mg / 100 mg)			
			ON WET BASIS		ON DRY BASIS		ON WET BASIS		ON DRY BASIS		ON WET BASIS		ON DRY BASIS	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	75.84± 0.20	74.56± 0.60	17.81± 0.25	18.63± 0.31	73.88± 1.13	73.38± 0.81	2.32± 0.18	3.16± 0.42	9.61± 0.73	12.22± 1.34	2.21± 0.07	2.11± 0.04	9.13± 0.25	8.34± 0.35
1.00	73.89*± 0.17	77.54± 0.55	19.19± 0.19	16.69± 0.25	73.44± 0.63	74.25± 1.06	1.88± 0.14	2.35± 0.29	7.10± 0.48	10.38± 1.10	2.38± 0.05	1.92± 0.12	9.13± 0.13	8.55± 0.48
2.50	75.62± 0.78	76.27± 4.59	17.06± 0.56	17.31± 3.44	70.00± 0.88	73.00± 1.50	1.83± 0.27	2.26± 0.48	7.43± 0.89	9.35± 0.39	2.22± 0.06	2.02± 0.31	9.13± 0.33	8.53± 0.52
5.00	74.66± 1.51	76.91± 0.45	17.50± 1.06	16.94± 0.19	68.94*± 0.38	73.44± 1.00	1.93± 0.20	2.04*± 0.14	7.63± 0.68	8.80*± 0.47	2.36± 0.09	2.13± 0.05	9.43± 0.76	9.20± 0.10
10.00	76.38± 0.71	76.72± 0.48	16.31*± 0.50	16.81± 0.19	68.94*± 0.75	72.19± 0.88	1.73*± 0.06	1.96*± 0.23	7.35± 0.37	8.40*± 0.90	2.11± 0.09	2.22± 0.05	8.93± 0.38	9.53± 0.14

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

4.1.7. Oxandrolone

4.1.7.1. Weight and Length Data

The data concerning weight and length for this steroid is given in Tables 57 and 58 and Figures 35 and 36. A steady increase in both weight and length was observed in fish receiving this drug. After sixty days, a significant ($P<0.001$) increase in both weight and length was observed. The fish groups receiving different doses of this drug were 251.96, 127.93, 164.80 and 250.28% heavier and 200.90, 109.91, 114.41 and 172.07% longer than the controls for 1.0, 2.5, 5.0 and 10.0 ppm dose respectively. In phase 2, one month after the withdrawal of the drug, the experimental groups receiving the 1.0 ppm (weight) and 1.0 and 10.0 ppm (length) were still significantly different from controls. These groups were still 95.46% heavier and 82.81 and 71.09% longer than the controls.

Although the SGR both for weight and length were higher in experimental groups during the drug feeding phase, after the removal of the drug, the experimental groups could not maintain the impetus of growth achieved during phase 1. The result was a decrease in SGR, both for weight and length which can also be seen in the form of decrease in percent weight and length gains, and a decrease in FCE (Tables 59 and 60).

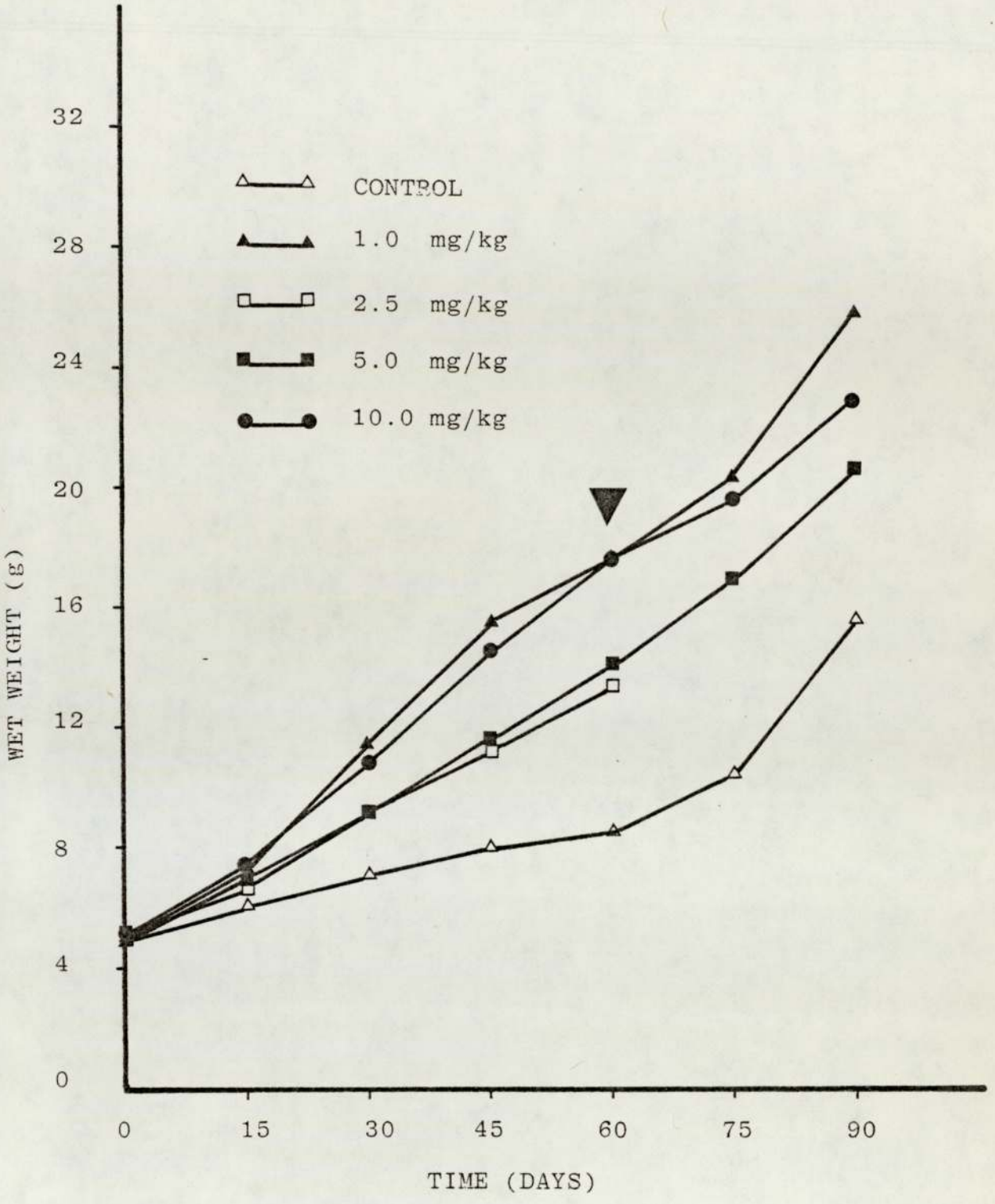


FIGURE 35 MEAN WEIGHTS OF CARP GIVEN OXANDROLONE *PER OS*. DRUG WAS WITHDRAWN AFTER 60 DAYS (ARROW).

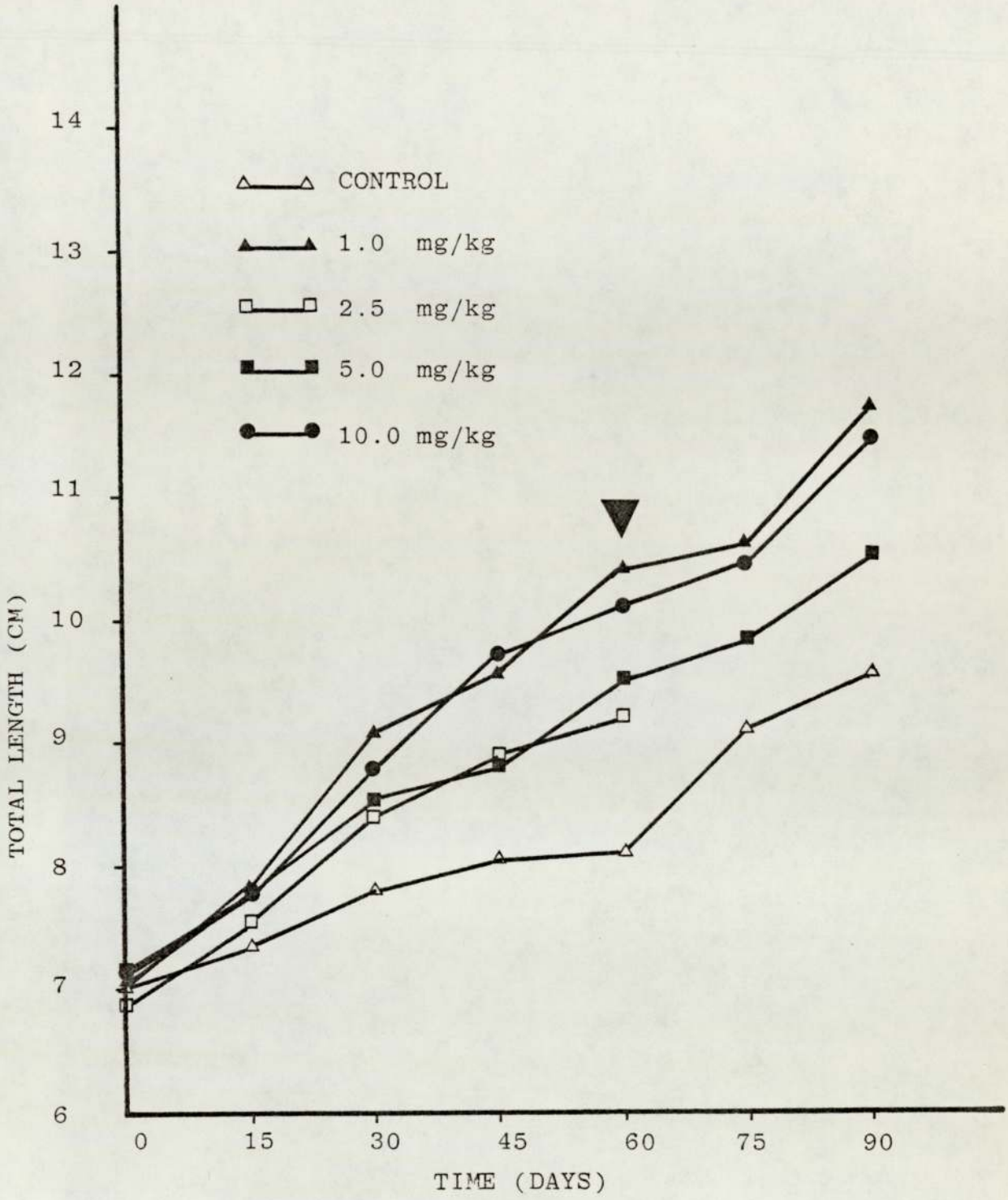


FIGURE 36 MEAN LENGTHS OF CARP GIVEN OXANDROLONE *PER OS*. DRUG WAS WITHDRAWN AFTER 60 DAYS (ARROW).

4.1.7.2. Tissue - Body Indices

A decrease in CSI (1.0 and 10.0 ppm; $P<0.001$), HSI (5.0 ppm; $P<0.01$) and VSI (10.0 ppm; $P<0.05$) was observed after sixty days of drug feeding. RSI recorded no change both after sixty and ninety days. CSI ($P<0.001$), HSI ($P<0.01$) and VSI ($P<0.05$) were lower than the control values in 1.0 and 10.0 ppm groups. These changes can be seen in detail in Table 61 and Figures 37 and 38.

4.1.7.3. Biochemical Changes

4.1.7.3.1. Liver (Table 62)

At the end of phase 1, total liver proteins recorded an increase ($P<0.05$) only in 5.0 ppm group, while a highly significant ($P<0.001$) increase in RNA/DNA and protein/DNA ($P<0.001$) was observed. Protein/RNA was decreased ($P<0.05$) in 1.0 and 10.0 ppm groups. Showing a proportionately higher increase in total RNA levels of the tissue. After ninety days, no differences were noted in total proteins, RNA/DNA and protein/DNA, but protein/RNA was higher ($P<0.01$) in 5.0 and 10.0 ppm groups.

4.1.7.3.2. Kidney (Table 63)

A decrease ($P<0.01$) in total proteins in 10.0 ppm group and a significant ($P<0.001$) increase in RNA/DNA and protein/DNA was noted after sixty days. No effect was recorded on the protein/RNA both at sixty and ninety days. The total proteins (1.0 and 10.0 ppm), RNA/DNA and protein/DNA (all experimental groups) were significantly ($P<0.001$) higher after ninety days.

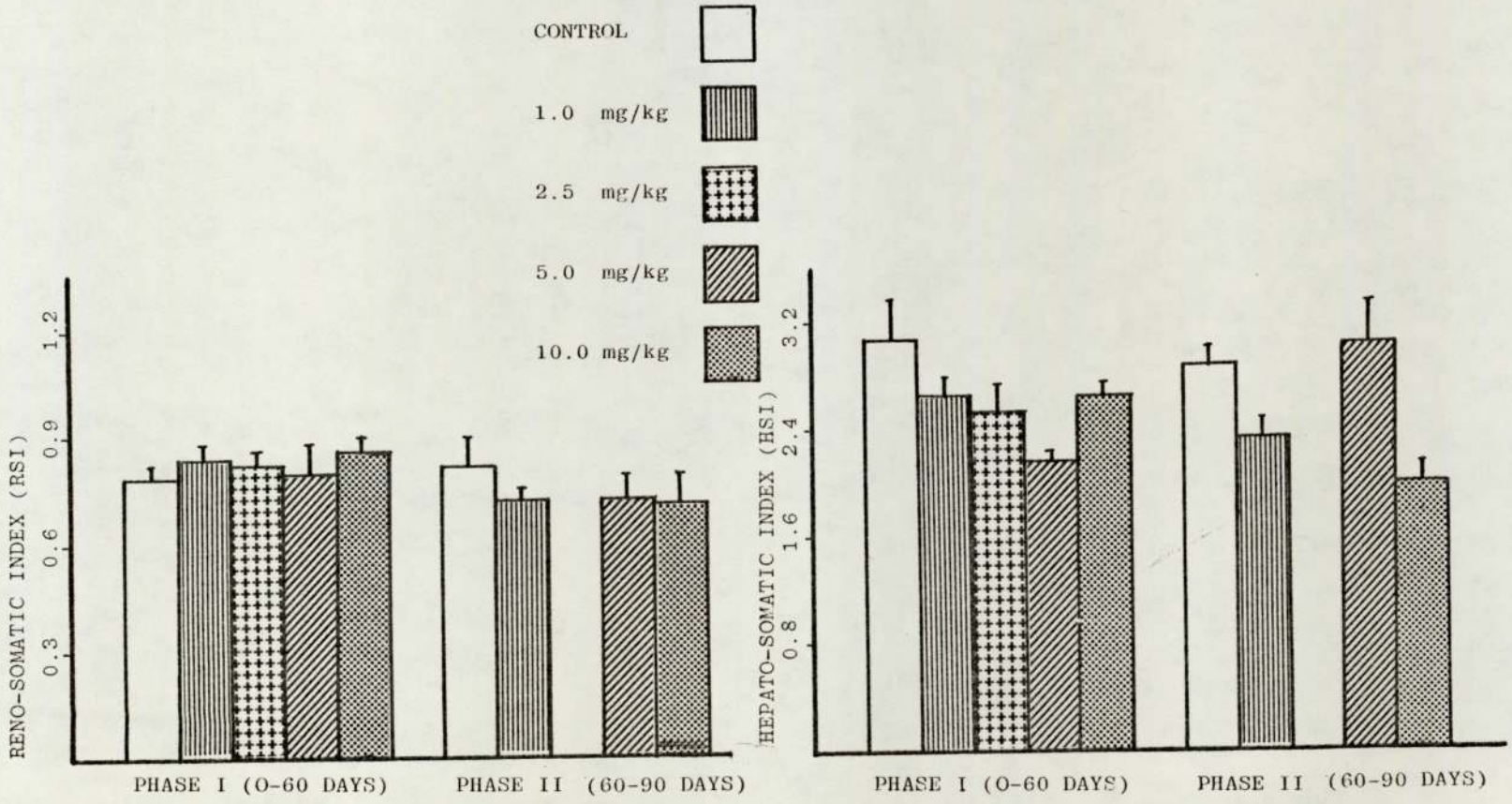


FIGURE 37 EFFECT OF OXANDROLONE GIVEN *PER OS* ON THE RSI AND HSI OF CARP. DRUG WAS WITHDRAWN AFTER 60 DAYS.

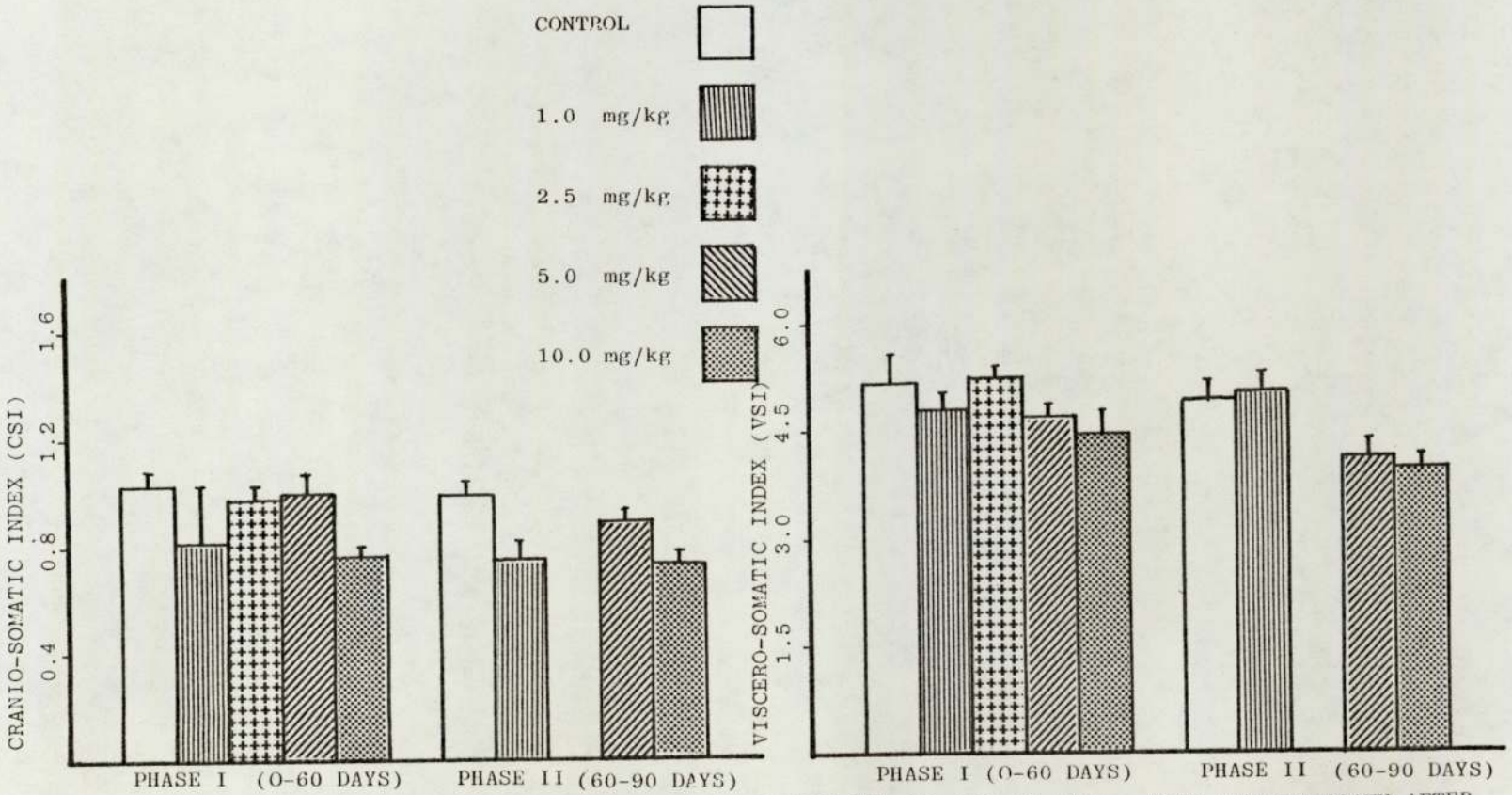


FIGURE 38 EFFECT OF OXANDROLONE GIVEN *PER OS* ON THE CSI AND VSI OF CARP. DRUG WAS WITHDRAWN AFTER 60 DAYS.

4.1.7.3.3. Brain (Table 64)

Total brain proteins were decreased in 1.0 and 10.0 ppm groups but RNA/DNA and protein/DNA were increased in phase 1. No change in protein/RNA was observed after sixty days. In phase 2, total proteins and proteins/DNA were significantly different only in 1.0 ppm group. No change was observed in any other parameter.

4.1.7.3.4. Muscle (Tables 65 and 66)

RNA/DNA ($P<0.01$), protein/DNA ($P<0.001$) and protein/RNA ($P<0.001$) were significantly different (Higher) than the controls. No effect on total proteins was noted after sixty days. At the termination of the experiment, total protein, RNA/DNA and protein/DNA exhibited no change from the control values. Protein/RNA ($P<0.01$) was lower in 5.0 and 10.0 ppm.

Muscle moisture (5.0 and 10.0 ppm) and crude proteins (2.5 ppm) were significantly ($P<0.05$) increased after sixty days. A significant decrease both on dry and wet bases was observed in total fat and ash content after sixty and ninety days. After ninety days, total moisture content increased ($P<0.001$) and protein content decreased ($P<0.01$) from the control values (Table 66).

TABLE 57 CHANGES IN BODY WEIGHT OF CARP FED OXANDROLONE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEAN (g) ± S.E. OF 25 FISH. PERCENT WEIGHT GAIN IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF OXANDROLONE mg/kg FOOD				
	0.00	1.00	2.50[a]	5.00	10.00
0	4.92± 0.32	5.04± 0.33	5.18± 0.40	4.94± 0.23	4.98± 0.17
15	6.10± 0.41 (24.06)	7.34± 0.45 (45.73)	6.73± 0.54 (29.88)	7.04± 0.37 (42.35)	7.38± 0.33 (48.15)
30	7.06± 0.50 (43.50)	11.47± 0.72 (127.64)	9.16± 0.71 (76.93)	9.21± 0.48 (86.35)	10.84± 0.49 (117.76)
45	7.99± 0.59 (62.35)	15.47± 1.07 (207.09)	11.16± 0.93 (115.46)	11.58± 0.73 (134.20)	14.54± 0.77 (192.07)
60*	8.50± 0.75 (72.72)	17.64± 1.25 (250.16)	13.34± 1.38 (157.51)	14.42± 1.44 (191.78)	17.52± 1.08 (251.86)
75	10.38± 0.77 (110.98)	20.29± 2.06 (302.86)	—	16.92± 1.73 (242.19)	19.52± 1.46 (291.95)
90	15.50± 2.89 (215.11)	25.72± 2.99 (410.62)	—	20.47± 3.66 (314.07)	22.78± 1.91 (357.52)

* DRUG WITHDRAWN AFTER 60 DAYS.

a DUE TO MECHANICAL FAILURE ALL FISH DIED IN THIS GROUP AFTER 60 DAYS.

STATISTICALLY SIGNIFICANT DIFFERENCES AMONG THE DOSES, ACCORDING TO SINGLE FACTOR ANALYSIS OF VARIANCE (SOKAL & ROHLF 1969)

60 DAYS

CONTROL VERSUS 1.0 = $P < 0.001$

CONTROL VERSUS 2.5 = $P < 0.01$

CONTROL VERSUS 5.0 = $P < 0.01$

CONTROL VERSUS 10.0 = $P < 0.001$

1.0 VERSUS 2.5 = $P < 0.05$

2.5 VERSUS 10.0 = $P < 0.05$

90 DAYS

CONTROL VERSUS 1.0 = $P < 0.05$

TABLE 58 CHANGES IN TOTAL BODY LENGTH OF CARP FED OXANDROLONE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEANS (cm) ± S.E. OF 25 FISH. PERCENT INCREASE IN LENGTH IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF OXANDROLONE mg/kg FOOD				
	0.00	1.00	2.50[a]	5.00	10.00
0	7.00± 0.15	7.05± 0.15	6.88± 0.17	7.14± 0.11	7.08± 0.08
15	7.36± 0.16 (5.16)	7.83± 0.15 (11.20)	7.56± 0.19 (9.96)	7.79± 0.13 (9.13)	7.80± 0.11 (10.12)
30	7.80± 0.18 (11.43)	9.07± 0.18 (28.82)	8.39± 0.21 (21.97)	8.54± 0.14 (19.61)	8.77± 0.13 (17.49)
45	8.03± 0.20 (14.66)	9.55± 0.17 (37.92)	8.89± 0.18 (27.85)	8.79± 0.24 (24.54)	9.71± 0.21 (34.83)
60*	8.11± 0.24 (15.91)	10.39± 0.23 (47.54)	9.21± 0.30 (34.01)	9.52± 0.28 (33.34)	10.10± 0.21 (42.66)
75	9.09± 0.22 (29.90)	10.61± 0.34 (50.59)	—	9.82± 0.33 (37.48)	10.45± 0.26 (47.63)
90	9.56± 0.61 (36.51)	11.73± 0.44 (66.60)	—	10.50± 0.59 (47.06)	11.46± 0.31 (61.89)

* DRUG WITHDRAWN AFTER 60 DAYS.

a DUE TO MECHANICAL FAILURE ALL FISH DIED IN THIS GROUP AFTER 60 DAYS

STATISTICALLY SIGNIFICANT DIFFERENCES AMONG THE DOSES, ACCORDING TO SINGLE FACTOR ANALYSIS OF VARIANCE (SOKAL & ROHLF, 1969)

60 DAYS

CONTROL VERSUS 1.0 = $P < 0.001$
CONTROL VERSUS 2.5 = $P < 0.001$
CONTROL VERSUS 5.0 = $P < 0.001$
CONTROL VERSUS 10.0 = $P < 0.001$
1.0 VERSUS 2.5 = $P < 0.001$
1.0 VERSUS 5.0 = $P < 0.05$
2.50 VERSUS 10.0 = $P < 0.05$

90 DAYS

CONTROL VERSUS 1.0 = $P < 0.01$
CONTROL VERSUS 10.0 = $P < 0.01$

TABLE 59 EFFECT OF FEEDING AND WITHDRAWAL OF OXANDROLONE ON THE SPECIFIC GROWTH RATE (SGR) OF CARP. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg FOOD	SPECIFIC GROWTH RATE (WEIGHT)			SPECIFIC GROWTH RATE (LENGTH)		
	0-60 DAYS	60-90 DAYS	0-90 DAYS	0-60 DAYS	60-90 DAYS	0-90 DAYS
0.00	0.92± 0.21	2.04± 0.48	1.29± 0.29	0.25± 0.07	0.55± 0.16	0.35± 0.10
1.00	2.13± 0.46	1.27± 0.23	1.84± 0.43	0.65± 0.13	0.41± 0.19	0.57± 0.14
2.50	1.60± 0.21	—	—	0.50± 0.10	—	—
5.00	1.81± 0.21	1.18± 0.08	1.60± 0.23	0.49± 0.08	0.33± 0.09	0.43± 0.08
10.00	2.13± 0.33	0.89± 0.11	1.72± 0.41	0.60± 0.09	0.43± 0.13	0.54± 0.10

TABLE 60 CHANGES IN THE CONDITION FACTOR AND FOOD CONVERSION EFFICIENCY OF CARP ADMINISTERED OXANDROLONE IN THE DIET FOR 60 DAYS.

CONCENTRATION OF DRUG mg/kg FOOD	CONDITION FACTOR			FOOD CONVERSION EFFICIENCY		
	0 DAYS	60 DAYS	90 DAYS	0–60 DAYS	60–90 DAYS	0–90 DAYS
0.00	1.43± 0.24	1.59± 0.27	1.78± 0.44	0.18	0.49	0.31
1.00	1.44± 0.26	1.57± 0.27	1.59± 0.30	0.44	0.28	0.36
2.50	1.59± 0.25	1.70± 0.28	—	0.34	—	—
5.00	1.38± 0.23	1.67± 0.26	1.77± 0.42	0.39	0.26	0.32
10.00	1.40± 0.24	1.70± 0.26	1.51± 0.33	0.45	0.18	0.32

TABLE 61 EFFECT OF FEEDING OXANDROLONE ON THE CRANIO-SOMATIC (CSI), HEPATO-SOMATIC (HSI), RENO-SOMATIC (RSI), AND VISCERO-SOMATIC (VSI) INDEX OF CARP. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg FOOD	CSI		HSI		RSI		VSI	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	1.03± 0.04	0.99± 0.05	3.07± 0.32	2.89± 0.13	0.79± 0.03	0.82± 0.08	5.19± 0.36	4.91± 0.25
1.00	** 0.82± 0.23	** 0.75± 0.06	2.66± 0.15	* 2.34± 0.14	0.84± 0.04	0.72± 0.03	4.80± 0.23	5.03± 0.27
2.50	0.98± 0.05	—	2.54± 0.20	—	0.83± 0.03	—	5.24± 0.11	—
5.00	1.00± 0.07	0.89± 0.04	** 2.16± 0.09	3.03± 0.27	0.80± 0.08	0.73± 0.06	4.69± 0.16	* 4.11± 0.21
10.00	*** 0.76± 0.03	*** 0.73± 0.04	2.67± 0.11	** 1.99± 0.13	0.86± 0.04	0.71± 0.08	* 4.45± 0.28	* 3.96± 0.14

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 62 EFFECT OF FEEDING OXANDROLONE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN LIVER OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	26.28± 2.41	31.28± 3.49	5.53± 0.48	8.88± 1.15	17.37± 1.26	16.37± 1.88	95.33± 9.69	142.11± 16.58
1.00	27.75± 1.12	32.72± 5.72	** 10.06± 1.20	11.84± 1.38	* 14.39± 0.62	15.61± 1.50	** 143.36± 15.50	180.62± 14.85
2.50	34.35± 6.71	–	6.72± 0.65	–	16.33± 0.21	–	109.43± 9.18	–
5.00	* 38.08± 6.11	23.85± 2.56	*** 11.56± 1.34	9.65± 1.08	14.86± 0.81	20.29± 1.21	*** 170.15± 17.97	* 192.30± 12.93
10.00	34.19± 3.25	27.95± 2.65	*** 14.02± 1.06	11.34± 0.57	** 11.97± 0.57	18.98± 0.97	*** 166.08± 5.40	** 215.69± 18.03

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL AND ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 63 EFFECT OF FEEDING OXANDROLONE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN KIDNEY OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	40.81± 3.50	26.19± 2.92	3.52± 0.26	4.14± 0.26	14.41± 0.72	13.16± 1.34	49.58± 2.34	54.34± 6.78
1.00	40.24± 4.82	50.39± *** 3.29	6.54± *** 0.61	11.22± *** 0.62	12.54± 1.83	11.21± 0.44	78.71± *** 2.99	125.09± *** 4.35
2.50	51.40± 3.14	—	5.29± ** 0.53	—	11.51± 0.64	—	59.93± 3.28	—
5.00	31.04± 4.48	27.33± 1.86	4.62± 0.77	8.44± *** 0.57	14.03± 0.66	12.48± 0.39	64.56± * 11.32	105.11± *** 6.63
10.00	23.42± ** 2.11	48.99± *** 5.53	6.62± *** 0.24	11.01± *** 0.92	12.63± 0.57	11.44± 0.22	83.98± *** 6.55	126.23± *** 11.59

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 64 EFFECT OF FEEDING OXANDROLONE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN BRAIN OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	41.36± 3.10	37.24± 1.27	2.73± 0.04	2.24± 0.04	18.81± 1.39	28.66± 0.57	51.56± 4.21	64.14± 1.09
1.00	** 23.59± 1.10	* 51.06± 3.55	* 3.53± 0.16	3.06± 0.55	20.98± 1.71	29.89± 0.96	** 74.00± 7.49	* 90.62± 14.12
2.50	42.44± 6.40	—	3.17± 0.22	—	16.43± 0.35	—	52.08± 3.56	—
5.00	38.01± 6.68	46.21± 6.42	3.24± 0.46	3.01± 0.16	19.48± 0.87	26.94± 2.04	62.29± 7.95	80.80± 6.00
10.00	* 25.16± 3.67	38.70±	** 3.94± 0.55	2.76± 0.23	18.03± 2.05	26.28± 1.54	* 68.25± 6.67	71.62± 3.09

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 65 EFFECT OF FEEDING OXANDROLONE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN MUSCLE OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg / kg	PROTEIN mg / 100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	13.60± 0.75	14.86± 0.59	10.51± 1.40	12.71± 1.87	36.94± 3.28	49.23± 3.15	375.67± 23.41	614.76± 69.43
1.00	16.44± 0.56	16.63± 0.57	** 16.52± 1.31	15.95± 2.66	* 55.56± 2.94	43.50± 1.09	*** 906.71± 25.67	694.60± 114.64
2.50	16.52± 0.66	—	* 14.31± 1.01	—	** 62.69± 2.97	—	*** 888.32± 30.91	—
5.00	16.72± 2.57	17.00± 1.26	* 14.00± 1.34	14.29± 1.39	** 62.70± 12.40	* 41.90± 2.62	*** 839.61± 102.71	588.10± 20.01
10.00	16.78± 1.16	15.91± 1.06	** 16.22± 0.97	15.00± 1.88	* 56.23± 3.09	** 36.49± 1.51	*** 928.40± 44.51	538.96± 50.47

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL AND ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 66 EFFECT OF FEEDING OXANDROLONE ON THE PROXIMATE COMPOSITION OF MUSCLE TISSUE FROM *Cyprinus carpio*. DRUG WAS FED FOR 60 DAYS ONLY.

CONCENTRATION OF DRUG mg/kg	MOISTURE mg/100 mg		CRUDE PROTEIN N x 6.25 (mg / 100mg)				TOTAL LIPIDS (mg / 100 mg)				ASH (mg / 100 mg)			
			ON WET BASIS		ON DRY BASIS		ON WET BASIS		ON DRY BASIS		ON WET BASIS		ON DRY BASIS	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	75.84± 0.20	74.56± 0.60	17.81± 0.25	18.63± 0.31	73.88± 1.13	73.38± 0.81	2.32± 0.18	3.16± 0.42	9.61± 0.73	12.22± 1.34	2.21± 0.07	2.11± 0.04	9.13± 0.25	8.34± 0.35
1.00	76.65± 0.16	78.22± *** 0.41	17.69± 0.19	17.25± ** 0.38	75.81± 0.69	79.25± ** 2.38	1.75± * 0.08	1.41± ** 0.21	7.48± * 0.42	6.43± ** 0.85	1.49± *** 0.11	1.31± *** 0.05	6.40± *** 0.46	6.03± *** 0.14
2.50	75.14± 0.70	—	18.94± * 0.31	—	76.25± * 0.75	—	1.74± * 0.12	—	7.0± * 0.49	—	1.57± *** 0.05	—	6.33± *** 0.18	—
5.00	76.89± * 0.42	77.32± ** 0.58	17.50± 0.56	18.44± 0.18	75.63± 1.75	79.25± ** 2.31	1.58± * 0.25	1.80± * 0.36	6.8± * 1.03	7.85± * 1.33	1.66± *** 0.10	1.44± *** 0.13	7.35± ** 0.47	6.33± ** 0.41
10.00	76.87± * 0.30	77.19± ** 0.27	17.00± 0.19	17.06± ** 0.19	73.44± 0.75	74.88± 1.38	1.16± *** 0.26	1.69± * 0.28	5.0± *** 1.07	7.38± * 1.15	1.43± *** 0.06	2.10± 0.15	6.18± *** 0.33	9.20± 0.60

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

4.1.8. Ethylestrenol

4.1.8.1. Weight and Length Data

The weight and length data accumulated over the period of ninety days (sixty days of drug feeding and thirty days of withdrawal) is presented in Figures 39 and 40. A clear cut growth response was observed in weight and length at sixty days. The detailed statistical analysis for weight and length is given in Tables 67 and 68. After sixty days the percentage weight and length gain over the controls for 1.0, 2.5, 5.0 and 10.0 ppm groups were 301.96, 343.58, 277.09 and 262.01 for weight and 220.72, 243.24, 189.19 and 202.70 for length respectively.

After the withdrawal, although the SGR for weight was lower than the corresponding values in phase 1, and from the control values for this phase, the SGR for length was still higher than the controls and resulted in a decrease of condition factor (Tables 69 and 70). The FCE in phase 1 was two to three times higher in experimental groups, but in phase 2, FCE was low than the control values, but the total FCE for ninety days was still higher than the controls.

4.1.8.2. Tissue - Body Indices

CSI was significantly less ($P<0.001$) than the control values both at sixty and ninety days. RSI also recorded a decrease ($P<0.01$) and a decrease also in HSI and VSI was noted in 10.0 ppm and 1.0 ppm groups respectively

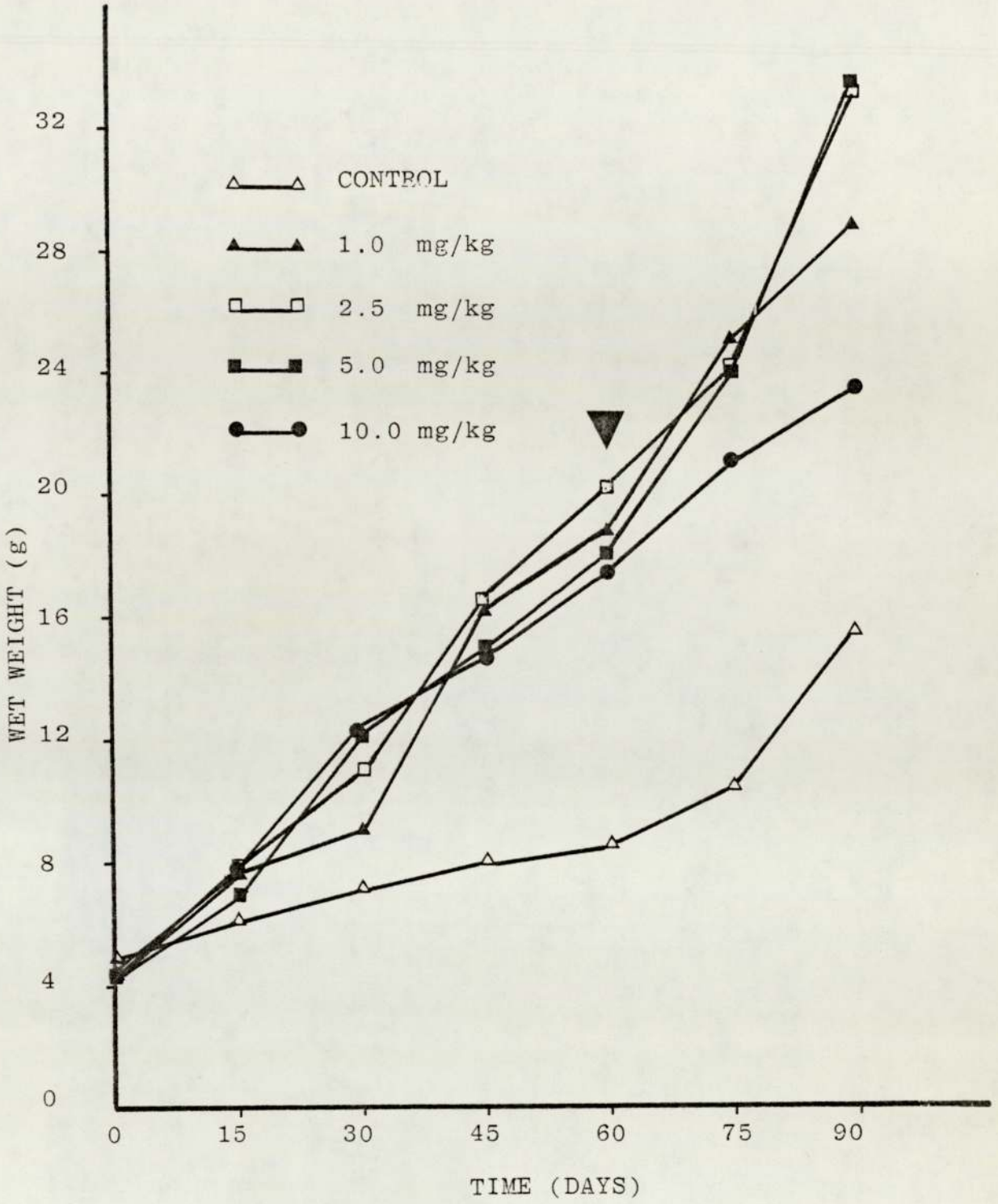


FIGURE 39 MEAN WEIGHTS OF CARP GIVEN ETHYLESTRENOL *PER OS*. DRUG WAS WITHDRAWN AFTER 60 DAYS (ARROW).

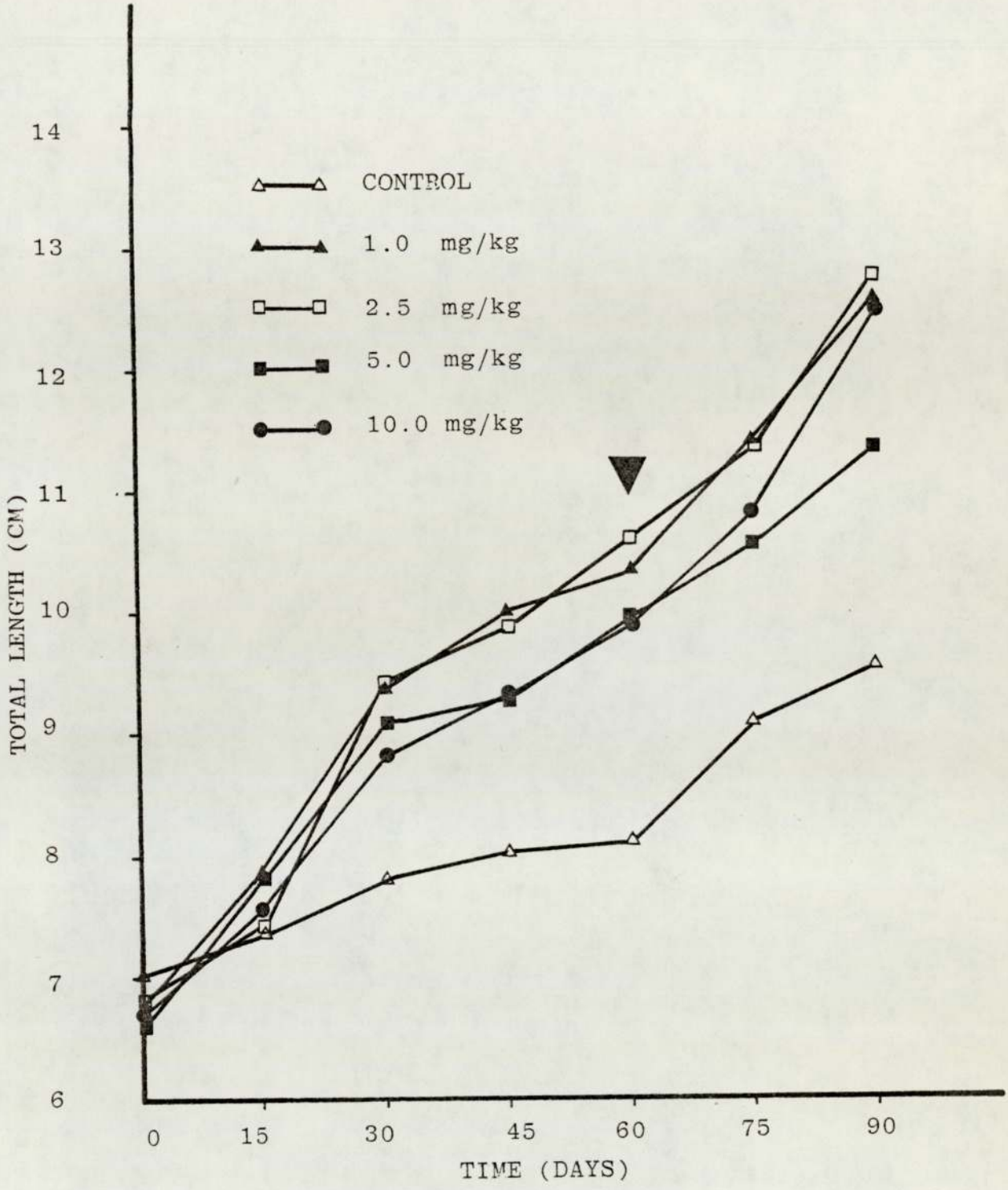


FIGURE 40 MEAN LENGTHS OF CARP GIVEN ETHYLESTRENOL *PER OS*. DRUG WAS WITHDRAWN AFTER 60 DAYS (ARROW).

after sixty days. After ninety days, HSI and RSI ($P<0.001$) were significantly lower, like CSI. No change, however, in VSI was noted. The changes can be seen in detail in Table 71 and Figures 41 and 42.

4.1.8.3. Biochemical Changes

4.1.8.3.1. Liver (Table 72)

In liver, after sixty days, an increase in RNA/DNA and protein/DNA (2.5 and 5.0 ppm only) was observed. Proteins were higher only in 2.5 ppm group (optimum dose). Protein/RNA was decreased in all except 1.0 ppm group. After ninety days, no change was observed in total proteins, protein/DNA. RNA/DNA was higher in 1.0 and 10.0 ppm groups and protein/RNA only in 2.5 ppm.

4.1.8.3.2. Kidney (Table 73)

A significant increase in total proteins was observed only in 1.0 ppm group both after sixty and ninety days. RNA/DNA and protein/DNA were significant for all the experimental groups after sixty days. No effect on protein/RNA was observed. After ninety days, RNA/DNA and protein/DNA were significant in 1.0 and 5.0 ppm groups while protein/RNA was increased in 1.0, 2.5 and 5.0 ppm groups.

4.1.8.3.3. Brain (Table 74)

Total proteins were significantly lower, while RNA/DNA and protein/DNA were statistically significant only in 2.5 ppm group. No change in protein/RNA was noted after

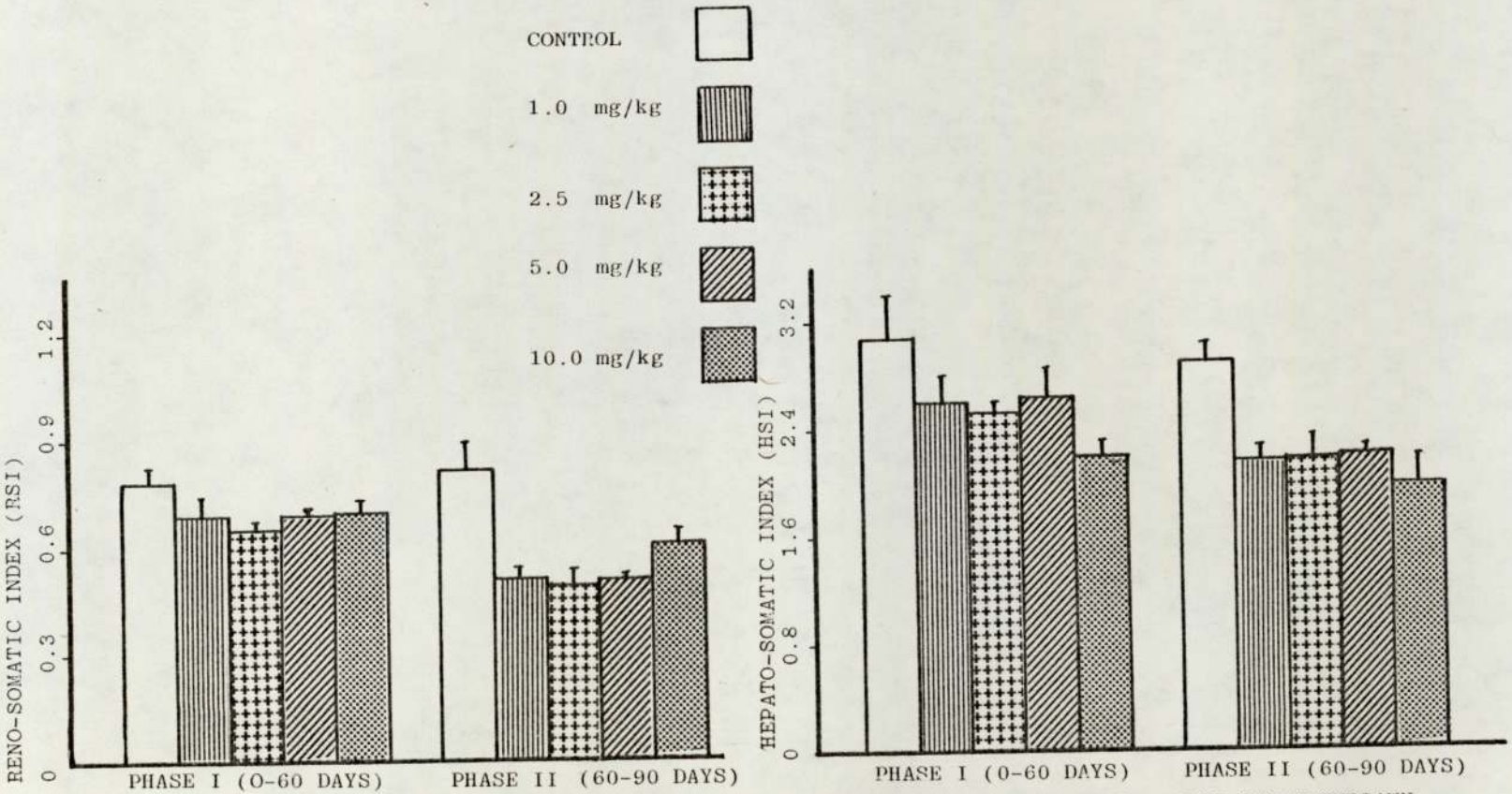


FIGURE 41 EFFECT OF ETHYLESTRENOL GIVEN *PER OS* ON THE RSI AND HSI OF CARP. DRUG WAS WITHDRAWN AFTER 60 DAYS.

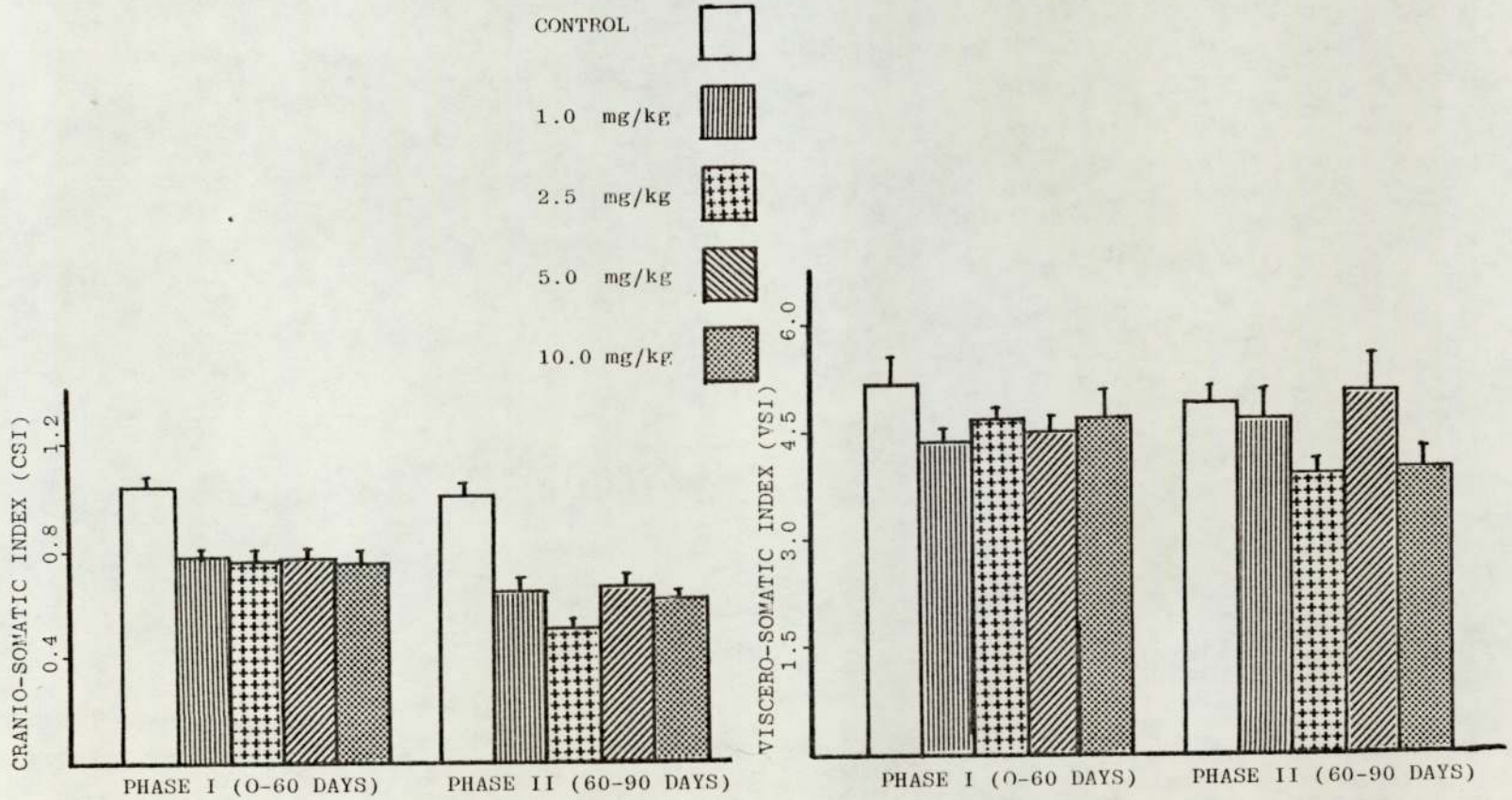


FIGURE 42 EFFECT OF ETHYLESTRENOL GIVEN *PER OS* ON THE CSI AND VSI OF CARP. DRUG WAS WITHDRAWN AFTER 60 DAYS.

sixty days. After ninety days, proteins were lower in 10.0 ppm group, RNA/DNA was elevated in all except 10.0 ppm group. Protein/RNA was less in all except 10.0 ppm group, while no effect was seen in protein/DNA.

4.1.8.3.4. Muscle (Tables 75,76,77,78, 79 and 80)
After sixty days, an increase was seen in all the four parameters, i.e. proteins, RNA/DNA, protein/RNA and protein/DNA of the muscle. After ninety days, none of these parameters was significantly different from controls (Table 75).

No change in moisture content was seen after sixty days. Crude proteins were significant in 1.0 ppm group only on the wet weight basis. Total lipids were lower in 10.0 ppm while ash content was lower ($P<0.001$). After ninety days, moisture content increased ($P<0.01$) in 2.5 and 10.0 ppm with a concomitant decrease in protein content ($P<0.001$). No change was seen in total lipids, but ash content was significantly lower ($P<0.001$; Table 76).

The effect of feeding and withdrawal of ethylestrenol on the muscle free and protein bound amino acids is given in Tables 77,78, 79 and 80.

TABLE 67 CHANGES IN BODY WEIGHT OF CARP FED ETHYLESTRENOL SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEAN (g) ± S.E. OF 25 FISH. PERCENT WEIGHT GAIN IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF ETHYLESTRENOL mg/kg FOOD				
	0.00	1.00	2.50	5.00	10.00
0	4.92± 0.35	4.36± 0.21	4.33± 0.17	4.52± 0.26	4.38± 0.21
15	6.10± 0.41 (24.06)	7.56± 0.38 (73.51)	7.91± 0.54 (82.25)	6.86± 0.48 (51.50)	7.80± 0.36 (78.11)
30	7.06± 0.50 (43.50)	9.00± 0.40 (106.54)	11.03± 0.82 (154.12)	12.16± 0.96 (168.75)	12.37± 0.61 (182.41)
45	7.99± 0.59 (62.35)	16.32± 0.76 (274.59)	16.69± 1.16 (284.59)	15.00± 1.14 (231.67)	14.63± 0.78 (233.88)
60*	8.50± 0.75 (72.72)	18.75± 1.09 (330.52)	20.21± 1.64 (365.81)	18.02± 1.49 (298.29)	17.34± 0.93 (295.76)
75	10.38± 0.77 (110.98)	24.96± 1.88 (472.95)	24.13± 2.51 (456.02)	23.88± 2.60 (427.66)	20.98± 1.40 (379.05)
90	15.50± 2.89 (215.11)	28.66± 2.31 (558.05)	32.97± 3.65 (659.93)	33.44± 3.65 (638.95)	23.40± 1.64 (434.13)

* DRUG WITHDRAWN AFTER 60 DAYS

STATISTICALLY SIGNIFICANT DIFFERENCES AMONG THE DOSES. STATISTICS ACCORDING TO SINGLE FACTOR ANALYSIS OF VARIANCE (SOKAL & ROHLF 1969)

60 DAYS

CONTROL VERSUS 1.0 = $P < 0.001$

CONTROL VERSUS 2.5 = $P < 0.001$

CONTROL VERSUS 5.0 = $P < 0.001$

CONTROL VERSUS 10.0 = $P < 0.001$

90 DAYS

CONTROL VERSUS 1.0 = $P < 0.01$

CONTROL VERSUS 2.5 = $P < 0.001$

CONTROL VERSUS 5.0 = $P < 0.001$

2.5 VERSUS 10.0 = $P < 0.01$

5.0 VERSUS 10.0 = $P < 0.01$

TABLE 68 CHANGES IN TOTAL BODY LENGTH OF CARP FED ETHYLESTRENOL SUPPLEMENTED DIETS FOR 60 DAYS. GIVEN ARE MEANS (cm) ± S.E. OF 25 FISH. PERCENT INCREASE IN LENGTH IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF ETHYLESTRENOL mg/kg FOOD				
	0.00	1.00	2.50	5.00	10.00
0	7.00± 0.15	6.75± 0.10	6.78± 0.09	6.68± 0.12	6.57± 0.10
15	7.36± 0.16 (5.16)	7.88± 0.13 (16.52)	7.93± 0.18 (16.99)	7.58± 0.18 (13.46)	7.82± 0.13 (19.10)
30	7.80± 0.18 (11.43)	9.38± 0.16 (38.84)	9.42± 0.23 (38.97)	8.83± 0.25 (32.20)	9.07± 0.16 (38.07)
45	8.03± 0.20 (14.66)	9.99± 0.16 (47.78)	9.88± 0.22 (45.69)	9.34± 0.26 (39.83)	9.33± 0.18 (42.07)
60*	8.11± 0.24 (15.91)	10.32± 0.21 (52.73)	10.59± 0.29 (56.30)	9.89± 0.30 (48.09)	9.93± 0.23 (51.21)
75	9.09± 0.22 (29.90)	11.40± 0.29 (68.68)	11.36± 0.39 (67.66)	10.81± 0.47 (61.92)	10.56± 0.28 (60.83)
90	9.56± 0.61 (36.51)	12.58± 0.36 (86.11)	12.75± 0.47 (88.14)	12.45± 0.53 (86.47)	11.37± 0.34 (73.16)

* DRUG WITHDRAWN AFTER 60 DAYS.

STATISTICALLY SIGNIFICANT DIFFERENCES AMONG THE DOSES. ACCORDING TO SINGLE FACTOR ANALYSIS OF VARIANCE (SOKAL & ROHLF 1969)

60 DAYS

CONTROL VERSUS 1.0 = $P < 0.001$
CONTROL VERSUS 2.5 = $P < 0.001$
CONTROL VERSUS 5.0 = $P < 0.001$
CONTROL VERSUS 10.0 = $P < 0.001$

90 DAYS

CONTROL VERSUS 1.0 = $P < 0.01$
CONTROL VERSUS 2.5 = $P < 0.01$
CONTROL VERSUS 5.0 = $P < 0.05$
CONTROL VERSUS 10.0 = $P < 0.05$
1.0 VERSUS 10.0 = $P < 0.05$
2.5 VERSUS 10.0 = $P < 0.05$

TABLE 69 EFFECT OF FEEDING AND WITHDRAWAL OF ETHYLESTRENOL ON THE SPECIFIC GROWTH RATE (SGR) OF CARP. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg / kg FOOD	SPECIFIC GROWTH RATE (WEIGHT)			SPECIFIC GROWTH RATE (LENGTH)		
	0-60 DAYS	60-90 DAYS	0-90 DAYS	0-60 DAYS	60-90 DAYS	0-90 DAYS
0.00	0.92± 0.21	2.04± 0.48	1.29± 0.29	0.25± 0.07	0.55± 0.16	0.35± 0.10
1.00	2.48± 0.83	1.43± 0.35	2.13± 0.58	0.71± 0.23	0.67± 0.01	0.70± 0.15
2.50	2.62± 0.59	1.66± 0.33	2.30± 0.44	0.75± 0.21	0.63± 0.11	0.71± 0.14
5.00	2.35± 0.63	2.09± 0.13	2.26± 0.41	0.66± 0.16	0.78± 0.12	0.70± 0.12
10.00	2.34± 0.71	1.01± 0.20	1.90± 0.54	0.70± 0.23	0.46± 0.03	0.62± 0.16

TABLE 70 CHANGES IN THE CONDITION FACTOR AND FOOD CONVERSION EFFICIENCY OF CARP ADMINISTERED ETHYLESTRENOL IN THE DIET FOR 60 DAYS.

CONCENTRATION OF DRUG mg/kg FOOD	CONDITION FACTOR			FOOD CONVERSION EFFICIENCY		
	0 DAYS	60 DAYS	90 DAYS	0-60 DAYS	60-90 DAYS	0-90 DAYS
0.00	1.43± 0.24	1.59± 0.27	1.78± 0.44	0.18	0.49	0.31
1.00	1.41± 0.24	1.71± 0.27	1.44± 0.28	0.52	0.30	0.40
2.50	1.34± 0.24	1.70± 0.30	1.59± 0.31	0.53	0.38	0.45
5.00	1.52± 0.26	1.86± 0.31	1.73± 0.34	0.47	0.49	0.48
10.00	1.54± 0.25	1.77± 0.27	1.59± 0.31	0.44	0.21	0.33

TABLE 71 EFFECT OF FEEDING ETHYLESTRENOL ON THE CRANIO-SOMATIC (CSI), HEPATO-SOMATIC (HSI), RENO-SOMATIC (RSI) & VISCERO-SOMATIC (VSI) INDEX OF CARP. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg FOOD	CSI		HSI		RSI		VSI	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	1.03± 0.04	0.99± 0.05	3.07± 0.32	2.89± 0.13	0.79± 0.03	0.82± 0.08	5.19± 0.36	4.91± 0.25
1.00	*** 0.77± 0.03	*** 0.63± 0.06	2.60± 0.19	** 2.17± 0.11	* 0.69± 0.05	*** 0.51± 0.03	* 4.44± 0.15	4.73± 0.38
2.50	*** 0.75± 0.04	*** 0.49± 0.02	2.53± 0.08	** 2.18± 0.17	** 0.65± 0.02	*** 0.49± 0.05	4.72± 0.10	3.92± 0.23
5.00	*** 0.76± 0.04	*** 0.65± 0.05	2.65± 0.22	** 2.19± 0.06	* 0.69± 0.01	*** 0.50± 0.01	4.54± 0.04	5.05± 0.53
10.00	*** 0.74± 0.05	*** 0.60± 0.01	** 2.20± 0.10	*** 1.98± 0.21	* 0.70± 0.03	** 0.61± 0.04	4.73± 0.34	4.03± 0.30

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 72 EFFECT OF FEEDING ETHYLESTRENOL ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN LIVER OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg / kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	26.28± 2.41	31.28± 3.49	5.53± 0.48	8.88± 1.15	17.37± 1.26	16.37± 1.88	95.53± 9.69	142.11± 16.58
1.00	34.50± 4.85	22.83± 3.92	5.76± 0.32	* 13.54± 0.94	17.57± 1.34	15.55± 1.58	100.82± 7.68	209.30± 21.74
2.50	* 41.67± 6.52	33.97± 5.04	*** 11.94± 2.32	8.00± 1.50	* 13.28± 2.13	* 26.56± 2.93	* 146.59± 16.09	214.50± 42.87
5.00	29.63± 3.51	24.40± 4.39	*** 13.05± 1.67	9.19± 1.63	** 10.86± 0.36	18.69± 1.45	* 142.06± 19.80	170.62± 30.21
10.00	36.44± 4.76	22.67± 2.13	* 9.26± 0.80	* 14.19± 1.15	* 12.66± 1.52	14.26± 0.95	114.70± 13.00	202.76± 22.47

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 73 EFFECT OF FEEDING ETHYLESTRENOL ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN KIDNEY OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	40.81± 3.50	26.19± 2.92	3.52± 0.26	4.14± 0.26	14.41± 0.72	13.16± 1.34	49.58± 2.34	54.34± 6.78
1.00	*** 65.12± 2.88	* 39.89± 4.77	4.61± 0.45	* 8.20± 0.76	16.88± 2.07	** 26.77± 4.06	* 75.14± 4.10	*** 210.68± 17.70
2.50	50.59± 5.67	26.61± 1.65	*** 6.59± 0.54	4.68± 0.71	12.08± 1.46	* 22.42± 3.21	** 79.61± 12.42	99.30± 8.84
5.00	50.18± 4.05	35.67± 4.36	*** 7.18± 1.01	*** 10.83± 2.37	12.93± 1.61	** 24.60± 1.59	*** 89.41± 8.20	*** 257.34± 46.47
10.00	40.80± 3.48	21.82± 2.80	* 5.36± 0.23	7.36± 0.45	16.57± 1.61	15.57± 1.39	*** 89.68± 11.82	112.77± 3.89

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P<0.05$

** = $P<0.01$

*** = $P<0.001$

TABLE 74 EFFECT OF FEEDING ETHYLESTRENOL ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN BRAIN OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	41.36± 3.10	37.24± 1.27	2.73± 0.04	2.24± 0.04	18.81± 1.39	28.66± 0.57	51.56± 4.21	64.14± 1.09
1.00	* 29.40± 3.87	29.35± 0.74	3.22± 0.17	** 3.46± 0.26	17.08± 1.10	*** 19.34± 0.65	54.68± 2.84	67.39± 7.25
2.50	31.32± 3.06	35.50± 3.40	** 4.74± 0.92	*** 3.62± 0.26	17.69± 2.01	** 20.73± 1.04	** 78.97± 8.73	74.69± 5.88
5.00	*** 23.23± 2.97	28.84± 3.44	3.73± 0.16	* 2.94± 0.32	14.95± 0.82	* 23.31± 2.41	55.76± 3.65	67.62± 9.20
10.00	** 25.56± 3.04	* 25.82± 4.84	3.89± 0.73	2.27± 0.17	17.75± 0.37	27.64± 1.59	68.81± 12.76	62.91± 6.19

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 75 EFFECT OF FEEDING ETHYLESTRENOL ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN MUSCLE OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	13.60± 0.75	14.86± 0.59	10.15± 1.40	12.71± 1.87	36.94± 3.28	49.23± 3.15	375.67± 23.41	614.76± 69.43
1.00	* 16.08± 0.92	16.15± 0.89	* 16.23± 1.44	14.17± 0.89	** 50.66± 2.99	49.19± 1.48	*** 824.96± 99.10	694.10± 31.21
2.50	*** 17.70± 0.23	15.85± 0.42	*** 21.36± 2.81	13.67± 1.39	43.35± 1.05	50.02± 1.50	*** 919.00± 104.90	681.74± 68.04
5.00	* 16.14± 0.66	16.95± 1.50	13.26± 2.11	15.43± 0.71	*** 60.62± 2.30	42.35± 0.87	*** 789.04± 106.01	652.34± 26.88
10.00	** 16.86± 0.25	16.50± 2.57	* 14.90± 0.10	16.45± 3.56	43.16± 1.88	43.02± 6.73	** 642.93± 25.97	646.51± 97.98

SIGNIFICANTLY DIFFERENT FROM CONTROLS * = $P < 0.05$

(SINGLE FACTOR ANALYSIS OF VARIANCE, ** = $P < 0.01$

ACCORDING TO SOKAL & ROHLF 1969) *** = $P < 0.001$

TABLE 76 EFFECT OF FEEDING ETHYLESTRENOL ON THE PROXIMATE COMPOSITION OF MUSCLE TISSUE FROM Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCENTRATION OF DRUG mg/kg	MOISTURE mg/100 mg		CRUDE PROTEIN N x 6.25 (mg/100 mg)				TOTAL LIPIDS (mg/100 mg)				ASH (mg/100 mg)			
			ON WET BASIS		ON DRY BASIS		ON WET BASIS		ON DRY BASIS		ON WET BASIS		ON DRY BASIS	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	75.84± 0.20	74.56± 0.60	17.81± 0.25	18.63± 0.31	73.88± 1.13	73.38± 0.81	2.32± 0.18	3.16± 0.42	9.61± 0.73	12.22± 1.34	2.21± 0.07	2.11± 0.04	9.13± 0.25	8.34± 0.35
1.00	75.25± 1.01	75.50± 0.54	* 19.25± 0.94	18.31± 0.13	78.00± 3.00	74.88± 1.50	2.05± 0.30	3.10± 0.36	8.23± 1.06	12.59± 1.29	*** 1.73± 0.09	*** 1.56± 0.15	*** 7.25± 0.35	*** 6.38± 0.54
2.50	76.64± 0.31	** 76.92± 0.37	17.75± 0.38	** 17.38± 0.19	75.81± 0.88	75.31± 0.81	2.45± 0.24	2.07± 0.28	10.50± 1.07	8.93± 1.1	** 1.86± 0.10	*** 1.73± 0.05	** 7.95± 0.38	*** 7.49± 0.26
5.00	76.60± 0.50	74.2± 0.45	18.19± 0.13	18.50± 0.31	77.81± 2.19	71.13± 0.81	2.04± 0.18	2.53± 0.28	8.68± 0.60	9.33± 0.81	2.09± 0.08	*** 1.70± 0.05	8.55± 0.26	*** 6.60± 0.26
10.00	77.04± 0.43	** 76.88± 0.53	17.69± 0.44	** 17.44± 0.38	76.88± 1.00	75.31± 0.63	** 1.50± 0.10	2.16± 0.19	** 6.45± 0.49	9.33± 0.76	*** 1.80± 0.05	*** 1.64± 0.09	*** 7.83± 0.30	*** 6.98± 0.24

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 77 EFFECT OF FEEDING ETHYLESTRENOL FOR 60 DAYS ON THE FREE AMINO ACIDS (n mole/mg) OF THE MUSCLE OF THE *Cyprinus carpio*.

AMNIO ACID	CONCENTRATION OF THE DRUG (mg/kg FOOD)				
	0.00	1.0	2.5	5.0	10.0
Taurine	14.07± 0.18	17.78± 1.48	16.21± 1.52	17.12± 0.18	17.60± 0.15
Aspartic	-	-	-	-	-
Threonine	1.36	4.21	3.24± 0.07	3.99± 0.03	3.84± 0.28
Serine	Traces	Traces	Traces	Traces	Traces
Glutamic	Traces	4.73	4.03± 0.09	4.14± 0.07	4.65± 0.38
Proline	-	-	-	-	-
Glycine	1.72± 0.16	4.52± 0.23	4.18± 0.12	5.14± 0.18	4.86± 0.30
Alanine	2.28± 0.10	4.41± 0.45	4.31± 0.08	4.31± 0.34	4.06± 0.21
Cysteine	-	-	-	-	-
Valine	-	-	-	-	-
Methionine	-	-	-	-	-
Isoleucine	-	-	-	-	-
Leucine	-	-	-	-	-
Tyrosine	-	-	-	-	-
Phenylalanine	-	-	-	-	-
Histidine	5.62 ± 0.07	10.32± 1.13	10.01± 0.55	9.84± 0.27	9.33± 0.31
Ornithine	Traces	-	-	-	-
Lysine	-	2.70 ± 0.42	2.25 ± 0.61	4.10 ± 0.56	3.83± 0.19
Ammonia	-	2.36 ± 0.98	2.57 ± 0.41	2.70	3.60
Arginine	-	6.35 ± 1.15	5.16 ± 0.78	7.29± 0.14	4.67± 0.05

TABLE 78 EFFECT OF WITHDRAWAL (30 DAYS) OF ETHYLESTRENOL ADMINISTERED *per os* for 60 DAYS ON THE FREE AMINO ACIDS (n mole/mg) OF THE MUSCLE OF *Cyprinus carpio*.

AMINO ACID	CONCENTRATION OF DRUG (mg/kg FOOD)				
	0.00	1.00	2.50	5.00	10.0
Taurine	12.51± 1.28	17.79± 0.02	16.87± 1.13	13.51± 0.73	13.91± 0.90
Aspartic	-	-	-	-	-
Threonine	2.09± 0.19	2.88± 0.10	3.16± 0.23	3.20± 0.05	2.54± 0.23
Serine	Traces	2.20± 0.41	2.67± 0.12	3.29± 0.08	2.17± 0.16
Glutamic	Traces	3.84± 0.01	3.78± 0.27	4.03± 0.03	3.09± 0.13
Proline	-	-	-	-	-
Glycine	2.30± 0.18	5.34± 0.07	4.74± 0.15	4.29± 0.06	3.00± 0.10
Alanine	2.01 ± 0.12	4.47 ± 0.13	5.84 ± 1.20	4.02 ± 0.24	3.24 ± 0.05
Cysteine	-	-	-	-	-
Valine	-	-	-	-	-
Methionine	-	-	-	-	-
Isoleucine	-	-	-	-	-
Leucine	-	-	-	-	-
Tyrosine	-	-	-	-	-
Phenylalanine	-	-	-	-	-
Histidine	9.34 ± 0.69	11.79± 0.44	11.10± 0.05	9.53 ± 0.68	7.62 ± 1.01
Ornithine	-	-	-	-	-
Lysine	3.16 ± 0.97	1.09 ± 0.24	1.48 ± 0.33	1.95 ± 0.45	4.48 ± 2.22
Ammonia	-	-	0.33 ± 0.12	-	-
Arginine	1.30±	10.71± 0.58	9.87± 0.74	8.72± 0.54	1.59± 0.42

TABLE 79 EFFECT OF FEEDING ETHYLESTRENOL FOR 60 DAYS ON THE PROTEIN BOUND AMINO ACIDS (n mole/mg) OF THE MUSCLE OF THE CARP *Cyprinus carpio*.

AMINO ACID	CONCENTRATION OF DRUG (mg/kg) FOOD				
	0.00	1.00	2.50	5.00	10.00
Taurine	-	-	-	-	-
Aspartic	112.66± 8.44	117.98± 1.50	95.47± 11.95	124.95± 6.45	124.34± 12.45
Threonine	70.60± 7.36	75.80± 2.34	66.13± 7.91	86.84± 6.62	77.74± 6.06
Serine	69.91± 8.97	72.75± 2.32	62.63± 7.36	84.81± 4.54	77.71± 8.99
Glutamic	152.33± 13.55	166.02± 2.99	134.41± 11.93	177.07± 5.96	175.88± 3.02
Proline	68.84± 9.14	71.93± 5.89	50.79± 5.01	82.34± 6.40	77.17± 10.78
Glycine	92.90± 13.32	94.83± 0.02	83.53± 6.97	105.29± 0.73	106.91± 11.48
Alanine	106.75± 18.77	106.83± 0.98	92.01± 9.29	110.20± 0.44	114.43± 2.13
Cysteine	-	-	-	-	-
Valine	55.01± 4.72	59.00± 1.60	48.92± 2.69	66.77± 5.69	62.96± 8.91
Methionine	38.84 ± 7.74	42.79± 3.49	39.88± 3.94	55.98± 4.88	49.33 ± 3.67
Isoleucine	66.89± 5.72	65.21± 3.83	57.09± 6.07	66.95± 7.72	67.37± 6.55
Leucine	92.34 ± 12.00	101.49± 0.49	85.15± 8.93	111.35± 5.95	98.79± 14.84
Tyrosine	32.90 ± 7.39	33.18± 0.73	28.63± 1.56	42.62± 2.38	35.81± 4.16
Pheylalanine	49.57 ± 9.56	50.17± 1.75	47.12± 6.02	55.57± 3.17	49.28± 1.69
Histidine	34.82± 5.49	41.23± 2.00	46.60± 5.04	49.65± 5.28	42.09± 3.42
Ornithine	-	-	-	-	-
Lysine	97.15 ± 10.86	110.95± 7.52	88.92 ± 14.21	113.40 ± 9.44	108.28 ± 11.89
Ammonia	106.21 ± 4.42	127.34 ± 12.77	104.68 ± 8.21	121.43 ± 3.78	128.18 ± 1.36
Arginine	84.78 ± 12.92	84.86 ± 4.08	90.47 ± 1.63	89.78 ± 5.28	89.50 ± 2.41

TABLE 80 EFFECT OF WITHDRAWAL (30 DAYS) OF ETHYLESTRENOL ADMINISTERED *per os* FOR SIXTY DAYS ON THE PROTEIN BOUND AMINO ACIDS (n mole/mg) OF THE MUSCLE OF *Cyprinus carpio*.

AMINO ACIDS	CONCENTRATION OF THE DRUG (mg/kg FOOD)				
	0.00	1.00	2.50	5.00	10.0
Taurine	-	-	-	-	-
Aspartic	144.54 ± 7.48	81.20± 11.3	102.38± 16.49	93.10± 1.67	116.82 ± 16.38
Threonine	95.89 ± 9.57	45.62± 8.03	62.65± 12.31	94.56± 0.63	74.84 ± 11.19
Serine	90.11 ± 3.79	47.38± 8.03	61.71± 10.21	61.71± 1.90	72.69 ± 13.93
Glutamic	200.41 ± 9.77	112.44± 16.74	141.53± 26.34	131.33± 2.41	160.64 ± 23.14
Proline	99.52 ± 3.61	37.77± 7.76	70.04± 10.83	54.25± 4.60	74.78 ± 19.12
Glycine	130.64 ± 15.47	67.60± 9.90	91.20± 14.64	79.28± 1.83	91.61 ± 10.55
Alanine	138.68 ± 12.10	76.31± 10.61	96.08± 20.59	85.87± 3.06	104.66 ± 16.34
Cysteine	-	-	-	-	-
Valine	77.38 ± 6.12	36.70± 5.57	67.84± 2.22	44.87± 0.69	60.37 ± 11.64
Methionine	54.48 ± 3.77	31.25± 4.30	40.78± 7.01	38.23± 0.11	57.66 ± 13.63
Isoleucine	77.79 ± 10.63	45.07± 7.09	61.56± 13.68	54.32± 0.93	66.14 ± 9.29
Leucine	121.93 ± 9.90	65.19± 7.46	85.93± 18.96	81.08± 0.92	99.93 ± 14.25
Tyrosine	47.26 ± 4.66	31.13± 0.08	30.53± 8.35	28.00± 0.65	34.88 ± 5.51
Phenylalanine	59.61 ± 6.20	34.46± 4.74	44.55± 10.32	37.56± 2.07	46.67 ± 8.51
Histidine	49.10 ± 2.67	30.57± 0.63	34.29± 6.56	30.12± 1.76	35.72 ± 5.72
Ornithine	-	-	-	-	-
Lysine	125.95 ± 8.21	80.57± 9.13	94.69± 22.03	87.12± 0.46	102.70 ± 11.47
Ammonia	129.77 ± 9.22	93.54± 1.59	116.75± 6.32	98.15± 0.52	116.71 ± 12.60
Arginine	88.16 ± 3.14	89.38± 5.82	91.04± 3.83	87.15± 5.57	89.26 ± 4.08

4.1.9. Effect of Re-Feeding of Hormones after Withdrawal Period on the Growth of Carp

In growth experiments with dimethazine, oxandrolone and ethylestrenol, after ninety days (thirty days after the drug was withdrawn), the fish which were on the optimum dose of these three steroids were fed again for another thirty days on the concentration of the hormone, on which they were before the withdrawal period. The doses which were selected for this experiment were, dimethazine (2.5 ppm), oxandrolone (10.0 ppm) and ethylestrenol (2.5 ppm). The results of this experiment on the growth of carp are presented in Table 81. There was no effect on the weight gain among all the groups (ANOVA, N.S.), but the FCE was higher in the experimental fish.

There was also no effect on tissue-body indices, except in the case of CSI which was higher ($P<0.05$) in the group fed oxandrolone (Table 82).

The plasma of these fish was analysed for total proteins and glucose levels. There was no effect on the total plasma protein levels. As far as plasma glucose is concerned, hypoglycemia was noticed in dimethazine ($P<0.001$) treated fish. In the other two experimental groups, no effect on the plasma sugar levels was observed (Table 82).

4.1.9.1. Biochemical Changes

4.1.9.1.1. Liver (Table 83)

Total proteins were significantly lower in oxandrolone and ethylestrenol groups, while no effect was seen in the dimethazine group. RNA/DNA was elevated ($P<0.001$) in all the treated groups and protein/RNA was decreased ($P<0.001$) in all the experimental groups. Protein/DNA was significant only in oxandrolone group.

4.1.9.1.2. Kidney (Table 83)

No effect on protein levels was observed but RNA/DNA ($P<0.001$) and protein/DNA ($P<0.05$) were elevated. Protein/RNA was lower ($P<0.001$) in oxandrolone and the ethylestrenol group.

4.1.9.1.3. Brain (Table 84)

No effect on protein and protein/DNA was noted. RNA/DNA ($P<0.001$) increased and protein/RNA ($P<0.001$) decreased in all the three experimental groups.

4.1.9.1.4. Muscle (Tables 84, 85 and 86)

No effect on proteins was observed. RNA/DNA and protein/DNA were elevated in oxandrolone and ethylestrenol. While protein/RNA ($P<0.05$) increased in the dimethazine group, it declined ($P<0.01$) in oxandrolone fed group (Table 84).

Muscle moisture content was not changed. Crude proteins were lower in dimethazine on wet weight bases but no

effect was seen on dry weight bases. In the ethylestrenol group, protein content of the muscle increased both on wet and dry bases ($P<0.001$). Total lipids were of the same magnitude in all the groups, but ash content was higher on wet as well as dry bases in oxandrolone and ethylestrenol groups (Table 85). The free amino acid levels in muscle and plasma are presented in Tables 86 and 87.

TABLE 81 EFFECT OF FEEDING (60 DAYS), WITHDRAWAL (30 DAYS) & REFEEDING FOR 30 DAYS OF DIMETHAZINE, OXANDROLONE & ETHYLESTRENOL ON THE WEIGHT, CONDITION FACTOR, SPECIFIC GROWTH RATES & FOOD CONVERSION EFFICIENCES OF CARP.

DRUGS mg/kg FOOD	INITIAL WEIGHT	FINAL WEIGHT	CONDIT-- ION FACTOR	SPECIFIC GROWTH RATE WEIGHT	SPECIFIC GROWTH RATE LENGTH	FOOD CONVER- SION EFFICIE- NCY
CONTROL	29.45± 1.57	49.32± 2.77	1.35± 0.26	1.73	0.63	1.16
DIMETH- AZINE 2.5	31.01± 2.36	46.41± 2.79	1.46± 0.28	1.35	0.41	1.31
OXANDRO- LONE 10.0	29.63± 2.30	50.87± 3.71	1.45± 0.28	1.82	0.52	1.35
ETHYLES- TRENOL 2.5	32.93± 2.17	50.82± 2.92	1.44± 0.28	1.46	0.44	1.30

TABLE 82 EFFECT OF FEEDING (60 DAYS), WITHDRAWAL (30 DAYS) & REFEEDING FOR 30 DAYS OF DIMETHAZINE, OXANDROLONE & ETHYLESTRONOL ON THE TOTAL PLASMA PROTEINS, GLUCOSE & HEPATO-SOMATIC (HSI), CRANIO-SOMATIC (CSI), RENO-SOMATIC (RSI) & VISCERO-SOMATIC (VSI) INDEX OF CARP.

DRUGS mg/kg FOOD	PLASMA PROTEIN g/100 ml	PLASMA GLUCOSE mg/100 ml	HSI	CSI	RSI	VSI
CONTROL	2.28± 0.24	92.50± 4.72	1.86± 0.15	0.51± 0.03	0.69± 0.04	4.83± 0.54
DIMETHAZINE 2.5	2.27± 0.14	57.50±** 7.80	1.85± 0.11	0.58± 0.03	0.62± 0.05	5.16± 0.50
OXANDROLONE 10.0	2.66± 0.13	88.75± 10.73	1.78± 0.26	0.60±* 0.03	0.70± 0.03	4.71± 0.33
ETHYLESTRENOL 2.5	2.41± 0.09	69.50± 12.02	1.84± 0.13	0.55± 0.03	0.58± 0.04	4.57± 0.22

SIGNIFICANTLY DIFFERENT FROM CONTROLS * = P< 0.05

(SINGLE FACTOR ANALYSIS OF VARIANCE ** = P< 0.01

ACCORDING TO SOKAL & ROHLF 1969) *** = P< 0.001

TABLE 83 EFFECT OF FEEDING (60 DAYS), WITHDRAWAL (30 DAYS) & REFEEDING FOR 30 DAYS OF DIMETHAZINE, OXANDROLONE & ETHYLESTRENOL ON PROTEIN, RNA & DNA IN LIVER AND KIDNEY OF CARP.

DRUGS mg/kg FOOD	LIVER				KIDNEY			
	PROTEIN mg/100 mg	RNA / DNA	PROTEIN/RNA	PROTEIN/DNA	PROTEIN mg/100 mg	RNA / DNA	PROTEIN/RNA	PROTEIN/DNA
CONTROL	19.73± 0.82	3.22± 0.12	22.38± 1.01	72,12± 4.93	17.60± 1.21	1.73± 0.30	27.08± 1.71	45.41± 4.25
DIMETH-AZINE 2.5	16.67± 1.62	*** 6.61± 0.30	*** 11.87± 0.40	78.37± 4.14	26.26± 7.51	* 3.30± 0.60	23.85± 4.11	* 63.94± 5.89
OXANDRO-LONE 10.0	* 15.50± 0.51	*** 8.16± 0.31	*** 12.20± 0.60	*** 99.57± 5.91	16.13± 0.73	*** 6.51± 0.33	*** 8.97± 0.52	58.07± 3.33
ETHYLES-TRENOL 2.5	** 13.55± 1.11	*** 5.05± 0.20	*** 12.31± 0.41	61.90± 1.17	16.66± 0.74	*** 7.47± 0.43	*** 8.43± 0.33	* 62.87± 3.88

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 84 EFFECT OF FEEDING (60 DAYS), WITHDRAWAL (30 DAYS) & REFEEDING FOR 30 DAYS OF DIMETHAZINE, OXANDROLONE & ETHYLESTRENOL ON PROTEIN, RNA & DNA IN BRAIN & MUSCLE OF CARP.

DRUGS mg/kg FOOD	BRAIN				MUSCLE			
	PROTEIN	RNA / DNA	PROTEIN/RNA	PROTEIN/DNA	PROTEIN	RNA / DNA	PROTEIN/RNA	PROTEIN/DNA
CONTROL	16.65± 3.99	1.54± 0.13	36.56± 2.58	56.08± 5.92	15.66± 1.46	6.87± 0.06	127.82± 8.28	877.21± 53.18
DIMETHAZINE 2.5	11.13± 0.33	5.79± *** 0.73	12.46± *** 0.94	73.75± 14.27	18.91± 1.49	6.72± 0.64	147.97± * 6.59	982.78± 58.38
OXANDROLONE 10.0	10.76± 0.30	4.01± ** 0.36	10.60± *** 1.66	40.79± 2.82	18.54± 0.86	12.68± *** 0.55	98.05± ** 3.09	1240.15± * 47.35
ETHYLESTRENOL 2.5	13.24± 1.33	6.34± *** 0.35	9.54± *** 0.50	60.02± 1.78	17.94± 1.01	9.36± * 1.17	110.97± 2.69	1045.74± * 149.02

SIGNIFICANTLY DIFFERENT FROM CONTROL * = $P < 0.05$

(SINGLE FACTOR ANALYSIS OF VARIANCE ** = $P < 0.01$

ACCORDING TO SOKAL & ROHLF 1969) *** = $P < 0.001$

TABLE 85 EFFECT OF FEEDING (60 DAYS), WITHDRAWAL (30 DAYS) & REFEEDING FOR 30 DAYS OF DIMETHAZINE, OXANDROLONE & ETHYLESTRENOL ON THE PROXIMATE COMPOSITION OF MUSCLE TISSUE OF CARP.

DRUGS mg/kg FOOD	MOISTURE mg/100 mg	CRUDE PROTEIN Nx6.25 (mg/100 mg)		TOTAL LIPIDS (mg/100 mg)		ASH (mg/100 mg)	
		ON WET WEIGHT	ON DRY WEIGHT	ON WET WEIGHT	ON DRY WEIGHT	ON WET WEIGHT	ON DRY WEIGHT
CONTROL	75.73± 0.39	16.63± 0.19	68.52± 0.78	2.73± 0.28	11.25± 1.15	1.32± 0.03	5.44± 0.12
DIMETH-AZINE 2.5	76.08± 0.54	*** 16.31± 0.19	68.19± 0.79	2.61± 0.03	10.91± 0.13	1.32± 0.04	5.52± 0.17
OXANDRO-LONE 10.0	76.53± 0.17	16.63± 0.25	70.86± 1.07	2.31± 0.20	9.84± 0.85	* 1.46± 0.04	* 6.22± 0.17
ETHYLES-TRENOL 2.5	75.63± 0.64	*** 17.75± 0.25	* 72.84± 1.03	2.84± 0.04	11.65± 0.16	** 1.47± 0.04	* 6.03± 0.16

STATISTICALLY DIFFERENT FROM CONTROL (ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 86 EFFECT OF FEEDING (60 DAYS), WITHDRAWAL (30 DAYS) & REFEEDING (30 DAYS) OF DIMETHAZINE, OXANDROLONE & ETHYLESTRENOL ON THE FREE AMINO ACIDS (n mole/mg) OF THE MUSCLE OF CARP.

AMINO ACID	CONCENTRATION OF DRUG (mg/kg FOOD)			
	CONTROLS	DIMETHAZINE 2.50	OXANDROLONE 10.0	ETHYLESTRENOL 2.50
Taurine	17.65± 1.34	17.09± 1.17	14.72± 0.40	16.69± 0.49
Aspartic	2.12± 0.06	1.58± 0.18	2.03± 0.10	1.86± 0.07
Therenonine	2.28± 0.15	2.97± 0.49	2.25± 0.01	1.93± 0.34
Serine	1.55± 0.18	2.12± 0.05	2.42± 0.01	2.05± 0.19
Glutamic	2.59± 0.12	2.65± 0.03	2.24± 0.15	2.35± 0.04
Proline	-	-	-	-
Glycine	2.81± 0.09	3.79± 0.63	3.94± 0.49	2.87± 0.05
Alanine	3.07± 0.16	3.86± 0.29	2.88± 0.11	2.66± 0.33
Cystein	-	-	-	-
Valine	Traces	Traces	Traces	Traces
Methionine	Traces	Traces	Traces	Traces
Isoleucine	Traces	Traces	Traces	Traces
Leucine	Traces	Traces	Traces	Traces
Tyrosine	Traces	Traces	Traces	Traces
Phenylalanine	Traces	Traces	Traces	Traces
Histidine	10.82± 0.87	10.95± 0.66	9.92± 0.95	9.98± 0.20
Ornithine	Traces	Traces	Traces	Traces
Lysine	6.84± 0.01	6.09± 0.63	5.09± 0.36	5.29± 0.54
Ammonia	8.61± 0.44	8.02± 0.35	7.65± 0.07	10.07± 0.77
Arginine	20.52± 2.00	29.28± 3.05	21.79± 3.22	21.34± 1.42

TABLE 87 EFFECT OF FEEDING (60 DAYS), WITHDRAWAL (30 DAYS) & REFEEDING FOR (30 DAYS) OF DIMETHAZINE, OXANDROLONE & ETHYLESTRENOL ON THE PLASMA FREE AMINO ACIDS (n mole/ml) OF CARP.

AMINO ACID	CONCENTRATION OF DRUG (mg/kg FOOD)			
	CONTROLS	DIMETHAZINE 2.50	OXANDROLONE 10.0	ETHYLESTRENOL 2.5
Taurine	903.08± 76.21	1106.37± 16.20	898.30± 102.51	1016.11± 69.13
Aspartic	90.68± 19.52	100.22± 8.52	104.86± 16.79	76.92± 9.45
Threnonine, Serine, Asparagine, Glutamine	169.66± 10.28	151.62± 0.82	161.51± 9.18	147.75± 9.34
Glutamic	239.98± 24.60	204.46± 18.00	198.49± 14.13	178.55± 10.10
Proline	329.50± 32.22	284.38± 17.95	276.16± 17.29	300.90± 50.19
Glycine	283.56± 52.34	236.49± 12.67	253.82± 26.95	233.31± 14.41
Alanine	636.05± 75.94	453.14± 11.45	425.35± 23.61	445.18± 37.73
Cysteine	97.64± 11.89	30.36± 1.60	70.88± 14.44	58.24± 10.67
Valine	196.30± 21.93	159.19± 10.80	180.07± 20.37	133.64± 8.77
Methionine	70.74± 8.86	38.02± 4.49	80.70± 7.56	74.91± 11.85
Isoleucine	173.71± 22.22	132.20± 6.48	159.38± 15.04	107.07± 12.15
Leucine	215.10± 23.08	170.38± 6.06	182.33± 19.13	117.30± 22.49
Throsine	208.86± 37.78	142.02± 23.31	150.78± 15.68	142.23± 7.40
Phenylalanine	140.21± 15.00	124.78± 8.60	145.80± 13.01	110.61± 5.19
Histidine	324.21± 38.03	302.19± 3.70	350.75± 27.13	274.78± 26.29
Ornithine	Present	Present	Present	Present
Lysine	386.96± 44.79	295.58± 24.67	326.89± 27.95	298.18± 32.20
Ammonia	1353.28± 83.74	796.67± 34.31	742.89± 69.71	879.39± 22.80
Arginine	252.85± 20.40	46.51± 11.64	193.88± 26.31	126.42± 19.36

4.1.10. Trenbolone Acetate

4.1.10.1. Weight and Length Data

The growth data for this drug is presented in Figures 43 and 44 for weight and length respectively. After sixty days of the drug feeding, there were no differences in weight but length in the 5.0 ppm group was significantly ($P<0.05$) lower as compared with controls and 10.0 ppm group. The 10.0 ppm group was also significantly longer ($P<0.05$) than 1.0 ppm group. After ninety days, the 2.5 ppm group gained maximum in weight and length and were significantly ($P<0.01$) heavier and longer than the controls and other experimental groups (Tables 88 and 89). The percentage weight and length gain for 2.5 ppm group over the controls was 46.89 and 32.31 respectively. The specific growth rate for weight and length also followed the same pattern (Table 90). The data regarding the condition factor and food conversion efficiency is given in Table 91.

4.1.10.2. Tissue - Body Indices

No effect on CSI was recorded after sixty or ninety days. HSI was lower after sixty days, but at ninety days only the 2.5 ppm group noted an increase ($P<0.05$) over the controls. RSI was not altered after sixty days but at ninety days a decrease ($P<0.01$) was observed. VSI increased in 1.0 and 2.5 ppm groups after sixty days, while no change was observed at ninety days (Table 92 and Figures 45 and 46).

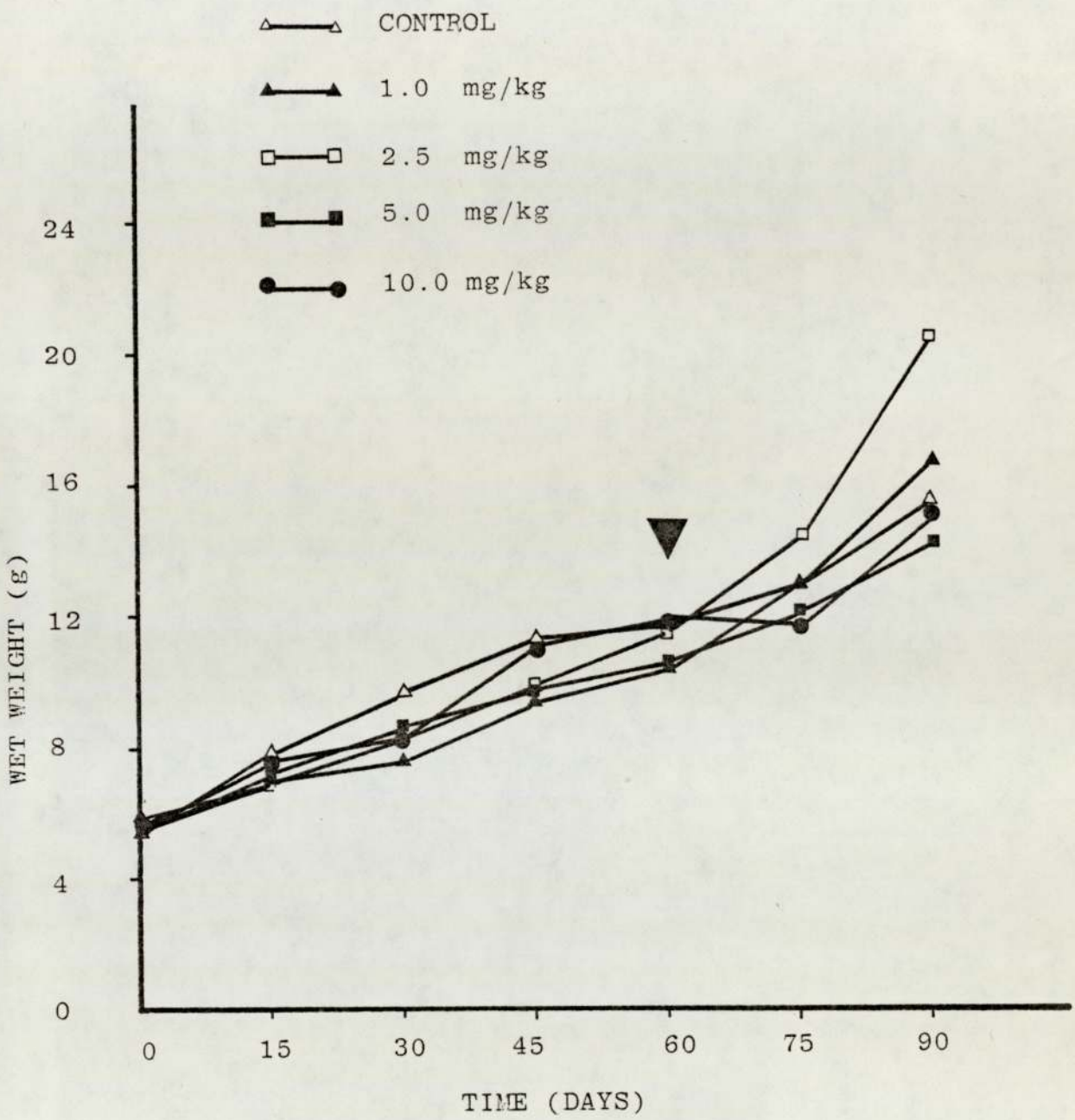


FIGURE 43 MEAN WEIGHTS OF CARP GIVEN TRENBOLONE ACETATE *PER OS*. DRUG WAS WITHDRAWN AFTER 60 DAYS (ARROW).

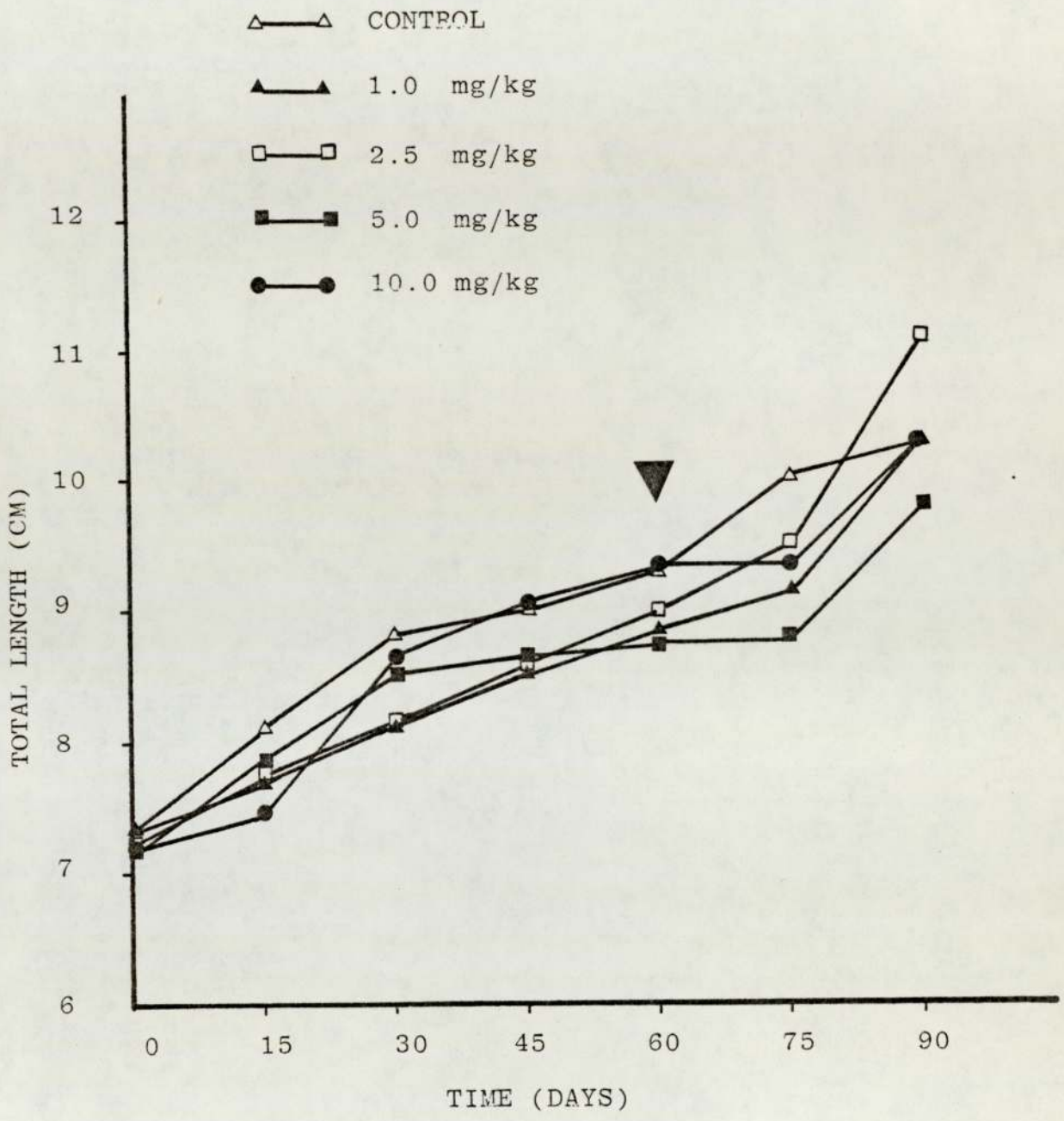


FIGURE 44 MEAN LENGTHS OF CARP GIVEN TRENBOLONE ACETATE *PER OS*. DRUG WAS WITHDRAWN AFTER 60 DAYS (ARROW).

4.1.10.3. Biochemical Changes

4.1.10.3.1. Liver (Table 93)

Total proteins were significantly elevated after sixty days ($P<0.001$), but at ninety days 2.5 and 5.0 ppm groups showed a decrease in protein content. No change was discerned in RNA/DNA after sixty days, but at ninety days, RNA/DNA decreased in 2.5 and 10.0 ppm groups. No change was discerned in protein/RNA after sixty and ninety days. Protein/DNA was lower in 5.0 ($P<0.05$) ppm and 2.5 ($P<0.01$) ppm after sixty and ninety days respectively.

4.1.10.3.2. Kidney (Table 94)

Total proteins were significantly ($P<0.001$) higher both at sixty and ninety days. RNA/DNA was lower in 2.5 ($P<0.01$) ppm; no change was detected after ninety days. Proteins/RNA increased ($P<0.01$) in 1.0 and 2.5 ppm in sixty days group and in 5.0 ppm ($P<0.001$) after ninety days. No change was noticed in protein/DNA after sixty or ninety days.

4.1.10.3.3. Brain (Table 95)

No change was detected in protein/DNA after sixty and ninety days. Proteins were higher ($P< 0.05$) only in 1.0 ppm group after sixty days, but they were lower ($P< 0.001$) in all but 1.0 ppm group, after ninety days. RNA/DNA decreased in the 1.0 and 2.5 ppm groups but increased ($P< 0.01$) in 10.0 ppm group after sixty days. In phase 2, RNA/DNA was significant only in 1.0 ppm group. After sixty days,

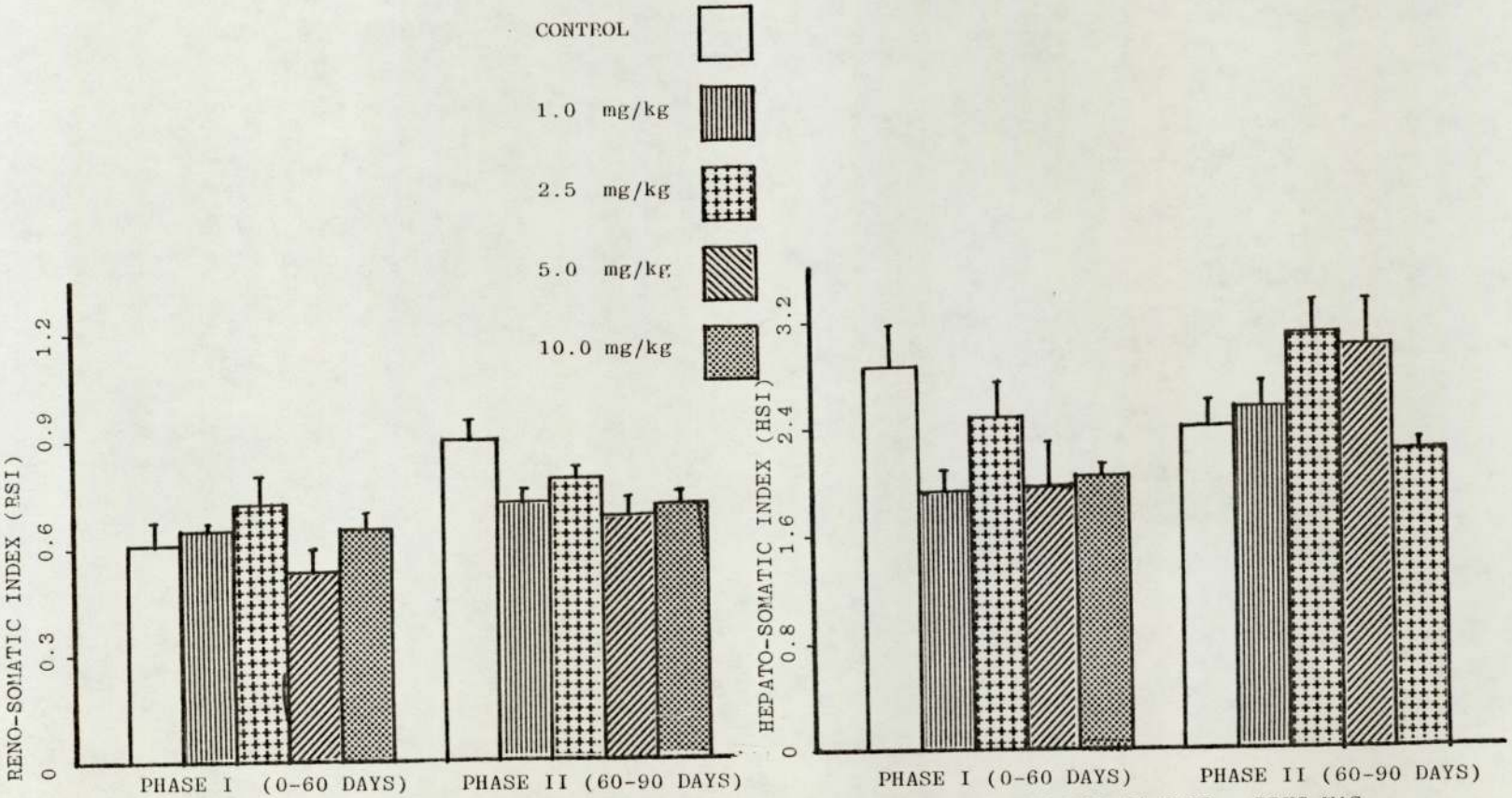


FIGURE 45 EFFECT OF TRENBOLONE ACETATE GIVEN *PER OS* ON THE RSI AND HSI OF CARP. DRUG WAS WITHDRAWN AFTER 60 DAYS.

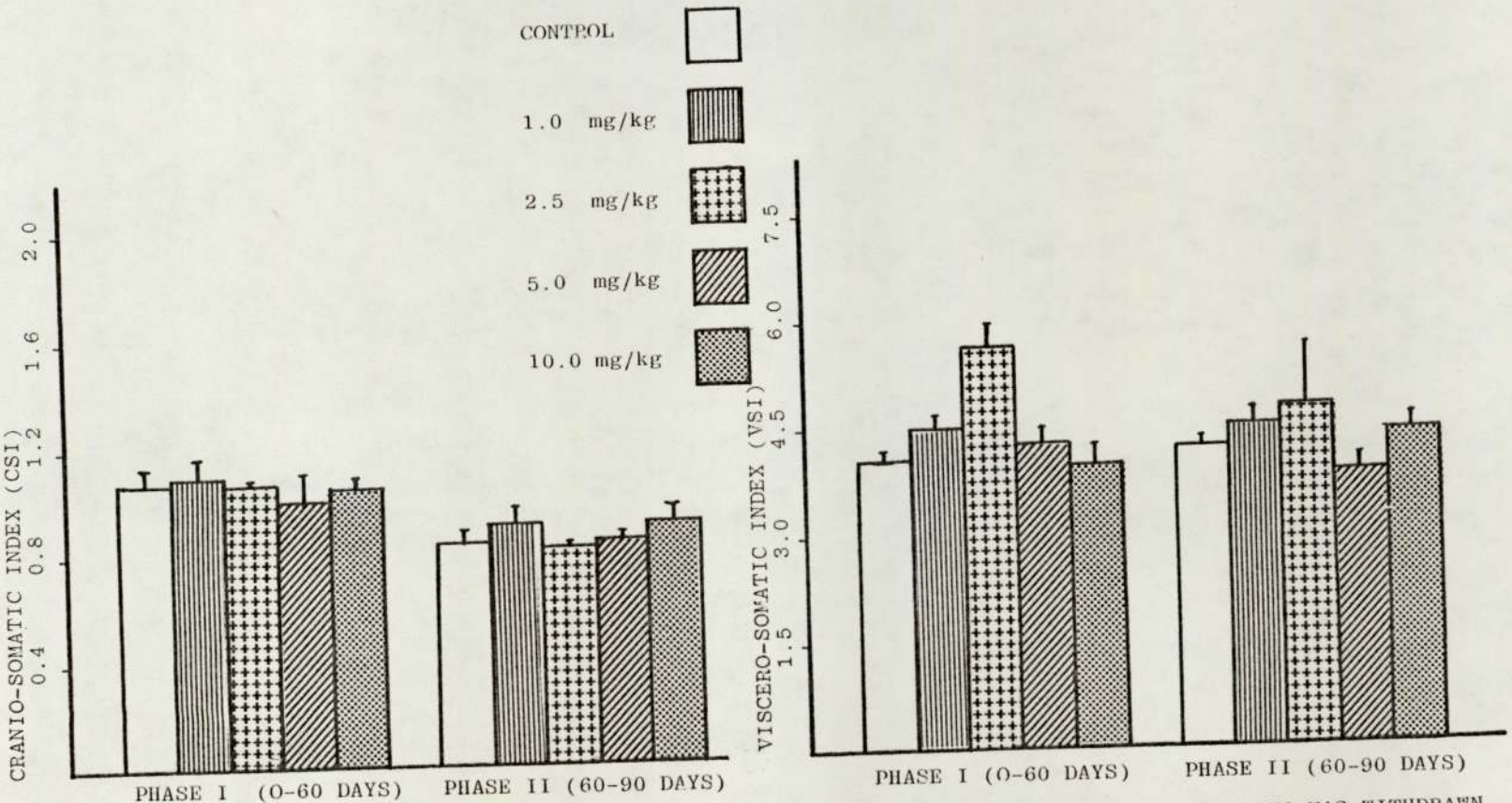


FIGURE 46 EFFECT OF TRENBOLONE ACETATE GIVEN *PER OS* ON THE CSI AND VSI OF CARP. DRUG WAS WITHDRAWN AFTER 60 DAYS.

protein/RNA increased in 1.0 and 2.5 ppm groups, but after ninety days, a decrease ($P<0.01$) was noted in all the groups.

4.1.10.3.4. Muscle (Tables 96 and 97)

Muscle proteins and protein/DNA were significantly higher ($P<0.01$) after sixty days but came to normal levels after ninety days. RNA/DNA decreased ($P<0.05$) in 2.5 and increased ($P<0.05$) in 5.0 ppm after sixty days, but after ninety days a decline in RNA/DNA was observed in all but 1.0 ppm group. Significant increase was noted in protein/RNA both at sixty and ninety days.

No change was seen in moisture and crude protein levels after sixty days of the drug feeding. Total lipids were lower ($P<0.01$) and ash content increased (2.5 ppm). After ninety days, moisture content of the muscle elevated with a concomitant decrease in crude protein, lipids and ash levels.

TABLE 88 CHANGES IN TOTAL BODY WEIGHT OF CARP FED TRENBOLONE ACETATE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEAN (cm) ± S.E. OF 25 FISH. PERCENT WEIGHT GAIN IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF TRENBOLONE ACETATE mg/kg FOOD				
	0.00	1.00	2.50	5.00	10.00
0	5.44± 0.16	5.88± 0.24	5.64± 0.22	5.39± 0.20	5.48± 0.14
15	7.84± 0.25 (44.17)	6.97± 0.38 (18.63)	6.92± 0.28 (22.75)	7.19± 0.27 (33.40)	7.56± 0.22 (37.94)
30	9.69± 0.35 (78.10)	7.61± 0.44 (29.52)	8.27± 0.42 (46.62)	8.55± 0.35 (58.77)	8.34± 0.25 (52.21)
45	11.03± 0.44 (102.67)	9.40± 0.58 (59.95)	9.89± 0.58 (75.37)	9.77± 0.45 (81.41)	11.11± 0.38 (102.88)
60*	11.67± 0.43 (114.57)	10.37± 0.75 (76.47)	11.48± 0.80 (103.59)	10.61± 0.55 (97.07)	11.86± 0.44 (116.57)
75	12.88± 0.39 (136.82)	12.98± 1.24 (120.87)	14.38± 1.25 (155.08)	12.08± 0.81 (124.25)	11.71± 0.56 (113.72)
90	15.57± 0.92 (186.18)	16.68± 1.59 (183.78)	20.52± 1.66 (263.92)	14.15± 1.40 (162.66)	15.12± 0.81 (175.94)

* DRUG WITHDRAWN AFTER 60 DAYS.

STATISTICALLY SIGNIFICANT DIFFERENCES AMONG THE DOSES, ACCORDING TO SINGLE FACTOR ANALYSIS OF VARIANCE (SOKAL & ROHLF 1969).

90 DAYS

CONTROL VERSUS 2.5 = $P < 0.01$

1 VERSUS 2.5 = $P < 0.05$

2.5 VERSUS 5.0 = $P < 0.01$

2.5 VERSUS 10.0 = $P < 0.05$

TABLE 89 CHANGES IN TOTAL BODY LENGTH OF CARP FED TRENBOLONE ACETATE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEAN (cm) ± S.E. OF 25 FISH. PERCENT INCREASE IN LENGTH IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF TRENBOLONE ACETATE mg/kg FOOD				
	0.00	1.00	2.50	5.00	10.00
0	7.33± 0.07	7.31± 0.09	7.22± 0.09	7.16± 0.09	7.28± 0.06
15	8.12± 0.08 (10.75)	7.71± 0.14 (5.48)	7.75± 0.10 (7.44)	7.86± 0.10 (9.75)	7.95± 0.08 (9.17)
30	8.82± 0.10 (20.35)	8.12± 0.15 (11.07)	8.25± 0.15 (14.31)	8.53± 0.12 (19.06)	8.66± 0.09 (18.89)
45	9.00± 0.12 (22.80)	8.52± 0.17 (16.54)	8.59± 0.18 (19.02)	8.62± 0.13 (20.28)	9.05± 0.10 (24.22)
60*	9.28± 0.11 (26.51)	8.85± 0.19 (21.00)	9.00± 0.22 (24.83)	8.75± 0.15 (22.11)	9.32± 0.12 (27.90)
75	10.03± 0.10 (36.80)	9.12± 0.26 (24.69)	9.50± 0.28 (31.63)	8.78± 0.19 (22.57)	9.36± 0.15 (28.54)
90	10.27± 0.20 (40.80)	10.30± 0.30 (40.85)	11.11± 0.31 (53.98)	9.80± 0.32 (36.80)	10.29± 0.18 (41.33)

* DRUG WITHDRAWN AFTER 60 DAYS

STATISTICALLY SIGNIFICANT DIFFERENCES AMONG DOSES, ACCORDING TO SINGLE FACTOR ANALYSIS OF VARIANCE (SOKAL & ROHLF 1969)

60 DAYS		90 DAYS	
CONTROL VERSUS	5.0 = $P<0.05$	CONTROL VERSUS	2.5 = $P<0.05$
1.0 VERSUS	10.0 = $P<0.05$	2.5 VERSUS	5.0 = $P<0.01$
5.0 VERSUS	10.0 = $P<0.05$	2.5 VERSUS	10.0 = $P<0.05$

TABLE 90 EFFECT OF FEEDING AND WITHDRAWAL OF TRENBOLONE ACETATE ON THE SPECIFIC GROWTH RATE (SGR) OF CARP. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg FOOD	SPECIFIC GROWTH RATE (WEIGHT)			SPECIFIC GROWTH RATE (LENGTH)		
	0-60 DAYS	60-90 DAYS	0-90 DAYS	0-60 DAYS	60-90 DAYS	0-90 DAYS
0.00	1.29± 0.45	0.97± 0.22	1.18± 0.30	0.40± 0.13	0.35± 0.13	0.38± 0.09
1.00	0.96± 0.20	1.61± 0.06	1.17± 0.19	0.32± 0.02	0.51± 0.22	0.38± 0.09
2.50	1.20± 0.08	1.97± 0.31	1.45± 0.20	0.37± 0.05	0.71± 0.25	0.49± 0.12
5.00	1.15± 0.30	0.97± 0.07	1.09± 0.19	0.34± 0.15	0.39± 0.25	0.35± 0.13
10.00	1.31± 0.44	0.83± 0.21	1.15± 0.38	0.41± 0.10	0.34± 0.22	0.39± 0.10

TABLE 91 CHANGES IN THE CONDITION FACTOR AND FOOD CONVERSION EFFICIENCY OF CARP ADMINISTERED TRENBOLONE ACETATE IN THE DIET FOR 60 DAYS.

CONCEN-TRATION OF DRUG mg/kg FOOD	CONDITION FACTOR			FOOD CONVERSION EFFICIENCY		
	0 DAYS	60 DAYS	90 DAYS	0-60 DAYS	60-90 DAYS	0-90 DAYS
0.00	1.38± 0.23	1.46± 0.24	1.44± 0.27	0.24	0.21	0.23
1.00	1.51± 0.25	1.50± 0.26	1.53± 0.30	0.20	0.36	0.27
2.50	1.50± 0.24	1.57± 0.27	1.49± 0.32	0.25	0.47	0.35
5.00	1.47± 0.24	1.59± 0.28	1.50± 0.32	0.23	0.21	0.22
10.00	1.42± 0.24	1.47± 0.24	1.39± 0.28	0.26	0.18	0.23

TABLE 92 EFFECT OF FEEDING TRENBOLONE ACETATE ON THE CRANIO-SOMATIC (CSI), HEPATO-SOMATIC (HSI), RENO-SOMATIC (RSI) & VISCERO-SOMATIC (VSI) INDEX OF CARP. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg FOOD	CSI		HSI		RSI		VSI	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	1.07± 0.06	0.83± 0.05	2.86± 0.32	2.41± 0.18	0.61± 0.04	0.89± 0.06	4.19± 0.09	4.20± 0.11
1.00	1.09± 0.08	0.90± 0.06	1.94± 0.13 **	2.55± 0.19	0.64± 0.02	0.72± 0.04 **	4.97± 0.19 *	4.49± 0.24
2.50	1.06± 0.02	0.81± 0.02	2.48± 0.23	3.08± 0.24 *	0.72± 0.07	0.79± 0.02	5.36± 0.32 **	4.77± 0.70
5.00	1.00± 0.12	0.84± 0.01	1.98± 0.20 **	2.99± 0.33	0.53± 0.06	0.68± 0.04 **	4.25± 0.23	3.82± 0.19
10.00	1.04± 0.05	0.90± 0.06	2.05± 0.06 *	2.22± 0.07	0.65± 0.05	0.71± 0.03 **	3.96± 0.26	4.39± 0.18

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 93 EFFECT OF FEEDING TRENBOLONE ACETATE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN LIVER OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	26.28± 2.41	31.28± 3.49	5.53± 0.48	8.80± 1.15	17.37± 1.26	16.37± 1.88	95.53± 9.69	142.11± 16.58
1.00	*** 51.72± 2.90	30.12± 2.76	4.49± 0.14	6.49± 0.85	18.27± 0.76	16.36± 1.85	81.88± 2.69	104.23± 13.14
2.50	** 42.99± 0.63	** 18.64± 3.13	5.02± 0.81	* 5.83± 0.36	18.98± 0.51	12.80± 0.44	95.63± 16.57	** 74.37± 4.10
5.00	** 41.83± 5.03	* 20.90± 2.44	4.25± 0.30	6.56± 0.86	15.51± 1.19	17.04± 1.30	* 65.12± 3.67	111.75± 16.94
10.00	* 37.33± 4.26	23.85± 1.56	5.31± 0.31	* 5.74± 0.41	16.95± 1.24	18.56± 0.82	90.94± 11.19	106.63± 8.63

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF, 1969)

* = $P<0.05$

** = $P<0.01$

*** = $P<0.001$

TABLE 94 EFFECT OF FEEDING TRENBOLONE ACETATE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN KIDNEY OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCENTRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	40.81± 3.50	26.19± 2.92	3.52± 0.26	4.14± 0.26	14.41± 0.72	13.16± 1.34	49.58± 2.34	54.34± 6.78
1.00	*** 64.93± 2.63	* 45.90± 5.10	2.89± 0.29	4.00± 0.05	*** 18.07± 0.43	12.80± 0.40	52.21± 5.27	51.20± 2.06
2.50	*** 59.27± 2.20	33.51± 8.55	** 2.45± 0.18	3.72± 0.25	** 17.64± 0.16	11.94± 0.67	43.16± 2.92	44.04± 1.95
5.00	*** 58.83± 2.53	** 50.99± 2.08	3.09± 0.15	3.76± 0.26	15.83± 0.19	** 16.56± 0.61	48.93± 2.46	62.20± 4.86
10.00	** 56.89± 2.63	* 44.33± 4.35	2.94± 0.13	3.72± 0.37	16.13± 0.51	13.81± 0.54	47.25± 1.63	51.39± 5.49

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 95 EFFECT OF FEEDING TRENBOLONE ACETATE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN BRAIN OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	41.36± 3.10	37.24± 1.27	2.73± 0.04	2.24± 0.04	18.81± 1.39	28.66± 0.57	51.56± 4.21	64.14± 1.09
1.00	* 50.23± 1.35	38.24± 0.49	*** 2.00± 0.08	* 2.99± 0.10	** 25.93± 0.34	*** 19.68± 0.37	51.82± 2.76	58.60± 0.83
2.50	46.34± 1.81	* 27.40± 4.59	*** 1.79± 0.08	2.71± 0.23	** 26.10± 0.90	*** 19.06± 1.02	47.98± 1.87	51.42± 3.97
5.00	44.45± 1.47	*** 14.38± 1.40	2.66± 0.34	2.73± 0.21	16.45± 1.78	* 24.56± 2.32	41.87± 1.28	68.05± 11.40
10.00	41.36± 3.83	** 21.33± 2.45	** 3.27± 0.02	2.53± 0.26	15.94± 2.22	* 24.12± 0.74	52.13± 7.64	60.70± 5.72

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 96 EFFECT OF FEEDING TRENBOLONE ACETATE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN MUSCLE OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	13.60± 0.75	14.80± 0.59	10.15± 1.40	12.71± 1.87	36.94± 3.28	49.23± 3.15	375.67± 23.41	614.76± 69.43
1.00	** 17.17± 1.13	15.78± 0.44	10.91± 1.37	10.36± 1.11	*** 62.16± 3.44	56.81± 3.10	** 666.46± 65.74	631.81± 79.63
2.50	** 18.02± 0.71	15.35± 1.04	* 5.96± 0.08	* 9.00± 1.18	*** 87.68± 6.38	** 70.98± 7.08	523.51± 43.71	631.78± 85.22
5.00	** 17.07± 0.96	13.96± 0.49	* 15.69± 2.02	** 7.38± 0.67	* 49.58± 2.30	*** 77.10± 5.50	*** 772.67± 91.98	537.06± 66.86
10.00	* 16.33± 0.55	15.92± 0.63	11.78± 2.38	* 8.53± 0.55	*** 64.60± 5.57	*** 86.27± 2.44	*** 724.10± 83.48	712.42± 41.70

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 97 EFFECT OF FEEDING TRENBOLONE ACETATE ON THE PROXIMATE COMPOSITION OF MUSCLE TISSUE FROM Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCENTRATION OF DRUG mg/kg	MOISTURE mg/100 mg		CRUDE PROTEIN N x 6.25 (mg/100 mg)				TOTAL LIPIDS (mg/100 mg)				ASH (mg/100 mg)			
			ON WET BASIS		ON DRY BASIS		ON WET BASIS		ON DRY BASIS		ON WET BASIS		ON DRY BASIS	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	75.84± 0.20	74.56± 0.60	17.81± 0.25	18.63± 0.31	73.88± 1.13	73.38± 0.81	2.32± 0.18	3.16± 0.42	9.61± 0.73	12.22± 1.34	2.21± 0.07	2.11± 0.04	9.13± 0.25	8.34± 0.35
1.00	75.54± 0.80	76.24*± 0.14	18.38± 0.50	17.56*± 0.37	75.00± 0.69	73.81± 1.44	1.36**± 0.13	2.27± 0.17	5.73**± 0.51	9.30± 0.64	2.35± 0.05	1.98*± 0.06	9.60± 0.42	8.35± 0.28
2.50	75.78± 0.52	76.39*± 0.58	18.06± 0.31	17.44*± 0.31	74.63± 0.31	73.88± 0.56	1.45**± 0.19	1.99*± 0.22	6.09**± 0.67	8.21*± 0.8	2.52**± 0.08	2.10± 0.05	10.40± 0.14	8.90± 0.41
5.00	74.84± 1.11	77.27**± 0.22	18.44± 0.50	16.69**± 0.31	73.44± 1.50	73.38± 1.69	1.72*± 0.25	2.24± 0.10	7.58*± 1.15	8.96± 0.64	2.25± 0.01	1.92**± 0.06	8.95± 0.39	8.43± 0.21
10.00	76.22± 0.39	76.68**± 0.40	18.50± 0.38	16.19***± 0.62	77.63± 0.94	71.75± 0.37	1.27***± 0.15	2.05*± 0.24	5.41***± 0.57	8.64*± 0.97	2.10± 0.08	1.99*± 0.02	8.80± 0.21	8.53± 0.11

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL AND ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

4.1.11. Cyproterone Acetate

4.1.11.1. Weight and Length Data

Weight and length data for this steroid is presented in Figures 47 and 48. Both weight and length were significantly higher than the controls after sixty days, but after the drug was withdrawn, the increase which was accumulated over phase 1 declined and after thirty days of drug withdrawal, there was no difference between experimental or control groups (Tables 98 and 99). The data concerning SGR, condition factor and FCE is presented in Tables 100 and 101.

4.1.11.2. Tissue - Body Indices

No change was observed in CSI and HSI both after sixty and ninety days. RSI was higher ($P<0.05$) only in 1.0 ppm group after sixty days; after ninety days, a drop in RSI was observed in 2.5 ppm group. VSI was higher both at sixty and ninety days (Table 102 and Figures 49 and 50).

4.1.11.3. Biochemical Changes

4.1.11.3.1. Liver (Table 103)

Protein increased ($P<0.05$) only in 5.0 ppm group, while RNA/DNA increased ($P<0.05$) only in 2.5 ppm group. No change was noted in protein/RNA and protein/DNA after sixty days of drug feeding. After ninety days, proteins were lower in 5.0 and 10.0 ppm ($P<0.05$ and $P<0.01$) group and protein/DNA in 10.0 ppm ($P<0.05$) group. Protein/RNA increased ($P<0.05$) in 1.0 and decreased in 2.5 ($P<0.01$) ppm group. No change was noticed in RNA/DNA.

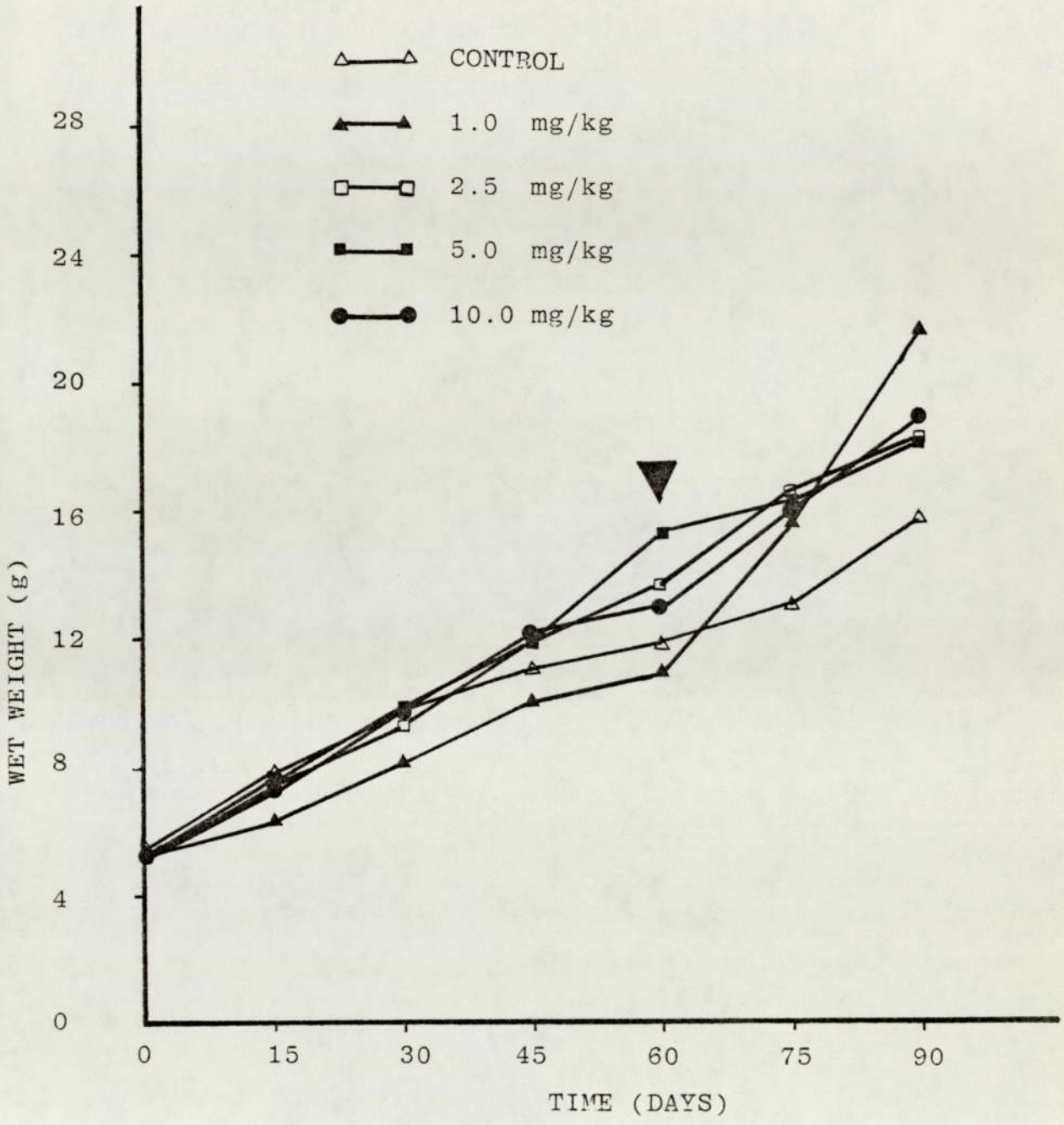


FIGURE 47 MEAN WEIGHTS OF CARP FED CYPROTERONE ACETATE *PER OS*. DRUG WAS WITHDRAWN AFTER 60 DAYS (ARROW).

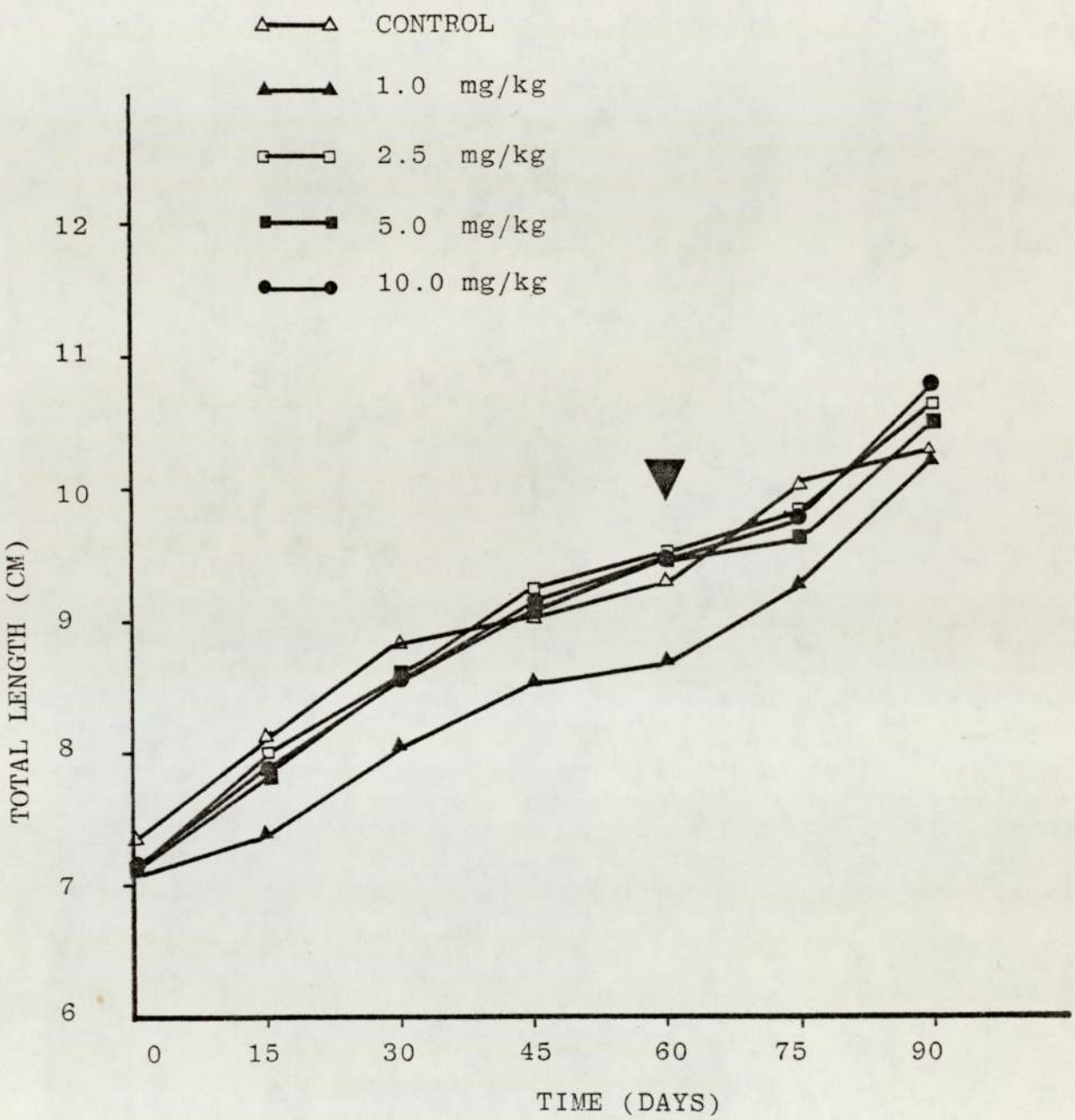


FIGURE 48 MEAN LENGTHS OF CARP FED CYPROTERONE ACETATE *PER OS*. DRUG WAS WITHDRAWN AFTER 60 DAYS (ARROW).

4.1.11.3.2. Kidney (Table 104)

Proteins increased in 2.5 and 5.0 ppm ($P<0.05$) but no change could be detected in RNA/DNA, protein/DNA and protein/RNA after sixty days. After ninety days, proteins were higher in 2.5 and 10.0 ppm, RNA/DNA and protein/DNA in 1.0 and 2.5 ppm while protein/RNA was lower in 10.0 ppm group.

4.1.11.3.3. Brain (Table 105)

Total proteins decreased ($P<0.01$) both at sixty and ninety (5.0 and 10.0 ppm) days. RNA/DNA decreased in 2.5 ($P<0.01$) and 10.0 ($P<0.001$) ppm but increased in 5.0 ($P<0.01$) ppm group. Protein/RNA increased in 2.5 and 10.0 ppm group after sixty days. No effect was seen in protein/DNA. In phase 2, RNA/DNA was increased in all but 10.0 ppm groups, while protein/DNA in 1.0 and 10.00 ppm groups. Protein/RNA decreased in all but 1.0 ppm group.

4.1.11.3.4. Muscle (Tables 106 and 107)

After sixty days, muscle proteins were higher in 1.0 and 2.5 ppm ($P<0.01$). RNA/DNA was significantly higher in 5.0 ($P<0.05$) ppm and protein/RNA in 10.0 ppm ($P<0.001$). Protein/DNA was higher in all experimental groups ($P<0.001$). After ninety days, no change was noted in proteins and RNA/DNA. Protein/RNA was higher in 1.0 and 5.0 ppm while protein/DNA in 1.0 ppm only.

Muscle moisture and crude protein content was higher in 1.0 and 5.0 ppm groups. Total lipids were lower in 10.0 ppm

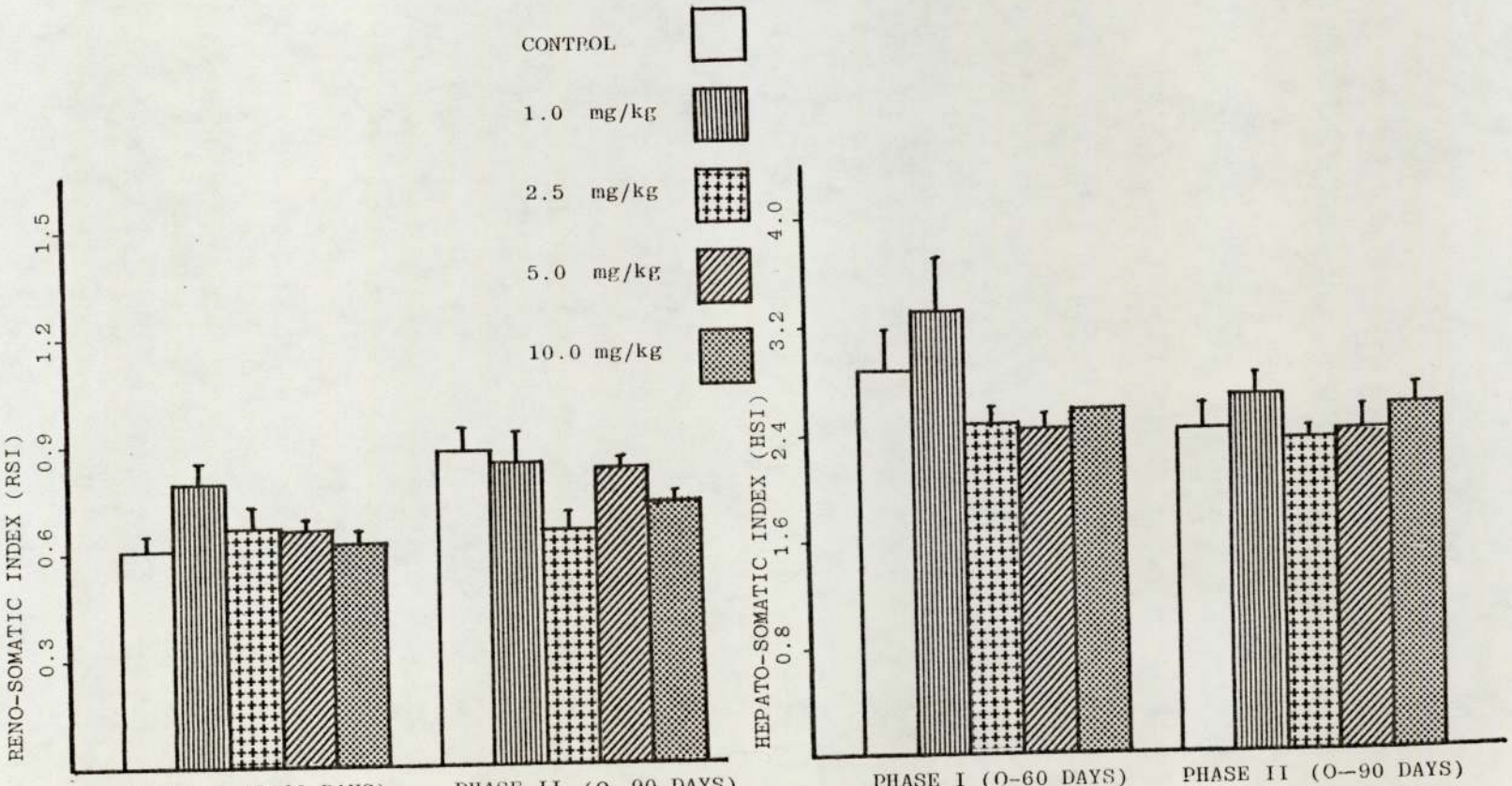


FIGURE 49 EFFECT OF CYPROTERONE ACETATE GIVEN *PER OS* ON THE RSI AND HSI OF CARP. DRUG WAS WITHDRAWN AFTER 60 DAYS.

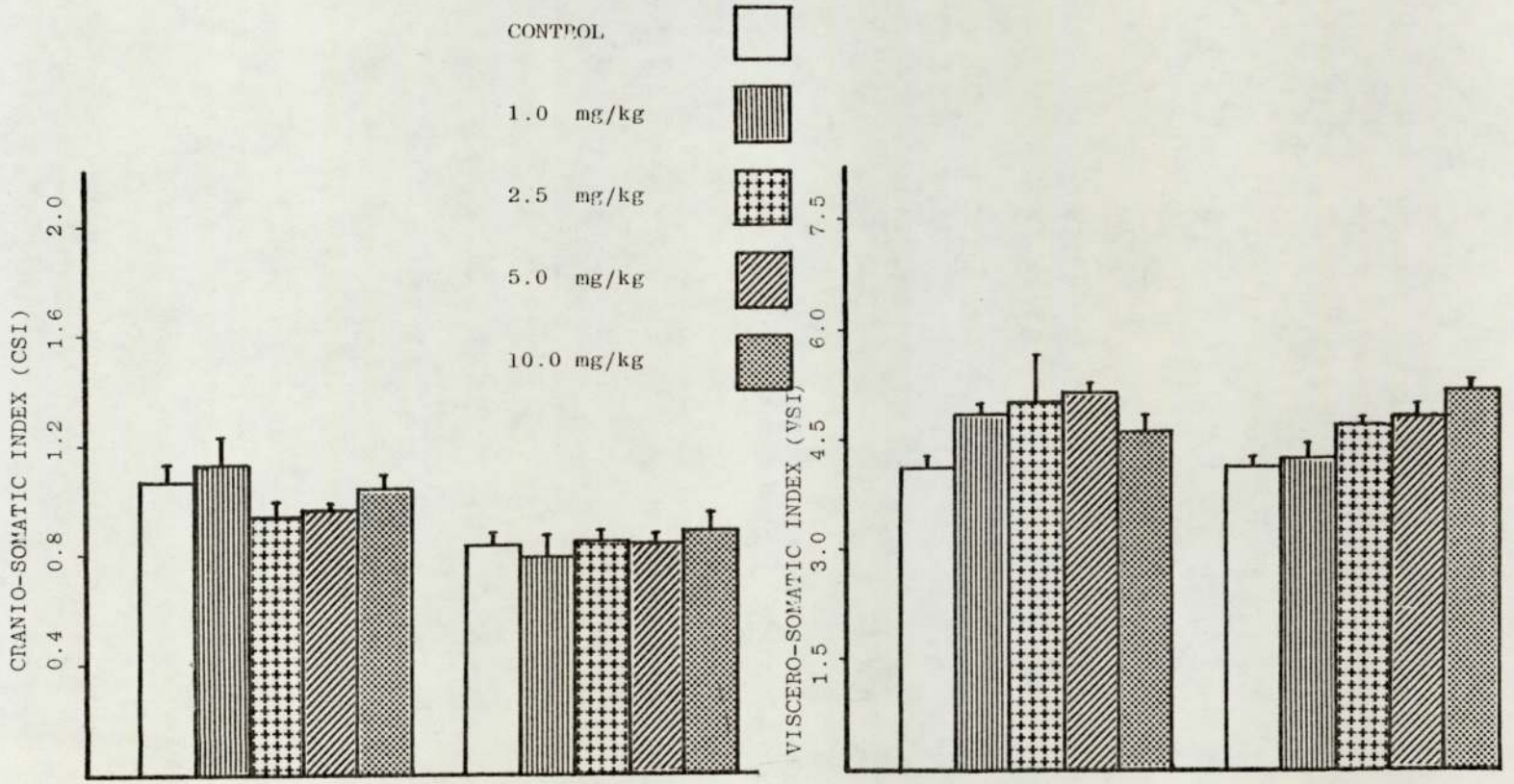


FIGURE 50 EFFECT OF CYPROTERONE ACETATE GIVEN *PER OS* ON THE CSI AND VSI OF CARP. DRUG WAS WITHDRAWN AFTER 60 DAYS.

and no effect on ash content was observed after sixty days. After ninety days, moisture was higher in 5.0 and 10.0 ppm (P 0.05). No change in crude protein and ash content was noticed, but total lipids were lower in 5.0 and 10.0 ppm groups.

TABLE 98 CHANGES IN BODY WEIGHT OF CARP FED CYPROTERONE ACETATE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEAN (g) ± S.E. OF 25 FISH. PERCENT WEIGHT GAIN IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF CYPROTERONE ACETATE mg/kg FOOD				
	0.00	1.00	2.50	5.00	10.00
0	5.44± 0.16	5.23± 0.42	5.06± 0.20	5.19± 0.17	5.13± 0.22
15	7.84± 0.25 (44.17)	6.30± 0.49 (20.48)	7.35± 0.30 (45.16)	7.56± 0.28 (45.65)	7.22± 0.33 (40.85)
30	9.69± 0.35 (78.10)	8.11± 0.74 (55.14)	9.19± 0.39 (81.65)	9.77± 0.39 (88.28)	9.69± 0.42 (88.96)
45	11.03± 0.44 (102.67)	9.99± 1.02 (91.14)	11.82± 0.51 (133.57)	11.77± 0.47 (129.42)	12.05± 0.62 (135.04)
60*	11.67± 0.43 (114.57)	10.75± 1.19 (105.56)	13.16± 0.66 (160.01)	15.15± 0.65 (191.95)	12.86± 0.73 (150.75)
75	12.88± 0.39 (136.82)	15.54± 2.48 (197.30)	16.45± 0.98 (224.98)	16.14± 1.37 (211.04)	15.94± 1.11 (210.73)
90	15.57± 0.92 (186.18)	19.61± 3.27 (275.00)	18.23± 1.19 (260.19)	17.95± 1.44 (246.00)	18.80± 1.45 (266.50)

* DRUG WITHDRAWN AFTER 60 DAYS.

STATISTICALLY SIGNIFICANT DIFFERENCES AMONG THE DOSES, ACCORDING TO SINGLE FACTOR ANALYSIS OF VARIANCE (SOKAL & ROHLF 1969).

60 DAYS

CONTROL VERSUS 5.0 = $P < 0.001$

1.0 VERSUS 2.5 = $P < 0.05$

1.0 VERSUS 5.0 = $P < 0.001$

5.0 VERSUS 10.0 = $P < 0.05$

TABLE 99 CHANGES IN TOTAL BODY LENGTH OF CARP FED CYPROTERONE ACETATE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEAN (cm) ± S.E. OF 25 FISH. PERCENT INCREASE IN LENGTH IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF CYPROTERONE ACETATE mg/kg FOOD				
	0.00	1.00	2.50	5.00	10.00
0	7.33± 0.07	7.08± 0.18	7.11± 0.09	7.10± 0.08	7.10± 0.10
15	8.12± 0.08 (10.75)	7.38± 0.17 (4.53)	7.90± 0.11 (11.14)	7.82± 0.09 (10.09)	7.84± 0.12 (10.48)
30	8.82± 0.10 (20.35)	8.05± 0.22 (14.06)	8.61± 0.13 (21.05)	8.61± 0.11 (21.17)	8.54± 0.13 (20.34)
45	9.00± 0.12 (22.80)	8.53± 0.28 (20.88)	9.23± 0.14 (29.80)	9.06± 0.12 (27.51)	9.16± 0.16 (28.96)
60*	9.28± 0.11 (26.51)	8.67± 0.31 (22.82)	9.50± 0.17 (33.59)	9.45± 0.13 (33.08)	9.46± 0.18 (33.24)
75	10.03± 0.10 (36.80)	9.26± 0.49 (31.23)	9.83± 0.21 (38.17)	9.61± 0.26 (35.25)	9.76± 0.23 (37.42)
90	10.27± 0.20 (40.80)	10.21± 0.55 (62.27)	10.68± 0.25 (50.24)	10.50± 0.28 (47.80)	10.77± 0.28 (51.71)

* DRUG WITHDRAWN AFTER 60 DAYS.

STATISTICALLY SIGNIFICANT DIFFERENCES AMONG THE DOSES, ACCORDING TO SINGLE FACTOR ANALYSIS OF VARIANCE (SOKAL & ROHLF 1969).

60 DAYS

1.0 VERSUS 2.5 = $P < 0.01$

1.0 VERSUS 5.0 = $P < 0.01$

1.0 VERSUS 10.0 = $P < 0.01$

TABLE 100 EFFECT OF FEEDING AND WITHDRAWAL OF CYPROTERONE ACETATE ON THE SPECIFIC GROWTH RATE (SGR) OF CARP. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg FOOD	SPECIFIC GROWTH RATE (WEIGHT)			SPECIFIC GROWTH RATE (LENGTH)		
	0-60 DAYS	60-90 DAYS	0-90 DAYS	0-60 DAYS	60-90 DAYS	0-90 DAYS
0.00	1.29± 0.45	0.97± 0.22	1.18± 0.30	0.40± 0.13	0.35± 0.13	0.38± 0.09
1.00	1.22± 0.26	2.04± 0.33	1.49± 0.27	0.35± 0.10	0.50± 0.04	0.41± 0.08
2.50	1.62± 0.37	1.10± 0.29	1.44± 0.28	0.49± 0.11	0.40± 0.12	0.46± 0.08
5.00	1.81± 0.27	0.58± 0.08	1.40± 0.31	0.48± 0.10	0.36± 0.17	0.44± 0.09
10.00	1.55± 0.41	1.28± 0.12	1.46± 0.27	0.48± 0.10	0.44± 0.16	0.47± 0.08

TABLE 101 CHANGES IN THE CONDITION FACTOR AND FOOD CONVERSION EFFICIENCY OF CARP ADMINISTERED CYPROTERONE ACETATE IN THE DIET FOR 60 DAYS.

CONCENTRATION OF DRUG mg/kg FOOD	CONDITION FACTOR			FOOD CONVERSION EFFICIENCY		
	0 DAYS	60 DAYS	90 DAYS	0–60 DAYS	60–90 DAYS	0–90 DAYS
0.00	1.38± 0.23	1.46± 0.24	1.44± 0.27	0.24	0.21	0.23
1.00	1.48± 0.24	1.65± 0.29	1.84± 0.36	0.28	0.45	0.34
2.50	1.41± 0.23	1.54± 0.26	1.49± 0.29	0.32	0.23	0.28
5.00	1.45± 0.25	1.79± 0.27	1.55± 0.36	0.39	0.12	0.26
10.00	1.43± 0.24	1.52± 0.25	1.50± 0.27	0.30	0.27	0.29

TABLE 102 EFFECT OF FEEDING CYPROTERONE ACETATE ON THE CRANIO-SOMATIC (CSI), HEPATO-SOMATIC (HSI), RENO-SOMATIC (RSI), AND VISCERO-SOMATIC (VSI) INDEX OF CARP. DRUG WAS FED FOR 60 DAYS ONLY

CONCEN-TRATION OF DRUG mg/kg FOOD	CSI		HSI		RSI		VSI	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	1.07± 0.06	0.83± 0.05	2.86± 0.32	2.41± 0.18	0.61± 0.04	0.89± 0.06	4.19± 0.09	4.20± 0.11
1.00	1.13± 0.10	0.79± 0.08	3.30± 0.39	2.24± 0.17	0.79± 0.06 *	0.84± 0.09	5.24± 0.12 **	4.65± 0.17 *
2.50	0.94± 0.05	0.85± 0.04	2.45± 0.11	2.32± 0.09	0.67± 0.06	0.65± 0.05 **	5.08± 0.65 **	4.72± 0.08 *
5.00	0.96± 0.02	0.84± 0.04	2.42± 0.10	2.39± 0.18	0.66± 0.02	0.82± 0.03	5.18± 0.16 **	4.83± 0.21 **
10.00	1.04± 0.05	0.89± 0.06	2.57± 0.28	2.10± 0.14	0.62± 0.03	0.73± 0.03	4.64± 0.24	4.85± 0.11 **

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 103 EFFECT OF FEEDING CYPROTERONE ACETATE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN LIVER OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	26.28± 2.41	31.28± 3.49	5.53± 0.48	8.88± 1.15	17.37± 1.26	16.37± 1.88	95.53± 9.69	142.11± 16.58
1.00	32.81± 9.52	26.73± 1.75	5.96± 0.78	8.16± 0.71	15.29± 1.56	* 19.89± 0.90	92.69± 16.43	162.68± 17.78
2.50	34.08± 4.72	27.49± 3.60	* 7.09± 0.40	10.49± 0.72	15.78± 0.95	** 11.22± 0.76	111.52± 8.13	119.25± 15.37
5.00	* 40.74± 5.22	* 22.14± 3.30	6.00± 0.33	7.98± 1.16	18.35± 1.15	13.10± 0.92	109.00± 2.73	102.75± 12.16
10.00	29.54± 2.99	*** 15.33± 0.16	5.50± 0.45	6.62± 0.63	16.27± 1.46	13.28± 0.88	84.59± 12.87	* 88.85± 12.37

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 104 EFFECT OF FEEDING CYPROTERONE ACETATE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN KIDNEY OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	40.81± 3.50	26.19± 2.92	3.52± 0.26	4.14± 0.26	14.41± 0.72	13.16± 1.34	49.58± 2.34	54.34± 6.78
1.00	49.54± 1.82	34.12± 5.49	3.63± 0.31	* 5.24± 0.54	15.00± 0.34	15.25± 0.28	54.11± 3.66	** 79.63± 7.32
2.50	* 51.63± 2.96	*** 53.59± 1.57	2.99± 0.35	** 5.72± 0.29	15.19± 1.16	12.63± 0.45	44.49± 3.96	* 72.18± 4.24
5.00	* 53.15± 2.10	28.16± 2.91	3.58± 0.15	4.12± 0.35	14.09± 0.69	12.14± 0.60	50.49± 3.29	52.81± 5.54
10.00	49.65± 3.78	* 40.49± 5.07	3.20± 0.48	4.21± 0.23	14.94± 0.96	* 10.20± 0.28	46.64± 4.41	43.07± 3.14

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 105 EFFECT OF FEEDING CYPROTERONE ACETATE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN BRAIN OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	41.36± 3.10	37.24± 1.27	2.73± 0.04	2.24± 0.04	18.81± 1.39	28.66± 0.57	51.56± 4.21	64.14± 1.09
1.00	** 24.75± 1.27	32.18± 3.28	2.79± 0.20	*** 3.58± 0.30	17.96± 0.32	26.95± 3.52	49.99± 3.46	*** 93.72± 5.84
2.50	*** 20.61± 2.26	35.87± 2.42	** 2.12± 0.10	* 2.85± 0.12	* 23.37± 1.20	** 19.21± 1.15	49.25± 0.46	54.79± 4.57
5.00	* 28.19± 5.45	** 24.24± 2.81	** 3.27± 0.26	** 2.97± 0.09	16.48± 0.91	** 19.67± 0.77	55.97± 6.17	58.58± 3.89
10.00	* 26.99± 4.39	*** 10.27± 1.54	*** 1.87± 0.09	2.35± 0.20	*** 30.79± 1.45	** 20.08± 0.93	57.44± 2.63	* 47.17± 4.56

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE, ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 106 EFFECT OF FEEDING CYPROTERONE ACETATE ON THE CONCENTRATIONS OF PROTEIN, RNA & DNA IN MUSCLE OF Cyprinus carpio. DRUG WAS FED FOR 60 DAYS ONLY.

CONCEN-TRATION OF DRUG mg/kg	PROTEIN mg/100 mg		RNA / DNA		PROTEIN / RNA		PROTEIN / DNA	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	13.60± 0.75	14.86± 0.59	10.15± 1.40	12.71± 1.87	36.94± 3.28	49.23± 3.15	375.67± 23.41	614.76± 69.43
1.00	** 16.88± 0.72	13.86± 0.86	12.06± 1.76	13.74± 2.23	68.81± 9.19	*** 90.48± 11.29	** 784.69± 61.29	*** 1219.19± 181.44
2.50	** 17.32± 0.58	13.11± 0.85	14.84± 2.30	14.16± 1.88	60.63± 2.78	54.28± 5.76	*** 904.85± 159.29	747.25± 70.62
5.00	14.73± 1.19	14.24± 0.84	* 17.24± 3.07	11.53± 0.62	48.36± 5.54	* 72.53± 4.56	** 743.86± 46.82	829.42± 33.69
10.00	15.09± 0.50	13.99± 1.17	5.97± 1.50	16.10± 0.46	*** 193.04± 30.77	42.83± 1.90	*** 1025.08± 111.48	690.09± 38.34

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 107 EFFECT OF FEEDING CYPROTERONE ACETATE ON THE PROXIMATE COMPOSITION OF MUSCLE TISSUE FROM *Cyprinus carpio*. DRUG WAS FED FOR 60 DAYS ONLY.

CONCENTRATION OF DRUG mg/kg	MOISTURE mg/100 mg		CRUDE PROTEIN N x 6.25 (mg/100 mg)				TOTAL LIPIDS (mg/100 mg)				ASH (mg/100 mg)			
			ON WET BASIS		ON DRY BASIS		ON WET BASIS		ON DRY BASIS		ON WET BASIS		ON DRY BASIS	
	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS	60 DAYS	90 DAYS
0.00	75.84± 0.20	74.56± 0.60	17.81± 0.25	18.63± 0.31	73.88± 1.13	73.38± 0.81	2.32± 0.18	3.16± 0.42	9.61± 0.73	12.22± 1.34	2.21± 0.07	2.11± 0.04	9.13± 0.25	8.34± 0.35
1.00	** 74.18± 0.44	75.50± 0.53	* 19.00± 0.38	18.56± 0.38	73.56± 1.06	75.88± 3.00	2.08± 0.08	2.20± 0.20	8.03± 0.23	8.95± 0.72	2.39± 0.16	1.89± 0.05	9.25± 0.57	7.75± 0.37
2.50	76.29± 0.43	75.82± 0.56	18.25± 0.33	18.56± 0.69	76.88± 1.31	76.69± 2.13	1.84± 0.23	2.25± 0.22	7.73± 0.85	9.28± 0.70	2.03± 0.04	2.12± 0.20	8.58± 0.13	8.73± 0.67
5.00	* 74.68± 0.23	* 76.66± 0.42	** 19.25± 0.50	18.81± 0.18	75.94± 1.38	* 80.63± 1.50	2.28± 0.13	** 1.34± 0.19	9.01± 0.51	** 5.75± 0.88	2.06± 0.08	2.16± 0.10	8.13± 0.33	9.25± 0.35
10.00	75.59± 0.05	* 76.53± 0.34	18.19± 0.38	17.38± 0.25	74.56± 0.25	74.13± 0.94	* 1.77± 0.07	* 2.05± 0.27	* 7.29± 0.40	8.72± 1.15	2.13± 0.05	1.99± 0.01	8.73± 0.11	8.50± 0.15

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

4.1.12. Effect of Combination of Steroids on the Growth of *Cyprinus carpio*

In this experiment, the optimum doses of those steroids (ethylestrenol (EE), oxandrolone (ON) and methyltestosterone (MT)) which gave maximum growth response in the present study in carp were combined and their effect monitored on the growth of carp. The experimental design was similar to the single hormone evaluation programme, but due to electricity failure all the fish died on the sixty-fourth day of the experiment, so the data presented is for sixty days only. After sixty days of feeding the steroids in different combinations, no effect on the growth was observed (Tables 108 and 109). By looking at Table 110, it appears that SGR for experimental groups were lower than the controls and there was also impairment of the food conversion efficiency. All these parameters were positive when the individual drugs were tried for growth promotion. It appears, then, that the steroids at the doses tested were becoming toxic to the fish.

Table 111 gives tissue - body indices for this experiment. CSI and RSI were higher ($P<0.05$) in EE-MT, EE-ON, EE-MT-ON but not in ON-MT. HSI was significantly lower ($P<0.01$) only in ON-MT. VSI was lower in ON-MT ($P<0.01$) and EE-MT-ON ($P<0.05$).

4.1.12.1. Biochemical Changes

4.1.12.1.1. Liver (Table 112)

Total proteins were significantly ($P<0.01$) higher only in EE-ON group. A significant decrease ($P<0.05$) in RNA/DNA was seen in EE-MT while protein/RNA was lower in EE-MT ($P<0.05$) and EE-MT-ON ($P<0.01$) groups. Protein/DNA was lower ($P<0.05$) in all groups.

4.1.12.1.2. Kidney (Table 113)

No changes in proteins and protein/DNA were noted. RNA/DNA was higher ($P<0.001$) in EE-MT-ON group while protein/RNA was lower in EE-MT ($P<0.001$) and EE-MT-ON ($P<0.001$).

4.1.12.1.3. Brain (Table 114)

Proteins were significantly lower ($P<0.01$) in EE-MT-ON groups. RNA/DNA was less ($P<0.05$) in ON-MT and higher ($P<0.001$) in EE-MT-ON. Protein/RNA (EE-MT and EE-MT-ON) and protein/DNA (all but ON-MT) were lower in experimental groups.

4.1.12.1.4. Muscle (Table 115)

No effect on proteins and protein/DNA was observed. RNA/DNA was higher ($P<0.01$) in EE-MT-ON. Protein/RNA was lower in all but ON-MT group.

TABLE 108 CHANGES IN BODY WEIGHT OF CARP FED ETHYLESTRENOL (EE) OXANDROLONE (ON) & METHYLTESTOSTERONE (MT) FOR 60 DAYS IN DIFFERENT COMBINATIONS. VALUES GIVEN ARE MEAN (g) ± S.E. OF 25 FISH. PERCENT WEIGHT GAIN IS GIVEN IN PARENTHESES UNDER THE ACTUAL VALUES.

DURATION (DAYS)	CONCENTRATION OF DRUGS mg/kg FOOD				
	CONTROL	EE-MT 2.5-2.5	ON-MT 2.5-2.5	EE-ON 2.5-2.5	EE-MT-ON 2-2-2
0	4.72± 0.12	4.46± 0.14	4.73± 0.22	4.78± 0.12	4.88± 0.27
15	7.04± 0.42 (49.15)	6.23± 0.23 (39.69)	7.07± 0.38 (49.47)	6.52± 0.34 (36.40)	7.04± 0.46 (44.26)
30	8.61± 0.63 (82.42)	7.53± 0.32 (68.83)	8.56± 0.51 (80.97)	7.49± 0.42 (56.69)	8.48± 0.54 (73.77)
45	10.25± 0.77 (117.16)	8.59± 0.36 (92.61)	10.20± 0.56 (115.64)	8.81± 0.48 (84.31)	10.63± 0.67 (117.83)
60	14.11± 1.23 (198.94)	10.58± 0.44 (137.22)	12.61± 0.70 (166.60)	11.58± 0.63 (142.26)	12.47± 0.77 (155.53)
75	—	—	—	—	—
90	—	—	—	—	—

DUE TO THE FAILURE OF ELECTRICITY ALL FISH DIED ON 64th DAY OF THE EXPERIMENT

TABLE 109 CHANGES IN TOTAL BODY LENGTH OF CARP FED ETHYLESTRENOL (EE) OXANDROLONE (ON) AND METHYLTESTOSTERONE (MT) FOR 60 DAYS IN DIFFERENT COMBINATIONS. VALUES GIVEN ARE MEAN (cm) ± S.E. OF 25 FISH. PERCENT INCREASE IN LENGTH IS GIVEN IN PARENTHESES.

DURATION (DAYS)	CONCENTRATION OF DRUGS mg/kg FOOD				
	CONTROL	EE-MT 2.5-2.5	ON-MT 2.5-2.5	EE-ON 2.5-2.5	EE-MT-ON 2-2-2
0	7.06± 0.12	7.05± 0.08	7.14± 0.12	7.12± 0.12	7.21± 0.14
15	7.95± 0.15 (12.61)	7.71± 0.09 (9.36)	8.00± 0.14 (12.04)	7.74± 0.13 (8.71)	8.00± 0.17 (10.96)
30	8.25± 0.19 (16.86)	8.21± 0.12 (16.45)	8.41± 0.16 (17.79)	8.09± 0.14 (13.62)	8.48± 0.18 (17.61)
45	8.83± 0.20 (25.07)	8.49± 0.12 (20.43)	9.02± 0.16 (26.33)	8.44± 0.15 (18.54)	8.97± 0.19 (24.41)
60	9.84± 0.26 (39.38)	9.10± 0.13 (29.08)	9.54± 0.18 (33.61)	9.16± 0.16 (28.65)	9.46± 0.20 (31.21)
75	-	-	-	-	-
90	-	-	-	-	-

DUE TO FAILURE OF ELECTRICITY ALL FISH DIED ON 64th DAY OF THE EXPERIMENT.

TABLE 110 EFFECT OF FEEDING ETHYLESTRENOL (EE), OXANDROLONE (ON) & METHYLTESTOSTERONE (MT) FOR 60 DAYS IN DIFFERENT COMBINATIONS ON THE CONDITION FACTOR, SPECIFIC GROWTH RATE AND FOOD CONVERSION EFFICIENCY OF CARP.

DRUGS mg/kg FOOD	CONDITION FACTOR	SPECIFIC GROWTH RATE (WEIGHT)	SPECIFIC GROWTH RATE (LENGTH)	FOOD CONVERSION EFFICIENCY
CONTROL	1.48± 0.24	1.88	0.57	0.41
EE-MT 2.5-2.5	1.40± 0.24	1.48	0.43	0.29
ON-MT 2.5-2.5	1.45± 0.24	1.68	0.49	0.32
EE-ON 2.5-2.5	1.51± 0.25	1.52	0.43	0.33
EE-MT-ON 2-2-2	1.47± 0.24	1.61	0.46	0.33

TABLE 111 EFFECT OF FEEDING ETHYLESTRENOL (EE), OXANDROLONE (ON) & METHYTESTOSTERONE (MT) FOR 60 DAYS IN DIFFERENT COMBINATIONS ON THE CRANIO-SOMATIC (CSI), HEPATO-SOMATIC (HSI), RENO-SOMATIC (RSI) & VISCERO-SOMATIC (VSI) INDEX OF CARP.

DRUGS mg/kg FOOD	CSI	RSI	HSI	VSI
CONTROL	0.82± 0.16	0.61± 0.03	2.32± 0.16	4.42± 0.23
EE-MT 2.5-2.5	* 1.02± 0.03	* 0.83± 0.12	2.00± 0.05	4.49± 0.15
ON-MT 2.5-2.5	0.87± 0.08	0.73± 0.03	** 1.58± 0.20	** 3.48± 0.17
EE-ON 2.5-2.5	** 1.05± 0.06	* 0.83± 0.02	2.06± 0.09	4.31± 0.14
EE-MT-ON 2-2-2	* 0.99± 0.05	* 0.79± 0.02	2.05± 0.17	* 3.81± 0.24

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 112 EFFECT OF FEEDING ETHYLESTRENOL (EE), OXANDROLONE (ON) & METHYLTESTOSTERONE IN DIFFERENT COMBINATIONS ON THE PROTEIN, RNA & DNA IN LIVER OF CARP. DRUGS WERE FED FOR 60 DAYS ONLY.

DRUGS mg/kg FOOD	PROTEINS mg/100 mg	RNA/DNA	PROTEIN/ RNA	PROTEIN/ DNA
CONTROL	19.94± 1.46	6.04± 0.58	19.70± 1.29	118.02± 11.14
EE-MT 2.5-2.5	20.30± 1.06	* 4.32± 0.46	* 15.89± 1.15	** 67.54± 5.24
ON-MT 2.5-2.5	21.71± 1.59	4.94± 0.53	18.57± 1.51	* 90.91± 8.73
EE-ON 2.5-2.5	** 26.20± 0.94	5.09± 0.24	16.02± 1.60	* 81.98± 10.69
EE-MT-ON 2-2-2	16.10± 1.82	6.25± 0.41	** 14.05± 0.44	* 88.32± 8.46

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS TO VARIANCE ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 113 EFFECT OF FEEDING ETHYLESTRENOL (EE), OXANDROLONE (ON) & METHYLTESTOSTERONE (MT) IN DIFFERENT COMINATIONS ON PROTEINS, RNA & DNA IN KIDNEY OF CARP. DRUGS WERE FED FOR 60 DAYS ONLY.

DRUGS mg/kg FOOD	PROTEINS mg/100 mg	RNA/DNA	PROTEIN/ RNA	PROTEIN/ DNA
CONTROL	40.36± 3.21	2.29± 0.10	14.67± 0.22	33.47± 1.26
EE-MT 2.5-2.5	38.92± 5.19	2.49± 0.10	*** 11.93± 0.49	29.59± 1.32
ON-MT 2.5-2.5	38.94± 1.82	2.27± 0.10	15.28± 0.52	34.74± 2.24
EE-ON 2.5-2.5	41.05± 2.26	2.44± 0.17	13.47± 0.38	32.99± 3.07
EE-MT-ON 2-2-2	45.66± 2.37	*** 3.40± 0.22	*** 10.24± 0.39	34.70± 2.17

SIGNIFICANTLY DIFFERENT FROM CONTROLS (SINGLE FACTOR ANALYSIS OF VARIANCE ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 114 EFFECT OF FEEDING ETHYLESTRENOL (EE), OXANDROLONE (ON) & METHYLTESTOSTERONE (MT) IN DIFFERENT COMBINATIONS ON PROTEINS, RNA & DNA IN BRAIN OF CARP. DRUGS WERE FED FOR 60 DAYS ONLY.

DRUGS mg/kg FOOD	PROTEIN mg/100 mg	RNA/DNA	PROTEIN/ RNA	PROTEIN/ DNA
CONTROL	20.36± 2.48	1.94± 0.05	19.41± 0.71	40.49± 3.76
EE-MT 2.5-2.5	17.75± 1.30	1.87± 0.06	*** 14.63± 0.63	** 27.33± 1.04
ON-MT 2.5-2.5	22.43± 3.36	* 1.72± 0.02	21.51± 1.02	36.93± 2.16
EE-ON 2.5-2.5	14.48± 0.95	1.83± 0.11	18.17± 0.65	* 33.16± 2.58
EE-MT-ON 2-2-2	** 11.26± 0.96	*** 2.56± 0.08	*** 12.66± 0.41	** 32.35± 1.29

SIGNIFICANTLY DIFFERENT FROM CONTROL (SINGLE FACTOR ANALYSIS OF VARIANCE ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 115 EFFECT OF FEEDING ETHYLESTRENOL (EE), OXANDROLONE (ON) & METHYLTESTOSTERONE (MT) IN DIFFERENT COMBINATIONS ON PROTEINS, RNA & DNA IN MUSCLE OF CARP. DRUGS WERE FED FOR 60 DAYS ONLY.

DRUGS mg/kg FOOD	PROTEINS mg/100 mg	RNA/DNA	PROTEIN/ RNA	PROTEIN/ DNA
CONTROL	16.82± 0.49	6.78± 0.79	70.85± 4.08	469.60± 26.72
EE-MT 2.5-2.5	16.21± 0.65	8.86± 1.43	* 60.21± 3.30	517.62± 56.50
ON-MT 2.5-2.5	16.83± 0.72	6.96± 0.76	67.50± 3.85	464.81± 41.01
EE-ON 2.5-2.5	17.58± 0.18	6.88± 0.60	*** 52.83± 0.79	361.68± 25.64
EE-MT-ON 2-2-2	17.20± 0.64	** 14.13± 3.02	*** 42.91± 2.32	598.50± 111.70

SIGNIFICANTLY DIFFERENT FROM CONTROL (SINGLE FACTOR ANALYSIS OF VARIANCE ACCORDING TO SOKAL & ROHLF 1969)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

4.1.13. Uptake and Disappearance of Radioactivity after Oral Administration of ^{3}H-testosterone

Table 116 gives the data concerning the uptake of the ^{3}H-testosterone after oral administration. The steroid was fed for twelve days. Fifteen hours after the last meal on the first day of the feeding of the label, the first sample was taken. It appears from the data presented in Table 116 that the steroid was readily absorbed from the gut because maximum levels of radioactivity were seen after one day of the feeding of the label. After this time the concentrations of the steroid came down and remained at that level up to four days of feeding, probably showing that isotopic equilibrium was reached. After twelve days of feeding of the label, little increases in label were seen in some tissue over the isotopic equilibrium values. These increases were probably due to the metabolites of the labelled steroid and not solely due to parent compound.

After twelve days of feeding of the radiolabel, the fish were returned to normal diet and disappearance of the label was follwed in fourteen different tissues of the body after one, three, five, seven, thirteen and twenty days. The data regarding this aspect is given in Table 117 and Figures 51, 52, 53, 54, 55, 56, 57, 58,59, 60, 61, 62, 63 and 64. The radioactivity of brain, heart, blood, gall bladder, anterior intestine and posterior intestine decreased exponentially throughout the twenty days of withdrawal period. While in liver, kidney, head kidney, spleen, testis, muscle, gill

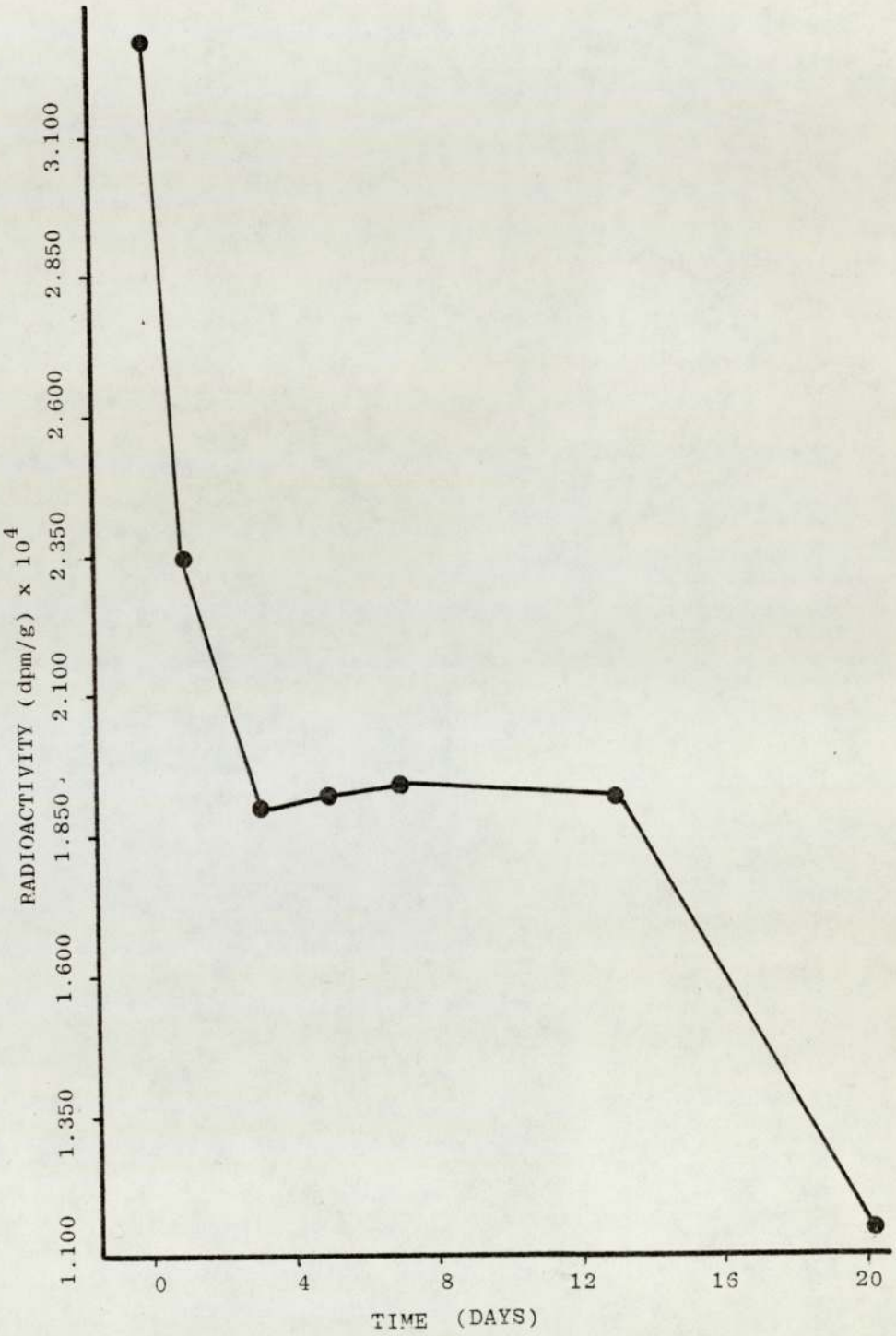


FIGURE 51 DISAPPEARANCE OF RADIOACTIVITY FROM THE LIVER OF CARP

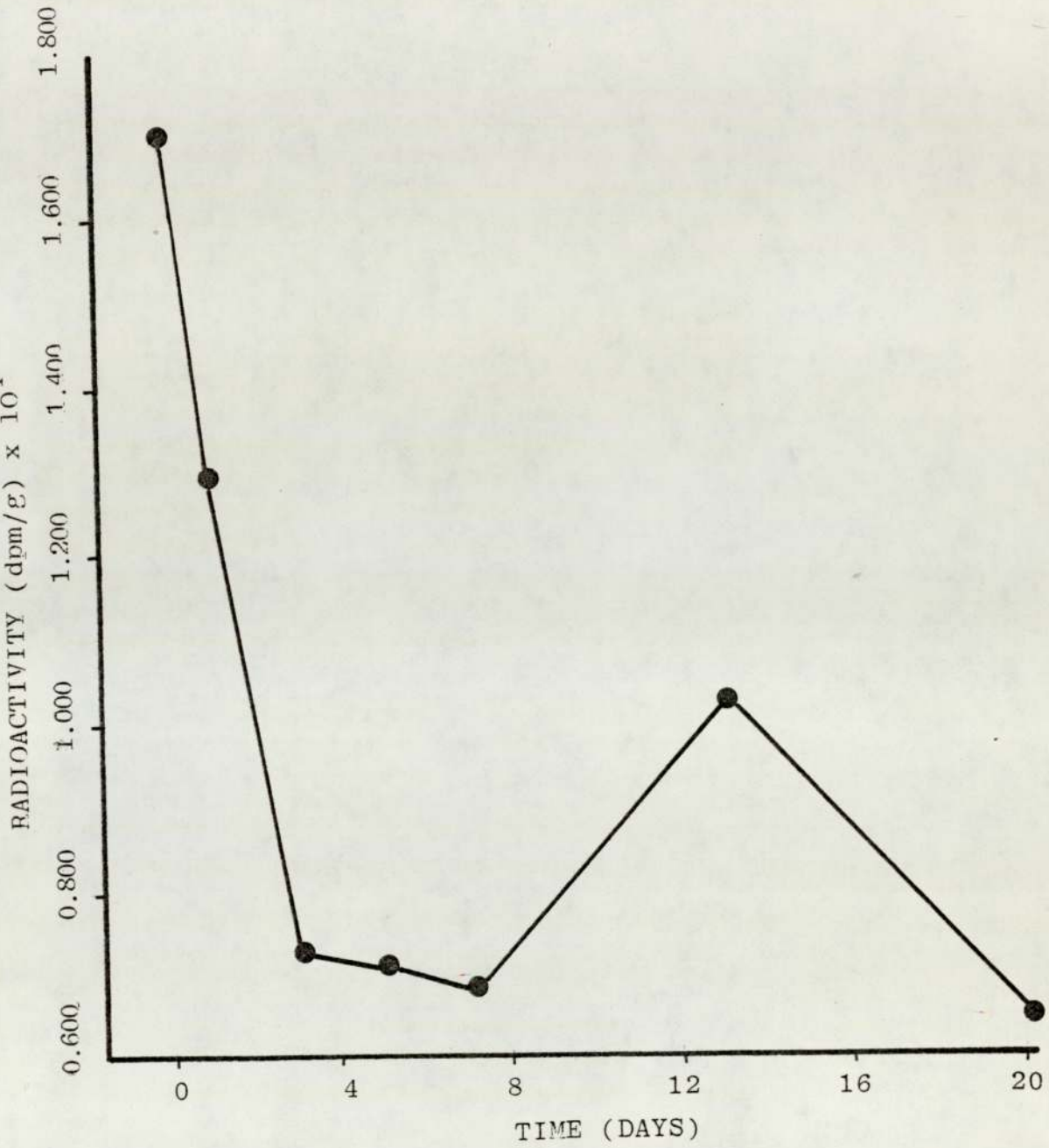


FIGURE 52 DISAPPEARANCE OF RADIOACTIVITY FROM THE KIDNEY OF CARP

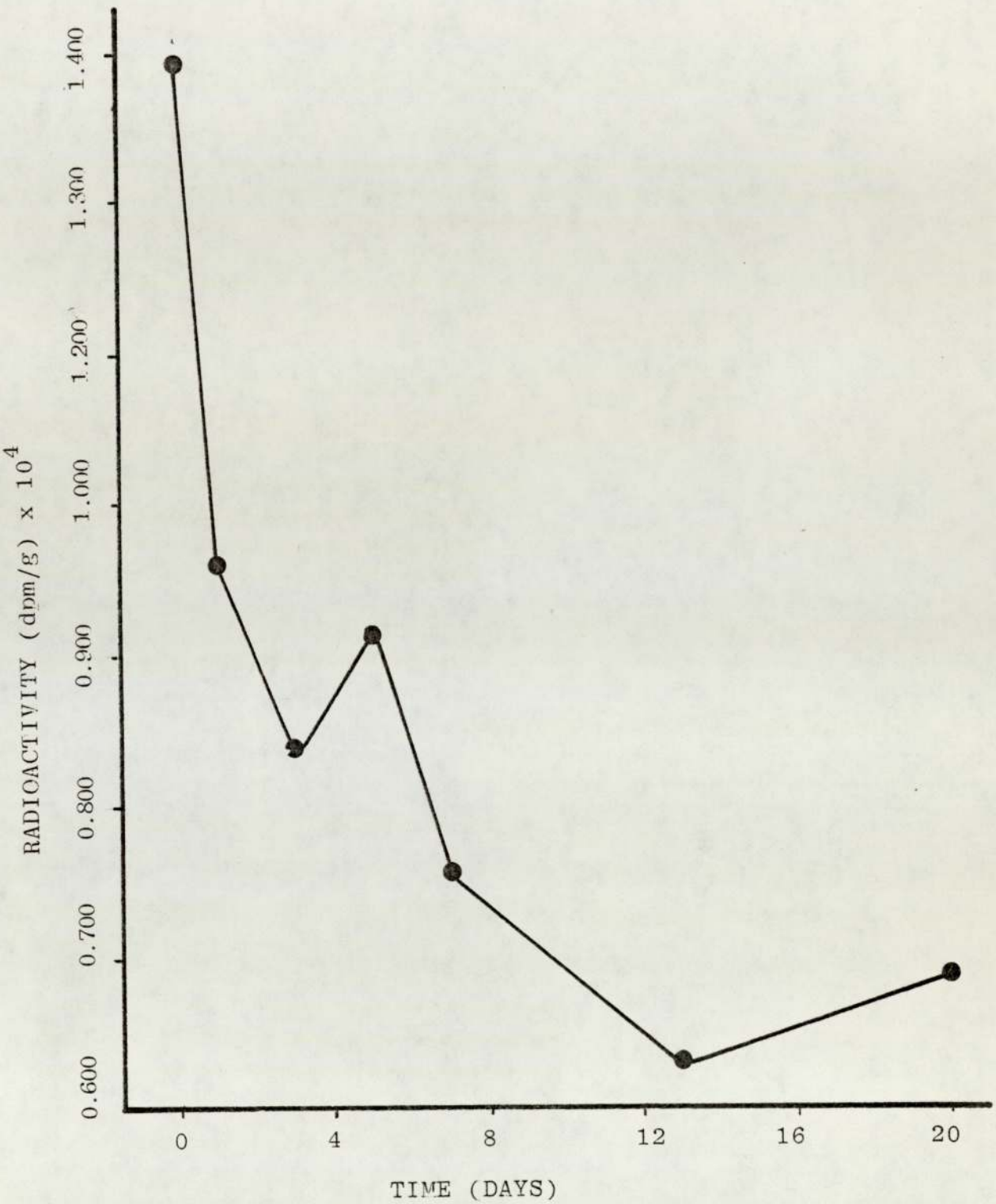


FIGURE 53 DISAPPEARANCE OF RADIOACTIVITY FROM THE HEAD KIDNEY OF CARP

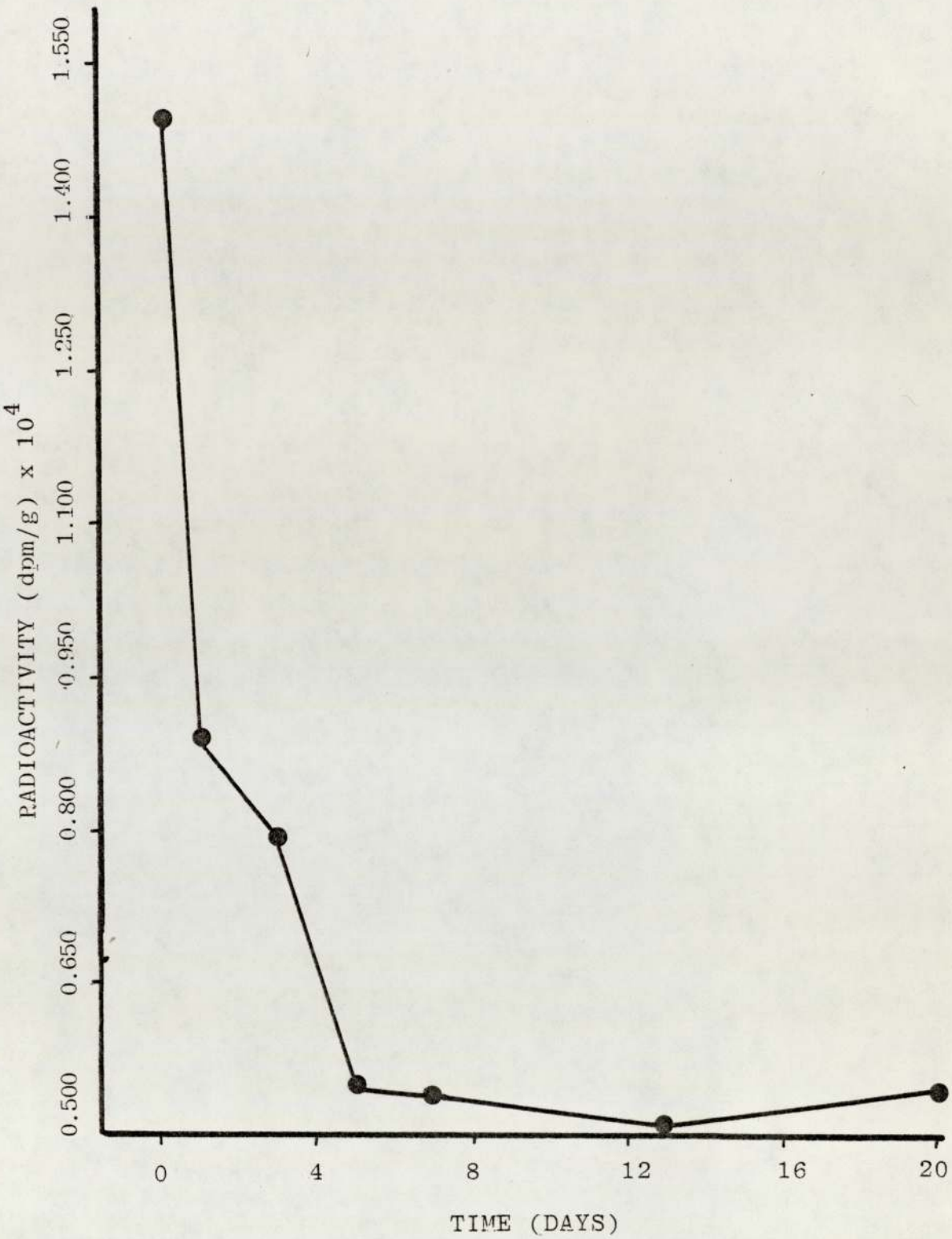


FIGURE 54 DISAPPEARANCE OF RADIOACTIVITY FROM THE BRAIN OF CARP

and skin the label decreased exponentially at least for the first three days. After three or five days, the radioactivity in some tissues (head kidney, spleen, testis and muscle) increased and then decreased. In liver after three days the level of radioactivity remained the same for up to thirteen days and at twenty days it declined drastically.

Three tissues behaved quite differently, they were kidney, gill and skin. In all these tissues the radioactivity was coming down smoothly when suddenly at the thirteenth day there was an increase (kidney and skin, Figures 52 and 58), in the label which came down with the same rapidity at twenty days. This can be due to odd samples at this point, but the steep in gill cannot be explained as odd, because there was a gradual increase in the radioactivity from three days onward which reached a maximum point on the thirteenth day, the concentration of the label at this point was even higher than the zero day values when the drug was withdrawn after feeding of twelve days (Figure 57).

The half-life of the radioactivity in different tissue is presented in Table 118. Maximum values for half-life were encountered in muscle (396 hours) followed by gill (175.07 hours), blood (151.20 hours) and head kidney (125.52 hours). The minimum values were observed for gall bladder (0.24 hour), posterior intestine (2.24) and heart (2.51 hours).

In Tables 119 and 120, the radioactivity in different

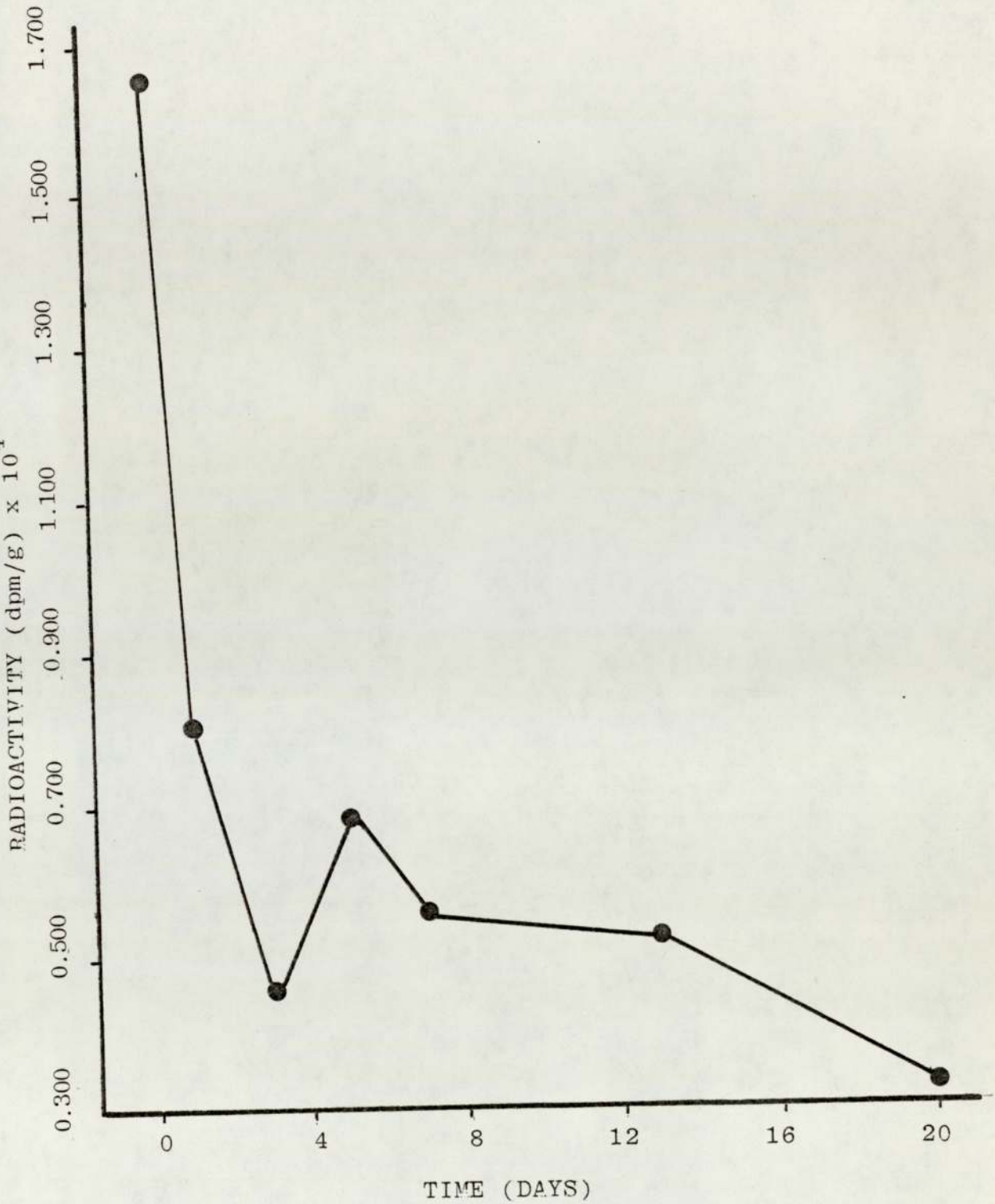


FIGURE 55 DISAPPEARANCE OF RADIOACTIVITY FROM THE SPLEEN OF CARP

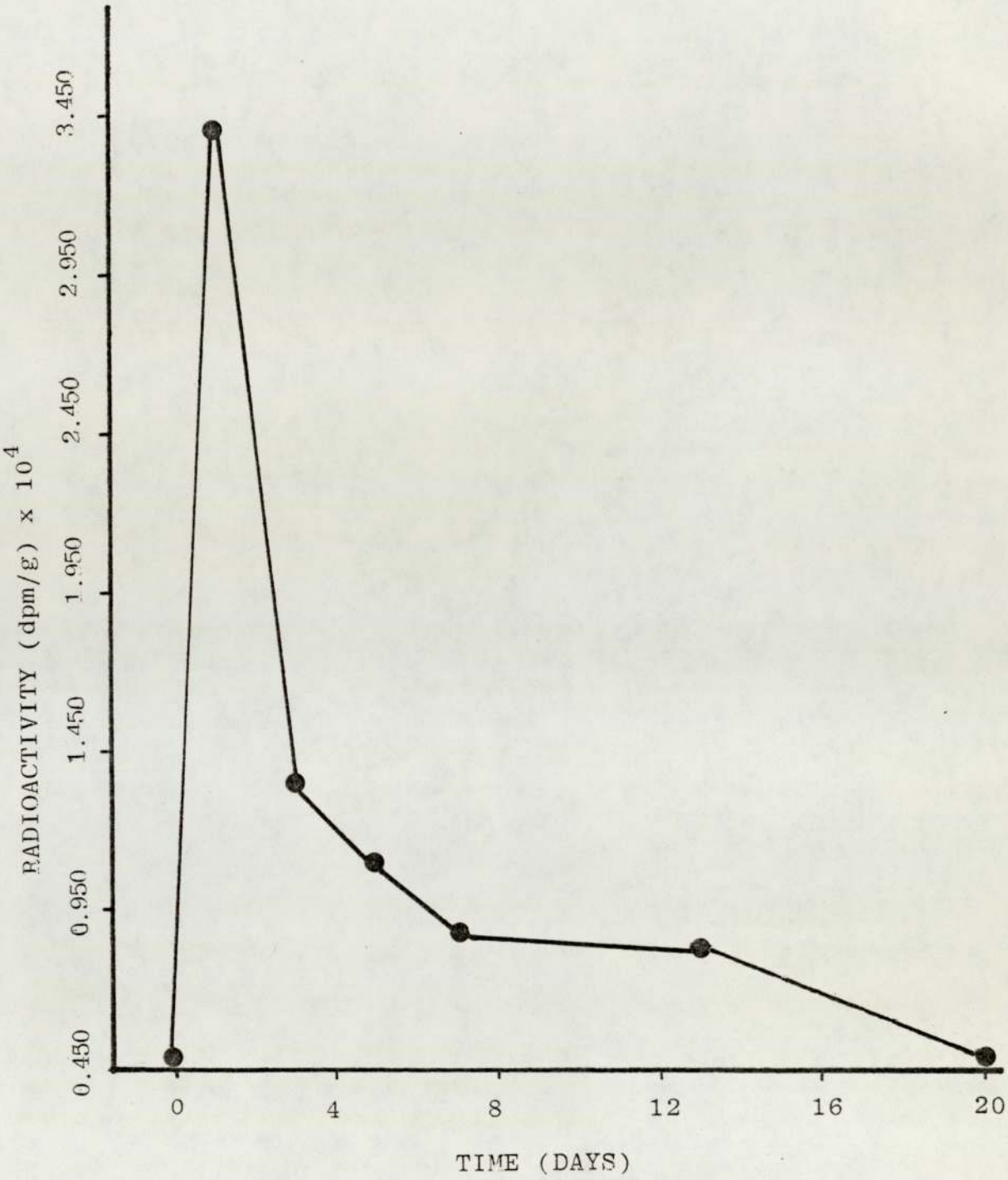


FIGURE 56 DISAPPEARANCE OF RADIOACTIVITY FROM THE HEART OF CARP

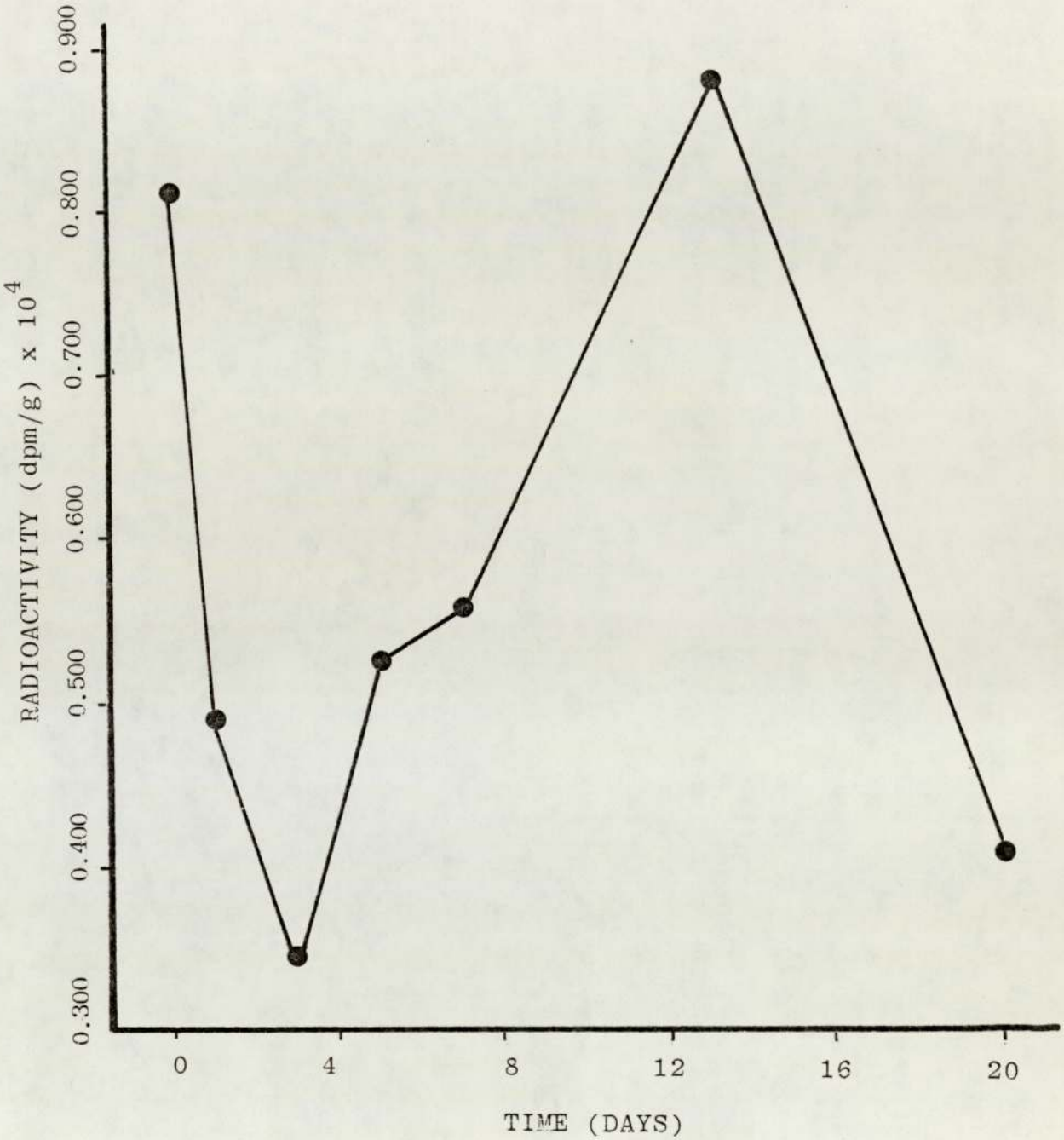


FIGURE 57 DISAPPEARANCE OF RADIOACTIVITY FROM THE GILL OF CARP

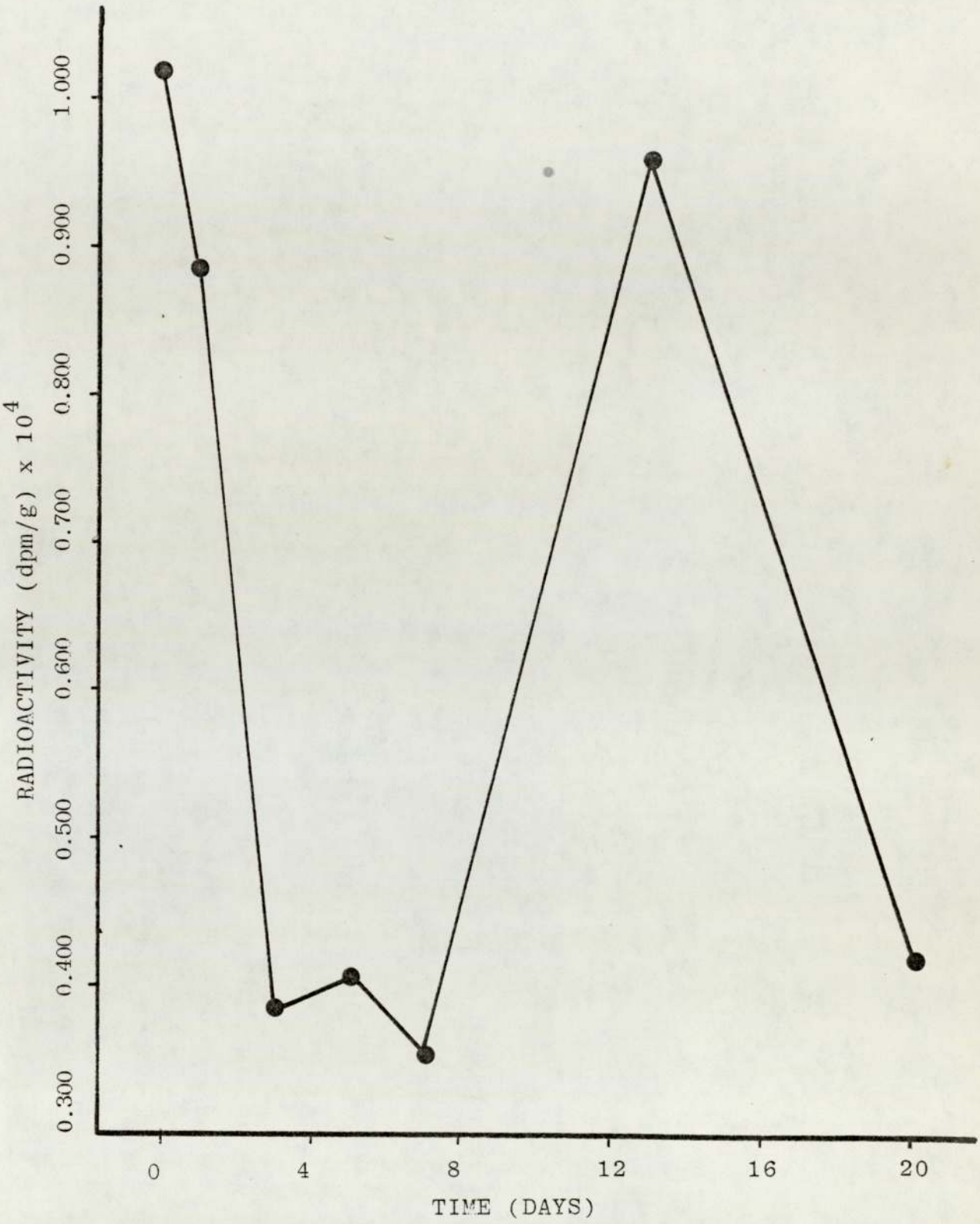


FIGURE 58 DISAPPEARANCE OF RADIOACTIVITY FROM THE SKIN OF CARP

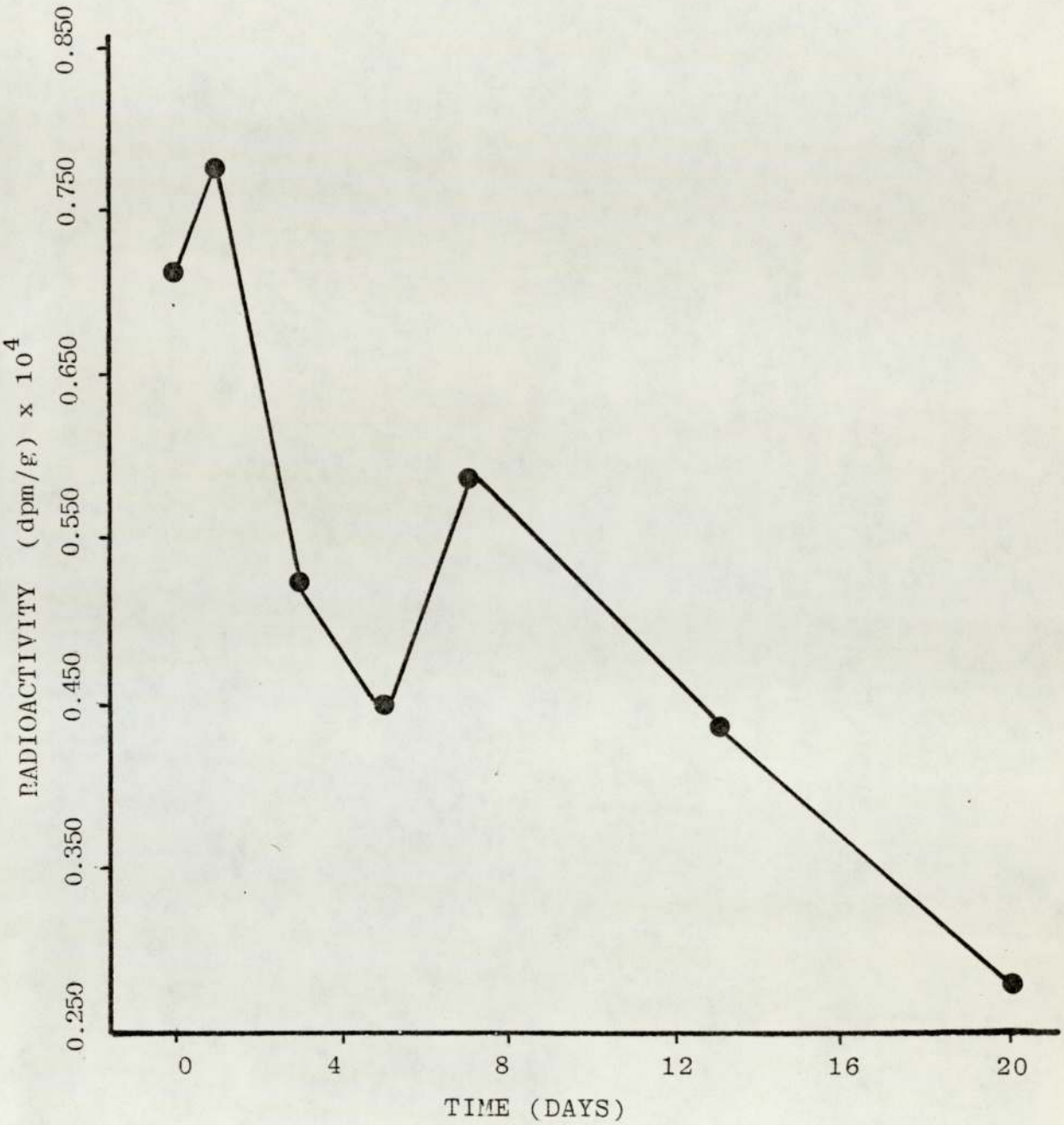


FIGURE 59 DISAPPEARANCE OF RADIOACTIVITY FROM THE TESTIS OF CARP

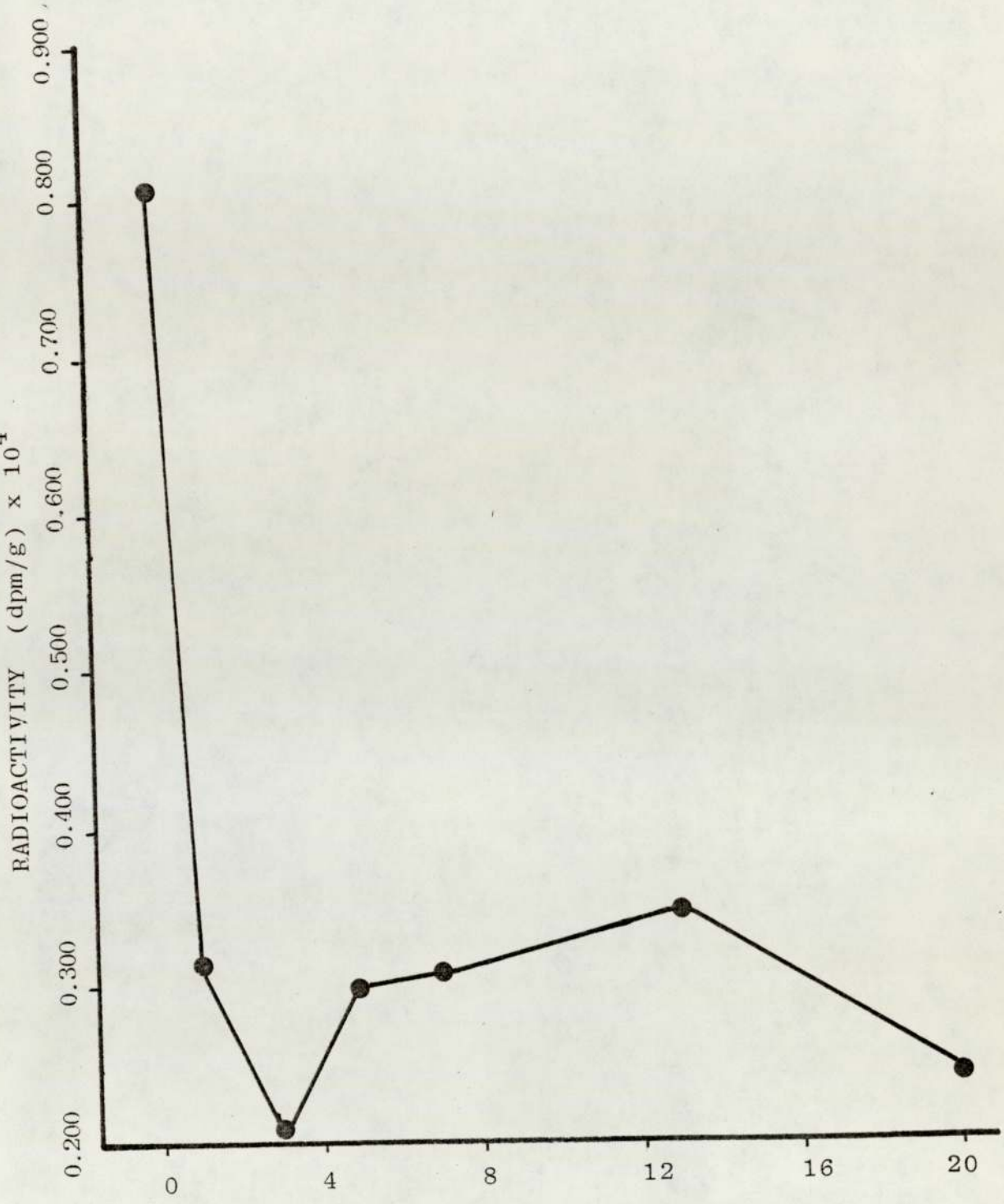


FIGURE 60 DISAPPEARANCE OF RADIOACTIVITY FROM THE MUSCLE OF CARP

tissues in percent of total radioactivity monitored, is presented with or without the gut and gall bladder. Of the total radioactivity at the isotopic equilibrium point 97.51% was associated with gut and gall bladder, of this 95.36% was in gall bladder only showing an enterohepatic secretion and faecal excretion of the steroid. Without the gall bladder and intestine, the maximum radioactivity at isotopic equilibrium was in liver (20.56%) followed by spleen (18.15%) and kidney (16.47%). The minimum label was in muscle (2.48%). Table 121 gives the tissue to blood ratio in different tissues of the carp.

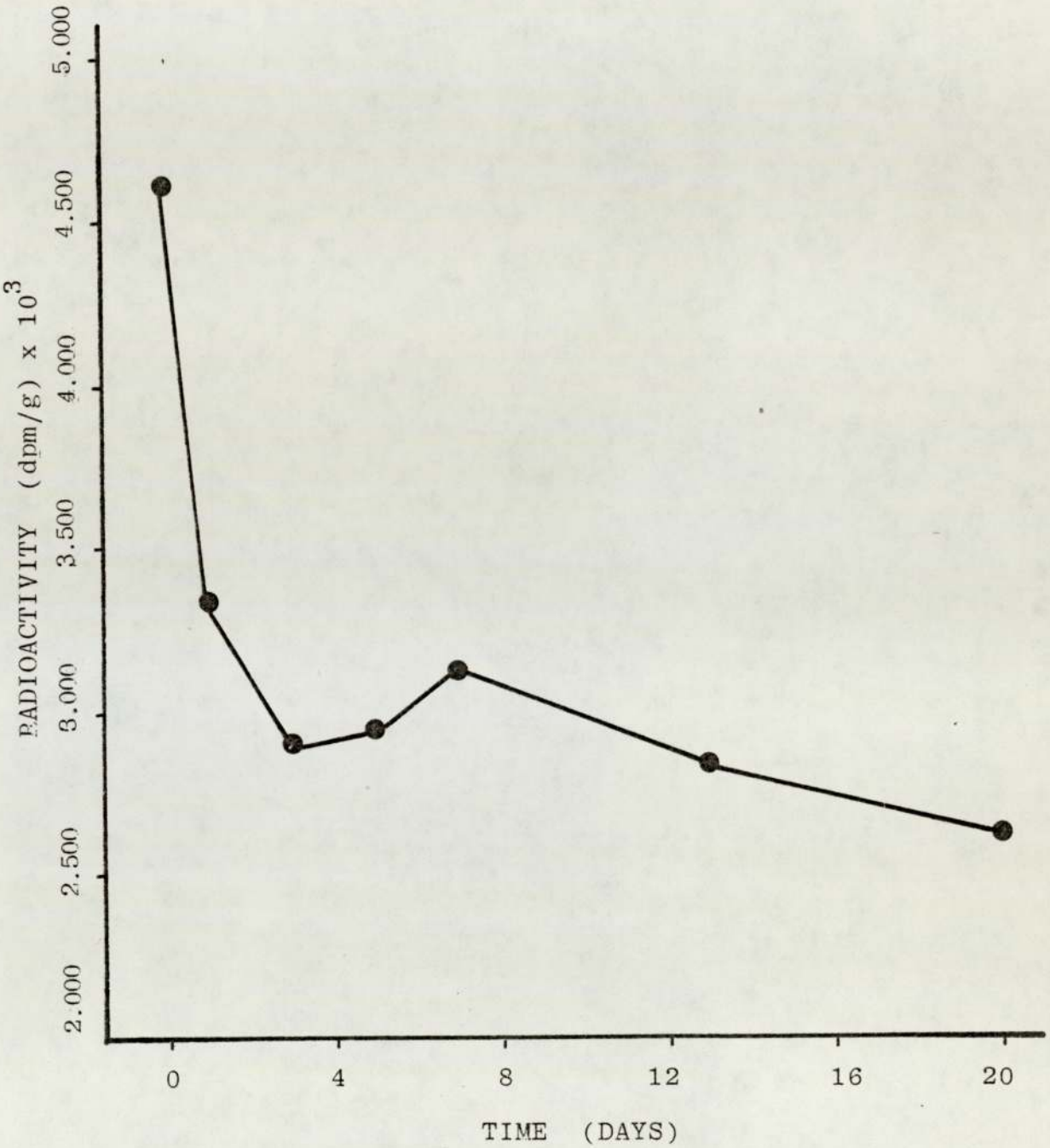


FIGURE 61 DISAPPEARANCE OF RADIOACTIVITY FROM THE BLOOD OF CARP

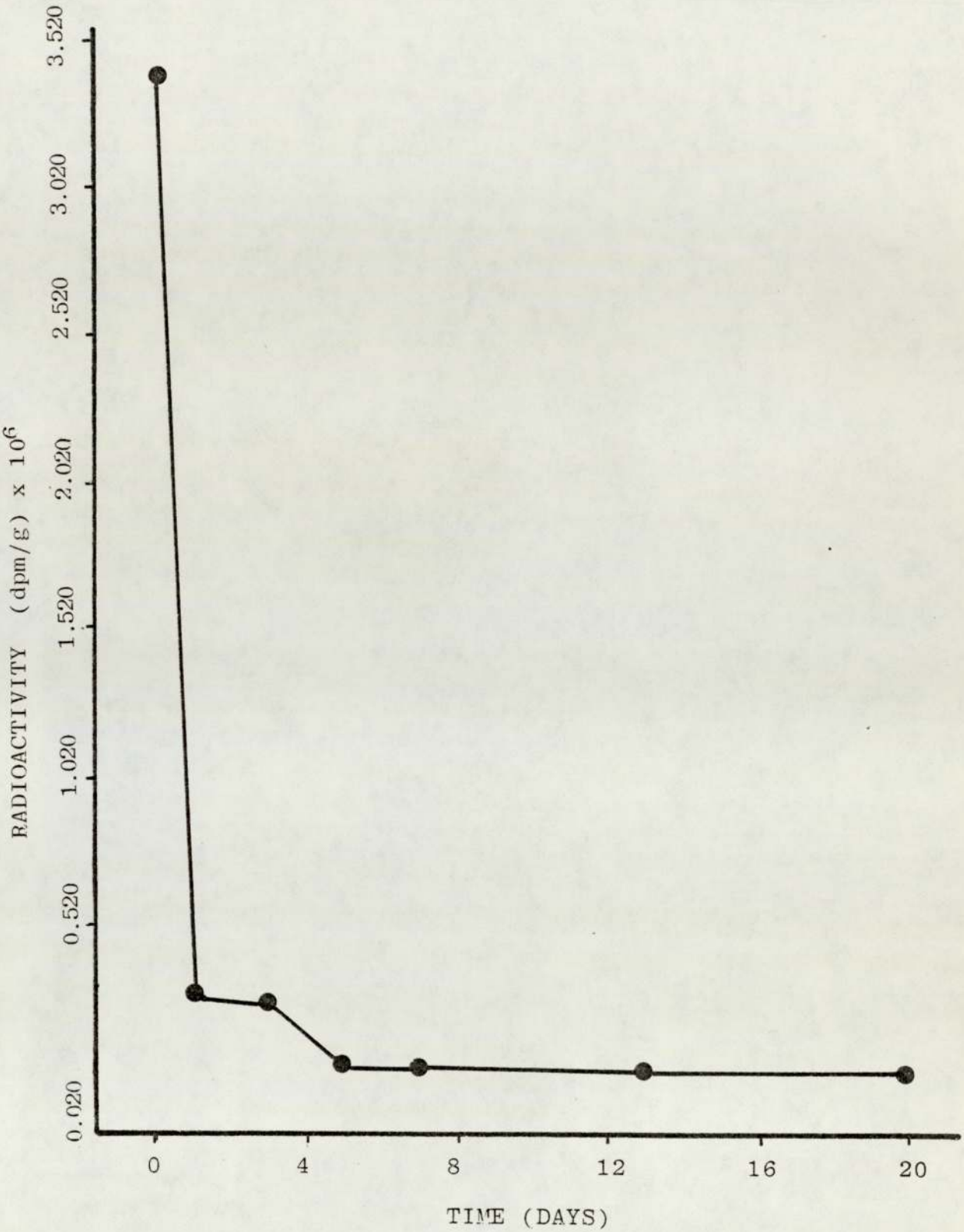


FIGURE 62 DISAPPEARANCE OF RADIOACTIVITY FROM THE GALL BLADDER OF CARP

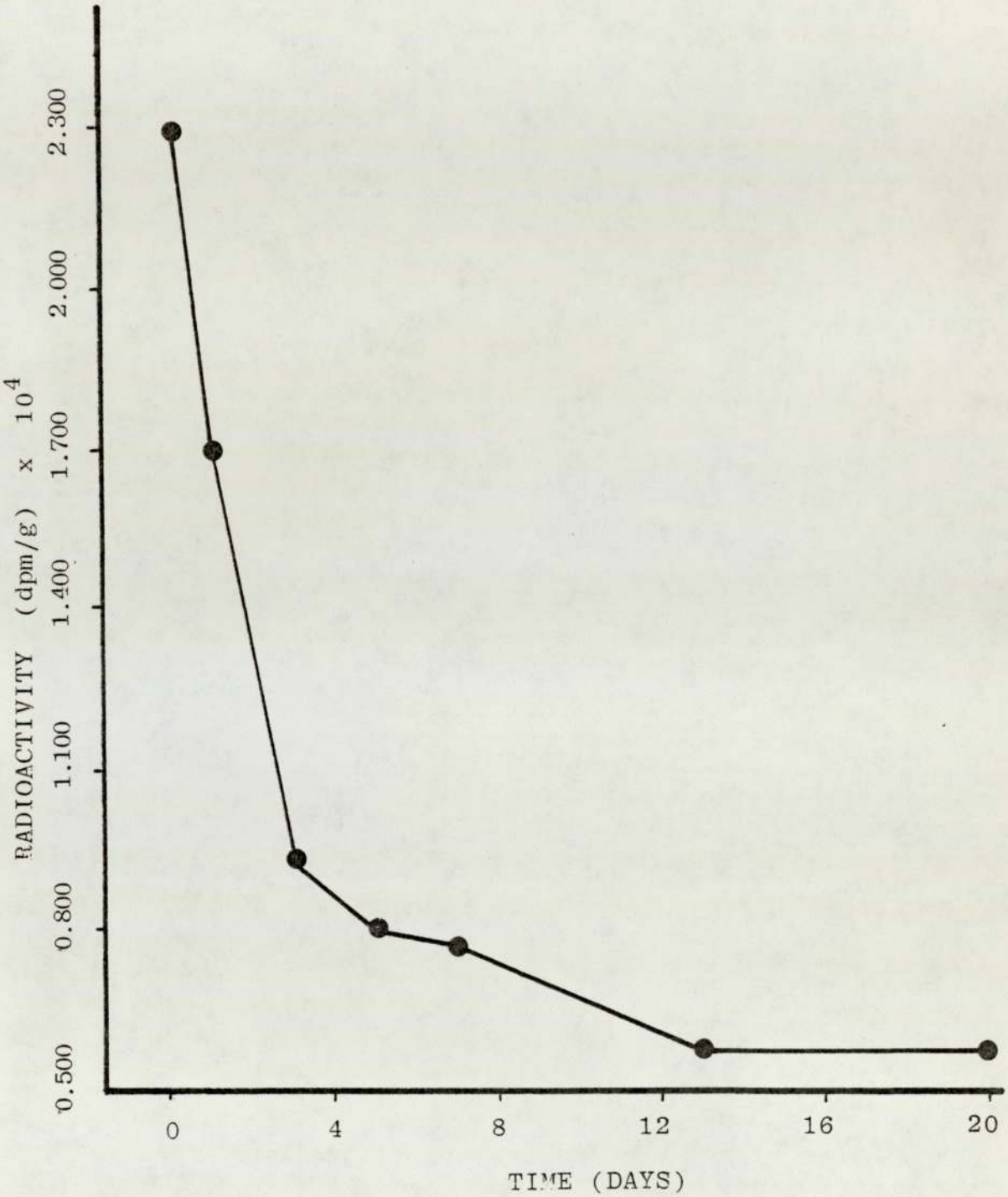


FIGURE 63 DISAPPEARANCE OF RADIOACTIVITY FROM THE ANTERIOR INTESTINE OF CARP

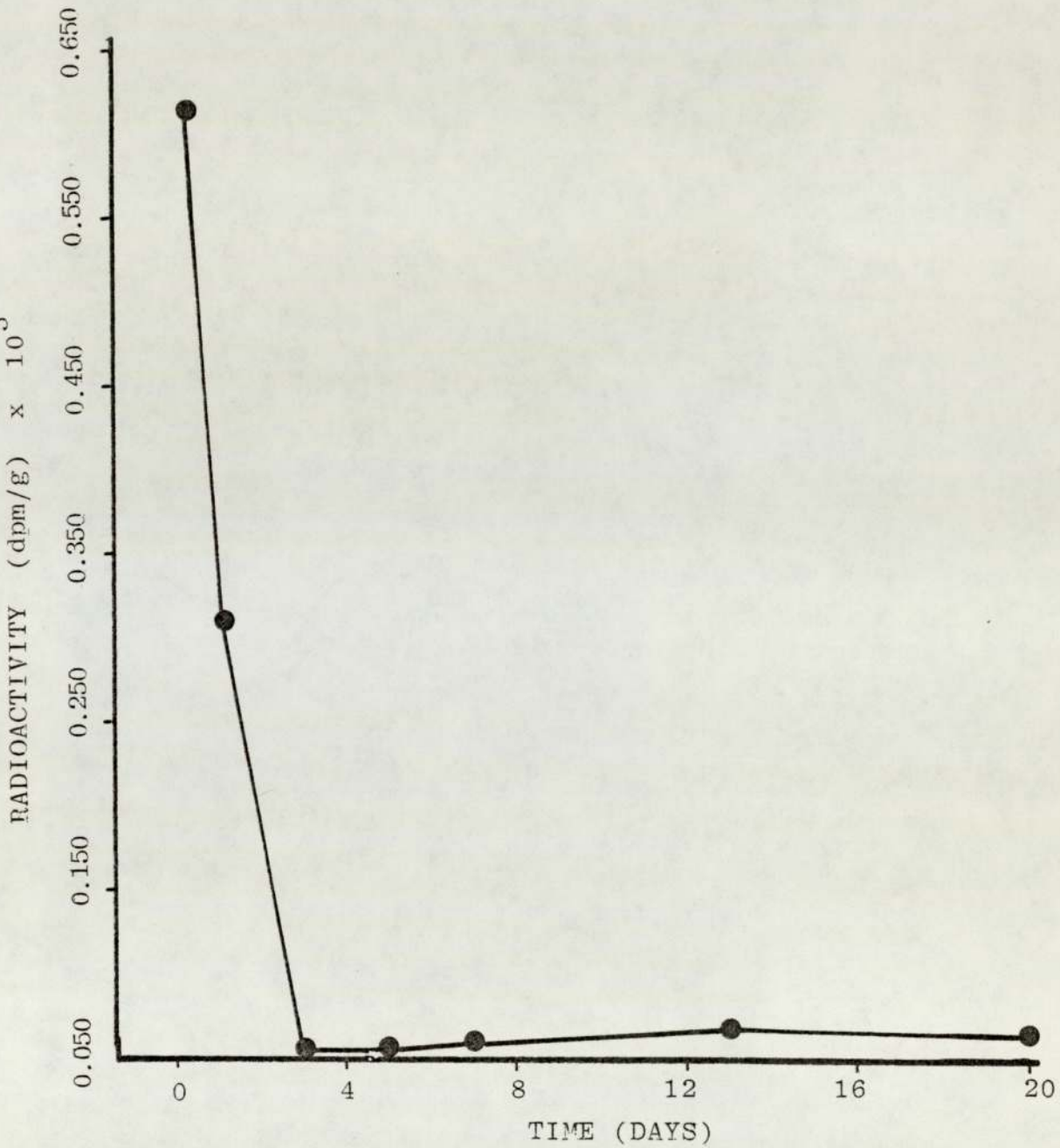


FIGURE 64 DISAPPEARANCE OF RADIOACTIVITY FROM THE POSTERIOR INTESTINE

TABLE 116 CONCENTRATION OF LABELLED SUBSTANCES IN TISSUES OF CARP (ng/g ± S.E.) FED RADIOACTIVE TESTOSTERONE. MOLECULAR WEIGHT OF METABOLITES WAS ASSUMED TO THAT OF TESTOSTERONE.

TISSUE	DAY 1	DAY 2	DAY 3	DAY 4	DAY 12
LIVER	95.41± 43.94	24.28± 4.32	25.97± 2.67	21.67± 4.22	37.78± 0.27
KIDNEY	36.92± 20.83	19.22± 7.41	17.62± 2.66	20.77± 9.54	19.68± 3.09
HEAD KIDNEY	26.03± 9.74	11.85± 2.46	11.07± 1.87	9.28± 1.42	14.89± 0.24
BRAIN	82.23± 63.80	9.50± 1.47	7.04± 1.20	6.63± 0.78	17.21± 4.84
SPLEEN	24.59± 8.32	25.88± 14.67	16.03± 4.03	21.58± 6.64	19.09± 1.81
HEART	10.52± 1.46	7.71± 2.14	4.08± 0.69	4.33± 0.75	5.59± 0.48
GILL	9.53± 1.69	4.66± 0.26	4.28± 0.45	4.71± 0.97	9.37± 2.22
SKIN	18.77± 5.17	4.96± 0.89	11.89± 5.50	8.14± 1.97	11.77± 2.07
TESTIS	11.33± 1.05	7.73± 2.88	6.04± 2.11	10.11± 4.92	8.23± 1.02
MUSCLE	21.30± 16.09	2.52± 0.49	2.39± 0.04	3.75± 1.39	9.32± 1.84
BLOOD	3.88± 0.20	7.62± 3.72	3.36± 0.38	3.15± 0.57	5.34± 0.32
GALL BLADDER	2091.56± 638.44	795.09± 125.65	6804.88± 479.29	5781.34± 1061.54	3942.23± 1427.31
ANTERIOR INTESTINE	85.85± 38.29	41.36± 19.23	44.13± 18.96	29.69± 3.97	26.46± 6.27
POSTERIOR INTESTINE	272.42± 77.72	83.54± 57.64	79.72± 27.11	23.49± * 8.45	71.10± 38.91

* SAMPLES WITHOUT ANY FAECES.

TABLE 117 EFFECT OF WITHDRAWAL OF RADIOACTIVE TESTOSTERONE FED FOR 12 DAYS ON THE CONCENTRATION (ng/g ± S.E.) OF LABELLED COMPOUNDS IN DIFFERENT TISSUES OF CARP.

TISSUE	DAY AFTER WITHDRAWAL						
	0	1	3	5	7	13	20
LIVER	38.78± 0.27	27.12± 3.92	21.86± 1.78	22.14± 2.60	22.37± 1.92	22.08± 1.62	13.08± 2.07
KIDNEY	19.68± 3.09	14.99± 4.13	8.42± 1.74	8.20± 0.80	7.90± 2.44	11.82± 1.84	7.41± 1.28
HEAD KIDNEY	14.89± 0.24	11.05± 0.84	9.65± 2.45	10.52± 1.73	8.72± 1.07	7.37± 0.85	8.06± 0.70
BRAIN	17.21± 4.84	10.21± 4.34	9.15± 2.90	6.28± 0.74	6.25± 0.57	5.87± 0.43	6.30± 0.80
SPLEEN	19.09± 1.81	9.27± 2.11	5.28± 0.75	7.88± 0.46	6.41± 0.39	6.00± 0.36	3.74± 1.07
HEART	5.59± 0.48	39.40± 12.83	15.71± 0.73	12.88± 6.10	10.16± 1.21	9.73± 0.92	5.53± 0.55
GILL	9.36± 2.22	5.64± 0.73	3.95± 0.45	6.05± 0.73	6.42± 0.45	10.13± 1.51	4.67± 0.58
SKIN	11.77± 2.07	10.23± 4.11	4.44± 0.69	4.68± 1.02	4.08± 1.47	11.07± 1.87	4.82± 0.50
TESTIS	8.23± 1.02	8.97± 2.94	6.05± 2.03	5.17± 2.89	6.77± 1.62	5.02± 0.43	3.22± 0.40
MUSCLE	9.32± 1.89	3.62± 0.37	2.37± 0.45	3.45± 0.81	3.55± 0.58	3.99± 0.40	2.79± 0.52
BLOOD	5.34± 0.32	3.86± 0.15	3.36± 0.40	3.41± 0.41	3.62± 0.23	3.28± 0.34	3.02± 0.58
GALL BLADDER	3942.23± 1427.31	337.33± 99.12	290.46± 5.20	52.48± 9.25	45.62± 3.27	33.38± 4.26	23.80± 0.87
ANTERIOR INTESTINE	26.46± 6.27	19.65± 4.38	10.80± 1.99	9.26± 3.11	8.90± 1.70	6.67± 1.55	6.70± 0.44
POSTERIOR INTESTINE	71.10± 38.91	36.08± 6.96	6.12± 1.0	6.43± 0.60	6.89± 1.01	8.04± 1.53	7.81± 2.96

TABLE 118 HALF-LIFE OF RADIOACTIVITY AFTER WITHDRAWAL OF STEROID FROM THE DIET

TISSUE	DAYS AFTER WITHDRAWAL	$T_{\frac{1}{2}}$ (hours)
LIVER	0-5 DAYS	13.36
KIDNEY	0-5 DAYS	9.80
HEAD KIDNEY	0-5 DAYS	125.52
BRAIN	0-5 DAYS	16.94
SPLEEN	0-5 DAYS	47.86
HEART	0-5 DAYS	2.51
GILL	0-5 DAYS	175.07
SKIN	0-5 DAYS	11.99
TESTIS	0-5 DAYS	17.51
MUSCLE	0-5 DAYS	396.00
BLOOD	0-5 DAYS	151.20
GALL BLADDER	0-5 DAYS	0.24
ANTERIOR INTESTINE	0-5 DAYS	6.40
POSTERIOR INTESTINE	0-5 DAYS	2.24

TABLE 119 DISTRIBUTION OF RADIOACTIVITY IN TISSUES OF CARP AS dpm, IN PERCENT OF TOTAL dpm OF ALL SAMPLED TISSUES. NUMBER OF ANIMALS ARE GIVEN IN PARENTHESES.

TISSUE	ISOTOPIC EQUILIBRIUM 2-4 DAYS OF FEEDING	12 DAYS OF FEEDING	20 DAYS AFTER REMOVAL OF DRUG	CONCENTRATION OF RADIOLABEL LEFT AFTER 20 DAYS. % 12 DAY
LIVER	0.51 (12)	0.90 (4)	12.96 (4)	34.62 (4)
KIDNEY	0.41 (12)	0.47 (4)	7.34 (4)	37.65 (4)
HEAD KIDNEY	0.23 (12)	0.35 (4)	7.98 (4)	54.13 (4)
BRAIN	0.17 (12)	0.41 (4)	6.24 (4)	36.61 (4)
SPLEEN	0.45 (12)	0.45 (4)	3.70 (4)	19.59 (4)
HEART	0.11 (12)	0.13 (4)	5.48 (4)	14.04 (4)
GILL	0.10 (12)	0.22 (4)	4.63 (4)	49.89 (4)
SKIN	0.18 (12)	0.28 (4)	4.77 (4)	40.95 (4)
TESTIS	0.17 (12)	0.20 (4)	3.19 (4)	39.12 (4)
MUSCLE	0.06 (12)	0.22 (4)	2.76 (4)	29.94 (4)
BLOOD	0.10 (12)	0.13 (4)	2.99 (4)	56.55 (4)
GALL BLADDER	95.36 (12)	93.91 (4)	23.58 (4)	0.60 (4)
ANTERIOR INTESTINE	0.82 (12)	0.63 (4)	6.64 (4)	25.32 (4)
POSTERIOR INTESTINE	1.33 (12)	1.69 (4)	7.74 (4)	10.98 (4)
TOTAL	100.00	99.99	100.00	

TABLE 120 DISTRIBUTION OF RADIOACTIVITY IN TISSUES OF CARP AS dpm IN PERCENT OF TOTAL dpm OF ALL SAMPLED TISSUES EXCLUDING INTESTINE AND GALL BLADDER. NUMBER OF ANIMALS ARE GIVEN IN PARENTHESES.

TISSUE	ISOTOPIC EQUILIBRIUM 2-4 DAYS OF FEEDING	12 DAYS OF FEEDING	20 DAYS AFTER REMOVAL OF DRUG
LIVER	20.56 (12)	23.87 (4)	20.88 (4)
KIDNEY	16.47 (12)	12.44 (4)	11.83 (4)
HEAD KIDNEY	9.21 (12)	9.41 (4)	12.87 (4)
BRAIN	6.62 (12)	10.87 (4)	10.06 (4)
SPLEEN	18.15 (12)	12.06 (4)	5.97 (4)
HEART	4.61 (12)	3.53 (4)	8.83 (4)
GILL	3.90 (12)	5.91 (4)	7.46 (4)
SKIN	7.14 (12)	7.44 (4)	7.49 (4)
TESTIS	6.83 (12)	5.20 (4)	5.14 (4)
MUSCLE	2.48 (12)	5.89 (4)	4.45 (4)
BLOOD	4.04 (12)	3.37 (4)	4.82 (4)
TOTAL	100.01 (12)	99.99	100.00

TABLE 121 TISSUE TO BLOOD RATIO OF RADIOACTIVITY IN DIFFERENT TISSUES OF CARP FED LABELED TESTOSTERONE. NUMBER OF ANIMALS ARE GIVEN IN PARENTHESES.

TISSUE	ISOTOPIC EQUILIBRIUM 2-4 DAYS OF FEEDING	12 DAYS OF FEEDING	20 DAYS AFTER REMOVAL OF DRUG
LIVER	5.09 (12)	7.07 (4)	4.33 (4)
KIDNEY	4.08 (12)	3.69 (4)	2.45 (4)
HEAD KIDNEY	2.28 (12)	2.79 (4)	2.67 (4)
BRAIN	1.64 (12)	3.22 (4)	2.09 (4)
SPLEEN	4.49 (12)	3.57 (4)	1.24 (4)
HEART	1.14 (12)	1.05 (4)	1.83 (4)
GILL	0.97 (12)	1.75 (4)	1.55 (4)
SKIN	1.77 (12)	2.20 (4)	1.60 (4)
TESTIS	1.69 (12)	1.54 (4)	1.07 (4)
MUSCLE	0.61 (12)	1.74 (4)	0.92 (4)
ANTERIOR INTESTINE	8.15 (12)	4.96 (4)	2.22 (4)
POSTERIOR INTESTINE	13.22 (12)	13.31 (4)	2.59 (4)
GALL BLADDER	947.01 (12)	738.25 (4)	7.88 (4)

CHAPTER FIVE

5.1. Discussion

The use of anabolic agents to promote the growth of livestock is not new. The effectiveness of the sex hormones and their synthetic derivatives as dietary supplements for finishing livestock has an obvious significance. The same principle can be applied to aquacultural practice. Most of the work on the regulation of growth in fish regarding anabolic-androgenic steroids has been on 17α-methyltestosterone, which is active orally apart from its parenteral actions, other natural and synthetic steroids have gained very little attention. In this study an effort was made on a systematic basis to evaluate the natural androgens with their "normal" androgenic and anabolic actions, as well as, the synthetic anabolic steroids, where the anabolic-androgenic properties are fairly disassociated.

As far as the stability of these compounds in the food is concerned, Simpson et al. (1974) has shown that tritiated ethylestrenol when incorporated into fish food and left in the laboratory in a transparent bag for one month, only 15% of the original compound was degraded. This shows that there are not many problems as far as the degradation of the active compound in the food is concerned.

5.1.1. Acceleration of Growth

The results of the present study clearly show that anabolic-androgenic steroids promote somatic growth when incorporated into the diet. Of the nine steroids tested, seven were able to induce positive growth responses as far as gain in weight and length was concerned. Two of them, trenbolone acetate and cyproterone acetate, were not very active in this regard. This can be due to the fact that trenbolone acetate is not active orally even in mammals (Scott, 1978) and secondly, the doses at which it was tried in the present study were probably not adequate for it to be active orally. This can be seen from the results (Tables 88 and 89) that while the lower doses (1.0, 2.5, 5.0 ppm) induced negative growth, the fish on the highest dose (10.0 ppm) were nearly equal to the control fish in their growth.

It appears from the results of the present study, that the growth promoting response of the steroids is dose-dependent. The seven steroids which induced positive growth in this study were all more active in this regard at the lower doses than at the higher ones. The optimum dose for all these steroids appear to be 1.0 or 2.5 ppm except in the case of adrenosterone, which induced maximum growth at the highest dose level (10.0 ppm). This is of interest to note, because adrenosterone is considered to be a metabolite of the natural sex hormones. This means that oxidation of the 17β-hydroxyl group of 11-ketotestosterone induced

better growth response than its parent compound. A better growth response to adrenosterone as compared with testosterone, 11-ketotestosterone, pregnenolone, androstenedione, methyltestosterone, 11α-hydroxymethyl-testosterone, fluoxymetesterone and cyproterone acetate have also been reported in *Tilapia nilotica* (Katz, et al., 1976).

It has been seen many times that natural or synthetic anabolic-androgenic steroids when given to the fish, although they increase the growth rate at lower doses their effect at higher doses become deleterious and detrimental to the growth. McBride and Fagerlund (1973) observed that 10.0 ppm of 17α-methyltestosterone induced an increase in the growth rate when fed to coho salmon, but no further gain was obtained if the dose was increased to a 50 ppm level. Yamazaki (1976) found the effective concentration of 17α-methyltestosterone to be 1.0 ppm when fed to goldfish. The growth rate of fish fed 10.0 ppm of this steroid was decreased and was parallel to the control fish and those on 30.0 ppm of the same steroid lost weight. Recently Matty and Cheema (1978) have shown that higher (10.0 and 20.0 ppm) doses of dimethazine did not induce any significant growth, while the same steroid at lower dose level (2.5 ppm) induced maximum growth response. Similar results have also been reported by Ashby (1957), Clemens et al., (1966) and Hirose and Hibiya (1968a, 1968b). In the present study 17α-methyltestosterone induced maximum

growth at 1.0 ppm level but at 10.0 ppm, it became deleterious to growth of carp, thus substantiating the results of other studies reported above.

The mechanism by which higher doses of androgens become deleterious to growth are not clear. In fact, no hypothesis in this regard has been put forth. According to the data available from mammalian and fish sources, it appears that probably two mechanisms are pertinent in this connection. Firstly, the higher doses of androgens decrease the appetite of the fish, resulting into decreased growth. Yamazaki (1976) has observed that total food consumption was proportional to the final body weight of *Carassius auratus* fed diets enriched with 1.0 - 30.0 ppm of 17α-methyltestosterone for fifty-six days, thus supporting our own view on appetite. Secondly, it has been shown many times that androgens increase the interrenal function in fish (Chester Jones et al., 1969, 1972, 1974; Fontaine, 1975; Simpson, 1978).

In mammals increased levels of glucocorticoids bring changes in the animal which are characterised by protein wasting due to reduction in protein synthesis and increase in intracellular proteolysis and retardation of the over all body growth (Ashmore and Morgan, 1967; Daughaday et al., 1975; Leung and Munck, 1975; Mayer and Rosen, 1977). According to Daughaday et al. (1975) the retardation of growth with excess glucocorticoids is probably due to

decreased secretion of growth hormone, antagonism of growth hormone action, direct effects on the muscle and cartilage and/or a combination of these effects. Simpson, (1978) has summarised the effects of glucocorticoids on protein metabolism and growth in fish and concluded that the effect of glucocorticoids are similar in mammals and fish. Recently, Kime (1978) has described an interesting adrenal-hepatic-gonadal-adrenal positive feedback loop in teleost fish. Further, it appears from the studies summarised by Simpson (1978), that in fish decrease in availability of food (natural or induced) increase the serum cortisol level and a concurrent decrease in T3 and T4, and in *Pleuronectes platessa* and rainbow trout the decrease in T3 and T4 is probably mediated through the inhibition of pituitary TSH.

It appears then, that the higher levels of androgens when fed, increase the interrenal activity with an increase in endogenous corticoids, which in turn bring changes manifested in lower growth of the fish.

Based on the per cent weight and length gains over the controls and the specific growth rate, it appears that ethylestrenol induced maximum growth. When all the steroids with their optimum dose were compared up to sixty days (drug feeding phase), the following sequence emerged:

Ethylestrenol (2.5) > Oxandrolone (1.0) > Dimethazine (2.5)

> Adrenosterone (10.0) > 11-ketotestosterone (2.5) > Testosterone (1.0) > Cyproterone acetate (5.0) > Trenbolone acetate (10.0).

17α-methyltestosterone is not compared with the above drugs, because this drug was fed for ninety days and food was given at 8% of the body weight of the fish as compared with 5% in all drugs.

The positive effect of anabolic-androgenic steroids on fish growth has been reported for goldfish (Hirose and Hibiya, 1968a; Yamazaki, 1976), salmon and rainbow trout (Hirose and Hibiya, 1968b; McBride and Fagerlund, 1973, 1976; Fagerlund and McBride, 1975b, 1977; Simpson, 1976; Higgs, et al., 1977; Matty and Cheema, 1978; Yu, et al., 1979). It appears from these studies and from the results of the present study that the optimum dose of these steroids are nearly the same (1.0 - 2.5 ppm) for goldfish, carp (present study), trout and salmon.

One very important aspect of the role of the anabolic-androgenic steroids on the growth of fish is to study the effect of acute withdrawal of the drug on the growth, as this is an obligatory process to avoid the accumulation of the steroid residues in different body organs, especially the muscle if the fish are to be used commercially. This means that a suitable schedule should be worked out by which the maximum advantage of the drug can be attained

by manipulating the duration of feeding of the drug. Keeping these factors in view, the acute withdrawal of the drug was undertaken to study the effect on growth.

It appears from the results that except in the case of 17α-methyltestosterone, all drugs when withdrawn from the food, could not maintain the impetus of growth, achieved during phase 1. There can be two reasons for this. Firstly, the duration; 17α-methyltestosterone was fed for ninety days while all other drugs were fed for sixty days. Secondly, there may be differences in the metabolism of the drug. Of the two reasons, the duration seems to play an important role. Another factor which might have an effect in this regard is the genetic background of the individual. The fish used in methyltestosterone experiments and in all other drugs were from two different farms. In mammals, the genetic background of the individual has a great influence on the ultimate growth of that species. No great emphasis has been given in aquacultural research on this important aspect of fish biology.

Although it was seen that withdrawal of the hormones induce changes in the percent weight and specific growth rate for weight, there is no such drastic effect, as far as the length is concerned. The result of this differential in response to withdrawal is the decrease in condition factor, which relates the length and weight of the fish. A decrease in condition factor has been seen after

treatment with anabolic steroids in salmon (McBride and Fagerlund, 1976; Fagerlund and McBride, 1975b, 1977; Higgs, et al., 1977).

Apart from acceleration of growth, another factor which has tremendous importance in aquaculture is the efficiency by which the fish convert the food into flesh. The animals with increased growth rate and better food conversion efficiency will be an ideal for cultivation purposes. In the present study, we have consistently seen that the concentrations of the hormones which induced growth also had better food conversion efficiencies. In fact, there is a direct correlation between the food conversion efficiency and specific growth rate for weight and length. For example, for 17α-methyltestosterone the correlation coefficient for weight and length was 0.9924 and 0.9595, which is near to the ideal value of 1.0. These values for some other steroids were, testosterone (weight, 0.9749; length, 0.8226), 11-ketotestosterone (weight, 0.9896; length, 0.9393), dimethazine (weight, 0.9952; length, 0.9417). From these values it appears that increase in weight is more closely related to food conversion efficiency, showing that these steroids are more potent in increasing weight changes than the length. This can also be seen in per cent increases over the controls and the fact that hormone withdrawal has less drastic effect on length. Increase in food conversion efficiency has been reported earlier after treatment with anabolic-androgenic steroids (Simpson, 1976;

Yamazaki, 1976; Matty and Cheema, 1978; Yu, et al., 1979).

5.1.2. Effect on the Tissues

In mammals apart from basic androgenic and anabolic effects, androgens bring about certain tissue changes which are most marked in liver and kidney (Kruskemper, 1968; Camerino and Sciaky, 1975; Kachakian, 1975, 1976; Nishino, 1975). It is obvious from the public health viewpoint that acceleration of growth should not accompany any tissue pathologies after treatment with anabolic steroids. In fish it has been reported that high doses of anabolic-androgenic steroids bring certain pathological changes like hypertrophy of the liver and kidney and in some severe cases haemorrhages have also been reported in the kidney. Thickening of the skin epidermis is also frequently reported in this connection (Hirose and Hibiya, 1968a, 1968b; McBride and VanOverbeeke, 1971; Yamazaki, 1972, 1976; Bulkley and Swihart, 1973).

In the present study at the end of phase 1 (sixty days of drug feeding), there was a consistent decrease in CSI, HSI and RSI except in the case of dimethazine where RSI was increased in higher doses. This effect of anabolic-androgenic steroids is different from some of the published reports cited above. Before commenting on this aspect, we must observe the normal growth pattern of the carp. By looking into the growth parameters (Tables 122, 123) in the present study (without any drug) it appears that

TABLE 122 GROWTH CHARACTERISTICS OF COMMON CARP OBSERVED IN THE PRESENT STUDY.

PARAMETER	DURATION (DAYS)			
	START	60	90	120
WEIGHT	4.70	10.09	15.54	49.32
LENGTH	6.51	8.70	9.92	15.39
CONDITION FACTOR	1.56	1.53	1.61	1.35
SPECIFIC GROWTH RATE (W)	-	1.11	1.24	1.97
SPECIFIC GROWTH RATE (L)	-	0.33	0.37	0.72
FOOD CONVERSION	-	0.21	0.27	0.72
CSI	1.58± 0.08	1.05± 0.06	0.91± 0.05	0.51± 0.03
HSI	2.11± 0.09	2.97± 0.32	2.65± 0.19	1.86± 0.15
RSI	0.68± 0.04	0.70± 0.05	0.86± 0.06	0.69± 0.04
VSI	6.60± 0.26	4.69± 0.27	4.56± 0.21	4.83± 0.54

TABLE 123 EFFECT OF NORMAL GROWTH ON THE PROTEIN, RNA & DNA CONTENT OF MAJOR ORGANS OF CARP.

ORGAN	PROTEIN (mg/100 mg)				RNA / DNA				PROTEIN / RNA				PROTEIN / DNA			
	START	60 DAYS	90 DAYS	120 DAYS	START	60 DAYS	90 DAYS	120 DAYS	START	60 DAYS	90 DAYS	120 DAYS	START	60 DAYS	90 DAYS	120 DAYS
LIVER	13.17± 0.52	26.28± 2.41	31.28± 3.49	19.73± 0.82	5.59± 0.82	5.53± 0.48	8.88± 1.15	3.22± 0.12	12.62± 0.43	17.37± 1.26	16.37± 1.88	22.38± 1.01	69.96± 9.52	95.53± 9.69	142.11± 16.58	72.12± 4.93
KIDNEY	11.82± 0.76	40.81± 3.50	26.19± 2.92	17.60± 1.21	2.18± 0.12	3.52± 0.26	4.14± 0.26	1.73± 0.30	9.32± 0.92	14.41± 0.72	13.16± 1.34	27.08± 1.71	20.05± 1.39	49.58± 2.34	54.34± 6.78	45.41± 4.25
BRAIN	9.43± 0.38	41.36± 3.10	37.24± 1.27	16.65± 3.99	1.97± 0.08	2.73± 0.04	2.24± 0.04	1.54± 0.13	17.92± 0.34	18.81± 1.39	28.66± 0.57	36.56± 2.58	35.31± 1.30	51.56± 4.29	64.14± 1.09	56.08± 5.92
MUSCLE	16.73± 0.91	13.60± 0.75	14.86± 0.59	15.66± 1.46	5.72± 0.39	10.15± 1.40	12.71± 1.87	6.87± 0.06	58.18± 2.75	36.94± 3.28	49.23± 3.15	127.82± 8.28	328.07± 31.39	375.67± 23.41	614.76 ± 69.43	877.21± 53.18

weight of the brain (relative to body weight) decreases as the weight of the fish increases. Liver and kidney weight increased to some extent and then declined, while VSI decreases for sixty days and then becomes steady. Now, if we take into consideration the changes observed in this study, it seems that there was no apparent effect on the CSI, HSI and RSI of the carp after steroid feeding. It appears from the Table 122 that tissue to body ratios depend on weight rather than the age of the fish. So, naturally, in a fast growing fish, the ratio of the organ to body weight will decline. This factor becomes all the more important when it is observed that in cases where there was no growth promotion in the experimental groups the CSI, HSI and RSI were comparable to the control values (see Table 102).

Secondly, it is also possible that increase in growth rate demands a high energy supply and that there is a modification of certain stores (particularly fat) from the tissue to the sight of protein synthesis (muscle) for energy supply, leading to the decrease in weight of these organs. Mobilisation of the fat from the viscera and liver has been reported in salmon by Simpson (1976).

5.1.3. Biochemical Changes

5.1.3.1. Proteolytic Enzymes

It was seen in the experiments with testosterone, 11-ketotestosterone and adrenosterone that feeding of these

steroids increased total proteases activity of the gut. This increase in activity of the gut was directly correlated in at least one case (11-ketotestosterone) to the total body weight and specific growth rate ($r = 0.7482$). After removal of the drug, the concentration of the proteases decreased in all experimental groups as compared with controls (Tables 32, 33 and 34). Whether the activity of the enzyme was a direct effect of steroids on the gut (active enzyme synthesis) or simply a product of increase in size is not clear. Although no study could be traced on the effect of androgens on the digestive enzymes in fish or for that matter in mammals, there are some indirect indications of the role of steroids in the activation of the digestive organs of the fish. For example, Yamazaki (1976) showed that in goldfish treated with 17α-methyltestosterone a marked increase not only in number (hyperplasia) but also in the granulation (hypertrophy) of the acinar cells occurred in the pancreas. The granular cells in the intestine also showed hypertrophy following treatment with methyltestosterone. Yamazaki concluded from these changes that methyltestosterone induces a higher rate of digestion or absorption of the food.

Conversely, the effect on the proteases may be a product of increase in size. It has been shown by Kawai and Ikeda (1971, 1973) that with the increase in size, there is an increase in the activity of the proteases of the gut. In fact, activity of proteases in a four month old carp was

shown to be only 25% of the adult. Recently, Dabrowski and Glogowski (1977) has also shown that the activity of proteases in the gut of carp (30 g) is four to six times higher than the activity of the carp fry gut.

5.1.3.2. Tissue Changes and Growth

Growth of any organism can be defined as protein accretion, and the growth of the whole organism or any organ, may be measured in terms of:

a) weight or liner dimensions;
b) feed consumption and feed efficiency;
c) tissue composition (proteins, fats, water, ash);
d) cell size and cell number (RNA and DNA); and
e) physiological development (protein and fat synthesis and turnover, enzymatic studies and hormone levels),

(Eisen, 1976). In mammals, growth takes place in three phases:

a) prenatal growth due to hyperplasia;
b) early postnatal growth is associated with hyperplasia and hypertrophy;
c) later growth due to hypertrophy.

(Winick and Noble, 1965; Winick, et al., 1972).

As the DNA content of the diploid cells in a species remain constant, and as the DNA is located almost entirely in the nucleus (Mirsky and Ris, 1951), this quantity is used as an index of cell number. Hyperplasia then means that there will be an increase in the total DNA content of

the organ. The ratio, protein/DNA, which is used as an index of cell size will obviously increase with hypertrophy or in older animals (Enesco and Leblord, 1962). There are numerous publications which support this general rule about cellular growth in different organs of the body (Medvedev, 1964; Priestley and Robertson, 1973; Bergen, 1974; Beecher, 1974; Thompson and Heywood, 1974; Sutherland, et al., 1974; Young, 1974, 1976; Eisen, et al., 1978; Millward and Waterlow, 1978; Allen et al., 1979).

As far as the studies on fish are concerned, Love (1970) is of the view that attainment of increased size will bring in changes in the chemistry of the fish. In cod (*Gadus morhua*) Love (1958a) has shown that with increase in size the muscle cell size rather than number increases and there is a direct relationship between the size of the muscle cell to the length of the fish. Love (1970) later on explains that as the fish doubles in length the DNA theoretically should become half of the original values, but this has not been seen, the reason for this is that some DNA is synthesised to control the "out of proportion" increase in cell size (Love, 1958b). This factor becomes all the more important due to the fact that fish continue to grow throughout their life, so there must be some additional synthesis of DNA for mitosis and for controlling the bigger cell size. Luquet and Durand (1970) has shown that increase in DNA content of the rainbow trout muscle during growth was mainly due to hyperplasia. Similar

results have been reported in growing fish (*Notemigonus crysoleucus* and *Lepomis macrochirus*, Bulow, 1970, 1971; *Cyprinus carpio* and *Micropterus dolomieu*, Haines, 1973; *Catla catla*, *Labeo rohita*, *Labeo bata* and *Clarius batrachus*, Mustafa, 1977a, 1977b; *Channa punctatus*, Mustafa and Jafri, 1977).

The most useful measure of the growth process seems to be a measure of the organiser of the protein synthesis i.e. of RNA. A measure of RNA will give us a direct approach to assess the growth rate at a given time. It has, though, been shown that the RNA/DNA ratio is a more accurate index of growth than RNA alone, firstly, because of the individual variations in RNA and DNA levels and secondly, that this ratio is not affected by the size of the tissue taken for analysis (Hotchkiss, 1955). As growth and its rate depends upon the food availability there should be a direct correlation between the food availability and RNA/DNA content of the muscle and other organs of the body. Bulow (1970, 1971) and Haines (1971) studied this possibility in golden shiners, blue gills, carp and smallmouth bass. They observed that with increasing level of food available to the fish, there was an increase in the RNA/DNA ratio in liver, stomach, intestine, anterior and posterior muscle and with overall increase in weight of the fish. These results are further supported by the studies of Bouche et al. (1977), who have shown that starvation decreases the turnover rate and half life of the total

nuclear and ribosomal RNA in carp liver.

In the present study, it was seen that with the increase in total weight and length there was an increase in the RNA/DNA and protein/DNA ratio of the liver, kidney and muscle. Although no study could be traced in fish describing the effect of androgen on RNA and DNA levels, many mammalian studies clearly show that androgens increase the RNA levels in different tissues of the body (DeLoecker, 1965; William-Ashman, 1965; Breuer and Florini, 1965, 1966; Bullock, et al., 1968; Grigsby, et al., 1976; Kochakian, 1975, 1976, 1977; Petrovic, et al., 1977). Treatment with other anabolic hormones, e.g. growth hormone and thyroxine have also been reported to increase the RNA and protein levels of the liver and muscle of the fish (Venogopalan, 1967; Medda and Ray, 1979).

Fish growth, as defined by increase in fish flesh, is mainly accompanied through the synthesis of protein. It is possible to measure both the basic building blocks (amino acids) and their products (proteins). In the present study both amino acids and proteins were determined. Although the amino acids did not give any encouraging results, the proteins were increased in many instances in all the tissues sampled. As far as the amino acids were concerned, the absence of a clear cut response can be due to the wide variations within the samples and the relatively few (four) samples tested. Nevertheless, by looking at

Table 45, it appears that some of the amino acids were lowered as compared with the controls.

Considerable information is available regarding the effect of growth on the protein content of the whole fish and/or different body organs (Love, 1970). It has been stated that the protein level of the muscle increases with growth (Shulman, 1976) in the prematurity stage, as before spawning a lot of energy is channelised towards the gonads, the amount of protein may show certain fluctuations during this time, but after the first or second spawning a sort of steady state is maintained (Mustafa and Jafri, 1977).

The effect of anabolic-androgenic steroids on the growth and protein content of fish has also been studied. Hirose and Hibiya (1968b) studied the effect of 4-chlorotestosterone on the growth of rainbow trout and observed a slight increase in muscle protein content and a decrease in non-protein nitrogen of the blood. But in the majority of cases, McBride and Fagerlund, 1976; Fagerlund and McBride, 1977; Simpson, 1976; Matty and Cheema, 1978; Yu, et al., 1979;, no effect on the muscle proteins was observed. The probable reasons for the difference in results are:

a) species differences; and

b) method of protein determination.

In all the studies reported above the proteins were determined by the Kjeldahl method, by multiplying the

total nitrogen with 6.25. In the present study, the proteins were determined by the Lowry et al., (1951) method and by the Kjeldahl method. The difference in proteins were only encountered when determined by Lowry method, which determines actual proteins and not in Kjeldhal method where nitrogen from amino acids, nitrogen bases and other sources are also determined. Since fish growth is generally due to the enhanced protein synthesis, a possible change in muscle protein content would seem likely and this factor becomes all the more important when growth is considered under the effect of anabolic steroids. Recently, it has been reported by Matty and Cheema (1978) that anabolic steroids (dimethazine and orethandrolone) increase the *in vivo* incorporation of ^{14}C-leucine in muscle proteins of rainbow trout.

In the experiment where total plasma proteins were determined, there was no statistically significant difference between the controls and experimental animals. The protein levels in the plasma in the present study were comparable to the previously reported values in carp (DeSmet, 1978).

It has been argued that muscle lipids increase during growth (Love, 1970) and that cholesterol content of muscle is independent of total lipids content (Wurtziger and Hensel, 1967). In the present study cholesterol was determined in fish treated with methyltestosterone only. It was seen that

while cholesterol decreased in liver, kidney and brain, it increased in muscle after ninety days of the feeding of the drug. As far as total muscle lipids are concerned, they were increased after treatment with methyltestosterone; with other drugs the total lipids either decreased or no effect was seen. Deposition of fat in muscle in response to steroid feeding has been reported in salmonids (Fagerlund and McBride, 1975b, 1977; McBride and Fagerlund, 1976; Higgs, et al., 1977). The reason why methyltestosterone behaved differently compared with other drugs is not know, but probably the deposition of fat in the muscle is a product of feeding level. Because, in methyltestosterone fish were fed rations at 8% of their body weight daily, in all other drugs, the ration level was 5% of the body weight daily.

5.1.3.3. Uptake and Disappearance of ^{3}H-testosterone

After feeding the ^{3}H-testosterone for twelve days, during which time samples were taken at one, two, three, four and twelve days for monitoring the uptake of the label. To study the withdrawal phenomenon, the samples were taken after one, three, five, seven, thirteen and twenty days in fourteen different tissues of the body after cessation of feeding tritiated testosterone. It appears that the steroid is readily absorbed from the gut, because after one day of feeding, the label was maximum in all the sampled tissues. After this initial pulse, the labelling declined and from two to four days the labelling was

nearly equal in all the tissues, showing that isotopic equilibrium had been reached. Isotopic equilibrium is defined as a state, reached by continuous administration of the isotope during which the concentration of the isotope is at is highest and constant level in all the tissues (Jacoby and Hickman, 1966). The fact that the levels of the isotope were higher in some tissues after twelve days of feeding of the label was probably due to the accumulation of metabolites, apart from the parent compound. In this connection, four tissues were most prominent, i.e. liver, brain, gill and muscle, and if we look at the half life of the radioactivity (Table 118) in these tissues, it certainly appears that there might be an accumulation of metabolites.

Metabolic products of the testosterone were not separated in this study. Therefore, it is not possible to demonstrate in what form the steroid was taken up by the cells and what were the metabolites. The metabolic rate of testosterone in fish has received in fact very scanty attention. The only paper that could be traced was that of Yano and Ishio (1978) who reported on the metabolites of testosterone in carp urine. Based on these studies, the authors concluded that in carp the metabolism of testosterone involves the following sequence:

Testosterone

or → androstenedione → androstanedione

Dehydroepiandrosterone androsterone

or

etiocholanone

In the present work after twelve days of feeding when the fish were put on normal diets, the maximum radioactivity was observed to occur in the gall bladder, posterior intestine and liver (Table 116). This observation supports the earlier findings of Schreck (1973) and Fagerlund and McBride (1978) that the entero-hepatic route is the major excretory organ for this steroid.

After removal of the drug, the disappearance of the label was exponential in all the tissues for the first five days. Based on the regression equations, half life of the radioactivity was calculated. Muscle, blood, head kidney and gill had the highest half life. Half life in the liver was 13.36 hours. After three days of the withdrawal the liver showed more or less constant values up to thirteen days, after which the radioactivity decreased. This plateau was probably due to the high metabolic activity of this organ because androgens have been shown to be metabolised efficiently in this organ of the fish (Hay et al., 1976; Inano et al., 1976; Hansson et al., 1979), and also probably due to the entero-hepatic excretion discussed above.

The behaviour of the kidney, gill and skin was different from all other tissues. They accumulated large quantities of the radioactivity at thirteen days. Although in kidney and gills this was a sudden increase, in skin a steady increase was noted, which started three days

after the withdrawal and reached a maximum at the thirteenth day. At this time, the radioactivity in skin was even higher than the zero day values. The reason for this strange behaviour is not know. Why were these organs accumulating the label when it was being actively excreted and when very small amounts were left inside the body? Further experiments are needed to explain this strange behaviour.

5.1.3.4. Probable Mechanism of Action of Anabolic-Androgenic Steroids

There are probably three aspects by which anabolic-androgenic hormones induce growth in fish. These are:

a) increased food conversion efficiency;
b) activation or secretion of other endogenous anabolic hormones;
c) direct effect of these steroids on gene expression in muscle cells.

Improvement of food conversion efficiency after feeding with anabolic steroids has consistently been seen in mammals and fish (Hirose and Hibiya, 1968b; Grigsby, et al., 1976; Simpson, 1976; Matty and Cheema, 1978). The process of increased food conversion in an individual can be achieved via two ways; firstly, there is an increase in the digestive enzymes helping with the digestion of the food and secondly, there is increased assimilation of the digested food. In the present study we have seen that

treatment with testosterone, 11-ketotestosterone and adrenosterone increase the total proteases activity of the gut, and this increase in proteases subsided after removal of the drugs. Hypertrophy and hyperplasia of acinar cells of pancreas and hypertrophy of the granular cells of the intestine has been observed earlier in rainbow trout treated with methyltestosterone. It was argued that this change brought increased digestion and/or assimilation of the food (Yamazaki, 1976).

The role of anabolic steroids (particularly estrogens) in the growth of mammals (ruminants) is fairly well established, and it has been argued that this effect is broughout about by endogenous growth hormone, insulin and thyroid hormones (Preston, 1975; Umberger, 1975; Trenkle, 1976; Trenkle and Burroughs, 1978; McMartin et al., 1978). Androgens have also been shown to increase endogenous thyroid hormones, insulin and prolactin (Grigsby et al., 1976; Herbert et al., 1977).

In fish there is evidence that androgens stimulate the thyroid (Sage and Bromage, 1970; Van Overbeeke and McBride, 1971; Higgs et al., 1977) and insulin from the pancreatic β-cells (Higgs et al., 1977). The role of these hormones in fish growth has been reviewed by Donaldson et al., (1979). In a study of the combination of growth hormone, thyroxine and 17α-methyltestosterone (Higgs et al., 1977) on the coho salmon, it was observed that these hormones acted

additively as far as growth was concerned and it was argued that these hormones mimicked the *milieu interiori* of the fish most conducive for the growth. The same process might be operating in carp.

The effect of anabolic-androgenic steroids on the gene expression in the receptive cells has gained a tremendous importance in the understanding of mechanism of action of these steroids. On the basis of these studies in mammals, a mechanism of action has been described for androgens (Minguell and Sierralta, 1975; Sluyser and Kassenar, 1975; Liao, 1977; Mainwaring, 1977). An important factor in this mechanism is the steroid-receptor complex which transports the active compound to nuclear sites for subsequent processes. Until recently these receptors were not recognised in skeletal muscle (Mainwaring, 1972; Giannopoulos, 1973). However, now it is clear from many studies that muscle do contain the cytoplasmic receptors for androgens, although their concentration as compared to other androgen dependent tissues is very low (Michel and Baulieu, 1974; Dube, et al., 1976; Kreig and Voigt, 1976, 1977; Tremblay, et al., 1977).

Recently, Powers and Florini (1975) has shown that testosterone has a direct effect on the skeletal muscle. They obtained a 25% stimulation by testosterone of the labelling index of rat muscle cells in culture and the average cell cycle time in testosterone treated cultures

was decreased by approximately nine hours as compared to the controls.

The role of androgens in stimulation of RNA polymerase and primining activity of the chromatin has been described (Breuer and Florini, 1965, 1966). The mode of action of growth hormone differs from androgens here at this point, as growth hormone activate only the RNA polymerase activity, this is probably the reason for having an additive effect of growth hormone with androgens. No comparable studies are available for fish, but it can be postulated that probably androgens induce the same type of change also in fish.

CHAPTER SIX

CONCLUSIONS

It is clear from the already published reports and the results of the present study that anabolic-androgenic steroids unlike estrogens, when incorportated in the diet increase growth rate and food conversion efficiency of salmonids and also of cyprinids (goldfish and carp). Although very few species and compounds have been tried in these studies, nevertheless, it appears that work with other species of fish and compounds will also lead not only to the confirmation of these effects in fish but also in better understanding of the role of these compounds in the growth of fish.

In this study, apart from length and weight increases, some biochemical indicators of growth were also studied. The role of RNA and DNA in protein synthesis is well established and this mechanism is considered universal in all animals. Haschemeyer (1978) has described the protein synthetic machinery as it is related to the fish studies. It was seen that cellular growth responses of carp (in the limited time of two-three months) were comparable to mammals. Systematic studies on the cellular responses to growth during the whole life of fish are badly needed.

Before applying these results at commercial levels, these hormones must be tried in hatchery conditions, as the

growth of fish is affected by a whole range of environmental factors from water quality to temperature. Apart from this, studies must be undertaken on the differential food requirements under the effect of anabolic-androgenic steroids with emphasis on different ratios of proteins, fats and carbohydrates.

One very important field of research, in order to understand the mode of action of these steroids is to study the effect of anabolic steroids on the activation and secretion of digestive enzymes and if possible on the gastro-intestinal hormones (secretin, gastrin, etc.).

A preliminary attempt was made to study the uptake and disappearance of the radioactive testosterone from the body of carp. This type of study is badly needed for monitoring the residues of drugs used, and also for identifying the metabolites of the compounds used. This should give further insight into the mechanism of action and physiology of these compounds in fish.

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