SOME ASPECTS OF ENDOCRINE CONTROL OF GROWTH AND PROTEIN METABOLISM IN RAINBOW TROUT, *SALMO GAIRDNERI*

BY

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SUMMARY

The influence of insulin, growth hormone and some synthetic anabolic steroids was studied on protein synthesis, indexed by incorporation of L-(^{14}C-U)-leucine into skeletal muscle protein and growth in rainbow trout. In rainbow trout, insulin tended to increase protein synthesis in skeletal muscle, decrease the excretion of ammonia in ambient water and induce an overall increase in muscle protein content. The incorporation of leucine-^{14}C increased in response to high protein feeding, while short term fasting and protein restriction decreased the incorporation. These changes in protein synthesis are probably related to altered plasma insulin levels during different nutritional states. An attempt was made in vain to measure plasma immunoreactive insulin levels in rainbow trout by using anti-mammalian insulin antibody. Although a single injection of a hypoglycemic sulphonylurea HB 419 (glibenclamide) stimulated protein synthesis, long term administration of glibenclamide and tolbutamide reduced growth rate of rainbow trout. Porcine and selachian growth hormone and rainbow trout pituitary extracts stimulated the rate of protein synthesis in skeletal muscle.

Three synthetic androgens, dimethazine, norethandrolone and methenolone, and an oestrogenic compound, diethylstilboestrol were administered orally at different concentrations in the diet to juvenile rainbow trout and their effect on protein metabolism, growth and food conversion was examined. Both dimethazine and norethandrolone, when given at low concentrations (2.5 and 5.0 mg/kg food) for 60 days significantly increased weight gain. These increases in weight gain were due to both the increased rate of

protein synthesis and improved food conversion efficiency. High concentrations of dimethazine (10 and 20 mg/kg food) produced an initial increase in weight gain but it was not significantly different from that of untreated fish after 48 days. There was no change in the hepatosomatic indices of the fish treated with dimethazine or norethandrolone. However, the renosomatic indices of rainbow trout fed dimethazine were significantly greater than those of the controls, apparently due to an increase in the renal haemopoietic tissue. Methenolone (10 mg/kg food) and diethylstilboestrol (1.2 mg/kg food) tended to depress growth rate and impair food conversion efficiency.

It is concluded that insulin and growth hormone can stimulate protein synthesis in rainbow trout. It appears that insulin plays a dominant role as a protein anabolic hormone in rainbow trout and it may, probably, be true for other carnivorous teleostei. Synthetic androgens, dimethazine and norethandrolone can manifest pronounced proteo-anabolic activity and have potential value as growth promoters in trout culture.

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2. METHODS AND RESULTS

1. INTRODUCTION

There has been a marked trend during the past two decades or so to formulate a sound scientific basis for fish culture in order to reduce the costs and make fish farming a profitable enterprise. The efforts made in this connection have been concentrated mainly around manipulation of environment, diet and feeding strategy to achieve acceleration of growth rate and enhancement of food conversion efficiency. Both growth rate and conversion efficiency can be greatly influenced and regulated within certain limits by controlling some environmental factors such as temperature , salinity and photoperiod. The research into the response of fish to proportioned rations and time feeding based on appetite schedule, qualitative and quantitative changes in the combination of various components in the diet and some ecological and genetic manipulation has led to large improvements in growth rates and food utilization. One area which has received little attention from fish nutritionists is the regulation of growth in fish by controlling the internal environment of the animal through manipulation of the endocrine system. The importance of such an approach becomes more evident when one observes that the greatest advances in the understanding of the growth process in mammals have come from studies of the function of endocrine factors in normal growth and that the application of the information gained from such studies, e.g. the use of sex steroids, has led to a distinct improvement in growth rates and food conversion efficiencies in livestock and veterinary industries. It was with this background that the present study involving the investigation of the effects of insulin, growth hormone, sex steroids and some other growth promoting substances on skeletal muscle protein synthesis and growth rate of rainbow trout, Salmo gairdneri, was undertaken. Protein synthesis in muscle, using incorporation of ^{14}C-leucine as its index was selected for detailed study since skeletal muscle constitutes the largest single tissue in the body; the bulk of the proteins

produced in it remain in situ, and perhaps muscle protein synthesis is in some way representative of somatic growth rate (Jackim and LaRoche, 1973).

A number of hormones seem to have an effect on whole body growth and protein metabolism in skeletal muscle in mammals, but no single hormone has absolute control of growth. Several hormones appear to act in concert to regulate the metabolism of muscle which results in the contributions of the tissue to the metabolic homeostasis of the body and growth within the limits of heredity and physical activity. Insulin, growth hormone and sex hormones were selected for investigation mainly due to two reasons: a. the role of these hormones in promoting growth of the whole animal and increasing the rate of synthesis of all proteins in mammals is well established (Snipes, 1968; Wool, Castle, Leader and Fox, 1972; Scow and Hagen, 1965), a similar role of these hormones was expected in Teleostei, particularly because of high content of protein in the natural diet in most species; b. there were strong indications of the involvement of these hormones in the regulation of various metabolic processes as shown in studies made in various salmonid species during later stages of their spawning migration during which the animals are in a state of restricted feeding and starvation. Several workers observed hypertrophy of pancreatic islets in migratory and non-migratory rainbow trout and Pacific salmon, Oncorhynchus tshawytscha (Hane and Robertson, 1959; Robertson and Wexler, 1960; Robertson, Kruff, Thomas, Favour, Hane and Wexler, 1961) and an increase in plasma growth hormone in migratory sockeye salmon, O. nerka (McKeown and Overbeeke, 1972).

Literature on the effect of insulin, growth hormone and sex steroids is limited to a few species and is fragmentary. Although a concept of hypoinsulinism in fish based on failure of insulin to modulate carbohydrate metabolism in some cases has been put forward by certain workers (Palmer and Ryman, 1972), there is evidence of an

important role of this hormone in regulation of protein metabolism. Sheshadri (1959) noticed an increase in nitrogen content of protein-bound amino acids fraction in skeletal muscle of (=Ophicephalus striatus) Channa striata after insulin injection. The effect of insulin to accelerate the incorporation of ^{14}C-labelled amino acids into skeletal muscle protein has been recorded in some fishes (Tashima and Cahill, 1968; Jackim and LaRoche, 1973; Ahmad and Matty, 1975a; Thorpe and Ince, 1976). Bovine and codfish insulin lowered plasma amino acid nitrogen in Anguilla anguilla and Esox lucius (Ince and Thorpe, 1974; Thorpe and Ince, 1974). The significance of the role of insulin in protein metabolism in fishes is further emphasized from the studies made by Patent and Foa (1971) who demonstrated that leucine (1 mg/ml) stimulated the release of insulin from incubated toadfish islets.

There is some evidence to suggest that mammalian and fish growth hormones have some influence on fish growth (reviews, Pickford and Atz, 1957; Pickford, 1959; Ball, 1969; Chester Jones, Ball, Henderson, Sandor and Baker, 1974). Fish growth hormone has been shown to stimulate linear growth in hypophysectomized male Fundulus heteroclitus (Pickford, 1954; Wilhelmi, 1955; Lewis, Singh, Seavey, Lasker and Pickford, 1972). The ability of mammalian pituitary extracts or hormones to induce growth has been demonstrated in Salmo trutta (Swift, 1954) and Salmo gairdneri (Chartier-Baraduc, 1959; Enotomo, 1964 a,b) Although there are quite a few observations of the function of growth hormone in relation to somatic growth in fishes, these studies are restrictive in nature and provide almost no information about the effect of this hormone on protein synthesis. Matty (1962) has reported nitrogen retention following injection of mammalian growth hormone into intact Cottus which is in agreement with the proteo-anabolic effects of this hormone in mammals (Evans, Briggs and Dixon, 1966; Pecile and Muller, 1968).

Certain steroids such as testosterone, its synthetic analogs and some steroid-like substances, e.g. diethylstilboestrol promote nitrogen conservation and body weight gains in mammals. Consequently these anabolic substances, for example methyl testosterone and diethylstilboestrol (Bidner, Merkel, Miller, Ullney and Hoefer, 1972; Clegg and Cole, 1954) are widely used in veterinary husbandry as a supplement in the food or as a pellet implant. A review of the literature shows that very few studies have been made to assess the anabolic role of sex steroids and similar substances in cultured fishes. Hutchison and Campbell (1964) reported negligible gains from treating Tilapia melanopleura with ethylestrenol. Hirose and Hibiya (1968a,b) noted a significant increase in the growth rate of goldfish and rainbow trout from 4-chloro-testosterone. Bulkley and Swilhart (1973) evaluated the effects of stanozolol on channel catfish and goldfish and recorded only slight improvements in weight gains. Recently Fagerlund and McBride (1975) observed that 17α-methyltestosterone supplementation in the diet of juvenile Coho salmon resulted in a significant increase in length and weight gains. Ashby (1957) noted that estradiol retarded, while progesterone accelerated, the growth of brown trout, Salmo trutta. Diethylstilboestrol, when given with food in low doses improved weight gains, food consumption and food conversion in plaice (Cowey, Pope, Adron, and Blair, 1973). Bulkley (1972), however, failed to achieve any weight gains in catfish with this oestrogen-like substance and reported a negative effect on protein synthesis confirming similar findings by Hoar (1958) in goldfish. Most of the workers mentioned above paid little attention to studying any change in protein metabolism under the influence of hormonal treatment.

The present studies were carried out to assess further the effects of insulin, insulinotrophic substances such as tolbutamide and glibenclamide, mammalian and fish growth hormone and pituitary extracts, some synthetic androgens and diethylstilboestrol on growth rate and protein

synthesis in rainbow trout. Also, an attempt was made to establish correlation between the rate of protein synthesis and insulin status of the fish by investigating the protein synthesis during high and low protein feeding and starvation. In addition to this, the effects of treating rainbow trout with goldthioglucose were examined.

1.1. THE PHYSIOLOGICAL ROLE OF INSULIN IN FISHES

Although the peripheral effects of islet secretions in mammals are well understood, the situation in fishes is at present vague and involves a number of problems and furthermore, the solutions to these problems are difficult to ascertain. The various methods of deducing the physiological role of insulin in fishes include the study of different physiological parameters after: a. the destruction of β-cells by employing alloxan or streptozotocin, b. islectomy and total or sub-total pancreatectomy, c. exogenous hormone treatment and metabolite loading, d. stimulation of endogenous insulin release using derivatives of sulphonylurea such as tolbutamide and glibenclamide.

Although hyperglycemia concurrent with β-cell destruction from alloxan administration to fish has been shown by a number of workers (Murrel and Nace, 1959; Chavin and Young, 1970b), this induction of hypo-insulinemia may be complicated by the toxic effect of alloxan at loci other than the islets and furthermore, any metabolic alterations may be transitory (Chavin and Young, 1970b). Several workers noticed a damaging effect of alloxan on liver, kidney and exocrine pancrease at doses which were effective to produce alterations in the morphology of β-cells (Epple, 1969). In view of these observations, it is difficult to draw any firm conclusion of the physiological role of insulin in fishes using β-cell cytotoxin, alloxan. Among other β-cell cytotoxins, streptozotocin has been tested in fewer species, and is known to have little if any effect on β-cell cytology of Anguilla rostrata (Brinn and Epple, 1972) and Gadus callarias (Thomas, 1971)

The concentration of pancreatic endocrine tissue into one discrete organ, the "principal islet" in some species of Teleostei and Cyclostomata prompted some workers (Chidambaram, Meyer and Hasler, 1973; Hardisty, Zelnik and Moore, 1975; Falkmer and Matty, 1966a, b; Falkmer, 1961, Schirner, 1963 , McCormick and Macleod, 1925; Simpson, 1926) to investi-

gate the physiological effects of islets after isletectomy. Isletectomy provides a useful tool in the hand of the fish physiologist but it is only of limited application.

The administration of exogenous insulin to study the endocrine effects has been a standard method but information received from such investigations needs careful interpretation because several factors such as purity of hormones (glucagon content), route of administration, dosage and species specificity of hormone, nutritional state of the animals and ecological conditions might influence the results.

The sulphonylurea hypoglycemic agents, tolbutamide and glibenclamide are known to exert their hypoglycemic action through stimulation of β-cells (Campbell, 1968; Breidahl, Ennis, Martin, Stawell and Taft, 1972). These drugs have also been employed in fishes to ascertain the role of insulin. However, it is doubtful whether the mechanism by which sulphonylureas exert their hypoglycemic action in mammals is also present in fish (Palmer and Ryman, 1972).

Besides the methods discussed above, the measurement of endogenous immunoreactive insulin under various natural and experimental conditions, and the study of the spontaneous diabetic state in the Japanese carp (Yokote, 1970a, b,c,d,e; Nakamura, Yamada and Yokote, 1971) to ascertain the role of islet physiology in fish has attracted some attention. The application of radioimmunoassay techniques, widely used in mammals, has not provided satisfactory results due to immunological differences between various vertebrate insulins (Tashima and Cahill, 1968). Although the spontaneous diabetic state in the Japanese carp exhibits decreased sensitivity to mammalian insulin and manifests symptoms similar to human diabetes such as hyperglycemia, altered glucose tolerance, lowered blood pH, glucosuria and ketonuria, further work is necessary in order to determine the usefulness of these animals as models for studying teleost islet physiology (Brinn, 1973).

1.1.1. CARBOHYDRATE METABOLISM

Several workers have investigated the role of insulin in modulating carbohydrate metabolism in fish by examining the effect of various treatments such as the administration of exogenous insulin, alloxan and tolbutamide, islectomy, pancreatectomy, and glucose loading on blood glucose or tissue glycogen content.

1.1.1.1. EFFECT OF EXOGENOUS INSULIN

BLOOD GLUCOSE:

The action of exogenous insulin on blood glucose in various chondrichthyean and teleost species is shown in Table I. The hypoglycemic response to insulin is virtually ubiquitous in cyclostomes (Bentley and Follett, 1965; Falkmer and Matty, 1966a; Plisetskaya, 1967; Leibson and Plisetskaya, 1967; Morris and Islam, 1969; Leibson and Plisetskaya, 1968; Falkmer, Emdin, Haveur, Lundgren, Marques, Ostberg, Steiner and Thomas, 1973), in chonrichthyans (Grant, 1964; Leibson and Plisetskaya, 1967, 1968; Patent, 1968, 1970; Grant et al. 1969) and actinopterygrians (Mann, Bollman and Magath, 1924; Olmstedt, 1924; Collip, 1925; McCormick and Macleod, 1925; Simpson, 1926; Gray, 1928; Gray and Hall, 1930; Root, Hall and Gray, 1931; Bruun and Hemmingsen, 1938; Vorhauer, 1938; Algauhari, 1958; Falkmer, 1961; Falkmer and Wilson, 1967; Leibson and Plisetskaya, 1967, 1968; Yanni, 1964; Seshadri, 1967; Young and Chavin, 1967; Khana and Mehrotra, 1969 ; Tashima and Cahill, 1968; Young, 1970; Wardle, 1972; Ince and Thorpe, 1974; Thorpe and Ince, 1974; Inui and Yokote, 1975b; Palmer and Ryman, 1972), though there appears to be great variability in species sensitivity to mammalian insulin. Whereas dogfish, ratfish (Patent, 1970), Anguilla japonica (Inui and Yokote, 1975b) and Raja erinacea (Grant, 1964) readily respond to a dosage of 5 U/kg, the teleost, Cottus scorpius (Falkmer, 1961) and the Atlantic hagfish, Myxine glutinosa (Falkmer and Matty, 1966a) respond slowly to doses of 1000 - 3000 U/kg. The fish's own insulin, however, is effective at con-

TABLE 1.: EFFECT OF EXOGENOUS INSULIN ON BLOOD GLUCOSE LEVELS IN FISHES

Species	Insulin injected	Dose and Route	Effect on Blood Glucose	References
Toadfish (Opsanus tau)	mammalian	100 U/kg	0	Tashima and Cahill (1964)
	Beef-Pork	" "	-	" (1968)
Goldfish (Carassius auratus)	Bovine	1 U/kg	- - -	Young and Chavin (1967)
	Bonito	"	- - -	"
	Tuna	"	- - -	"
	Hydrolagus	"	- - -	"
	Squalus	0.5 U/kg	- -	"
	Trypsin treated	10 U/kg	- -	Young (1970)
	Bovine (glucagon content 0.0003%)	1-100 U/kg 1000+5000+	- 0	" " " "
	Bovine(glucagon content 0.8%)	1 and 10 U/kg 100 U/kg 1000-5000 "	- - 0 + +	" " " " " "
Scorpion fish (Scorpaena porcus)	Mammalian			
Carp (Cyprinus carpio)	"	20-60 U/mg	- -	Leibson and Plisetskaya (1967 and 1968)
Goby (Gobiidae sp.)	"	"	- -	"
Sea bass (Spicara smaris)	"	"	- -	"
Horse mackerel (Trachurus mediterraneus ponticus	"	"	- -	"
Eel (Anguilla anguilla	Codfish	2 U/kg(I.C)	- -	Ince and Thorpe "(1974)
	Bovine	" " "	0	
Pike (Esox lucius)	Bovine	50,25, 10 U/kg (I.C)	- - -	Thorpe and Ince (1974)
		2 U/Kg	- -	"
	Cod fish	2 U/kg (I.C)	- - -	"

Table 1. (continued)

Species	Insulin injected	Dose and route	Effect on Blood glucose	References
Eel (Anguilla japonica)	Pork	5 U/kg	- -	Inui and Yokote (1975b)
Rainbow trout (Salmo gairdneri)	mammalian	4 U/fish(I.C) + 1 g glucose	-	Palmer and Ryman (1972)
Eel (Clarias lazera)	"	10 U/kg	-	Yanni (1964)
	"	10 " + glucose 4 g/kg	-	"
	"	4 U/kg (sub.cut)	-	Al-Gauhari (1958)
Sculpin (Cottus scorpius)	ox	1,5 U/kg (I.M)	0 0 (fed fish)	Falkmer and Wilson, 1967
		20,100 " "	- -(Fasted)	"
		1000 " "	0 (fed)	"
	cod	1,5,20 " "	0 (fed)	"
		100 U/kg "	- - (fasted)	"
	sculpin	1,5,20 " "	- - (fasted)	"
Dogfish (Squalus acanthias)	Bovine	5 U/Kg(I.U)	- -	Patent (1967 and 1970)
	dogfish	0.3 " "	- -	" "
	ratfish	2 " "	- -	" "
Ratfish (Hydrolagus colliei)	Bovine	5 U/kg(I.U)	- -	" "
	dogfish	0.3 " "	- -	" "
	ratfish	2 " "	- -	" "
Skate (Raja erinacea)	Bovine	5 U/kg (I.M and I.U)	- -	Grant (1964)
	"	0.2 mg/kg)	- -	Grant, Hendler and Banks (1969)
Skate Trygon(=Dasyatus) pastinaca	mammalian	20-60 U/kg	- -	Leibson and Plisetskaya (1967 and 1968)
				Leibson, Plisetskaya and Stabrovski (1963)

siderably lower doses than the mammalian preparations (Falkmer and Matty, 1966b; Falkmer and Wilson,1967; Thorpe and Ince, 1974; Ince and Thorpe, 1974). There are also indications of the species specificity of the hormone among different groups of fishes (Young and Chavin, 1967; Young, 1970; Patent, 1967, 1970). A number of factors, e.g. nutritional state of the animal (Falkmer and Wilson, 1967), habitat of the species (Patent, 1970) and purity of the hormone i.e. glycogen content (Young and Chavin, 1967) influence the glycemic levels. In general, the hypoglycemic responses to exogenous insulin in fishes are noted to be slow, the peak of hypoglycemia or convulsions occurring 1 or 2 days after injection (Brinn, 1973; Epple, 1969). Seshadri (1967), however, reported a mammalian-like response in (=Ophicephalus striatus) Channa striata with return to normal blood sugar levels within three hours.

Tashima and Cahill (1964) could not observe any hypoglycemia in the toadfish, Opsanus tau, at a very high dosage of bovine insulin. However, in a later study (1968) 100 U/kg dose of beef-pork insulin was shown to have a hypoglycemic action in toadfish caught during fall but not in the early summer. Insulin accelerated the disappearance of U-^{14}C glucose from the blood and increased the incorporation of glucose carbon label into muscle glycogen.

These different responses of fish to insulin, according to Thorpe and Ince (1974) could be due to differences in the biological half-life of the administered insulin. They also emphasized the initial blood glucose value of the animal under test and suggested that insulin-induced hypoglycemia could be more difficult, or even impossible to detect in those species which have low resting levels of blood glucose. Leibson and Plisetskaya (1968) related the variations in responses to insulin in fish to their phylogenetic position and mode of life. According to these workers there is a trend for a reduction in the duration of the hypoglycemia from the cyclostomes through cartilaginous fishes to teleostei.

They also considered the mobility of the fish to be an important factor in determining its response to insulin, quoting the bottom-dwelling scorpion fish, Scorpaena porcus, in which hypoglycemia lasts longer than in the more active sea-bass, Spicara smaris. Gray (1938) and Gray and Hall (1930) had earlier suggested that insulin sensitivity may be dependent upon mobility.

GLYCOGEN CONTENT

The effect of exogenous insulin administration on the glycogen content of muscle and liver in Chondrichthyes and Teleostei appears to be correlated with the animals' mode of life (Patent, 1970). In Squalus acanthias and Hydrolagus colliei, liver glycogen content was unaffected by insulin. However, there was a small amount of glycogen deposition in Squalus muscle. Leibson et al. (1963) and Leibson and Plisetkaya (1968) reported glycogen deposition in liver and muscle of Trygon (=Dasyatus) pastinaca after insulin injection. The effect on liver glycogen content was variable (Leibson et al. 1963). In Teleostei the effect of insulin on glycogen content in liver and muscle varies considerably. Root et al. (1931) noticed an increase in liver and muscle glycogen in Stenotomus chrysops. However, Tashima and Cahill (1964) failed to observe any effect of insulin (0.1 U/ml) on U-^{14}C-glucose incorporation into heart, liver and skeletal muscle in vitro in toadfish. In Ophicephalus striatus repeated injections of insulin (5 U/fish) caused a maximum increase in liver and muscle glycogen content within 90 minutes (Seshadri, 1967). In Clarias lazera, Yanni (1964) observed that the effect of insulin (0.01 U/g) on glycogen deposition in various tissues becomes more pronounced in the presence of glucose. A higher dose of insulin (0.02 U/g) in the presence of high levels of glucose or insulin alone in a smaller dose (0.01 U/g) produced a decrease in liver glycogen content. These observations suggest that at least in some Teleostei higher levels of insulin

may cause hepatic glycogenolysis to provide substrate for enhanced glycogenesis in muscle and other tissues. Among cyclostomata, the reports on effects of insulin on liver glycogen levels appear to be far from convincing and this may be attributed to the vast variations found in glycogen and lipid contents in the liver of different sexes (Hardisty et al., 1975). In lampreys, mammalian insulin led to an increase or ambiguous behaviour of liver glycogens; it caused little or no increase in muscle glycogen (Bentley and Follet, 1965; Leibson and Plisetskaya, 1967). In Petromyzon, exogenous insulin caused a decrease of the high glycogen content of the brain (Plisetskaya, 1967). In Myxine, a decrease in the liver glycogen level was noticed after insulin treatment (Falkmer and Matty, 1966a). Although in experiments on river lamprey, conducted over four successive years, Leibson and Plisetskaya (1968) obtained evidence of a rise in liver glycogen levels in one season only, the same authors (Plisetskaya and Leibson, 1973) however, detected increase glycogen synthetase activity in the liver of the adult Lampetra fluviatilis after the administration of insulin and a decrease in liver glucose with raised glycemia levels was reported after treatment of river lampreys with an anti-lamprey-insulin serum (Plisetskaya and Leibush, 1972). The failure to observe similar responses in Myxine (Falkmer and Wilson, 1967) may be due to the use of antisera to mammalian insulin.

1.1.1.2. EFFECT OF β-CELL CYTOTOXINS AND ISLETECTOMY

ALLOXAN:

An indication of the role of endocrine pancreas in carbohydrate metabolism in fish is evident in studies dealing with induction of "alloxan diabetes" in fishes. Although diabetogenic action of alloxan has been shown by a number of workers in cyclostomata (Ermisch, 1966; Windbladh and Biuw, 1970), Chondrichthyes (Saviano, 1946, 1947a; Clausen,

1953, Patent, 1968) and teleostei (Saviano, 1947b; Lazarow and Berman, 1947; Doerr, 1950; LaGrutta,1950;Grosso, 1950; Mosca, 1959; Clausen, 1953; Schatzle, 1954; Nace, 1955, 1960; Murrel and Nace, 1959; Nace, Schuh, Murrel and Dingle, 1958; Nace, Yip, Silkovskis, Moule and Moule, 1959; Falkmer,1961; Falkmer and Olsson, 1962; Moule and Nace, 1963; Young and Chavin, 1963, 1966; Chavin and Young, 1970b; Khana and Mehrotra, 1968, Minick and Chavin, 1972b; Inui and Yokote, 1975a), considerable variation has been reported about the effect of the drug on β-cell morphology. Among cyclostomata only high doses of alloxan were found to destroy β-cells in lampreys (Ermisch, 1966; Winbladh, 1967) and hagfish (Falkmer and Winbladh, 1964). In hagfish, there was no correlation between β-cell destruction and hyperglycemia. In the Chondrichthyean group, Selachii, intraperitoneal injection of alloxan produced hyperglycemia but no islet damage (Saviano, 1946). However, Kern (1966) was unable to produce any alterations in the blood sugar level or the islet organs in Scyliorhinus canicula, Raja asterias and Torpedo mormorata. Similarly Grant et al. (1969) did not observe any change in blood glucose levels in Raja erinacea and suggested that this could be due to rapid deterioration of alloxan before reaching the islet tissue. Subcutaneous administration of low doses of alloxan caused β-cell destruction in Scyliorhinus canicula (Clausen, 1953). Similarly in ratfish, Hydrolagus colliei, Patent (1968) noticed selective damage of β-cells after injection of alloxan (300 mg/kg). The actions of alloxan upon islet tissue in teleostei are variable. No apparent effects are produced in some species (Doerr, 1950; Pallot and Schatzle, 1953) while β-cells degeneration may occur in other species (Lazarow and Berman, 1948; Grosso, 1950; Nace et al., 1958; Murrel and Nace, 1959; Falkmer, 1961). Alloxan has been shown to induce necrotic changes in liver, kidney and exocrine pancreas (Saviano, 1947b; Doerr, 1950; Grosso, 1950; Mosca, 1959; Schatzle, 1954; Murrel and Nace, 1959; Falkmer, 1961; Young and Chavin, 1966; Chavin

Young, 1970b). Although alloxan has been shown to produce hyperglycemia in most Teleostei, the time of onset, duration and severity of the hyperglycemic response were variable (Saviano, 1947a; Lazarow and Bergman, 1947, 1948; Murrel and Nace, 1959; Nace, 1955; Falkmer, 1961). It appears that the inconsistency observed in these studies may have originated in the extreme instability of alloxan at physiological pH, its toxicity for tissues other than β-cells of the islets, and the inherent variability in the response of different species to alloxan. As pointed out earlier, the alloxan-induced hypoinsulinemia may be complicated by the toxic effect of alloxan at loci other than the islets, therefore information received from these investigations does not permit any firm conclusions to be made regarding islet physiology.

ISLETECTOMY

In most cases, surgical removal of islet tissue or isletectomy in fish has been reported to enhance blood glucose levels, though there are considerable species differences in the isletectomy-induced hyperglycemia (Epple, 1969). In Cottus scorpius, the occurrence of hyperglycemia was more evident in fed than in starved animals; insulin alleviated hyperglycemia (Falkmer, 1961; Falkmer and Matty, 1966b). In other teleosts hyperglycemia, glucosauria and an increase in lipids occurred (McCormick and Macleod, 1925; Simpson, 1926). In Ictalurus melas, hyperglycemia was unmistakable in all isletectomized fish (Chidambaram et al., 1973). These findings indicate a positive role of islet hormones in homeostasis of blood glucose in teleostei. In hagfish, Myxine glutinosa, isletectomy had no effect on the blood sugar level (Schirmer, 1963; Falkmer and Matty, 1966a). However, in lamprey (Lampetra fluviatilis), total extirpation of islet tissue caused a significant increase in blood glucose levels (Hardisty et al., 1975). The later report is in agreement with that of Barrington (1942) who observed hyperglycemia in the larval Petromyzon marinus, unicolor, after destruction of the

islet tissue by cautery. The discrepancy in response of lamprey and hagfish to removal of islet tissue might be the result of scattered β-cell -like elements in the bile duct and possibly also in the gut of the later species (Falkmer and Matty, 1966a).

PANCREATECTOMY:

The glycemic response of fish to pancreatectomy also exhibits species differences. The removal of pancreas caused hyperglycemia in elasmobranchs (Diamare, 1906; Diamare and Montuori, 1907) and eel (Lewis and Epple, 1972). Grant (1964) did not observe any change in blood glucose levels in pancreatectomized skate, Raja erinacea. In a later communication, however, Grant et al. (1969) reported the occurrence of hypoglycemia following pancreatectomy in this species and suggested that blood sugar regulation in skate might be primarily mediated by glucagon.

1.1.1.3. EFFECT OF GLUCOSE LOADING

As reviewed in the preceding pages, the carbohydrate metabolism in fish seems to resemble that of mammals in the sense that blood glucose is subject to control by exogenous insulin, pancreatectomy, islectomy or treatment with islet cytotoxins. The widely reported observation that fish are generally intolerant to high levels of administered glucose (Epple, 1969) indicates a fundamental difference between carbohydrate metabolism in fish and that in mammals. In mammals, it is well established that glucose loading results in an initial hyperglycemia which is rapidly disposed of due to endogenous insulin release ;the increase in insulin synthesis due to glucose stimulation has been shown both in vitro and in vivo (Metz, 1958; Randle, 1966; Hahn, Lippman, Knopse and Michael, 1970; Hermansen, Pruett, Osnes and Giere, 1970; Hellman, 1970). In fish, Young and Chavin, (1965) reported a short mammalian like response to glucose injections in goldfish, Carassius auratus,kept at a rather high temperature (25°C), all other investigators observed pronounced and persistent hyperglycemia following

glucose administration, with a rather slow return to normal levels. In elasmobranchs, Raja erinacea (Hartman, Lewis, Brownell, Angerer and Shelden, 1944; Grant, 1964), Squalus acanthias (Patent, 1967 and 1970; Oppelt, Bunim and Rall, 1963) in the holocephalian, Hydrolagus colleie (Patent, 1967, 1970), and in the teleosts ,Cottus scorpius,(Falkmer, 1961), trout (Phillips, Tunison and Brockway, 1948; Palmer and Ryman, 1972), Clarias batrachus (Khana and Mehrota, 1969; Bhatt, 1974), Clarias lazera (Yanni, 1961, 1964), Heteropneustes fossilis (Khana and Rekhari, 1972), Esox lucius (Thorpe and Ince, 1974) and Anguilla anguilla (Ince and Thorpe, 1974), glucose tolerance was considerably lower than in mammals and glucose levels did not return to normal before a period of 9 hours to several days. A similar response to glucose loading was reported in the case of cyclostomes (Bentley and Follett, 1965; Falkmer and Matty, 1966) and other poikilotherms (e.g. Coulson and Hernandez, 1953; Wright, 1959; Penhos, Wu, Reitman, Sodero, White and Levine, 1967). As pointed out by Hardisty et al, 1975, the large individual variations in blood sugar levels especially in the early periods after glucose loading might be attributed to a number of factors, among them the rate of uptake of the injected glucose into the extracellular compartment, the intensity of insulin secretion, metabolism of glucose, the storage of glycogen in the liver or the rate of renal excretion of glucose.

1.1.1.4. CONCEPT OF HYPOINSULINISM

The incidence of glucose intolerance in fish has been suggested to be the result of insufficient circulating insulin (Falkmer, 1961; Palmer and Ryman, 1972) or due to proportionately low carbohydrate content in the natural diet (Tashima and Cahill, 1968). This concept of hypoinsulinism is further supported by the effect of exogenous insulin administration. Insulin, when administered in conjunction with glucose, markedly improved tolerance (Palmer and Ryman, 1972;

Hendler, 1965). Further the inability of tolbutamide to modulate carbohydrate metabolism in goldfish, Carassius auratus, (Kohler and Lippman, 1963) and rainbow trout, Salmo gairdneri (Palmer and Ryman, Ryman, 1972) was also considered to imply a low insulin status and total exhaustion of endogenous insulin supplies.

It seems rather drastic to imply a low insulin status in fish, however the primary importance of insulin in glucose homeostasis in fish appears to be somewhat questionable (Tashima and Cahill, 1968; Falkmer and Wilson, 1967). Contrary to the observation that glucose stimulated the release of insulin from islet tissue in vitro in Opsanus tau (Watkins, Leonards, Dixit, Cooperstein and Lazarow, 1964; Patent and Foa, 1971) , Tashima and Cahill, (1968) could not observe any change in immunoreactive insulin after glucose loading in the same species. Falkmer and Wilson (1967) were unable to provoke hyperglycemia in Cottus scorpius with highly potent guinea pig and horse antisera to ox-insulin. Although the potency of antisera to mammalian insulin to neutralize endogenous insulin in fish is questionable due to immunological differences between the fish and mammalian insulin, Falkmer and Wilson (1967) found enough evidence from their observations of the physiological effects of various vertebrate insulins to suggest that "insulin may be involved only to a small extent in the blood sugar regulation of teleosts". Lewis and Epple (1972), finding that partial pancreatectomy of the eel did not produce a hyperglycemia concluded that "a strict homeostatic control of glucose, as in mammals, is not present, at least in many species". The reported blood sugar values of 0 mg% or almost 0 mg% in goldfish (Chavin and Young, 1970a) and the fact that Yokote (1970c) induced a blood glucose level of 0 mg% in normal carp without observing convulsions suggests that glucose may not be the main energy source in fish. This observation together with the findings that in Anguilla anguilla bovine insulin at 2 mg/kg lowered the plasma

amino acid nitrogen without affecting the plasma glucose (Ince and Thorpe, 1974) and that various vertebrate insulins induced a decline in FFA and phospholipids in Carassius auratus (Minick and Chavin, 1972a) indicates a much wider role of insulin in fish, particularly in relation to lipid and protein metabolism.

1.1.2. INSULIN AND PROTEIN SYNTHESIS

The role of insulin in the control of protein metabolism has not been as widely appreciated as its effect on glucose and fat metabolism. An investigation into this subject is of particular importance in fish in which a. the growth of body, the bulk of which is protein, continues throughout life, and b. skeletal muscle, unlike that in mammals, serves as a primary fuel depot in addition to its role in locomotion. During a period of undernutrition protein is lost from the skeletal muscle, which contains up to 80% of total amino acids, and provides amino acids for protein synthesis in other tissues and gluconeogenic substrate to provide energy for those tissues metabolizing glucose. Thus, the role of skeletal muscle, the largest tissue in the body, in maintaining metabolic homeostasis and growth of body is very important in fish. In view of the aforementioned reasons and also since the major content of the natural diet of the majority of fishes is protein, the assessment of the actions of insulin on protein synthesis in fish is expected to carry a great deal of significance.

1.1.2.1. ROLE OF INSULIN IN PROTEIN SYNTHESIS IN MAMMALS

In mammals, insulin has been shown to influence nitrogen metabolism in three ways. They are (1) the lowering by insulin of blood amino acid levels, (2) the stimulation of the incorporation of labelled amino acids into protein both *in vivo* and *in vitro*, and (3) stimulation of the synthesis of various specific enzymes that play important roles in regulating the direction of metabolism. These last effects are largely in the liver and also in adipose tissue, but do not appear to occur in kidney and have not been demonstrated for identifiable enzymes in muscle (Manchester, 1970a). In this review, therefore, the current information on the role of insulin in relation to the first two effects has been summarised. As first suggested by Fuller Albright (1942 - 43) the hormonal effects on

nitrogen metabolism can be separated into anabolic and catabolic components, therefore an attempt has been made to distinguish insulin's effects on protein metabolism into these two categories. The effect of insulin in triglyceride metabolism in adipose tissue demonstrates both these actions (Cahill, Aoki and Marliss, 1972). In high physiological doses, insulin augments glucose uptake and lipogenesis, constituting an anabolic effect, whereas in low as well as high physiological doses, it exhibits anticatabolic effect by inhibiting lipolysis.

A. INSULIN AND PROTEIN ANABOLISM

It has been shown that in mammals, the stimulatory effect of insulin on protein synthesis involves an increase in the transport of amino acids into skeletal muscle cells and a simultaneous stimulation of the incorporation of the amino acids into peptide chains (Snipes, 1968).

(i). ACCUMULATION OF AMINO ACIDS BY MUSCLE

Although free amino acids constitute 0.5% of total body source of amino acids (Munro, 1970), they play a key role in overall nitrogen metabolism since it is in this form that most of the metabolically important nitrogen is absorbed from the intestinal tract and the form in which it is transported to and from the various organs. Since most cell membranes contain an active transport system for amino acids, the concentration of individual amino acids in the intracellular water is generally higher than the plasma. There is sufficient evidence to indicate that insulin might influence the flux of free amino acids between the major compartments in the body (Lacy, 1973). The lowering by insulin of blood amino acid levels has been shown by a number of workers (Carlsten, Hallgren, Jagenburg, Svanborg, Werko, 1966; Crofford, Felts and Lacy, 1964; Debarnola, 1965; Harris and Harris, 1947; Swenseid, Tuttle, Drenick, Joven and Massey, 1967; Zinneman, Nuttall and Goetz, 1966). Most of them assumed that insulin worked by enhancing muscle uptake of amino acids, supporting the hypothesis of Russel (1955) which she derived from her

earlier experiments (Frame and Russel, 1946), showing that insulin prevented the rise in alpha amino-nitrogen levels in eviscerated rats. The above mentioned studies described marked sensitivity of the branched chain amino acids (valine, leucine and isoleucine) to insulin. Crofford et al.(1964) achieved similar results by utilizing glucose infusion as a stimulus to endogenous insulin secretion and demonstrated a significant decline for 6 essential amino acids - valine, leucine, isoleucine, threonine, phenylalanine and tyrosine. The influence of the accumulation of amino acids in the muscle was studied on non-metabolizable amino acids. The transport of non-metabolizable amino acids, α-aminoisobutyric acid and cycloleucine, into muscle was observed to be stimulated by insulin (Kipnis and Noall, 1958; Manchester and Young, 1960a; Akedo and Christensen, 1962; Manchester and Wood, 1963; Arvill and Ahren, 1967a, b; Guidotti, Borghetti, Gaja, Loreti, Ragnotti and Foa, 1968). This also helped to disassociate the effects of insulin on transport of amino acids from its effects on protein synthesis, i.e. incorporation of amino acids into peptide chains in the normal cells. Among the natural amino acids, the accumulation of glycine, proline and methionine in the muscle was enhanced by insulin, but, with the possible exception of serine, threonine, histidine and alanine, the accumulation of none of the others was observable (Manchester, 1970a). This is in contrast to their incorporation into protein , which insulin enhances in every case. There seems to be no obvious reason that might explain why the accumulation of glycine, proline, alanine, serine, histidine and methionine is influenced by insulin whereas uptake of other amino acids is not (Manchester, 1970a). As pointed out by Cahill et al. (1972) one of the problems in this connection could be in characterizing the process, since many amino acids, particularly the branched-chain group, are metabolized directly in the muscle (Manchester, 1965) and these may be actively accumulated and subsequently oxidized. Manchester (1970a,b,c), Munro (1970), Wool (1969)

and Wool, Stirewalt, Kurihara, Low, Bailey and Oyer (1968) have reviewed this topic extensively. Another factor which makes characterization of intracellular accumulation of amino acids even more difficult is the incorporation of labelled extracellular amino acids into muscle cell protein directly from extracellular pool. Hider, Fern and London (1969) demonstrated the incorporation of labelled extracellular amino acids into protein in the extensor digitorum longus muscle of rats without equilibration with the intracellular pool of amino acids. The independent nature of the action of insulin on transport of amino acids into the muscle cells has been demonstrated by inhibition of protein synthesis by actinomycin D, puromycin, or cycloheximide (Castle and Wool, 1964; Wool, Castle and Moyer, 1965; Manchester, 1970b; Scharff and Wool, 1965). The inhibition of protein synthesis permitted insulin to initiate amino acid accumulation. It has been concluded by Cahill et al. (1972) that de novo protein synthesis is not needed for the insulin effect on the cell membrane for amino acid accumulation, nor is removal of intracellular amino acids by their incorporation into protein necessary.

(ii). INCORPORATION OF AMINO ACIDS INTO PROTEIN

The first evidence of direct control by insulin of protein synthesis was furnished by Krahl (1952, 1953) and Sinex, Macmuller and Hastings (1952) who demonstrated that insulin enhanced the incorporation of labelled amino acids into rat diaphragm muscle in vitro. Insulin, since then, has been shown to stimulate the incorporation of all the naturally occurring amino acids into protein (Manchester, 1970a). Manchester and Young (1958) and Wool and Krahl (1959) showed that increased incorporation of labelled amino acids into protein was not dependent on glucose. The presence or absence of glucose in the medium neither ameliorated nor adversely affected the stimulatory effect of insulin on the incorporation of amino acids into protein. Manchester (1970a) inferred from these observations that the action of insulin on amino acid incorporation did not appear to depend on stimulation by the hormone

of the glucose uptake, and furthermore, that availability of energy derived from glucose catabolism was not specifically important for protein synthesis or its enhancement by insulin. The independence of insulin action on protein synthesis from effects on sugar metabolism was also shown in an investigation carried out by Battagalia, Manchester and Randle (1960) in which inhibition of glucose uptake by phloridzin was seen to be without any effect on glycine incorporation into protein. Converse to this, Manchester (1970a) demonstrated that inhibition of protein synthesis by cyclohexamide in rat diaphragm *in vitro* did not affect insulin's effect on stimulation of glucose uptake and glycogen synthesis; thus confirming the mutual independence of effects of insulin on carbohydrate and protein metabolism.

Although it is well established that insulin is capable of accelerating both amino-acid transport into the cells (*in vitro* as well as *in vivo*) and can independently increase the incorporation of intracellular amino acids with protein, it is not possible to state with certainty which of these processes is quantitatively more important. This would depend on which process is rate limiting under a given process. Unlike glycogen synthesis in muscle, in which intracellular free glucose level is normally low (Randle and Morgan, 1962) and the rate of transport of glucose into the cell is thus a potentially rate-limiting step in allowing glycogen synthesis to proceed, it is difficult to ascertain the rate-limiting process in protein synthesis in muscle. However, there are several points which suggest that the transport step is not normally the predominant point of action of insulin (Lacy, 1973). The magnitude of increase in the accumulation of amino acids within the cell due to the influence of insulin is small and in many cases it can only be demonstrated after inhibition of protein synthesis by puromycin (Wool et al., 1965). Furthermore, the intracellular accumulation of all the amino acids is not enhanced by insulin. These observations

appear to minimize the importance of the amino acid transport step. Therefore, Lacy (1973) regarded stimulation of protein synthesis as a major site of action of insulin at the cellular level in muscle with its effects on amino acid transport serving a facilitative role. Recently Singh, Chausouria and Udupa (1975) have confirmed this view. They demonstrated that insulin stimulated transport of glycine-^{14}C into normal rat muscle was independent of its incorporation into protein in the early phase, but in the later stage, acceleration of the transport of the amino acid was dependent on synthesis of some protein, perhaps a membrane carrier.

B. INSULIN AND PROTEIN CATABOLISM

Apart from inducing anabolic activity in muscle, insulin also inhibits proteolysis both in muscle(Pozefsky, Felig, Tobin, Soeldner and Cahill, 1969) and liver (Mortimer, 1963; Exton et al., 1970). Exton et al. (1970) demonstrated that insulin decreased proteolysis by inhibiting the incorporation of amino acids into glucose. Similarly Mortimore (1963) observed inhibitory action of insulin on proteolysis in rat liver in vitro. Pozefsky et al. (1969) infused insulin in high physiological levels into skeletal muscle and noted an acute decrease in the rate of release of all amino acids, particularly the branched chain group. The effect was not so pronounced on lysine and alanine release. The pattern of amino acid release from the muscle protein after insulin administration differed considerably from the pattern of uptake during protein anabolism (Lotspeich, 1949) and likewise differed greatly from the pattern of amino acids stored in the muscle protein. Cahill et al. (1972) suggested that the pattern was more similar to the pattern of free amino acids stored inside the muscle cells. This view explained the low rate of release of branched chain amino acids since their concentration was also noted to be low inside the muscle water.

C. INTERRELATIONSHIP BETWEEN INSULIN AND NUTRITION

It has been observed that many of the changes in growth of muscle during malnutrition are similar to those changes taking place after induction of diabetes (Trenkle, 1974). If the intracellular events are similar in nutrients and insulin deficiency, nutrients and insulin might be closely interrelated in some fashion. Either insulin controls the internal nutrient environment of the muscle cells, which in turn maintains the machinery of protein synthesis within the cell, or the nutrients may regulate synthesis and secretion of insulin. A correlation seems to exist between insulin concentration and nutritional state of the animal; this could permit a precise regulation of amino acid metabolism by insulin. The concentrations of insulin during fasting state in normal mammals were noted to be low (Cahill, 1970, 1971; Cahill, Herrera, Morgan, Soeldner, Steinke , Levy, Richard and Kipnis, 1966; Grey, Goldring and Kipnis, 1970; Trenkle, 1972). Protein deficiency also decreased plasma insulin levels in rats (Young, Villaire Newberne and Wilson, 1973) and in man (Becker, Pimstone, Hansen and Hendricks, 1971). Similarly Trenkle (1974) observed that plasma insulin levels increased in proportion to the amount of protein in diets fed ad libitum. Young et al.(1973) reported that plasma insulin levels were significantly lower in protein deprived rats. The decreased levels of insulin during fasting (or protein restriction) act as a signal to both muscle and adipose tissue to release amino acids and free fatty acids (Cahill, 1970, 1971; Cahill et al. 1966). This release facilitates the flow of most of amino acids from the muscle to liver where they undergo deamination and are either oxidized or converted to glucose or fat. This flow of amino acids from muscle to liver is under the influence of insulin in the sense that the low, but not absent, insulin levels allow for the orderly release of amino acids (in contrast to the uncontrolled release in untreated juvenile diabetes)(Lacy,

1973). Therefore, insulin levels in fasting serve to control and interrelate muscle nitrogen with the energy needs of the remainder of the body. A further evidence of insulin's control of nitrogen metabolism during the state of fasting is provided by the findings of Buse and Buse (1970) and Buse, Friderici, Biggers and Buse (1970). They showed that elevated levels of free fatty acids resulting from low insulin during fast suppress protein synthesis in skeletal and cardiac muscle, increase the oxidation of branched chained amino acids and inhibit the oxidation of alanine and pyruvate in the muscle. This later reaction permits sparing of alanine which serves as substrate for hepatic gluconeogenesis.

The evidence that low levels of insulin during malnutrition exert a marked effect on the release of amino acids from muscle has led many observers to conclude that the major action of insulin in sparing protein is brought about by its controlling the flow of substrate from muscle to liver (Lacy, 1973). Other workers such as Felig and Wahren (1971) have emphasised the action of insulin in decreasing the uptake and destruction of amino acids by the liver as the important mechanism for sparing protein. It is likely that both of these organs are responsible in part for the effects of insulin, and that they are, in fact, complementary.

Contrary to the situation in fasting, the high insulin levels during the fed state are considered to trigger accelerated synthesis of protein in the muscle (Munro, 1964). It has been pointed out that, in mammals, the increased synthesis of protein during the fed state depends on muscle activity (Goldberg, 1968) and protein content of the muscle (Cahill et al. 1972). According to these authors, both these factors could alter protein synthesis and override the influence of insulin. In case of optimum nitrogen reserves in the muscle, the increasing amino acids would be metabolized in the liver to glucose and/or fat.

1.1.2.2. EFFECT OF INSULIN ON PROTEIN METABOLISM IN FISHES

There is little information available on the influence of insulin on the proteins in fishes. Despite the obvious significance which an investigation into this topic might have in the study of evolutionary development of insulin and its metabolic effects through vertebrates, relatively few workers have given any consideration to it. As long ago as 1959, Seshadri demonstrated that in Ophicephalus striatus, insulin injections resulted in a decrease of free amino acids in the skeletal muscle and an increase of protein-bound amino acids. Almost no work had been done in this field until recently, when it was suggested that glucose homeostasis may not be as important in fishes as in higher vertebrates, and a few studies were initiated to assess the effect of pancreatic hormones on proteins and lipids. Tashima and Cahill (1968) noted that insulin increased the incorporation of glycine-^{14}C into muscle protein in toad fish, Opsanus tau, and suggested that one of the prime effects of insulin is on the incorporation of amino acids into skeletal muscle protein. A similar effect of insulin on incorporation of labelled amino acids into skeletal muscle has been observed in other teleosts, Carassius auratus (Ahmad and Matty, 1975[a]), Fundulus heteroclitus, (Jackim and LaRoche, 1973) and Esox lucius (Thorpe and Ince, 1976: personal communication). Jackim and LaRoche (1973) reported a decrease in the incorporation of leucine-^{14}C into skeletal muscle in Fundulus heteroclitus after fasting and this related with Patent and Foa's (1971) finding of low insulin levels in goldfish after 5 days' fast.

Thorpe and Ince (1974) examined the effect of insulin on blood metabolites in Northern pike, Esox lucius and observed that both bovine and cod insulin (2 U/kg) induced a decline in plasma amino acids. Insulin produced similar effects in the European silver eel, Anguilla anguilla (Ince and Thorpe, 1974). These workers observed a rapid

clearance of amino acid load as compared to that of glucose load in both the teleost species and concluded that insulin in fish has the regulation of protein metabolism as one of its primary functions.

Another evidence of interrelationship between insulin and protein in fish was shown by Tashima and Cahill (1968) who noted that a protein meal but not oral glucose load of 1 g significantly increased the insulin levels in toadfish and suggested that in the toadfish, which are essentially protein eating animals, amino acids and not glucose may serve as the major stimulus for insulin secretion. Patent and Foa (1971),however, showed that in goldfish both leucine and glucose had a marked stimulatory effect upon insulin release from toadfish islets in vitro.

There is some evidence to indicate that insulin inhibits amino acid and protein catabolism in fish. Inui and Yokote (1975a) noted that alloxan-induced hypoinsulinemia in Japanese eel, Anguilla japonica, produced a pronounced elevation of plasma amino nitrogen whereas insulin injection had the opposite effect.

The up-to-date information on insulin's action in fishes suggests its central role in controlling nitrogen metabolism and this appears to have a phylogenetic precedent. As pointed out by Cahill et al. (1972) the protein anabolic effect of insulin appears to have come early in vertebrate evolution. This view is supported by certain observations made on ontogenetic stages of mammals. Clark, Cahill, and Soeldner (1968) showed that insulin had little effect on fat and carbohydrate metabolism in the rat foetus and likewise the primary stimulus for insulin release in the premature human infant (Grasso, Saporito, Messina and Reitano, 1968; Buchanan and Schwartz, 1967) or foetal monkey (Mintz, Chez and Horger, 1969) appeared to be amino acids and not glucose.

1.1.1.3. IMMUNOLOGY OF INSULIN

The immunological properties of fish insulin have been reviewed in detail by Falkmer and Wilson (1967), Epple (1969) and Falkmer and Patent (1972). Although the hypoglycemic action of teleost insulin in mammals is well-known (McCormick and Noble, 1925; Vincent, Dodds and Dickens, 1925) as is hypoglycemic effect of mammalian insulin in fishes (Section 1.1.1.1), there are considerable biological and antigenic disparities, not only between mammalian and teleost insulin but also among teleost insulins (Wilson, 1966; Falkmer and Wilson, 1967). The blood glucose of teleosts showed marked resistance to both bovine and fish's own insulin (Falkmer, 1961; Ince and Thorpe, 1974; Tashima and Cahill, 1964; Falkmer and Wilson, 1967). The antisera raised against beef insulin failed to neutralize endogenous insulin in Cottus scorpius since no hyperglycemia could be detected after antiserum injection by Falkmer and Wilson (1967). Fish insulins show a number of distinct differences from those of mammals. Amino acid substitutions occur at more than 15 loci. Thus, the differences in immunological and biological properties of the insulins, perhaps, result from these molecular differences (Wilson, 1966; Smith, 1966; Wilson and Dixon, 1961; Wilson, Dixon and Wardlaw, 1962; Grant and Reid, 1968; Eck and Dayhoff, 1966).

Due to the great immunological differences between fish and mammalian insulins, the quantitation of islet secretions by radioimmunoassay has been hampered significantly and use of antisera to mammalian insulin, labelled mammalian insulin or mammalian insulin standards has failed to produce satisfactory results (Tashima and Cahill, 1968; Ahmad and Matty, 1975; Thorpe and Ince, 1976 (personal communication)). Patent and Foa (1971) solved this problem by detaining a guinea pig antibody against codfish insulin, and were successful in measuring insulin levels in gold fish and toadfish, both in vitro and in vivo. Brinn (1973) has suggested that despite the immunological differences between mammalian and fish

insulins, the biological activities of mammalian insulins in fish, shown by Young and Chavin (1965, 1967) and Minick and Chavin (1972a) are sufficient at physiological doses to justify their continued use as a means of studying islet physiology.

1.2. GROWTH HORMONE

It is generally accepted that pituitary growth hormone (GH) plays an important role in the regulation of overall growth of the body in most vertebrate species (Daughaday, Herington and Phillips, 1975). The influence of purified mammalian or fish GH in stimulating body growth in fishes has been reviewed by Ball (1969), Pickford (1959), Pickford and Atz (1957) and Chester Jones et al. (1974). Several workers have demonstrated the ability of GH to induce body growth both in intact and hypophysectomized fish. Injections of mammalian GH evoked a positive growth response in intact Fundulus heteroclitus (Pickford and Thompson, 1948), Salmo trutta (Swift, 1954), Salmo gairdneri (Chartier-Baraduc, 1959), Salmo irideus (Enotomo, 1964a) and Oncorhynchus kisutch (Higgs, Donaldson, Dye and McBride, 1975).

Swift (1954) administered 10 µg GH/g body weight/week to four year old intact Salmo trutta for four weeks and observed higher specific growth rates in length in the treated fish. Young Salmo irideus treated with bovine growth hormone (15 µg/g/week) for 27 days showed an improvement in weight gains (Enotomo, 1964a). Chartier-Baraduc (1959) noted that in rainbow trout injected with GH (approximately 15 µg/g/week) for two weeks, the mean weights were significantly greater than those of controls. In yearling Coho salmon, Oncorhynchus kisutch, administration of 10 and 100 µg bovine GH/g/week by intraperitoneal injection or by pellet implantation for eight weeks caused a marked increase both in mean weights and length (Higgs et al., 1975). Mean weights for treated fish were 44 - 60% higher than those for respective controls. The method of hormone administration did not show any influence on the growth of fish. Higgs et al. (1975) considered that the optimum dose of GH for inducing maximum growth response in Coho salmon was between 0 - 10 µg/g/week.

In a second series of experiments, Higgs, Donaldson, Helen, Fagerlund

and McBride (1975) investigated the influence of bovine GH, alone and in various combinations with thyroxine and 17α-methyltestosterone on growth rate in O.kisutch. They noted the following sequences of individual and synergistic responses.

(GH + MT + T4) > (GH + MT) > (GH + T4) =
(GH) > (MT + T4) > (MT) > (T4) > (controls)

Another evidence of the ability of GH to produce a positive growth response in fishes is found in the observations that hypophysectomy arrests linear growth in certain species whereas administration of GH or pituitary extracts promotes growth in hypophysectomized fish. Cantilo and Regalado (1942) reported the growth-promoting properties of pituitary extracts in Salvelinus fontinalis. Pickford (1953a) was the first to demonstrate the growth-suppressing effects of hypophysectomy in Teleostei. She noted that hypophysectomy in killifish, Fundulus heteroclitus, was followed by cessation of linear growth and irregular changes in weight. These observations were confirmed by Ball (1962, 1965) and his colleagues who found that hypophysectomy not only arrested linear growth but also caused a regression in length in Poecilia formosa and P. latipinna. Similar effects of hypophysectomy were observed in dogfish by Vivian (1941). Replacement therapy with GH in hypophysectomized fish results in stimulation of growth. In a series of investigations, Pickford (1953b, 1954, 1957, 1959) showed that purified beef GH promoted growth in hypophysectomized male killifish. Temperature was seen to influence the growth response of Fundulus to mammalian hormone (Pickford, 1957, 1959). The maximum growth response was noted between 20 - 25°C whereas almost no effect occurred below 15°C. Pig, sheep, monkey and human GH also produced linear growth in hypophysectomized killifish (Pickford et al. 1959). and a similar response was manifested with pig and beef GH in hypophysectomized

Poecilia formosa and P.latipinna (Ball, 1968).

Fish growth hormone, purified from hake, Urophysis tenuis, pollack, Pollachius virens, and cod, Gadus morhua pituitaries (Pickford, 1954; Wilhelmi, 1955) and from the par distalis of blue shark, Prionace glauca (Lewis, Singh, Seavey, Lasker and Pickford, 1972) stimulated growth in length in hypophysectomized killifish. However, Pickford, (1954) found that even the most highly purified fish GH was less potent than the equivalent dose of beef GH. It was pointed out by Pickford (1954) that the lesser response to fish GH could have been caused by the presence of an inactive component or correlated with total lack of thyroid stimulation.

It is possible to measure mammalian GH due to its ability to increase growth of the tibia of young hypophysectomized rats. Pituitary extracts from all the tetrapods including pituitary extracts from lungfish (Dipnoi), the bowfin and garpike (Holostei) and sturgeon and paddlefish (Chondrostei) as observed by Hayashide and Lagios (1969) and Hayashida (1971) exhibit this effect, but it is not manifested by those of Teleostei or Chondrichthyean fish (Geschwind, 1967). This fact, along with the observation that a standard beef GH caused a linear log response in the length increase of hypophysectomized killifish (Pickford, 1959) over a dose range of 3 - 30 µg/g body weight enabled Swift and Pickford (1965) to establish a bioassay of fish GH in hypophysectomized Fundulus heteroclitus. The "Fundulus test" still remains as the principal bioassay for testing teleostean GH preparations (Pickford, 1973).

An indirect evidence of role of GH in the regulation of growth in fish is provided by the observation that GH content of pituitary of the perch, Perca fluviatilis, coincided with the growth period (Swift and Pickford, 1965). During the winter, when the perch do not grow appreciably, the pituitary contained a low resting level of GH. The

GH content of the pituitary was noted to be maximum in June, at the time of onset of natural growth period.

It is well established that in mammals GH stimulates protein synthesis and cell division (Cheek and Hill, 1970; Pecile and Muller, 1968, 1972; Kostyo and Nutting, 1973; Knobil, 1966; Manchester, 1970 c; Tata, 1970) and plays an important role in the continuous regulation of protein and energy metabolism by stimulating protein synthesis, lipolysis and glucose conservation. However, little information is available on the effects of mammalian or fish GH on fish metabolism. Bovine GH enhanced growth by improving appetite in Coho salmon (Higgs et al. 1975) and in hypophysectomized killifish (Pickford, 1957). These observations are in agreement with those of Cheek (1968) who noted stimulation by GH of food intake and growth in mammals.

Growth hormone has been reported to manifest protein anabolic activity in fishes. Higgs et al.(1975) found that despite the higher percentage of muscle water in Coho salmon treated with bovine GH, the amount of protein in the treated fish was 37 - 59% higher than that of control fish. Enotono (1964b) showed that the ratios of crude protein to crude ash in the whole body of Salmo irideus, treated with GH, were larger than those of control fish. A similar protein anabolic effect of GH was reported by Matty (1962) who noted a marked increase in nitrogen retention following injections of GH into intact Cottus scorpius.

The action of GH on lipolysis and carbohydrate metabolism has been shown in some fishes. GH decreased the percentage of muscle lipid as well as ratios of lipid to dry body weight in hormone treated fish (Higgs et al. 1975a). In mammals, GH is known to stimulate fat mobilization; the energy thus released from lipid oxidation spares dietary protein for growth (Frye, 1967). In Oncorhynchus nerka high levels of serum growth hormone (McKeown and Overbeeke, 1972) and in O. tshawytscha, hyperglycemia (Robertson et al. 1961)

has been reported during spawning migration. This observation gives some indication of the role of GH in energy metabolism in fish. Matty (1962) reported an increase in plasma glucose in Cottus scorpius foll- GH injection. Enotomo (1964b) found that a single injection of beef GH caused transient glucosauria, but no elevation of blood glucose, which suggests a renal effect of the GH rather than an action on tissue utilization of glucose. The pituitary extracts do not appear to have a diabetogenic action in higher vertebrates (Pickford, 1957). Similarly purified fish GH showed no effect on blood glucose in higher groups (Wilhelmi, 1955). These findings along with Wilhelmi's (1955) ob- servation that fish GH failed to stimulate nitrogen retention in rat suggests that fish growth hormone is antigenic towards mammals but mammalian preparations are not antigenic towards fish (Pickford and ATZ, 1957).

The actions of GH on carbohydrate metabolism are substantiated by the effects of hypophysectomy in fish. Hypophysectomy in Poecilia (Ball et al, 1965,1966) and in killifish (Pickford, 1953a) was followed by an increase in size of the liver due to deposition of fat and glycogen. In elasmobranchs, hypophysectomy ameliorated pancreatectomy-induced diabetes (Orias, 1932; Abramowitz, Hisaw, Bettiger and Papandrea, 1940).

It appears from the current information on the effects of GH in fish, that this hormone has a great potential to promote growth and manifest proteo-anabolic activity in fishes.

1.3 SEX HORMONES

1.3. SEX HORMONES

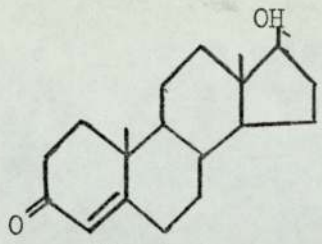
1.3.1. CURRENT STATUS

As early as 1935 (Kochakian and Murlin, 1935), the growth promoting effects of the androgenic steroids were recognised and use was promptly made of these properties. The promotion of muscle protein synthesis and reduction of nitrogen loss were particularly useful applications.

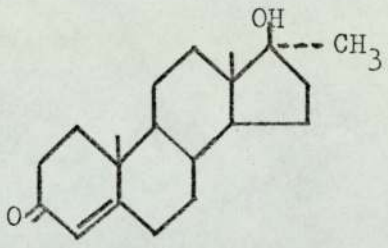
Several of the naturally occurring steroid hormones such as testicular androgens in the male and oestrogens, progesterone and relaxin in the female possess anabolic activity. But the androgens are by far the most effective of these. In naturally occurring androgens, the steroids with 19 carbon atoms having an oxygen function (ketonic or hydroxyl) at C-3 and C-17 and partial unsaturation of the A-ring, the anabolic and androgenic properties are combined to a varying degree in the same molecule, as for example in testosterone.

Some of the earlier studies clearly showed that administration of testosterone propionate to mammals increased nitrogen retention due to an increase in synthesis of proteins accompanied by a decrease in the rate of amino acid catabolism (Kenyon, 1942; Hamilton, 1948; Bartlett, 1953). However, routine administration of testosterone as an anabolic agent was limited by its concurrent and conspicuous androgenic effects. This led to an intensive research to develop new steroids where anabolic actions were potentiated concurrent with reduction of androgenic properties. Numerous compounds have now been synthesized, by modification of testosterone molecules, which embody the largest possible separation of the components of anabolic and androgenic activity. Figure I. depicts the formulae of a few of these synthetic anabolic agents. In these compounds the basic four-ring steroid nucleus has been retained along with some degree of unsaturation in ring A. The major changes involve the addition of unsaturated groups at position 1 in the A-ring, the addition of

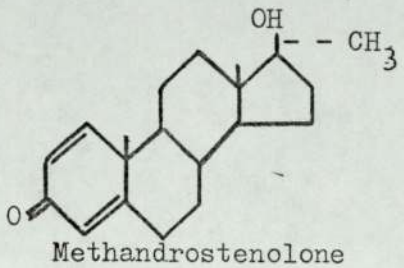
Fig.1. Formulae of testosterone and nine of the commercially available anabolic steroids



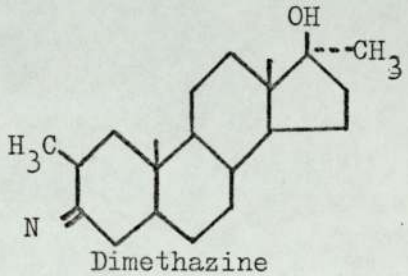
Testosterone



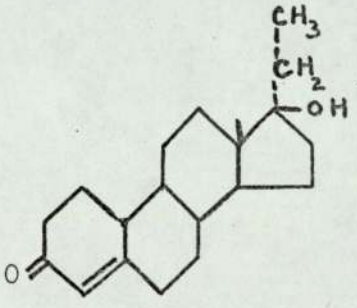
17α-methyltestosterone



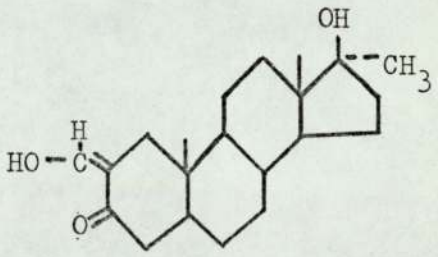
Methandrostenolone



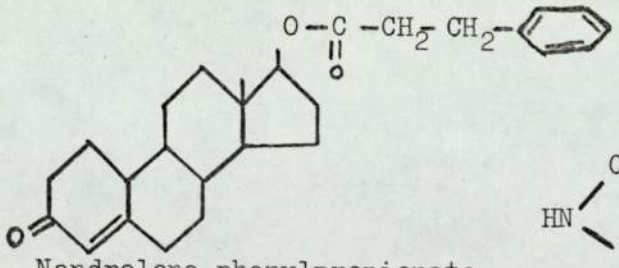
Dimethazine



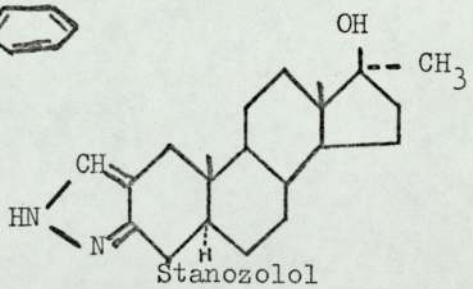
Norethandrolone



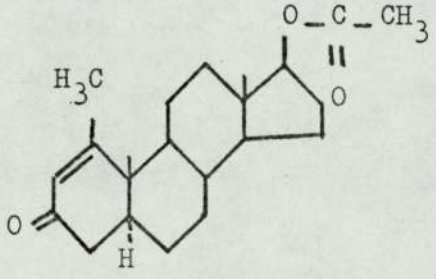
Oxymetholone



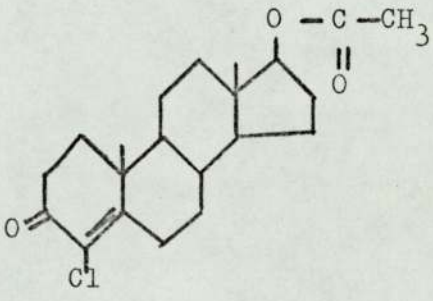
Nandrolone phenylpropionate



Stanozolol



Methenolone acetate



4-Chlorotestosterone

methyl, ethyl or phenylpropionate radicles at the 17 position in the D-ring and the formation of 19-nor compounds by the elimination of the angular methyl group at position 10. The relationship between molecular structure of anabolic steroids and their biological activity has been discussed by Anstall (1974), whereas the methods of synthesis of these compounds and various experimental techniques to assess their anabolic/androgenic ratio are admirably reviewed by Kruskemper (1968). Basically, the anabolic/androgenic index, as determined by these techniques, is expressed as the ratio of the weight of the levator ani muscle in rat to the prostate or seminal vesicle after administration of a test steroid in carefully standardised dosage for a standard time period. As regards the molecular structure and biological activity relationship, it is clear that the presence of certain groups such as 17β-hydroxyl group and Δ^4-3 one system and the inhibition of aromatisation of the A-ring are necessary for biological activity, hence some modifications of the steroid molecule tend to protect these from metabolic attack. Examples of these are alkylation of the 17α- position, 4-chloro substitution for the protection of 17β-hydroxyl group, 2α-methylation and 6α-methylation to protect the 4,5 double bond in the A-ring and 1α-methylation or removal of the angular methyl group at C-10, as in the 19-nortestosterone series, to prevent aromatisation of the A-ring, which would lead to oestrogen formation. Some modifications facilitate uptake of the steroid from the alimentary tract. These include esterification of the 17β-hydroxyl group, e.g. with acetate, propionate, deconate etc., which both protects the group from oxidative attack and prolongs the biological activity. Besides these two types of structural alterations, substitution of certain groups at various carbon atom positions brings about a fairly high degree of disassociation of androgenic and anabolic effects. 1α,2α, 4α and 4β substitutions generally decrease the androgenic activity markedly while the anabolic effects are depressed only slightly, thus bringing about a

useful degree of disassociation of anabolic from androgenic properties. 1β, 2β, 5α. 6β, 7β, 8β, 10α or β, 11β and 16α or β substitutions diminish overall biological activity, decreasing almost to the same degree both anabolic and androgenic activity. 7α, 11α substitutions and 17α-methylation augment both anabolic and androgenic potency. Substitution at 3α position leads to enhanced androgenic activity, whereas 3β and 9-C substitutions have somewhat variable effects. This knowledge of the relationship between molecular structure and biological activity led to a great advance in the synthesis of anabolic steroids and several of these compounds have been shown to promote net retention of protein in mammals (Zafar, Mellinger, Wolf, Morrow, Whitten, Dejongh and Hills, 1974; Daughaday et al., 1975) and are unique in the sense that the dosage which promotes maximum anabolism causes few, if any, undesirable androgenic side effects. These compounds have obvious significance for all branches of animal production and studies on the anabolic effects of some of these steroids on fish have been initiated.

1.3.2. ACTION ON MAMMALIAN AND AVIAN GROWTH

As discussed in the section 1.3.1, the development of inexpensive sources of synthetic hormones and substances with hormonal activity such as diethylstilboestrol (DES), a synthetic organic compound with oestrogenic properties, led to their use in animal husbandry to achieve faster growth rates and improved food conversion efficiency. The hormones used and tested in particular for this purpose had been testosterone and its analogues and synthetic "oestrogens" - diethylstilboestrol and hexoestrol.

The effects of testosterone propionate on beef cattle were examined by Burris, Bogart and Oliver (1953). They showed that weekly injection of this hormone in calves resulted in greater daily increments in weight and an increase in muscle protein content as compared with control animals. A similar effect of testosterone was observed by Burgess and Lamming (1960b) who fed testosterone mixed in feed to steers and noticed 21% additional weight gain and improved food conversion efficiency in the treated animals over a period of 154 days. The level of hormone administered was 20 mg per day per animal on the first day and increased gradually to 40 mg/day/animal by the end of the experiment. This confirmed an earlier observation made by Burris, Bogart, Oliver and Mackay (1953) which indicated that only those workers who had increased the level of hormone with the increase in weight of the animals had obtained positive growth response.

The use of androgens in sheep has been reported with conflicting results. Andrews, Beeson and Harper (1949) and Means, Andrews and Beeson (1953) found that implants of 10 mg and 30 mg testosterone respectively increased growth rate and the former investigation showed that the treatment appeared to improve carcass quality. However, O'Mary, Pope, Wilson, Bray and Casida (1952) and Pope, O'Mary, Batter-

man, Bray and Casida (1950) were unable to show any increase in weight gains over controls, using testosterone. Similarly subcutaneous implant of testosterone (30 mg per animal) in suckling lambs weighing 40 pounds each, failed to produce any significant improvement in growth rate (Burgess and Lamming, 1960a).

Bidner, Merkel, Ullney and Hoefer (1972) evaluated the effect of methyl-testosterone in swine and noticed faster growth rates and better food conversion when androgenic effects of methyltestosterone were reduced by the addition of a counter balancing amount of oestrogenic diethylstilboestrol (2.2 mg DES + 2.2 mg methyltestosterone per kilogram of diet). Similar results were obtained with this combination of hormones by Jordan, Waitt and Scholz (1965) and Thrasher, Vincent, Scott and Mullins (1967) in pigs.

The use of oestrogens and oestrogenic compounds, DES and hexoestrol to improve growth rate and food conversion efficiency has been very successful. There are numerous reports in literature about the effects of these substances in avian and mammalian species. Trenkle (1969) reviewed the role of oestrogens in feeds on mammalian and avian growth and held the view that factors such as species of animal, age, diet and dosage of oestrogens could have considerable influence on the net effect of oestrogens on somatic growth and metabolism; thus the administration of oestrogens might not always result in stimulation of growth. As shown in the case of DES (Table II) different species did not respond in a similar manner to oestrogenic substances and also, considerable variation was noticed in how a given species responded to different dosages of treatment. In general, action of oestrogens in mammals appears to be anabolic, depending of course, on the factors suggested above. This has resulted in widespread use and evaluation of these substances in poultry and livestock production. Almquist and Merrit (1962)

TABLE 2. GROWTH RESPONSE IN DIFFERENT SPECIES FED DIETHYLSTILBOESTROL

Species	Quantity administered	Approximate Daily dosage (µg/kg/day	Growth Response	Reference
Cattle	10 mg/day	25 to 75	+	Burroughs et al.(1955)
	10 mg/day	0 - 30	+	Burgess and Lamming(1966a)
Sheep	12 mg/day	30	+	Burgess and Lamming(1966b)
	2 mg/day	50 - 100	+	Hale et al (1955)
Turkeys	15 mg(implant)	-	+	Almquist and Merrit(1962)
Chickens	1 mg/day	1,000	0	Lorenz(1954)
Pigs	2 mg/day	25 to 50	0	Beeson,Andrews, Perry and Stob (1955)
Rats	1.00 µg/g of diet	100	-	Preston,Cheng and Burroughs (1960)
	0.10 µg/g of diet	10	-	" "
	0.01 µg/g of diet	1	0	" "
Mice	100 µg/day	5000	-	Wilbur,Eggert and Linken-heimer(1967)
	10 µg/day	500	+	" "
Guinea Pigs	1.00 µg/g of diet	100	-	Preston et al. (1960)
	0.10 µg/g of diet	10	0	" "
	0.01 µg/g of diet	1	+	" " "

noticed that subcutaneous implantation of a pellet containing 15 mg DES in turkeys resulted in up to 14% increase in weight gains. The anabolic effect was manifested in the males as well as females. In chickens, implanting DES increased food consumption and fat deposition (Lorenz, 1954). Eaton, Nalbandov and Forbes (1955) observed that in growing chicks, oestrogens did not exhibit a proteo-anabolic effect. Similarly in cockerels, DES failed to increase non-fat carcass weight (Baum, Geneva and Meyer, 1960). Trenkle (1969) suggested that increase in body weight gains following administration of oestrogens to poultry, as observed by various workers, was probably a result of fat deposition. He reported that dienestrol diacetate, given in low doses, tended to give superior results as compared with DES. The action of DES and hexoestrol in ruminants (cattle and sheep) differs from that in poultry, in that ruminants respond to these substances with pronounced increase in weight gains (Burroughs, Culbertson, Cheng, Hale and Homeyer, 1955; Hale, Homeyes, Culbertson and Burroughs, 1955). These species respond in a similar manner to either implanted or orally fed DES. Burroughs, Culbertson, Kastelic, Cheng and Hale (1954) and Burroughs et al. (1955) demonstrated that oral administration of DES (25-75 µg/kg/day) to cattle produced significant increases in weight gains and food utilization. Similarly, Burgess and Lamming (1960b) exhibited positive growth response and an increase in muscle protein content in cattle fed DES (20 - 30 µg/kg/day) or implanted with hexoestrol (30 mg pellet per animal). Although sheep exhibits a positive growth response to administration of oestrogens, there is a confusing picture of the influence of age and sex on this response. Perry, Andrews and Beeson (1951) reported that implantation of either 12 or 24 mg of DES in young suckling lambs produced significant increases in rates of weight gains. Clegg, Albaugh, Lucas and Weir

(1955) also found that DES was effective in increasing gains in lambs of this age. However, Jordan and Dinusson (1950) and Jordan (1953a, b) were unable to produce significant increases in rates of weight gains in suckling lambs with a 12 mg implant of DES nor did Wilkinson, Carter and Copenhaver (1955) achieve satisfactory results with a combination of oestradiol and progesterone. Burgess and Lamming (1960a), however, showed that lambs implanted with 12 mg DES gained 33% more weight than the controls. Both in sheep and cattle, hexoestrol and DES have a strong proteo-anabolic effect. The muscle protein content of the animals treated with these compounds showed a significant increase over that of controls (Clegg and Carroll, 1956; Gee and Preston, 1957; Hale et al. 1955). An increase in retention of nitrogen (Struempler and Burroughs, 1959; Whitehair, Gallup and Bell, 1953) following DES treatment also confirms the anabolic nature of oestrogens in ruminants. There has been no report on the effect of these substances on appetite but often there is a small increase of about 2% in feed intake by ruminant animals fed oestrogens (Trenkle, 1969).

1.3.3. GROWTH PROMOTING EFFECTS IN FISHES.

1.3.3.1. EFFECT ON BODY GROWTH AND FOOD CONVERSION

Although the use of sex hormones to enhance growth rate, protein synthesis and efficient utilization of feeds in animal husbandry has been very successful and has realized substantial savings in production costs, it has received little attention in the field of fisheries management and aquaculture. The research on the response of fish to sex steroids has been limited to attempts at sex reversal or the inducement of sterility (Chan, Tang and Lofts, 1972; Clemens, McDermitt and Inslee, 1966; Clemens and Inslee, 1968; Takahashi, 1975; Okada, 1975; Eversole, 1941; Berkowitz, 1941). An indication of the effectiveness of sex steroids to induce growth in fishes is found in the studies dealing with the effect of these hormones on unpaired fins and their supporting skeleton. Scott (1944) observed that in the young poecillid fish, Lebistes reticulatus, both oestrogens (oestradiol) and androgens (pregneninolone) altered the skeletal structures associated with the anal fin. Implantation of a pellet of oestrone, oestradiol, testosterone or testosterone propionate induced an elongation of fin rays of unpaired fins in gobiid fish, Chaenogobius annularis (Egami, 1960). Testosterone produced similar results in two other gobiid species and stimulated elongation of the spines of dorsal fin in Monacanthus cirrhifer (Ishii and Egami, 1957) and and Petrogobius zonoleucus (Egami, 1959). All these workers did not investigate any direct effect of the hormone on body growth. However, Cohen (1946) in an investigation dealing with the influence of oestrogens and androgens on gonads and certain skeletal structures in platyfish, Platypoecilus maculatus, noted that feeding immature male and females on alpha-oestradiol benzoate (65 µg/fish/week) and pregneninolone (260 µg/fish/week) respectively for a period of up to 20 weeks resulted in acceleration of growth in females, whereas in the males the growth was suppressed.

A screening of the communications dealing with the effect of steroid hormones on sex reversal also reveals a few observations of the effect of these substances on growth in fishes. Berkowitz (1938) showed that feeding or injection of crystalline oestrogens increased the size of Lebistes reticulatus males beyond that usually attained by them. On the contrary, Eversole (1941) found that androgens, whether fed orally in crystalline form (Pregneninolone, 0.3 mg - 0.8 mg or 1 mg/fish/week) or injected (Testosterone propionate, 0.025 mg - 0.1 mg/fish/week) for a period of 8 weeks suppressed the growth of both mature and immature Lebistes reticulatus. The effect of pregneninolone was more severe and 42 days old fish were noted to be 25% shorter than control fish of the same age. Progesterone and pregnandiol were reported, in the same study, to have no effect on somatic growth. Ashby (1957) reported that alevins of brown trout, Salmo trutta, when treated with progesterone (50 γ - 100 γ / litre in culture medium) grew more quickly in length as compared with the control group; testosterone (50 γ - 100 γ /litre) and oestradiol (50 γ and 300 γ /litre) on the other hand, retarded the growth rate. A similar growth depressing effect of methyltestosterone when given in feeds at a concentration of 20, 25 and 30 ppm for 60 days to newly born guppies, Lebistes reticulatus, was observed by Clemens et al. (1966). All these earlier investigations appear to project a rather confusing picture of the effect of sex hormones on growth and it seems that, perhaps, the dose of hormone, duration of treatment and age of animal, particularly in the case of androgens, has some significance in their findings. During the past few years, however, some attempts have been made to evaluate, in a more systematic manner, the anabolic role of certain synthetic steroids in cultured fishes. Some of these studies have exhibited positive growth response of fishes to the administration of androgens and indicated a potential

role of these growth promoting substances in aquaculture.

Hirose and Hibiya (1968a, b) noted a significant increase in the growth rate of goldfish and rainbow trout from 4-chloro-testosterone. When given by intramuscular injection, this steroid induced maximum growth at dosage levels of 0.5 mg/fish/week in goldfish and 2.5 mg/fish/week in rainbow trout, both in sexually active and inactive seasons. The administration of higher doses resulted in diminished growth promoting potency due to the androgenic side effects of the steroid such as hypertrophy of liver and kidney and suppression of gonads. The increase in growth rate seemed to be mainly due to an increase in the utility of food as indicated by a tendency of increase in digestibility of protein and low non-protein nitrogen in serum in the trout treated with 4-chloro-testosterone. The chemical composition of the muscle remained unchanged, suggesting that 4-chlorotestosterone promotes normal growth of fish. For the sake of comparison with 4-chlorotestosterone, two other androgens, methylandrostenediol and testosteronepropionate, were tested. Of these, the former was effective in promoting weight increases, although both hormones caused undesirable renotrophic changes.

The anabolic activity of another synthetic androgen, stanozolol was evaluated by Bulkley and Swilhart (1973) in immature goldfish and channel catfish. These workers added the steroid to the diet by mixing the hormone first with the gelatine and then spraying the gelatine mixture on the ration before feeding. This hormone, tested at three levels of treatment (0.25, 2.5 and 25.0 mg/kg body weight daily) caused only slight improvements in weight gains. This slight response in weight gains which occurred shortly after feeding of the drug was not noticeable after 28 days and therefore it was concluded that stanozolol, at the dosages tested, did not seem to be efficacious in producing long term weight gains among immature goldfish and

channel catfish. The failure of Bulkley and Swilhart to obtain any significant positive growth response to the administration of stanozolol appears to be due to the small number of fish tested (12 per group), great size variations within each group and limited feeding of ration (3% of body weight per day). Like 4-chlorotestosterone, this steroid hormone also showed androgenic side effects, causing renal hypertrophy.

McBride and Fagerlund (1973) examined the growth stimulating effect of 17α-methyltestosterone supplementation in the diet of juveniles of two species of Pacific salmon, Coho, Oncorhynchus kisutch and Chinook, O. tshawytscha. In short-term experiments of six weeks (Coho) and twelve weeks (Chinook), significant increases in weight gains and length were obtained in groups of fishes fed this steroid at a concentration of 1, 10 and 50 ppm (Coho) and 0.2 and 1 ppm (Chinook). The hormone when fed at 50 and 10 ppm concentration to Coho salmon was more effective during period I (0 - 14 days) than during period II (14 - 42 days). During period I the fish receiving 50 and 10 ppm of methyltestosterone grew at a rate of 2% per day while the control group gained only 0.9%. During period II, however, the group receiving 1 ppm of steroid were the fastest growers. Low doses of 1 ppm (Coho) and 1 and 0.2 ppm (Chinook) were not effective in promoting significant weight gains until after 28 days in Cohos and 56 days in Chinooks. No explanation has been given by the authors about this delayed response to low doses and faster initial response to high doses of hormone treatment. Although McBride and Fagerlund examined the effect of methyltestosterone on the histology of skin and gonads, they did not assess the androgenecity of the hormone by comparing its effect of ratios of liver, kidney and gonads to body weights of treated and control groups. In a second series of experiments, Fagerlund and McBride investigated the growth

rate of juvenile Coho salmon which were given diets supplemented with 17α-methyltestosterone, for a prolonged period (1) while kept first in fresh water and then in salt water (1975a) and (2) when kept exposed to different constant temperatures, 11.5 and 16.5°C (1975b). Successively larger increases relative to the control were noted during the period of culture in fresh water (57 weeks) in the groups receiving 10 ppm and 1 ppm of steroid, the weight gain over control being 120% and 70% respectively. The hormone was not apparently effective at a lower dose of 0.2 ppm. On transfer to salt water, the growth rate of the two groups retained on high doses of the steroid declined; the decrease being more pronounced in the fish receiving 10 ppm of hormone. This group also suffered a large number of mortalities. It was suggested that these changes were perhaps associated with an upset of the physiological balance caused by the steroid which interfered with the organism's capacity to adapt to a change in the external environment. An analysis of the flesh in this study showed no significant difference in moisture content due to hormonal treatment. Feeding 1 ppm 17α-methyltestosterone raised the fat content by 43.6% as compared to the control group. The protein content was not estimated. In the experiment dealing with the exposure of fish receiving 17α-methyltestosterone at 0.2 ppm and 1 ppm concentration to two different constant temperatures, 11.5 and 16.5°C, it was noted that the growth response to the steroid became more pronounced at higher temperature. It was pointed out by the authors that the fish fed 0.2 ppm steroid ration showed a significant increase in weight gains as compared to the control group, although in the earlier investigations carried out at ambient temperatures, this concentration of the steroid failed to evoke a response. An objection, which might be raised against these observations is that the authors did not take into account the possibility of accumulation of excreted metabolites

of 17α-methyltestosterone in water in the closed system, which could have exerted their influence on the growth rate, thus making 0.2 ppm dosage level also apparently effective. It is clear from the findings of McBride and Fagerlund that 17α-methyltestosterone, incorporated in low doses in the feed, has a strong potential as a growth promoter in the fresh water culture of Pacific salmon. High doses, however, can lead to marked androgenic effects.

Since higher androgenic potential of 17α-methyltestosterone appeared to be a setback for its use in commercial fisheries, a few other less harmful substances were also tested by some workers. These included ethylestrenol and stanozolol. Stanozolol, as already discussed, did not produce sufficient anabolic response in goldfish and Channel catfish (Bulkley and Swilhart, 1973). Similarly with ethylestrenol, there were no noticeable differences in weight gains in Tilapia melanopleura (Hutchison and Campbell, 1964). These workers have not mentioned the dosage and duration of the hormonal treatment; hence the failure of the hormone to produce significant growth responses may be attributed to an improper level of one or both of these factors. This speculation finds some basis in a preliminary study made by Simpson, Wright and Fraser (1974), who reported significant weight gains and improved food conversion efficiency in rainbow trout and Atlantic salmon under the influence of ethylestrenol. These workers fed steroid supplemented diet to the fish for 3 months at the dosage level of 0.5, 2.5 and 12.5 mg/kg food (ethylestrenol) and 2.5 and 12.5 mg/kg food (methyltestosterone). Although ethylestrenol in this study was shown to increase weight gains and improve food utilization efficiency at all levels of treatment, there were conflicting responses exhibited by rainbow trout and Atlantic salmon to different dosage levels. In rainbow trout higher doses (12.5 mg) were seen to be most effective, whereas in Atlantic salmon

the maximum growth response was exhibited by the lower doses (0.5 and 2.5 mg/kg food). Considering this difference in optimum dose of ethylestrenol for rainbow trout and Atlantic salmon and also recalling the higher optimum dose of 4-chlorotestosterone for rainbow trout as compared to that of goldfish, it can be implied that the level of a hormone capable of producing maximum growth response in different teleostean species depends upon the interspecific physiological differences. Coming back to the findings of Simpson et al. (1974), methyltestosterone, being more androgenic in nature, improved growth response at lower doses only; higher doses showed growth depressing activity. Analysis of carcass and viscera at the end of the experiment revealed that increase in growth rate and food conversion efficiency did not appear to be achieved at the expense of muscle quality; mean values of carcass nitrogen, dry weight and lipids for treated groups were not significantly different from those of the control. As expected, ethylestrenol did not produce any noticeable damaging side effects. The stability of the steroid hormones, when incorporated into feeds, has always been suspected particularly due to the presence of autoxidising lipids in the fish food. In view of this, Simpson et al. (1974) were particularly careful in the incorporation of steroid into the commercial food. These workers incorporated the hormone into rations by spraying them in the form of ethanolic solution on the commercial pelleted food and then evaporated the alcohol before storing the food under low temperature. The stability of ethylestrenol incorporated into the food was checked after one month's storage period, using isotopically labelled material, and 85% undegraded steroid was found. The ingested ethylestrenol, as examined in one experiment, appeared to be quickly metabolised and excreted. It was suggested that this rapid metabolism and elimination of the steroid was consistent with its effectiveness as an oral

anabolic agent and high enough to ensure that residues would not remain in body tissues.

Simultaneously with the trials of androgen analogues relatively devoid of androgenic properties, fish nutritionists have also been looking into the feasibility of the use in aquaculture of some steroid-like compounds in which the growth promoting actions are divorced from hepatotrophic and renotrophic side effects. One such compound is diethylstilboestrol (DES), a substance with oestrogenic properties. As reviewed in the Section 1.3.2, diethylstilboestrol when added in minute quantity to the feeds of cattle, markedly increased the rate of weight gain and food conversion efficiency. The research on the response of fish to DES has been limited to investigations dealing with its efficiency to stimulate sex reversal (Berkowitz, 1938 and 1941; Castel-nuovo, 1937). The effect of DES on nitrogen metabolism was examined by Hoar (1958) who found that at a concentration of 0.4 ppm in aquarium water, the nitrogen excretion was increased in goldfish, Carassius auratus. A similar negative growth response was observed by Ghittino (1970) who fed rainbow trout on feeds containing DES at levels of 5g and 500 g/100 kg of food. This was equivalent to 500 µg and 50 mg/kg body weight per day when fed at the rate of 1% body weight of dry ration. The growth of trout receiving these levels of DES was lower than that of controls.

Because loss of nitrogen and reduced growth rate is a normal response to excessive doses of steroid hormones (Rubenstein, Kurland and Goodwin, 1939; Kruskemper, 1968) and oestrogenic substances such as DES (Wilbur, Eggert and Linkenheimer, 1967), lower doses of DES (0.6, 1.2 and 2.4 mg/kg wet diet or 1.2 to 5.00 mg/kg dry food) were used by Cowey, Pope, Adron and Blair (1973) in experiments with 0-group plaice, Pleuronectes platessa. At the lowest treatment level, there was a marked growth promoting action of DES with no undesirable side effects apparent during a 10 week feeding period. The higher doses caused a

slight suppression of growth. The growth promoting action of DES was ascribed to a greater voluntary intake of food and an improved utilisation of food protein. The efficiency of food conversion for the lowest level of treatment was 8.3% greater than that for the control. In this communication, Cowey et al. (1973) also noted that the total weight gains over the whole experimental period varied considerably between individual fish, even on the same diet, and were found to be highly correlated with initial weight. It was, therefore, emphasised that in order to make correct comparisons between diets it is necessary to adjust the weight gains to correspond to a common initial weight.

The anabolic response to DES as observed by Cowey et al.(1973) in 0-group plaice could not be confirmed by Bulkley (1972) in Channel catfish, Ictalurus punctatus. DES, when given in feeds (0.1, 1.0 and 10 mg/lbs food) to catfish fingerlings depressed appetite and caused a reduction in growth rate during 25 days experimental period. An inverse relation was noted between growth and amount of DES received by different groups of fish; thus the fish receiving no DES grew at the fastest rate whereas those receiving the highest level of dosage showed minimum growth. An analysis of visceral fat also indicated catabolic effect of DES on protein synthesis. An inverse relation between DES levels and the amount of visceral fat was attributed to appetite suppression. The decrease in visceral fat was considered to have resulted due to mobilisation of fat stores to maintain body energy in a condition of depleted food intake and high loss of nitrogen. DES caused hypertrophy of the kidneys but did not affect liver. Fagerlund and McBride (1975b) also tested DES and oestradiol on Coho parr. Oestradiol was found to be ineffective whereas DES produced undesirable changes, namely reduction in growth rate and increased mortalities. Recently Simpson (1976) in an attempt to delay maturation of male salmon by treatment of eggs and alevins with oestradiol, has shown that, although the hormonal treatment resulted in

producing entirely female populations, it suppressed somatic growth, largely defeating the objects of the exercise.

A review of the literature on DES and growth in fish appears to suggest that response of fish to this oestrogenic substance depends on dosage of DES and physiological differences between various species.

1.3.3.2. EFFECT ON TISSUES

Several workers have reported the influence of sex hormones, particularly androgens on various organs and tissues of the salmonids. These reports fall mainly into two categories. First, there are the observations, made mainly on gonads, liver and kidney, in order to study the side effects of sex hormones as a part of an evaluation of their growth promoting properties. The second category includes the communications which were undertaken because of indications of the involvement of androgens as causative agents in histological changes in various tissues of salmonids during upstream or spawning migration. One example of these is the examination of the effect of methyl testosterone on skin and gonads of salmonids, by Yamazaki (1972) who carried out this investigation in the aftermath of the observation that the skin of Pacific salmon, genus Oncorhynchus, (Robertson and Wexler, 1960) and trout, Salmo trutta (Stoklosowa, 1966, 1970) becomes harder and much thicker during upstream or spawning migration and gonadal maturation, probably as an adaptation, influenced by sex steroids, against the various physical obstacles during migration and the special spawning habit of digging a spawning nest in the gravel bottom of rivers. Although several reports in the second category cover the effect of sex steroids in a variety of organs and tissues, notably gastrointestinal tract, cardiovascular system, skin, gonads and some endocrine glands, these are not very purposeful from the point of view of present studies because dosage level of hormones used in these was very high. In view of this, the present review of the influence of sex hormones on various tissues has been confined mainly to those effects which were observed during the studies of their growth promoting properties.

Kidney:

The most conspicuous effect of both androgens and oestrogens on the teleost kidney seems to be an increase in the ratio of kidney to

body weight, renosomatic index (RSI), caused mainly due to hypertrophy of the epithelial cells and nuclei of tubules, dilation of glomerulus, sclerosis of glomerular capillaries and a thickening of Bowman's capsules. 17α-methyltestosterone, 11-ketotestosterone and oestradiol were noted to cause these deleterious changes in gonadectomized adult sockeye salmon, Oncorhynchus nerka (McBride and Overbeeke, 1971). Hirose and Hibiya (1968a, b) found hypertrophied and degenerated kidney tubules in goldfish and rainbow trout treated with methyl-androstenediol and 4-chlorotestosterone. This response was more pronounced with higher doses of the hormone, particularly in sexually active seasons. Ashby (1957) noticed that administration of testosterone (60 γ/litre) or oestradiol (50 γ and 300γ /litre)into the aquarium produced similar effects in the kidney in alevins of brown trout, Salmo trutta. The pronephric tubules were much enlarged whereas those of the mesonephros were increased in diameter and reduced in number in fishes treated with the higher concentration of oestradiol. A powerful hypertrophic effect of an oestrone or progesterone injection on the "anterior interrenal" of a teleost, Gasterosteus aculeatus was observed by Stanworth (1953). The anabolic steroid, stanozolol also induced hypertrophy of the kidney in goldfish and altered the renosomatic index in fishes fed this hormone at a concentration of 25 mg/kg body weight. Bulkley (1972) demonstrated the renotrophic effect of diethylstilboestrol in channel catfish and pointed out that a comparison of renosomatic indices of groups of fish fed different levels of diethylstilboestrol, indicated a direct relationship between renal hypertrophy and amount of diethylstilboestrol received.

Liver:

Although androgens, oestrogens and other sex hormones are known to cause degeneration or hypertrophy of hepatic cells, these changes

have not been reported as frequently in fishes as one would expect, even after treatment with slightly higher doses of these hormones. The hormones with predominant myotrophic properties, such as ethyl-estrenol and stanozolol, did not produce any significant difference in the ratio of liver to body weights, hepatosomatic index (HSI) in salmonids (Simpson, 1974) and goldfish (Bulkley and Swilhart, 1973) respectively. In rainbow trout, 4-chlorotestosterone showed an increasing tendency in HSI (Hirose and Hibiya, 1968a, b). This tendency seemed to be related to hypertrophy of liver cells, which were noticed to be laden with glycogen reserves. An increase in the glycogen content of liver in response to sex hormone treatment in fishes is not an uncommon phenomenon and has also been observed in Oryzias latipes (Egami, 1955) and in Misgurnus anguillicaudatus (Kobayashi, 1953) after oestrogen treatment. McBride and Overbeeke (1971) demonstrated that two androgens, 17α-methyltestosterone and 11-ketotestosterone produced degenerative changes in the liver of adult sockeye salmon. The influence of oestrogen, oestradiol on liver in this fish was complex. There was a marked difference in the condition of the liver between the two sexes. Only the females exhibited hypertrophied cells containing finely granulated cytoplasm and clearly enlarged nuclei with conspicuous central nucleoli. McBride and Overbeeke ascribed this oestrogen induced hypertophy of the liver cells in females to an enhanced yolk production together with an inhibited release of this product. The synthetic oestrogenic compound, diethylstilboestrol, however, did not produce any liver hypertrophy in immature channel catfish (Bulkley, 1972).

Skin:

Changes in skin colouration and texture with gonadal maturation in Teleostei have been observed quite frequently and associated with the variations in the levels of endogenous gonadal hormones. These

changes were exhibited under captivity also by adult sockeye salmon(Idler, Bitners and Schmidt, 1961) injected with 11-ketotestosterone, an androgen present in the plasma of this species (Idler, Schmidt and Ronald, 1960). Fagerlund and Donaldson (1969) observed changes in the external colouration of castrated sockeye salmon, treated with 11-ketotestosterone or 17α-methyltestosterone. The effect of oral administration of the latter on the skin was observed in sexually immature pink salmon, Oncorhynchus gorbuscha and Chum salmon, O. nerka, (Yamazaki, 1972) and Coho salmon, O. kisutch (McBride and Fagerlund, 1973). Both in pink and chum salmon, feeding of 17α-methyltestosterone in the diet (50 and 100 mg/kg dry food) caused a thickening of the skin. The hormone affected both epidermis and dermis. In the epidermis, contents of the mucous cells increased and the cells constituting stratum germinativum hypertrophied and changed into columnar shape. The collagenous fibres in the dermis were observed to be more dense and cells also increased in number. A similar response in the skin of Coho salmon was observed. A pronounced increase in epidermal thickness was clearly evident in those fish fed the hormone at concentrations of 10 and 50 mg/kg diet. McBride and Overbeeke (1971) noticed a marked increase in thickness of skin in both sexes of adult gonadectomized sockeye salmon, O.nerka after injection of 17α-methyl-testosterone and 11-ketotestosterone. The epidermis as well as dermis were involved in this response but it was more conspicuous in the former. The injection of oestrogens (oestradiol and oestradiol cypionate) also evoked similar changes in the skin of females. As for the mode of actions of sex hormones on skin, it is generally regarded that they act directly on the skin and not through the stimulation of the pituitary which might secrete some hormones as prolactin or TSH (Yamazaki, 1972).

Gonads:

The suppressive action of synthetic androgens and oestrogens on the gonads has been demonstrated by a number of workers. 17α-methyl

testosterone induced degenerative changes in the testes of various salmonids. In pink and chum salmon, Yamazaki (1972) showed that oral administration of 17α-methyl-testosterone resulted in great suppression of spermatogenesis and induced atretic changes in spermatogonia. Some spermatogonia hypertrophied and developed vacuoles in the cytoplasm before disintegration. The degeneration and cessation of spermatogonial division caused a reduction in the number of spermatogonia. Similar degenerative changes were noticed in the testes of juvenile coho and chinook salmon fed 17α-methyltestosterone supplemented diet at concentrations of 2.5, 10 or 50 ppm (McBride and Fagerlund, 1973; Fagerlund and McBride, 1975). Hirose and Hibiya (1968a, b) noticed a suppressive action of testosterone, methylandrostenediol and 4-chlorotestosterone on the gonads in goldfish and rainbow trout. The ratio of gonad to body weight, gonadosomatic index (GSI) decreased in groups of fish treated with these androgens, especially in sexually active season. Testosterone and oestradiol caused a strong inhibition of the development of germinal tissue and increased vascularity of the gonadal region in brown trout (Ashby, 1957). A similar suppressive action on testes of an androgen, pregneninolone in platyfish, Platypoecilus maculatus (Cohen, 1946) and diethylstilboestrol and oestradiol benzoate in Lebistes reticulatus (Berkowitz, 1941) was shown earlier. Contrary to these observations, there is also evidence that androgens stimulate spermatogenesis as in hypophysectomized Fundulus (Lofts, Pickford and Atz, 1966) and in catfish (Sundararaj and Nayyar, 1967). Eversole (1941) reported a stimulation of testis in Lebistes reticulatus, after treatment with pregneninolone. The testis in the treated fish were at a more advanced stage of maturation as compared to that of controls; there was an abundance of precocious spermatocytes in the treated testes whereas in untreated ones only spermatogonea were noticeable. Simpson et al.(1974) found hypertrophy of the sperm ducts and a general advancement in the

development of testis in rainbow trout treated with 17α-methyl testosterone and ethylestrenol. The GSI of the steroid treated fish was also greater than that of controls.

A review of the literature reveals a controversy regarding the action of androgens on the ovaries. Contrary to the degeneration of ova as a result of treatment with androgens as described by Yamazaki (1972), Hirose and Hibiya (1968a,b), Eversole (1941) and Cohen (1946), the ovary showed no suppressive response to 17α-methyltestosterone (McBride and Fagerlund, 1973; Fagerlund and McBride, 1975) in Pacific salmons and to ethylestrenol or methyltestosterone (Simpson et al.,1974) in rainbow trout. The suppressive action of androgens on the ovary included an inhibition of development of oocytes due to suppression of yolk deposition followed by atresia of the oocyte. These effects agree with the conclusion of Pickford and Atz (1957) that the mammalian or synthetic androgens are almost entirely inhibitory on the piscine ovary. These workers suggested that degeneration of the ovary might be direct or indirect through the inhibition of gonadotrophic secretion from the pituitary gland. Hirose and Hibiya (1968a, b) indicated that effects of androgens were mediated through the pituitary, whereas Yamazaki (1972) was of the view that the effect seemed to be direct since the response of the ovary was quick and all the ovarian eggs, irrespective of their size, were disintegrated. There is some evidence that the pituitary involvement in the piscine oocyte development does not occur until the yolk deposition stage (Dodd, 1972). Therefore, Fagerlund and McBride (19 75) considered that if the effect of androgens were mediated through the pituitary, the lack of response of the ovary (at the pre-yolk stage) to androgens in their investigation would not be unexpected.

2. METHODS AND RESULTS

2.1. EFFECT OF INSULIN AND INSULINOTROPIC SUBSTANCES ON PROTEIN SYNTHESIS AND GROWTH IN RAINBOW TROUT

2.1.1. INSULIN AND PROTEIN SYNTHESIS IN THE SKELETAL MUSCLE

A: MUSCLE PROTEIN CONTENT

MATERIALS AND METHODS

Rainbow trout (Salmo gairdneri) obtained from Vortex Fish Farms, Donnington, Stow-on-the-Wold, were acclimatized for at least three weeks in small hatchery troughs supplied with running dechlorinated Birmingham city water at a flow rate of 0.5 litre/minute. The ammonia levels throughout the experimental period were kept below toxic levels. The water temperature during the experiment varied from 12 - 15°C. The fish were fed a commercial pelleted food (Beta Trout Pellets, Cooper Nutrition Products Limited, Essex) to satiation twice daily during a natural photo-period.

Three groups of six rainbow trout each, weighing 10 - 12 gms were injected intramuscularly with porcine insulin (Actrapid, Novo Industri A/S, Copenhagen, Denmark). The hormone was diluted with 0.7% NaCl and administered in two doses of 3 I.U and 5 I.U/Kg body weight. Control fish were injected with an appropriate volume of 0.7% saline. The fish were killed 36 hours after insulin injection with a blow on the head and muscle samples were quickly frozen and stored at -20°C for subsequent analysis.

ANALYTICAL PROCEDURE

Protein content of rainbow trout muscle was determined by the standard aqueous Biuret reaction method. Tissue protein was extracted by a modification of Schmukler and Yiengst (1968) procedure. A 100 mg portion of skeletal muscle was homogenized with an all glass homogenizer in cold 0.7% saline solution (1 : 10 w/v). The homogenate was mixed

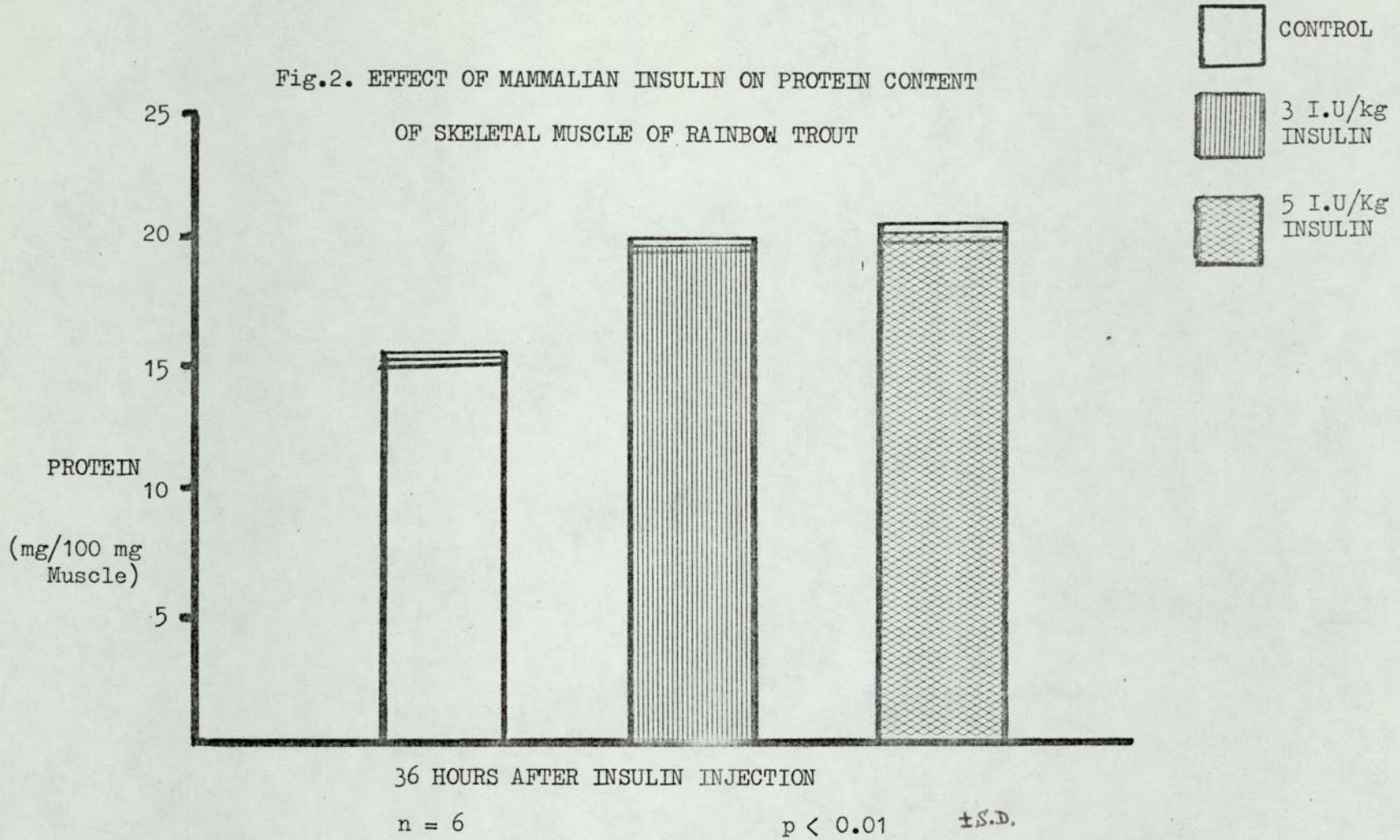
with 2 ml of 10% trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 15 minutes. The precipitate was further washed with 2 ml of 5% TCA and centrifuged again for 15 minutes. Lipids were removed from the precipitate by sequential extractions with 10 ml 95% ethanol, 10 ml 1/1 acetone-ether and 5 ml ether. The precipitate was then vacuum dried. A few mgs of dry precipitate were hydrolyzed in 4 ml of 0.3N KOH during 1 hour of incubation at 37°C. An aliquot (1 ml) of KOH hydrolysate was mixed with 4 ml Biuret reagent (Boehringer Mannheim GmbH,Mannheim, Germany) and the optical density was measured on a Beckman D.B. spectrophotometer (Beckman Instruments Limited) at 540 mμ. Fish muscle protein was used as protein standards in these experiments. Protein was isolated from the rainbow trout muscle in the manner described above. Quantity of protein was determined by the microkjeldahl procedure. Using this protein as standards, an appropriate standard curve was prepared (Fig.3).

RESULTS

Fig.2. shows the effect of a single injection of insulin on skeletal muscle protein content in rainbow trout during a 36-hour period. Insulin at both dosage levels tested produced a significant rise ($p<0.001$) in the muscle protein content. The mean values were 19.46 $\pm$ 1.25 (S.D) mg/100 mg muscle and 20.48 $\pm$ 1.89 mg/ 100 mg muscle for insulin treated groups (3 I.U/Kg and 5 I.U/Kg respectively) and 16.06 $\pm$ 1.06 mg/100 mg muscle for the control group.

Fig.2. EFFECT OF MAMMALIAN INSULIN ON PROTEIN CONTENT

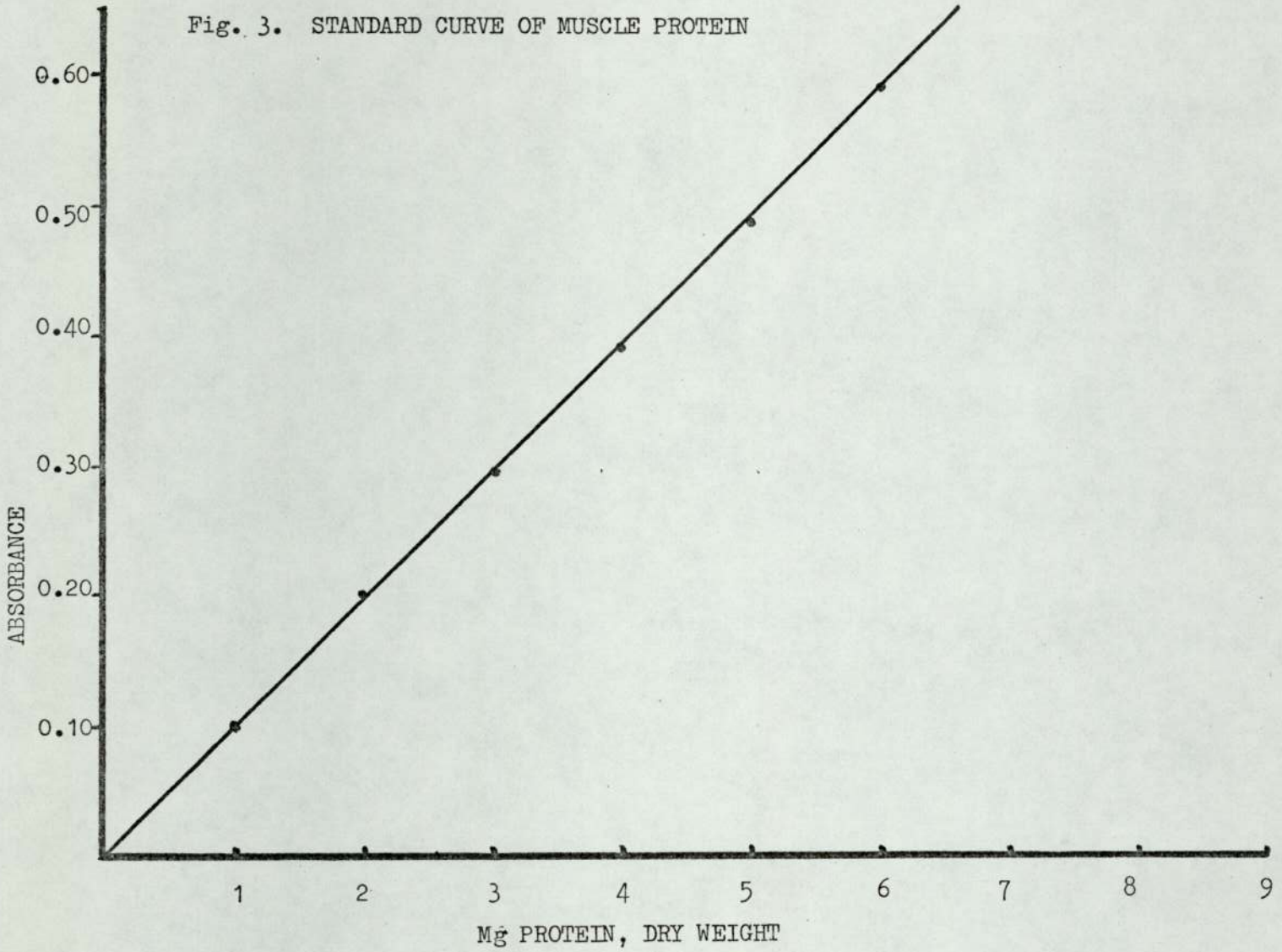
OF SKELETAL MUSCLE OF RAINBOW TROUT



36 HOURS AFTER INSULIN INJECTION

n = 6 $p < 0.01$ ±S.D.

Fig. 3. STANDARD CURVE OF MUSCLE PROTEIN



B. IN VIVO L-LEUCINE-^{14}C INCORPORATION INTO SKELETAL MUSCLE PROTEIN

MATERIALS AND METHODS

ANIMALS

Young rainbow trout (Salmo gairdneri) purchased from Bibury Trout Farm, Gloucestershire were maintained in small hatchery troughs kept within the laboratory and supplied with well aerated running tap water. The fish were fed commercial pelleted diets to satiation, twice daily during a natural photoperiod. Food was withdrawn 18 hours before insulin treatment.

INSULIN TREATMENT

In the first experiment, fish weighing from 16 to 20 gms were treated with insulin (0.2 I.U./fish or 0.7% saline)through the intramuscular route. L-(U-^{14}C)-leucine (The Radiochemical Centre, Amersham, Bucks) with a specific activity of >270 mci/mmoles was made up as a 10 µci/ml solution with 0.7% saline and an appropriate dose of radioactivity (5 µci/100 gm body weight)was injected intraperitoneally 18 hours after insulin/saline administration. Exactly 24 hours later, the fish were killed with a blow on the head and were placed in plastic bags and frozen immediately at - 20°C for subsequent determination of radioactivity in the muscle protein.

In the second experiment insulin (3 I.U/kg) and L-Leucine-^{14}C (5 µci/ 100 gm body weight) were injected simultaneously through the intra-peritoneal route into rainbow trout (10 - 15 gms in weight). The controls were given an appropriate volume of 0.7% saline in place of insulin. Fish were sacrificed at 3, 6, 9, 12, 24 and 48 hours post injection and were frozen at -20°C.

TISSUE PREPARATION AND MEASUREMENT OF PROTEIN AND RADIOACTIVITY,

Skin was stripped with forceps from the caudal area of fish and a sample of skeletal muscle was removed from the dorso-lateral section adjacent to the adipose fin. 30 - 100 mg portions of muscle were homo-

genized and protein was prepared from these by the method described in Section 2.1.1.(A). After every washing and centrifugation of homogenate the supernatant was carefully removed with a Pasteur pipette. All washings were pooled for counting of TCA soluble radioactivity. An aliquot (0.2 ml) of the supernatant was added to glass vials containing 10 ml of scintillation cocktail (Kostyo and Schmidt, 1963), a mixture of 600 ml Dioxan, 100 ml anisole, 100 ml 1,2-dimethoxyethane, 4.8 g 2,5-diphenyloxazole (PPO) and 120 mg 1,4-Bis-2-(4-methyl-5-phenyloxazolyl) benzene (dimethyl POPOP). The radioactivity in the sample was measured for 10 minutes on a β-counter (Beckman L.5 230).

A portion of dry protein precipitate was used for the determination of protein content by the Biuret method as described in Section 2.1.1 (A), whereas the remainder was prepared for measurement of radioactivity. A few mgs of this dry protein precipitate were hydrolysed in 1.0M methanolic solution of hyamine hydroxide (1 ml/10 mg protein) at 37°C for 1 hour. The hydrolysate was cooled and then mixed with 10 ml of toluene-phosphor solution, containing 0.5% PPO and 0.01% dimethyl POPOP (Herberg, 1960) in a glass vial. The radioactivity in the sample was counted for 10 minutes.

Incorporation of leucine-^{14}C into muscle protein was calculated as

1. % incorporation= $\frac{\text{c.p.m. in precipitate x 100}}{\text{c.p.m. in precipitate + c.p.m. in supernatant}}$

(Jackin and LaRoche 1973)

2. specific activity = c.p.m. in precipitate/mg protein in precipitate

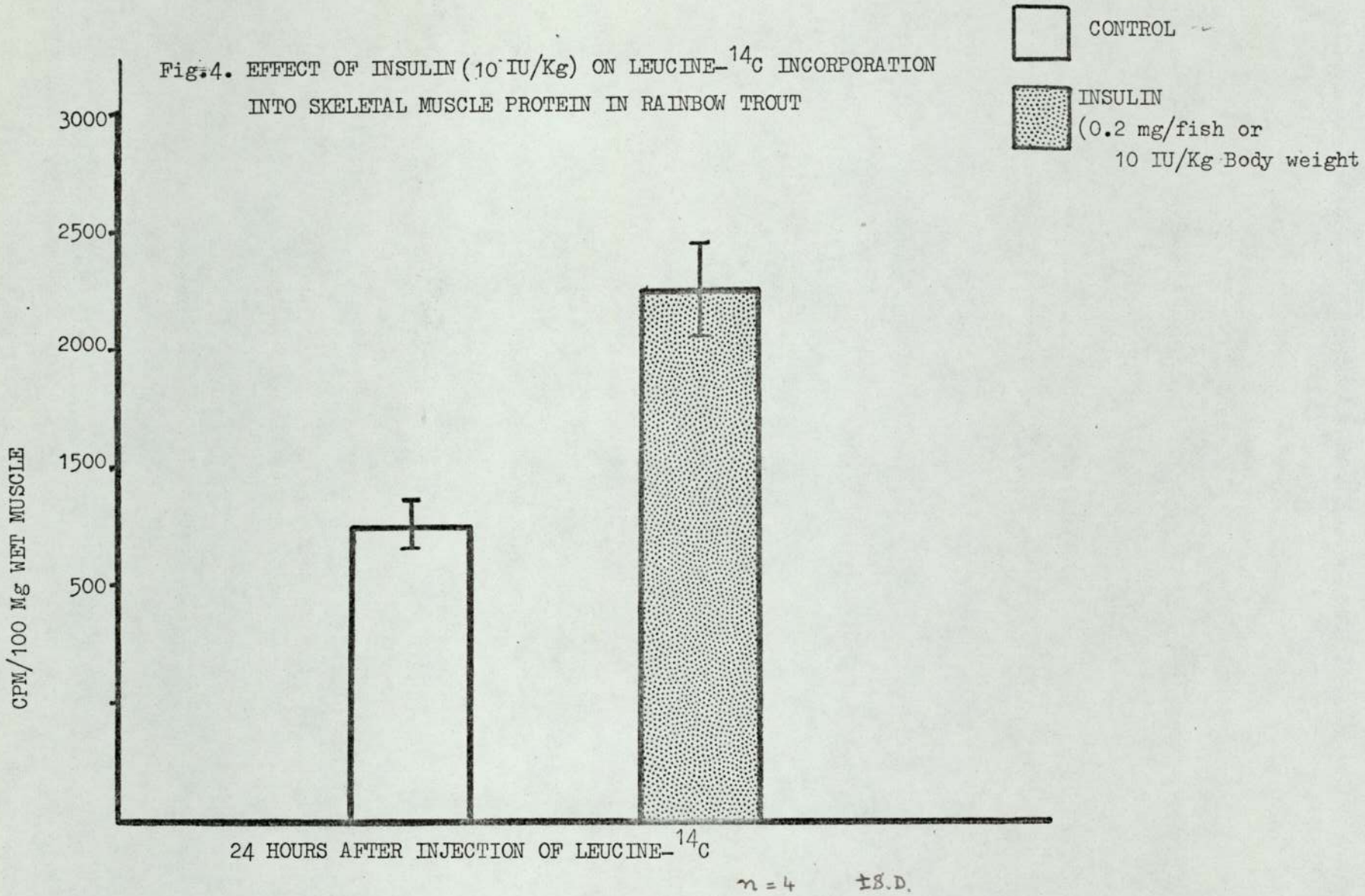
3. Incorporation = c.p.m./g wet muscle.

RESULTS

The influence of insulin (approximately 10 I.U/Kg body weight) on the 24 hour leucine-^{14}C incorporation into skeletal muscle protein in rainbow trout is shown in Table 3 and Fig.4. It can be seen that insulin had induced a distinct anabolic response in rainbow trout and a marked

stimulation of leucine-^{14}C incorporation into skeletal muscle protein was evident. When insulin (3 I.U/Kg) and leucine-^{14}C were injected at the same time, again a marked increase in the rate of leucine-^{14}C incorporation occurred (Figs.5 and 6). Levels of radioactivity in the precipitate (protein specific activity) as well as its percentage incorporation (Tables 4 and 5) show a significant effect ($p < 0.01$) at 6, 9, 24 and 48 hours after simultaneous injection of insulin and tracer. Fig. 7 shows the levels of radioactivity in the supernatant. Insulin treatment did not produce a significant alteration in the concentration of leucine in the intracellular and intercellular pool. Thus it appears that the acute changes in the incorporation of leucine-^{14}C into the skeletal muscle protein are not entirely due to increased transport of the tracer into the muscle cells.

Fig. 4. EFFECT OF INSULIN (10 IU/Kg) ON LEUCINE-^{14}C INCORPORATION INTO SKELETAL MUSCLE PROTEIN IN RAINBOW TROUT



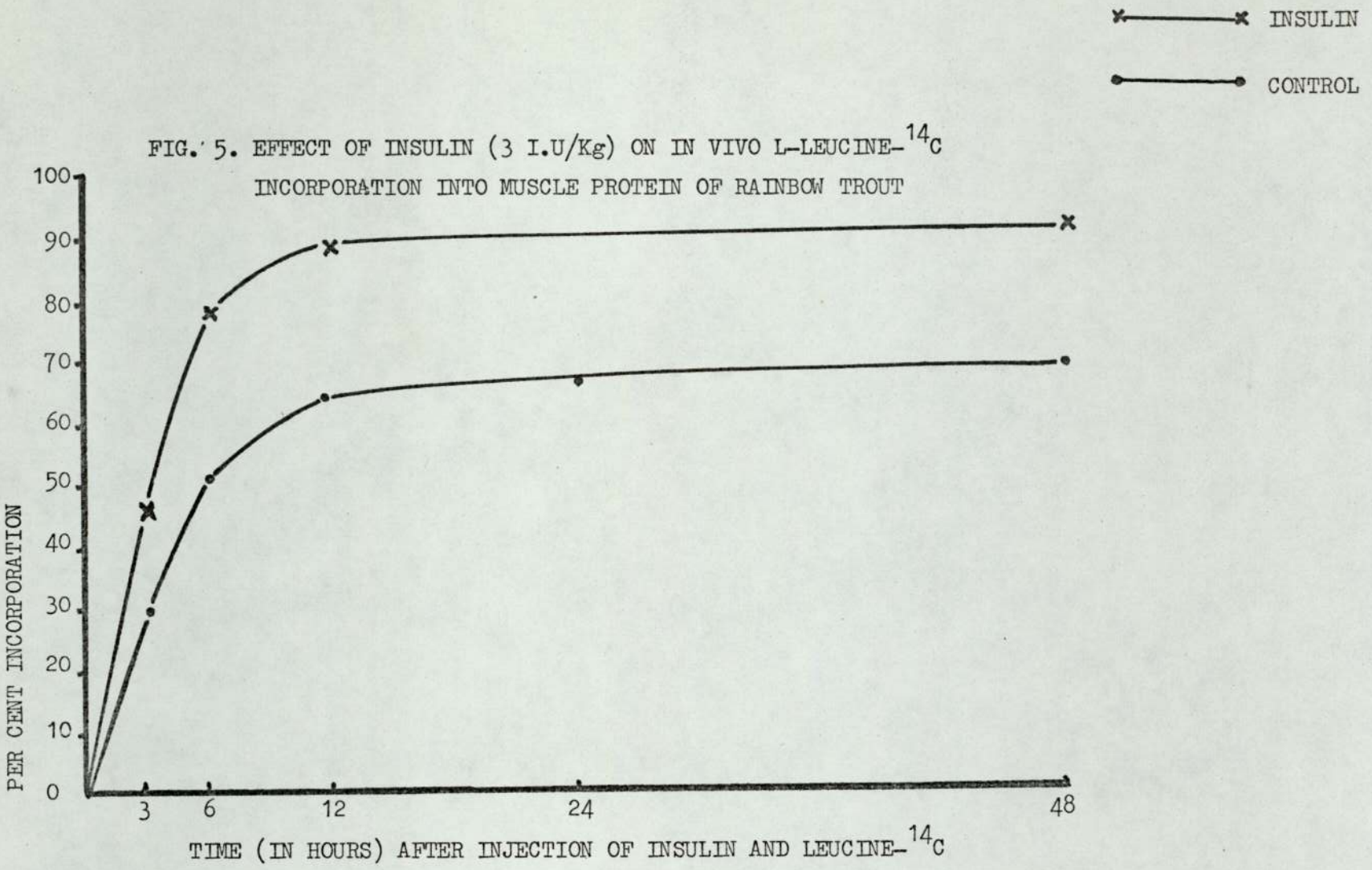
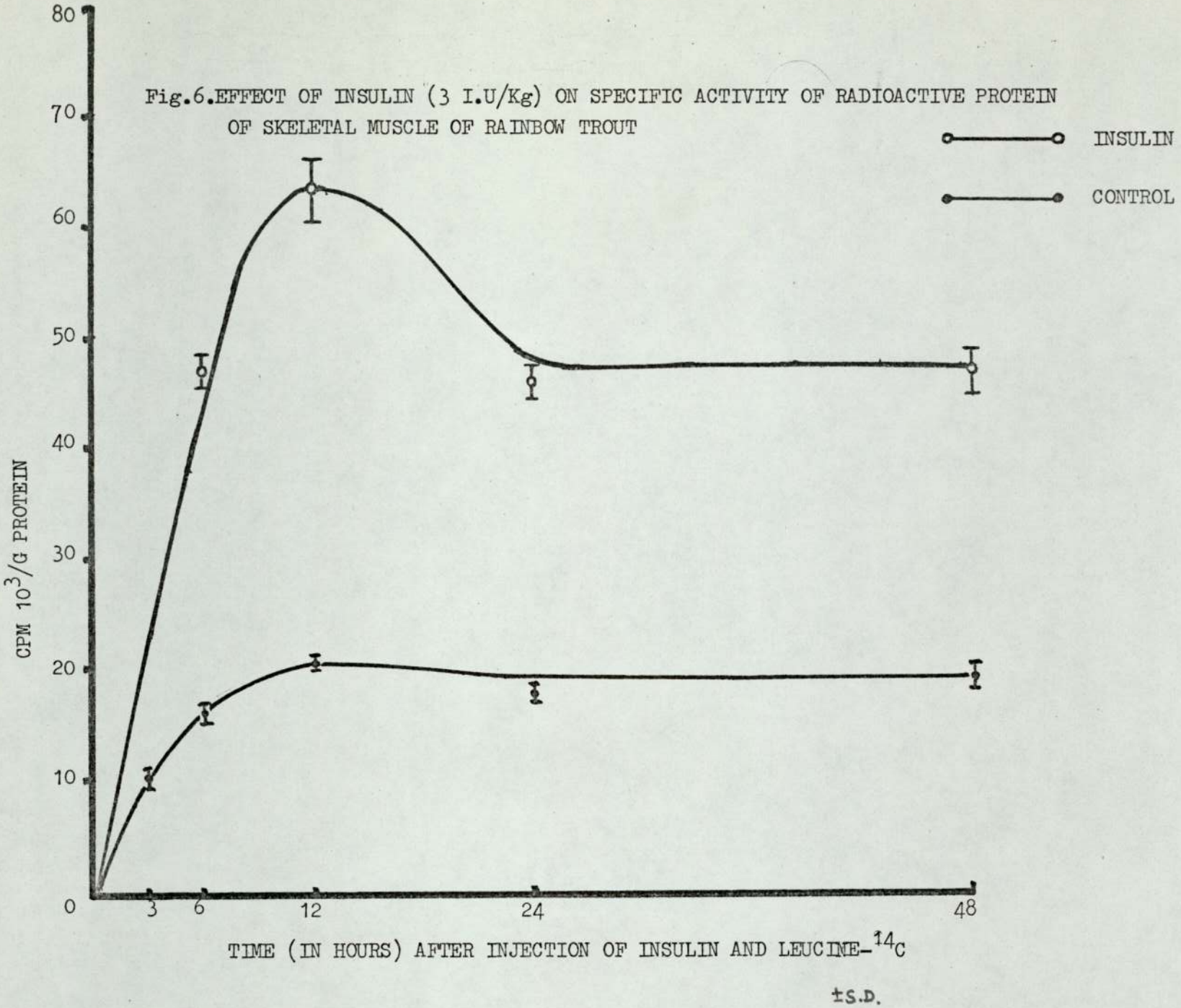


FIG. 5. EFFECT OF INSULIN (3 I.U/Kg) ON IN VIVO L-LEUCINE-^{14}C INCORPORATION INTO MUSCLE PROTEIN OF RAINBOW TROUT

Fig.6.EFFECT OF INSULIN (3 I.U/Kg) ON SPECIFIC ACTIVITY OF RADIOACTIVE PROTEIN OF SKELETAL MUSCLE OF RAINBOW TROUT



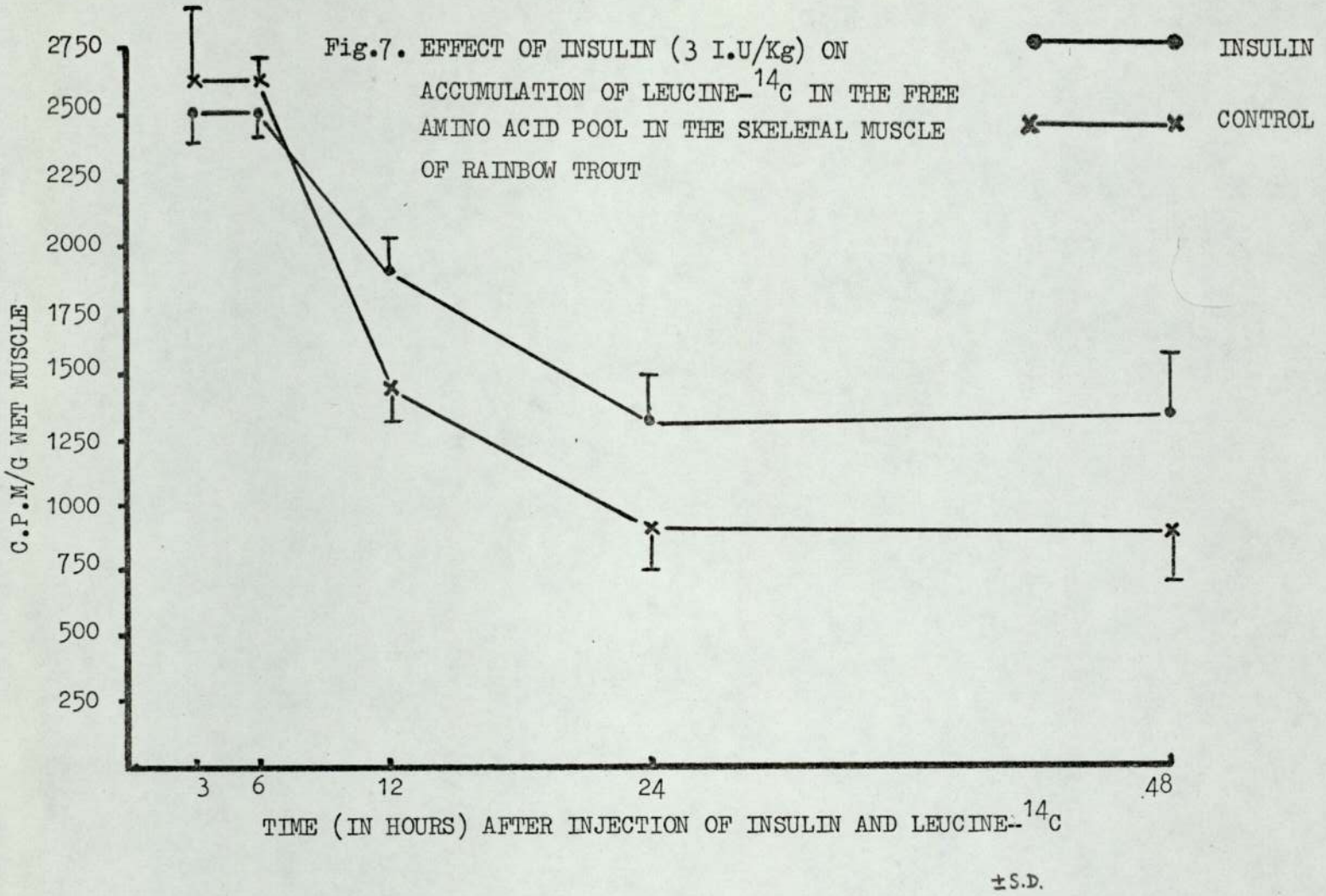


Fig.7. EFFECT OF INSULIN (3 I.U/Kg) ON ACCUMULATION OF LEUCINE-^{14}C IN THE FREE AMINO ACID POOL IN THE SKELETAL MUSCLE OF RAINBOW TROUT

TABLE 3. IN VIVO EFFECT OF PORCINE INSULIN ON LEUCINE-^{14}C INCORPORATION INTO MUSCLE PROTEIN OF RAINBOW TROUT

Fish were injected with insulin (approx. 10 I.U/Kg body weight). After 18 hours they were injected intraperitoneally with leucine-^{14}C (5 µc/100 gm body weight) and sacrificed 24 hours later. The values given for leucine incorporation are means $\pm$ S.D. of 4 fish

Treatment	Leucine-^{14}C incorporation c.p.m/100 mg wet muscle	P
SALINE	1246 $\pm$ 200	
INSULIN	2257 $\pm$ 401	$P < 0.01$

TABLES 4 and 5

IN VIVO EFFECT OF PORCINE INSULIN ON L-(U-^{14}C)-LEUCINE INCORPORATION INTO SKELETAL MUSCLE PROTEIN OF RAINBOW TROUT

Fish were injected intraperitoneally with leucine-^{14}C (5 µc/100 gm body weight) and insulin (3 I.U/Kg body weight) or saline at 0 time. The values given for leucine incorporation are means ± S.D of 3 fish.

Table 4

Time after injection (hours)	LEUCINE-^{14}C INCORPORATION (% incorporation)	
	SALINE	INSULIN
3	39.06 ± 3.33	45.03 ± 4.91
6	51.03 ± 1.51	77.33 ± 0.85 *
12	64.10 ± 2.00	88.33 ± 2.31 *
24	66.68 ± 5.82	89.83 ± 3.40 **
48	69.45 ± 6.14	90.80 ± 3.53 **

Table 5

Time after injection (hours)	LEUCINE-^{14}C INCORPORATION (PROTEIN SPECIFIC ACTIVITY)	
	SALINE	INSULIN
3	10.08 ± 0.86	11.70 ±0.97
6	16.01 ± 1.08	47.38 ±2.38 *
12	20.08 ± 0.88	63.56 ±4.57 *
24	15.47 ± 1.35	45.81 ±1.75 *
48	19.28 1.36	48.67 3.98 *

* $P < 0.001$
** $P < 0.01$

C. REGULATION OF PROTEIN SYNTHESIS IN RESPONSE TO STARVATION AND LOW AND HIGH PROTEIN FEEDING

MATERIALS AND METHODS

Rainbow trout, obtained from Bibury Trout Farm, Gloucestershire were maintained in 3 outdoor polythene tanks (capacity 70 litres) supplied with aerated running tap water. The fish weighed from 30 - 35 gms. Temperature during the experimental period varied from 14 - 16°C. After 3 weeks' acclimatization period, the fish were distributed into 3 groups of 20 fish each. These three groups of trout were either starved or fed a low (25%) or a high protein ($55\frac{1}{2}$) diet for a period up to 10 days. At the end of 4 and 10 days, 4 fish from each group were taken at random and injected with L-(U-^{14}C)-Leucine (3 µC/100 gm body weight). The percentage incorporation of the radioactivity into skeletal muscle protein was determined by the method described in Section 2.1.1.(A and B).

Two diets using different levels of protein were formulated in the following way.

HIGH PROTEIN DIET

Dry Ingredients	gms
Herring Meal	70
Wheat Middlings	23
Cod Liver Oil	5
Alpha Cellulose	4
Mineral Premix (A)	4
Vitamin Premix (B)	1
Durabond Binder	1

LOW PROTEIN DIET

Dry Ingredients	gms
Herring Meal	30
Wheat Middlings	23
Cod Liver Oil	5
Alpha Cellulose	38.5
Mineral Premix (A)	4
Vitamin Premix (B)	1
Durabond Binder	1

(A) MINERAL PREMIX

Mineral	Quantity
Calcium Orthophosphate	13.58 gms
Calcium lactate. $5H_2O$	32.70 "
Ferric citrate. $5H_2O$	2.97 "
Magnesium sulphate. $7H_2O$	13.20 "
di-potassium hydrogen ortho-phosphate	23.98 "
di-sodium hydrogen ortho-phosphate	8.72 "
Sodium chloride	4.35 "
Aluminium chloride (anhydrous)	8.3 mgs
Potassium iodide	15 mgs
Manganese sulphate. H_2O	80 "
Cobalt chloride. $6H_2O$	100 "
Zinc sulphate. $7H_2O$	150 "

(B) VITAMIN PREMIX obtained from Cooper Nutrition Products, Essex, contained the following vitamins:

Vitamin	mgs/Kg food (except where otherwise shown)
A	0.012 M.I.U
D_3	0.015 M.I.U

E	60
K	15
B_1	10
B_2	25
B_6	15
Nicotonic acid	150
Calcium pentothenate	50
Folic acid	4
Biotin	0.6
B12	0.02
Choline chloride	1.5 gms
BHT (antioxidant)	113

The vitamin premix contained some trace elements, therefore mineral premix was adjusted accordingly.

All vitamins and minerals were mixed into dry wheat middlings. The fish meal, wheat middlings and alpha cellulose were thoroughly mixed by stirring them together in a Kenwood Food Mixer. Cod liver oil was then added to the dry mix. After mixing it thoroughly, binder and water (150 ml/ 100 g diet) were added until a stable consistency was obtained. The diets so prepared were then pelleted by extruding them through a sponge mincer. The finished pellets were air dried at room temperature for 3 days. The percentage protein in each diet was determined using the macrokjeldahl method. Since food protein may have a higher metabolizable energy content than carbohydrate in fish (Cowey, Pope, Ardon and Blair, 1972) the two diets were not made isoenergetic. Fish were fed twice daily at the feeding level of 1% of the body weight.

RESULTS

A comparison of 6 hour leucine-^{14}C incorporation into skeletal muscle protein in the fasted fish was made to that of fish fed high or low protein diet for 4 and 10 days (Fig.8, Table 6). At the end of a

10-day period, a marked contrast was evident in the values of percentage leucine incorporation in the 3 groups and the following sequence of percentage of radioleucine was observed:

Fasted < Low Protein < High Protein

A similar trend was noticeable after a 4 days period, though the percentage leucine incorporation in the fasted group was not different from that of the group fed low protein. The results indicate the influence of different levels of dietary protein and starvation on amino acid metabolism at the two time points tested and point towards the key role of proteins in fuel metabolism and its regulation by insulin in fishes.

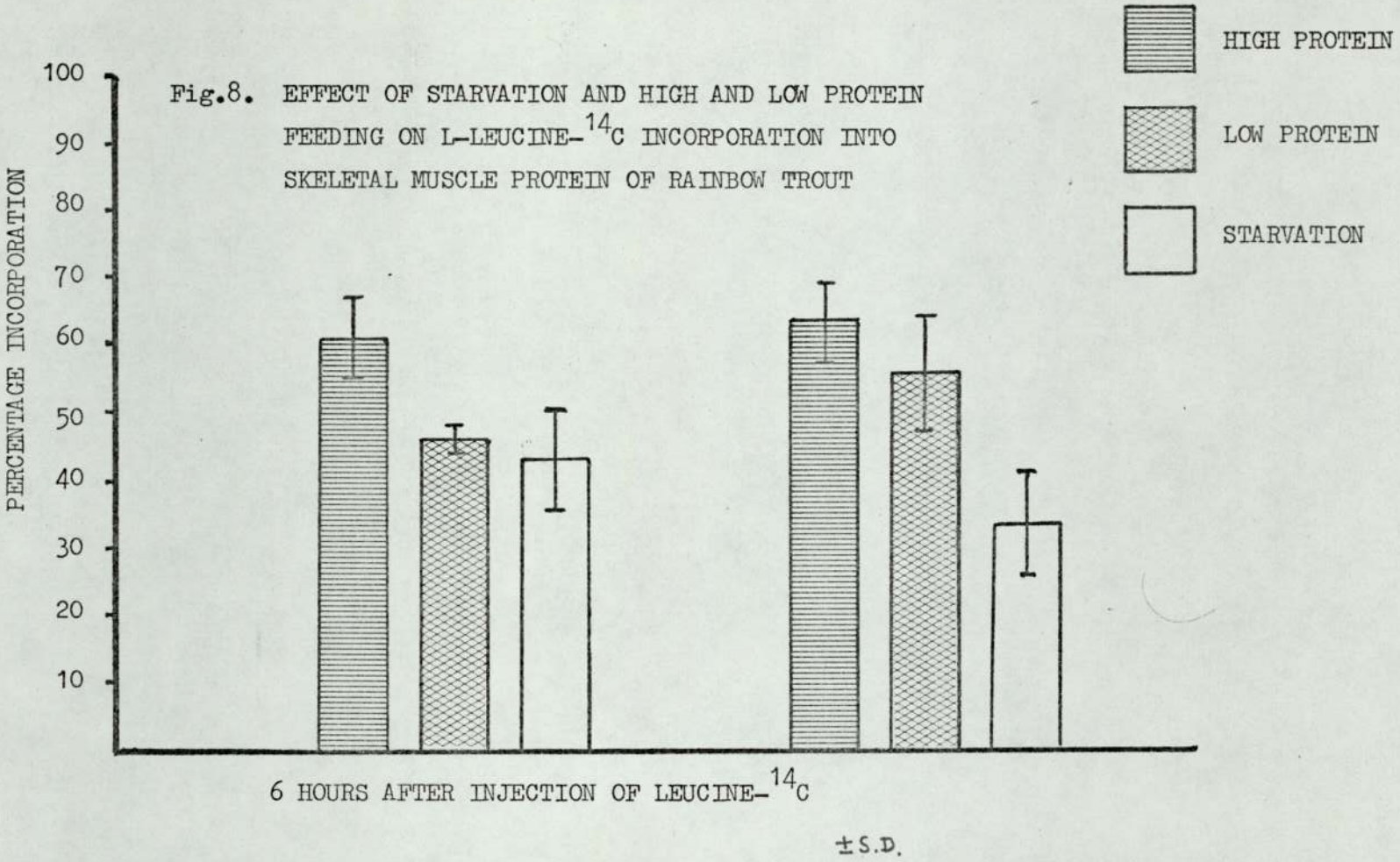


Fig.8. EFFECT OF STARVATION AND HIGH AND LOW PROTEIN FEEDING ON L-LEUCINE-^{14}C INCORPORATION INTO SKELETAL MUSCLE PROTEIN OF RAINBOW TROUT

TABLE 6

PERCENTAGE INCORPORATION OF RADIOACTIVITY FROM L-(U-^{14}C) LEUCINE IN VIVO INTO SKELETAL MUSCLE PROTEIN OF RAINBOW TROUT EITHER STARVED OR GIVEN HIGH OR LOW PROTEIN DIET

Fish were starved/fed either high- or low protein diet. At the end of the period indicated they were then injected intraperitoneally with L-leucine-^{14}C (3 µci/100 g body weight) and sacrificed 6 hours later. The values given for leucine incorporation are means ±S.D. The numbers in parentheses indicate the number of fish used.

TREATMENT	% LEUCINE-^{14}C INCORPORATION INTO MUSCLE PROTEIN	
	AFTER 4 DAYS	AFTER 10 DAYS
HIGH PROTEIN (55%)	60.9 ± 11.4 (4)	63.6 ± 11.4 (3)
LOW PROTEIN (25%)	45.9 ± 4.1 * (4)	55.7 ± 17.0 (3)
STARVATION	43.4 ± 15.0 (4)	33.4 ± 14.5 * (3)

* $P < 0.05$

2.1.2. INSULIN AND PROTEIN CATABOLISM: EFFECT ON AMMONIA PRODUCTION

MATERIALS AND METHODS

Rainbow trout (Salmo gairdneri) obtained from Bibury Trout farm, Gloucestershire were split into four groups and acclimatised in small (8 litre) glass aquaria at 14°C. These aquaria were kept in a large containment vessel filled with circulating water kept at a constant temperature by using circatherms. Fish were fed standard commercial trout diets once in a day during a natural photoperiod. Ammonia produced by a definite weight of rainbow trout (usually 30 gms) during a 24 hours period was measured.

Individual groups of fish were placed in 5 litres of water in separate tanks which were covered on top. A gentle flow of compressed air was maintained in each tank. Each day, at the same time, a sample of water was taken for analysis. The fish were then quickly transferred to an adjacent identical tank which had already been filled with 5 litres of well aerated clean water of the same temperature. The original tank was thoroughly washed and was used for maintaining fish for the next day. This procedure enabled change of water and removal of excreta without causing too much stress to the fish. After one week's acclimatization period, resting levels of ammonia were determined and fish were injected intraperitoneally with insulin (3 I.U/kg body weight). Ammonia excreted by different groups of fish in ambient water during the following 5 days was measured.

The ammonia concentration in the ambient water was determined by direct nesslerization. 1 ml of a freshly-prepared mixture of equal volumes of Nessler's reagent and 50% sodium potassium tartrate was added to a 25 ml sample, and the absorbance measured on a Beckman spectrophotometer (Beckman Instruments Limited) at 500 µm. To the tartrate had previously been added 1% of Nessler's reagent and after standing for

a few days, the clear solution was decanted off and used. Standard solutions of ammonium chloride were treated in exactly the same manner as described above for the samples and a standard curve was prepared by diluting the stock ammonium chloride solution (Fig.9)

RESULTS

As ammonia, rather than either urea or uric acid, is the main end product of nitrogen metabolism in fish (approximately 80% of the nitrogen eliminated in fish is ammonia) and most of this is said to be excreted extra-renally through the gills (Smith, 1929; Wood, 1958; Fromm , 1963; Black, 1957), it should be possible, then, to measure changes in protein metabolism by analyses of ammonia in the ambient water. The data summarized in Fig.10 illustrates that insulin produces a marked decline in ammonia excretion in ambient water by rainbow trout. The administration of insulin depressed the ammonia excreted by rainbow trout at 14°C from a mean resting level of 36.97 $\pm$ 1.04 mg/100 g fish/24 hours to 31.42 $\pm$ 0.50 during the first day. On the second day, the ammonia level was further decreased to 25.40 $\pm$ 1.04. After that the ammonia excretion tended to return towards normal state and on the 5th day resting level of 36.22 $\pm$ 0.41 was recorded. Thus the effect of insulin administration on ammonia excretion in rainbow trout suggests an anticatabolic role of insulin in protein metabolism. Since diet, age and activity of the fish were expected to affect the actual amount of ammonia excreted in this test, therefore, instead of carrying out a control experiment side by side with the hormone treated fish, firstly, the normal resting levels of ammonia excretion were determined in each group of fish and then the changes were observed in the same fish after insulin administration.

Fig.9. STANDARD CURVE OF AMMONIA

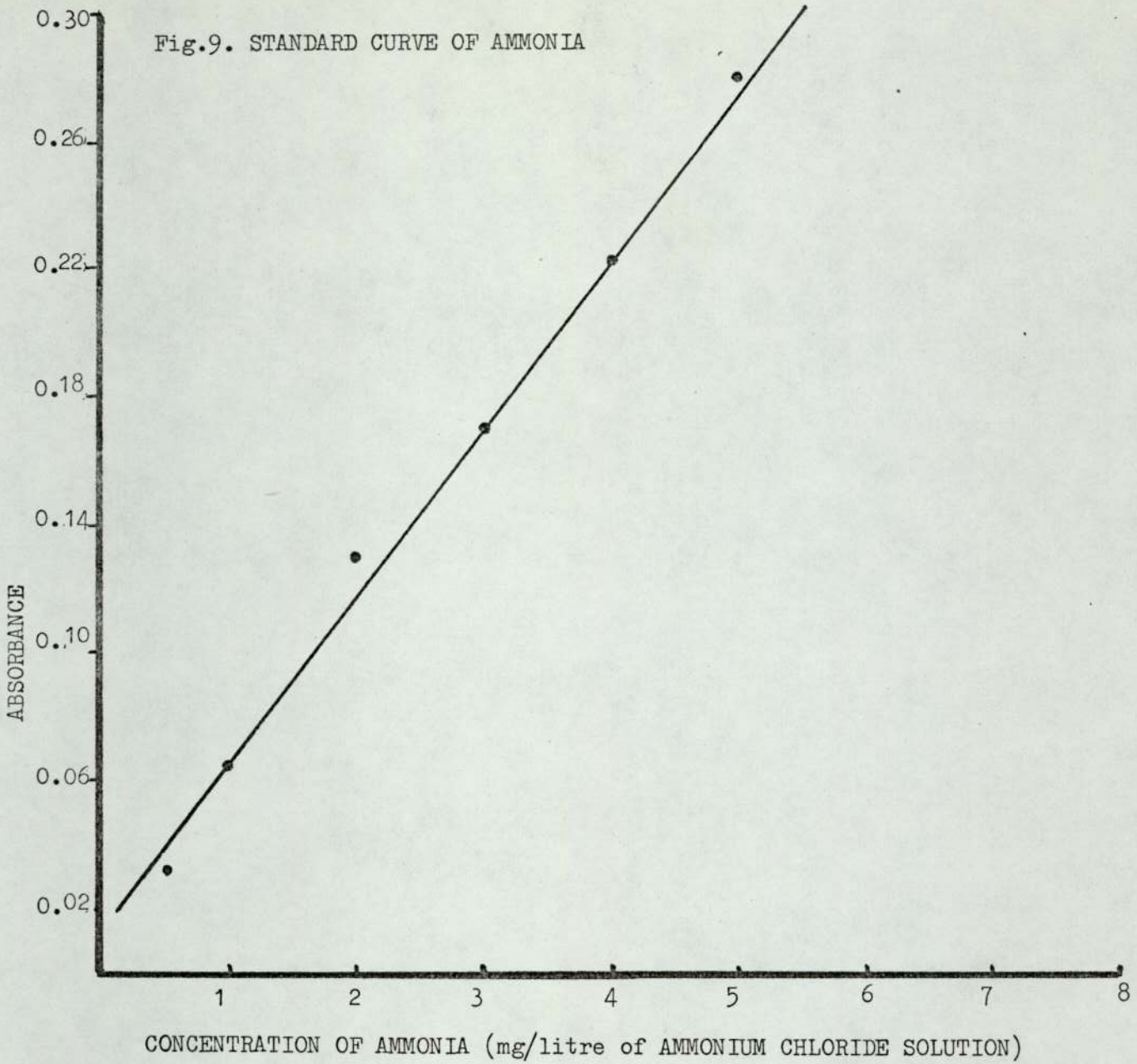


Fig.10. EFFECT OF INSULIN (3 I.U/KG BODY WEIGHT) ON AMMONIA EXCRETION BY RAINBOW TROUT IN AMBIENT WATER

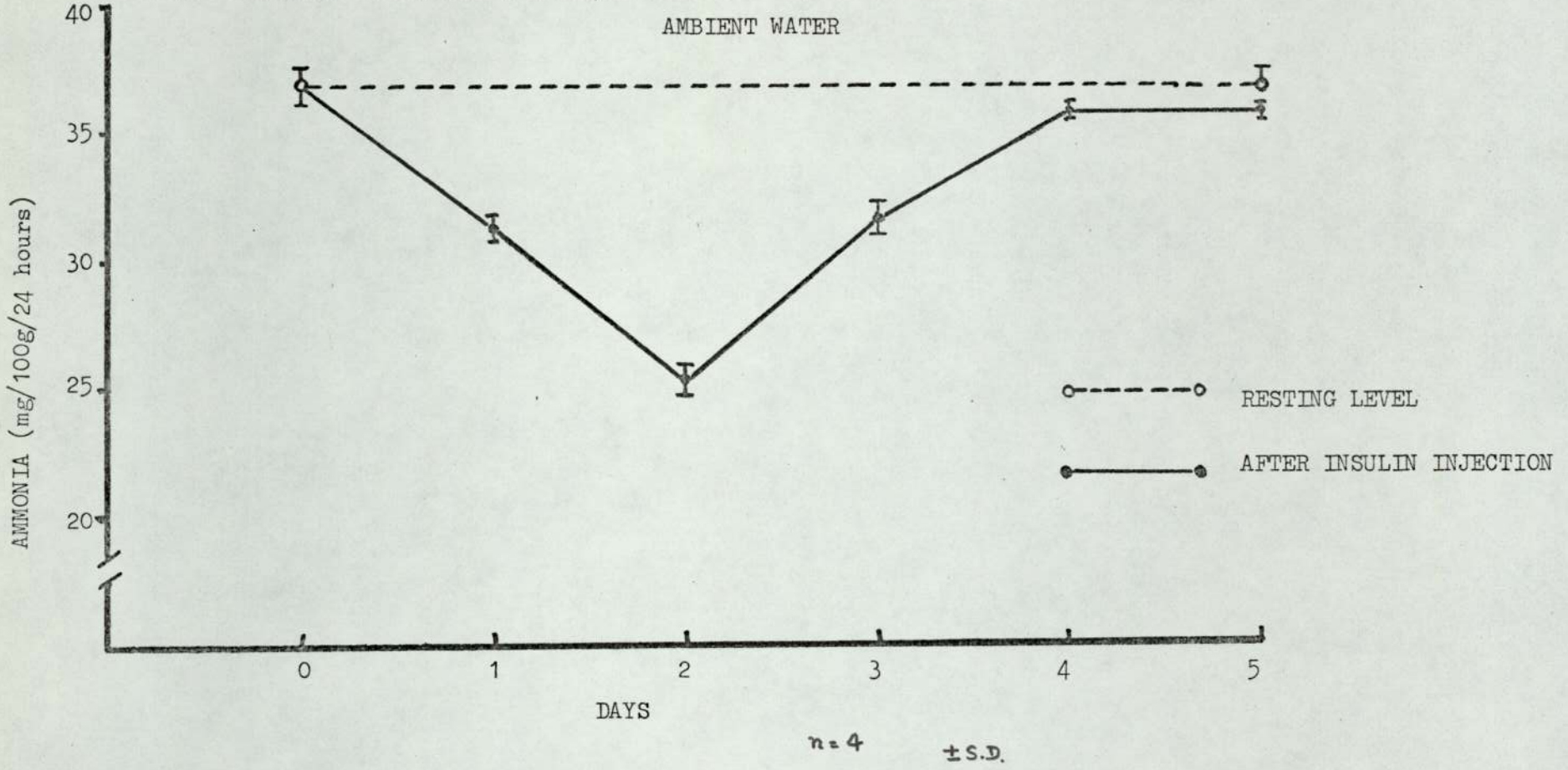


Table 7. EFFECT OF INSULIN (3 I.U/KG BODY WEIGHT) ON AMMONIA EXCRETION BY RAINBOW TROUT IN AMBIENT WATER

TIME (IN HOURS) AFTER INSULIN INJECTION	AMMONIA EXCRETED mg/100g fish/24 hours
Resting level	36.97 ± 1.04
24	31.42 ± 0.50 *
48	25.40 ± 1.01 *
72	31.75 ± 1.04 *
96	36.20 ± 0.36
120	36.27 ± 0.41

Values given are means ± S.D. of 4 groups

* $p < 0.01$

2.1.3. EFFECT OF GLIBENCLAMIDE AND TOLBUTAMIDE ON PROTEIN SYNTHESIS AND GROWTH IN RAINBOW TROUT

MATERIALS AND METHODS

Leucine-^{14}C Incorporation Experiment

Glibenclamide

Two groups of hatchery reared rainbow trout (Salmo gairdneri) weighing from 16 to 20 gms were acclimatized for 2 - 3 weeks at ambient temperature (15 - 17°C) in small hatchery troughs.

A few mgs. of glibenclamide (HB419), a gift from Farbwerke Hoechst AG, Frankfurt (M)-Hoechst, were dissolved in a minimum amount of N/50 sodium hydroxide prepared in deionised water. This solution was further diluted with deionised water to make a final concentration of 1 mg/ml. For intraperitoneal injection, the concentration of this stock solution was adjusted so that the appropriate dose of glibenclamide was contained in 0.1 ml. The fish were injected with glibenclamide (5 mg/kg body weight) through the intraperitoneal route. The controls were injected with the solvent (NaOH) used for dissolving glibenclamide. Four hours later, the fish were injected with L-leucine-^{14}C (5 µci/100 gm body weight). The fish were sacrificed exactly 4 hours later and samples of skeletal muscle were stored at -20°C. The incorporation of radioleucine into skeletal muscle protein was determined by methods described in Section 2.1.1(A and B)

Growth Experiments

Glibenclamide:

Rainbow trout (Salmo gairdneri) purchased from Vortex Fish Farms, Donnington, Stow-on-the Wold, were divided into 4 groups of 30 fish each. These were kept in 4 similar outdoor tanks supplied with running tapwater at a flow rate of 0.3 gallons/minute. Fish were individually marked with liquid nitrogen so that successive measurements on the same individual

could be linked. A commercial pelleted food (Beta Trout Pellets, Cooper Nutrition Products Ltd., Essex) was used as the basic stock in all diets. Glibenclamide was incorporated into the food in the following manner.

Glibenclamide was dissolved in a minimum amount of N/50 NaOH as described above and its concentration was adjusted with deionised water so that appropriate amounts (10,25 or 50 mg) were contained in 50 ml. This 50 ml solution was dispersed over 1000 gms of evenly spread pellets by means of a spray bottle connected to a pressure pump. The food was mixed thoroughly to ensure equal distribution of glibenclamide. The food was air dried to evaporate moisture and was stored at -20°C.

4 groups of rainbow trout were fed diets supplemented with either 0, 10, 25 or 50 mg/kg of glibenclamide for a period of 4 weeks. Fish were fed 3 times daily during a natural photoperiod; feeding was stopped when the fish ceased to eat the food and the daily food intake of each group was calculated. Fish were weighed individually at bi-weekly intervals.

Tolbutamide

Two groups of 25 rainbow trout each (average weight 81 gms) were maintained at 10°C to 12°C temperature in large fibre glass tanks supplied with continuously running well aerated tap water. The ambient ammonia levels were always kept well below toxic levels. During the experiment, which lasted 4 weeks, fish were fed "Beta Trout Pellets" supplemented with tolbutamide (0 or 2.5 gm/kg diet). Tolbutamide was dissolved in ethanol and incorporated into the diet by methods described above. Fish were fed to satiation at least twice daily during a natural photoperiod. Fish in each group were weighed individually at bi-weekly intervals.

RESULTS

Glibenclamide (5 mg/kg body weight) stimulated the incorporation of leucine-^{14}C into skeletal muscle protein in rainbow trout. 4 hour incorporation of radioleucine into skeletal muscle protein was significantly greater ($p < 0.05$) in glibenclamide treated group as compared to that in the control group (Fig.11, Table 8).

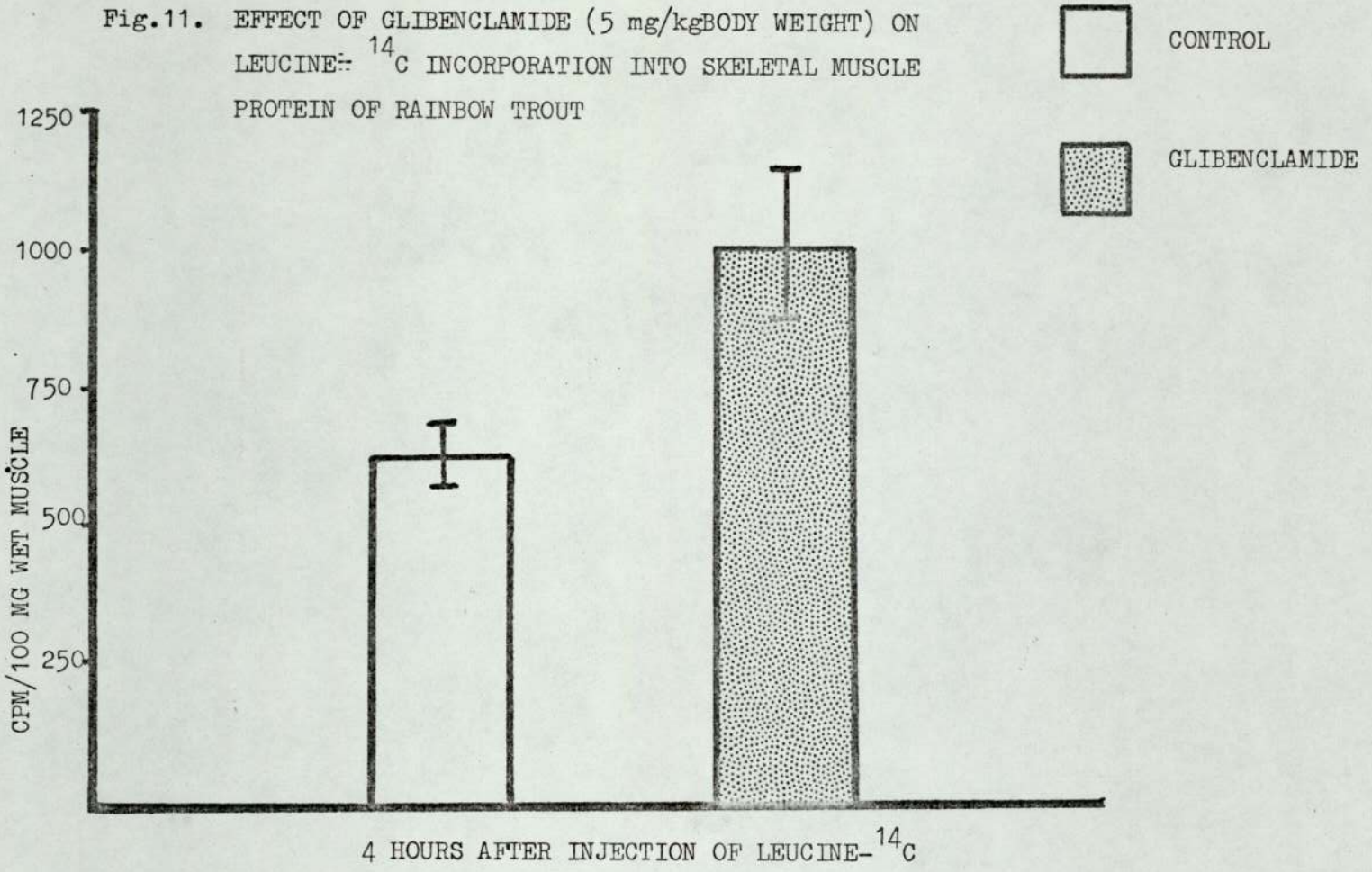
Rainbow trout fed glibenclamide supplemented diets at a concentration of 10, 25 and 50 ppm did not show the expected improvement in growth rate. Instead the growth was retarded. The mean weights along with per cent weight gains in four groups of fish after 14 and 28 days are shown in table 9. Fig.12 illustrates the inverse relation between growth and the amount of glibenclamide received. Since weight gains vary considerably between individual fish even on the same diet and appear to be correlated with initial weight (Cowey et al. 1973), therefore comparison was also made of weight gains between different groups at low, medium and high initial weights of < 25 gms, < 30 gms and < 35 gms. The results showed that growth depressing effects of glibenclamide were almost identical on small and large trout.

All four groups of fish seemed to be feeding at the start of the experiment but by the end of the second week, a relation between food consumption and dosage of HB419 was noticeable. Fish in the control group appeared to be eating actively whereas glibenclamide treated fish exhibited reduced appetite. The amount of food consumed and its conversion efficiency in control and glibenclamide-receiving groups are shown in Table 11. It is clear that glibenclamide not only depressed appetite but also impaired efficiency of food conversion.

The effect of feeding tolbutamide supplemented diets to rainbow trout is shown on Table 12. Like glibenclamide, this sulphonylurea hypoglycemic agent also exhibited deleterious effects on

the growth rate in one year old rainbow trout. The weight gains over a four week period in tolbutamide treated fish were significantly less than those in the control group ($p < 0.001$). The percentage increase in weight (g/100 g initial weight) over the entire experimental period (4 weeks) was 37.03% as compared to 12.19% in the group receiving tolbutamide.

Fig.11. EFFECT OF GLIBENCLAMIDE (5 mg/kgBODY WEIGHT) ON LEUCINE-^{14}C INCORPORATION INTO SKELETAL MUSCLE PROTEIN OF RAINBOW TROUT



n = 5 $p < 0.02$ $\pm$ S.D.

Fig.12. EFFECT OF FEEDING GLIBENCLAMIDE SUPPLEMENTED DIETS TO RAINBOW TROUT

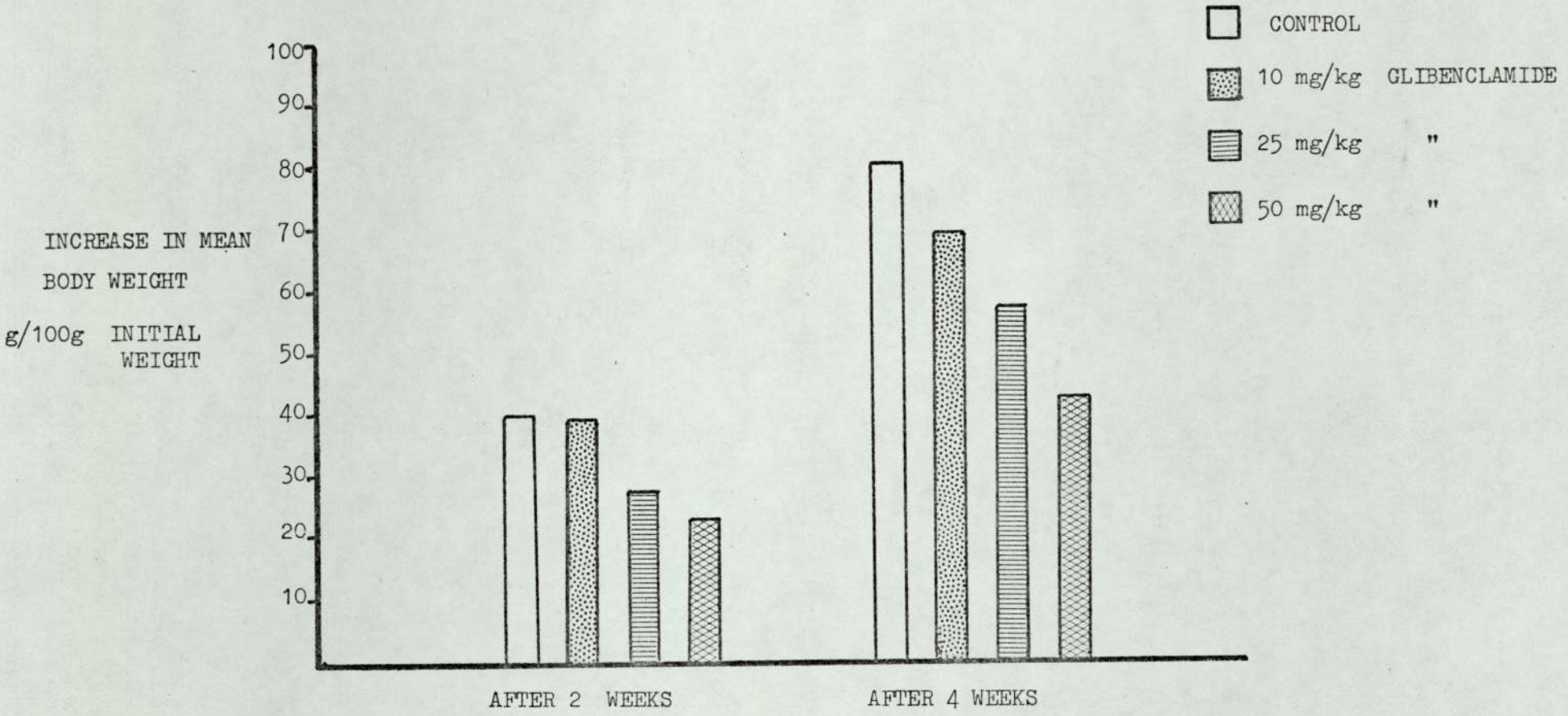


Fig.13. EFFECT OF FEEDING TOLBUTAMIDE SUPPLEMENTED DIETS TO RAINBOW TROUT

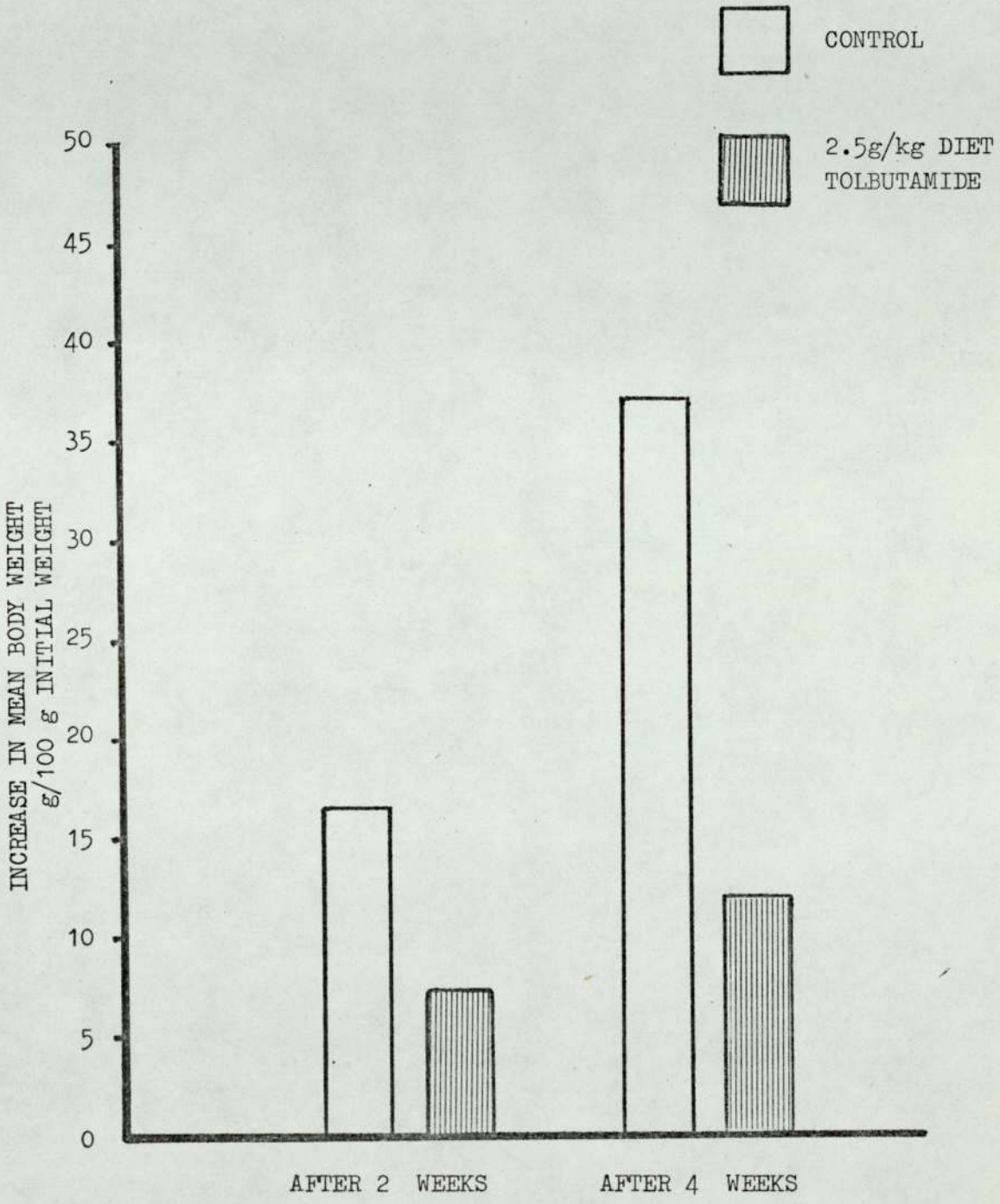


Table 8. IN VIVO EFFECT OF GLIBENCLAMIDE ON LEUCINE-^{14}C INCORPORATION INTO SKELETAL MUSCLE PROTEIN OF RAINBOW TROUT

Fish were injected with glibenclamide (5 mg/kg body weight). After 4 hours they were injected intraperitoneally with leucine-^{14}C (5 µci/100 gm body weight) and sacrificed 4 hours later. The values given for leucine-^{14}C incorporation are means ± S.D. of 5 fish.

TREATMENT	LEUCINE-^{14}C INCORPORATION c.p.m/100 gm wet muscle	P
CONTROL	606 ± 107	
INSULIN	1006 ± 284	<0.02

Table 9. EFFECT OF FEEDING GLIBENCLAMIDE SUPPLEMENTED DIETS TO RAINBOW TROUT.

Mean weights(g±S.D) of rainbow trout fed glibenclamide supplemented diets. Percentage increase in weight is shown in parentheses.

DAYS	0	14	28
Group I CONTROL	26.07 ± 4.06	36.58 ± 5.67 (40.3%)	47.05 ± 7.46 (80.4%)
Group II 10 mg glibenclamide/ kg food	25.35 ± 3.74	35.45 ± 5.84 (39.8%)	42.91 ± 7.70* (69.2%)
Group III 25 mg glibenclamide/ kg food	27.27 ± 4.79	34.77 ± 6.90 (27.5%)	42.85 ± 8.63* (57.1%)
Group IV 50 mg glibenclamide/ kg food	26.29 ± 3.58	32.36 ± 4.64** (23.0%)	37.55 ± 5.78** (42.8%)

n = 25

* $p < 0.05$

** $p < 0.01$

Table 10. AMOUNT OF FOOD CONSUMED AND FOOD CONVERSION EFFICIENCY OF RAINBOW TROUT RECEIVING DIFFERENT LEVELS OF GLIBENCLAMIDE

AMOUNT OF GLIBENCLAMIDE GIVEN	AMOUNT OF FOOD CONSUMED PER DAY (g/100 g initial weight)	FOOD CONVERSION EFFICIENCY (%)
CONTROLS	4.29	66.66
10 mg/kg food	3.89	63.64
25 mg/kg food	3.63	56.36
50 mg/kg food	3.31	46.10

Table 11. EFFECT OF FEEDING TOLBUTAMIDE SUPPLEMENTED DIETS ON RAINBOW TROUT.

Mean weights ($g \pm S.E$) of rainbow trout fed tolbutamide supplemented diets. Percentage decrease in weight gains over control group is shown in parentheses.

DURATION (WEEKS)	0	2	4
CONTROL	89.0 ± 3.8	103.7 ± 4.4	121.9 ± 5.4
TOLBUTAMIDE (2.5g/kg food)	83.8 ± 2.4	89.9 ± 2.7* (9.29%)	94.0 ± 3.1** (24.81%)

n = 25

* $p < 0.01$

** $p < 0.001$

2.2. EFFECT OF MAMMALIAN GROWTH HORMONE, FISH GROWTH HORMONE AND FISH PITUITARY EXTRACTS ON PROTEIN SYNTHESIS IN RAINBOW TROUT.

MATERIALS AND METHODS

Rainbow trout (Salmo gairdneri) obtained from Bibury Fish Farm, Gloucestershire were acclimatized for 2 weeks at ambient temperature (13 - 14°C) in small hatchery troughs supplied with running dechlorinated tap water. Fish were fed commercially available pelleted diet to satiation twice daily and were exposed to a natural photoperiod. At the time of the experiment fish weighed from 20 - 30 gms. Food was withdrawn 18 hours before the experiment.

GROWTH HORMONE

Porcine growth hormone (Ferring AB Malmö, Sweden) and shark growth hormone (Scripps Clinic and Research Foundation, California, U.S.A) were dissolved in 0.005 M hydrochloric acid (0.5 mg/ml). Porcine growth hormone was injected intraperitoneally in two doses (0.25 mg and 0.1 mg per fish) whereas shark growth hormone was given only in one dose of 0.1 mg/fish. The control fish were given appropriate volumes of 0.005M hydrochloric acid. After a period of 16 hours following hormone administration, the fish were injected intraperitoneally with 1-(U-^{14}C)-leucine (5 µci/100 gm body weight). Exactly 6 hours later fish were sacrificed and were frozen at -20°C. The incorporation of radioleucine into skeletal muscle protein was determined and was interpreted as percentage incorporation.

PITUITARY EXTRACTS

In another experiment fish were treated with pituitary extracts prepared from pituitaries of mature rainbow trout. Pituitary glands were collected from freshly caught rainbow trout (weighing from 300 - 400 gms) in the following manner. Each fish while still alive, was placed on a wooden table and held firmly. Using a sharp knife, the head was cut open transversely about $\frac{1}{2}$ inch behind the eyes. The cut extended

half way through the head and passed through the brain. The pituitary gland, exposed by lifting the brain, was picked up with a pair of fine forceps. The glands were quickly frozen by keeping them in a glass tube suspended by means of a wire in a flask containing liquid nitrogen. Eventually the glands were stored at -20°C in a deep freeze. Extracts of whole pituitary were prepared in the following manner. One gram of frozen pituitary glands were at first lyopholised and then homogenized in 0.7% saline solution. The homogenate was then suitably diluted for intraperitoneal injection. Each fish received 0.2 ml of pituitary extract equivalent to 100 mg of frozen pituitary. Fish in the control group were injected with an appropriate volume of 0.7% saline. After a period of 16 hours following the injection of pituitary preparation, fish were injected with l-leucine-^{14}C (5 µci/100 gm body weight). Exactly 6 hours later fish were killed by a blow on the head and were stored at -20°C. The incorporation of leucine into skeletal muscle protein was determined by the method described in section 2.1.1.(A and B) The data was interpreted as % incorporation.

RESULTS

Porcine growth hormone, at both the dosage levels tested, exerted an acute in vivo effect on protein synthesis in young rainbow trout. The hormone caused a significant increase in 6 hour leucine-^{14}C incorporation into skeletal muscle protein (Fig.14, Table 12). Mean values for uptake of radioleucine were 71.74 $\pm$3.83 (S.E.M) in the fish treated with 0.25 mg of GH and 33.69 $\pm$ 6.9 in the control group. At the lower dose (0.1 mg/fish) % incorporation was 68.17 $\pm$ 7.4 as compared to 30.5 $\pm$ 4.9 in the controls (Fig.15).

Shark GH (0.1 mg/fish) produced a trend towards increased protein synthesis (Fig.16) but it was not statistically significant probably due to large individual variations, and the small number of animals per group. Mean values of % incorporation of leucine in the hormone

treated and control group were 62.15 ± 7.3 and 56.82 ± 12.0 respectively.

The effect of administration of pituitary extracts on protein synthesis were similar to those observed after GH treatment. At the dosage level (100 mg/fish) tested, pituitary preparation induced an increase in the incorporation of leucine-^{14}C into skeletal muscle protein of rainbow trout (Fig.17). In the treated group mean % incorporation of radioleucine was 62.06 ± 2.59 (S.E.M) whereas in the control group it was 47.2 ± 4.09.

Fig.14. EFFECT OF PORCINE GROWTH HORMONE ON L-leucine-^{14}C INCORPORATION INTO THE PROTEIN OF SKELETAL MUSCLE OF RAINBOW TROUT

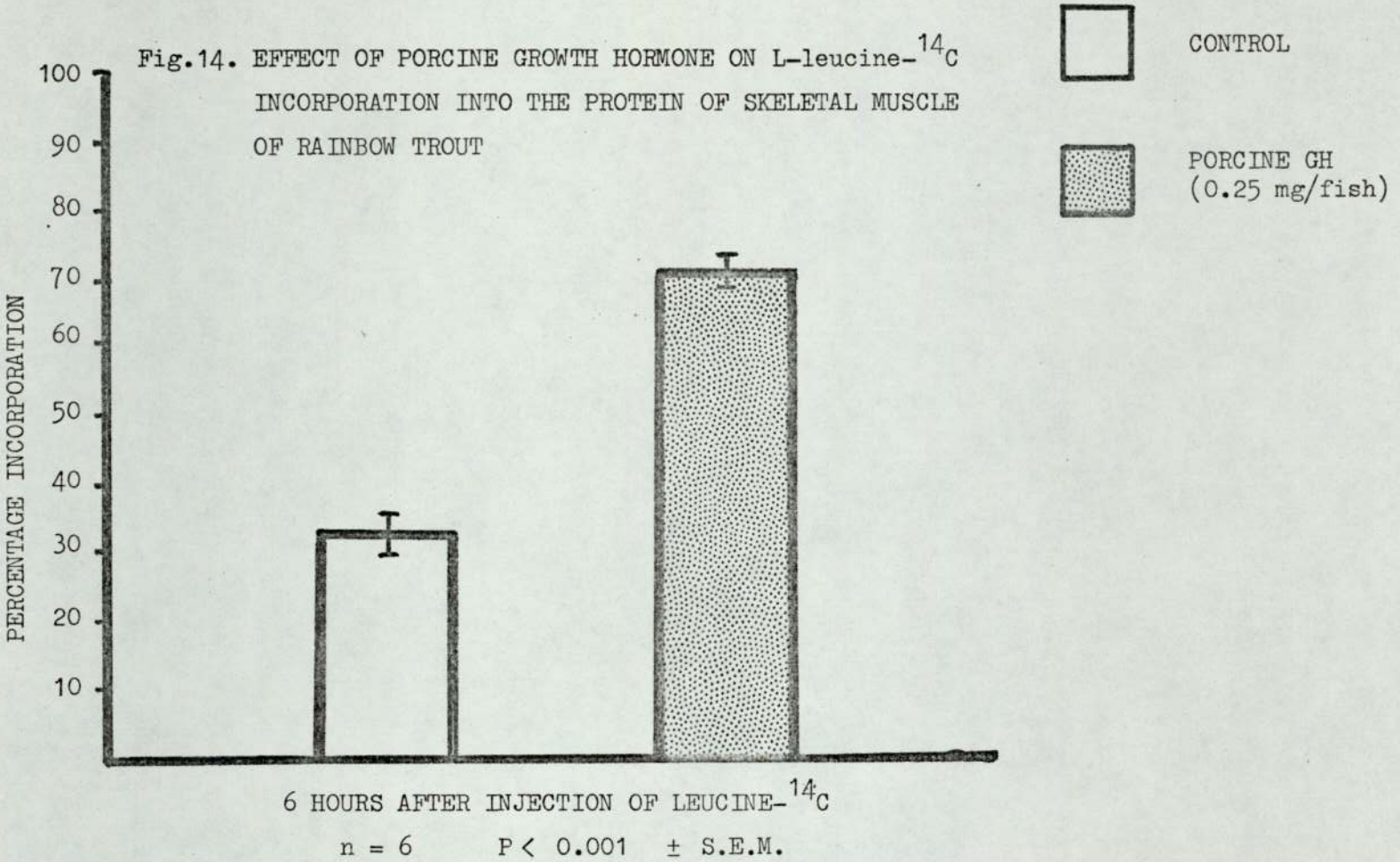


Fig.15. EFFECT OF PORCINE GROWTH HORMONE ON L-LEUCINE-^{14}C INCORPORATION INTO THE PROTEIN OF SKELETAL MUSCLE OF RAINBOW TROUT

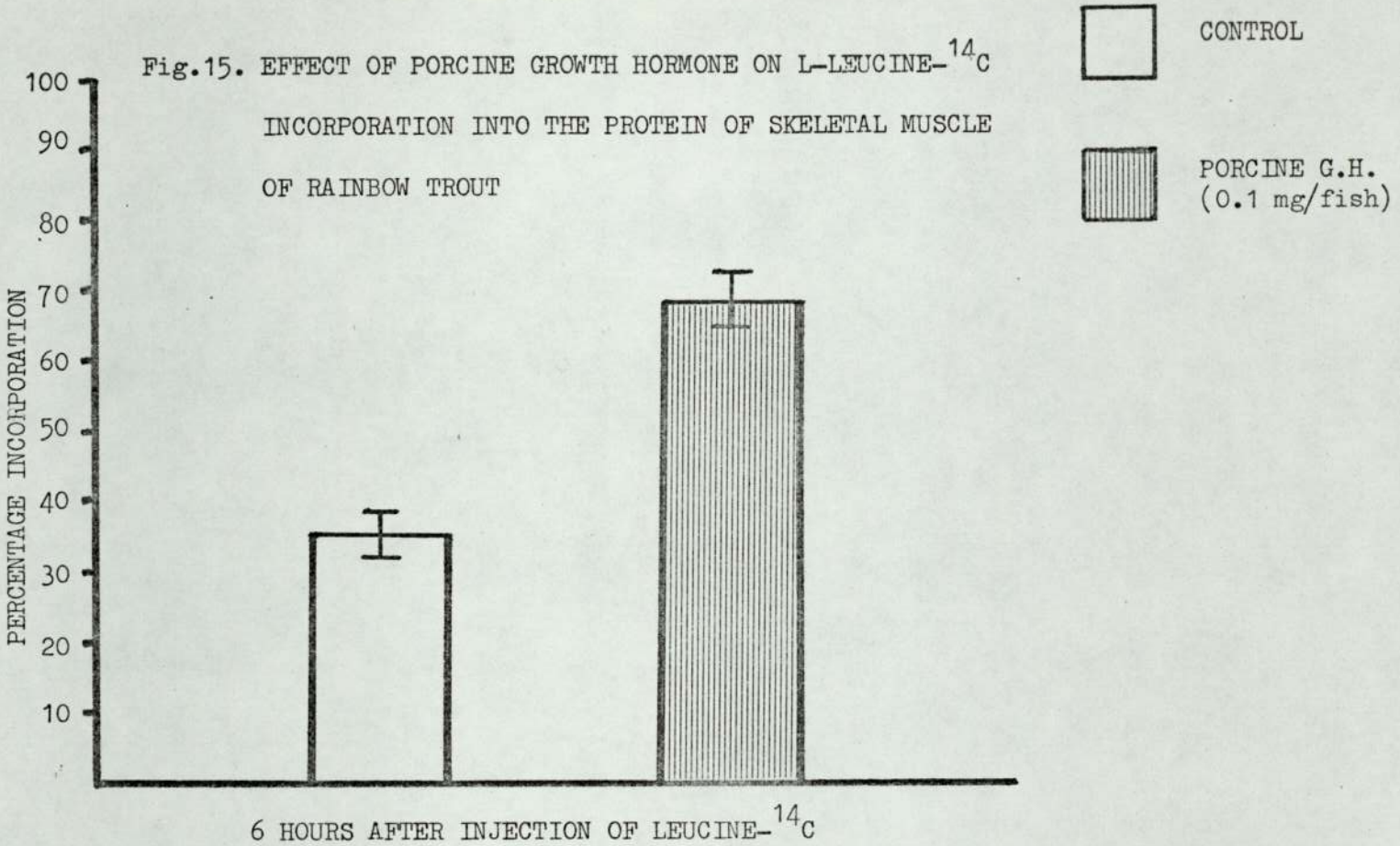
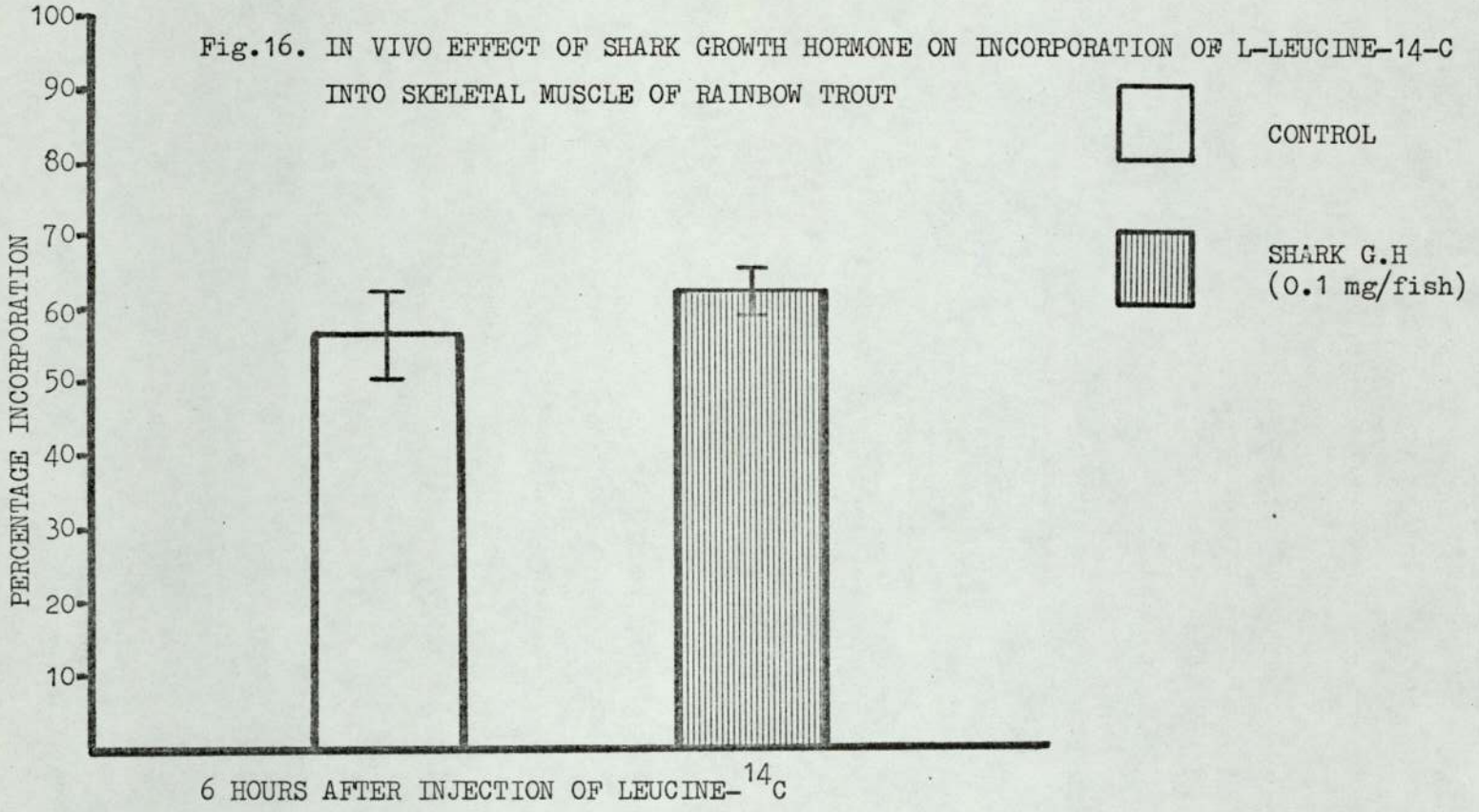
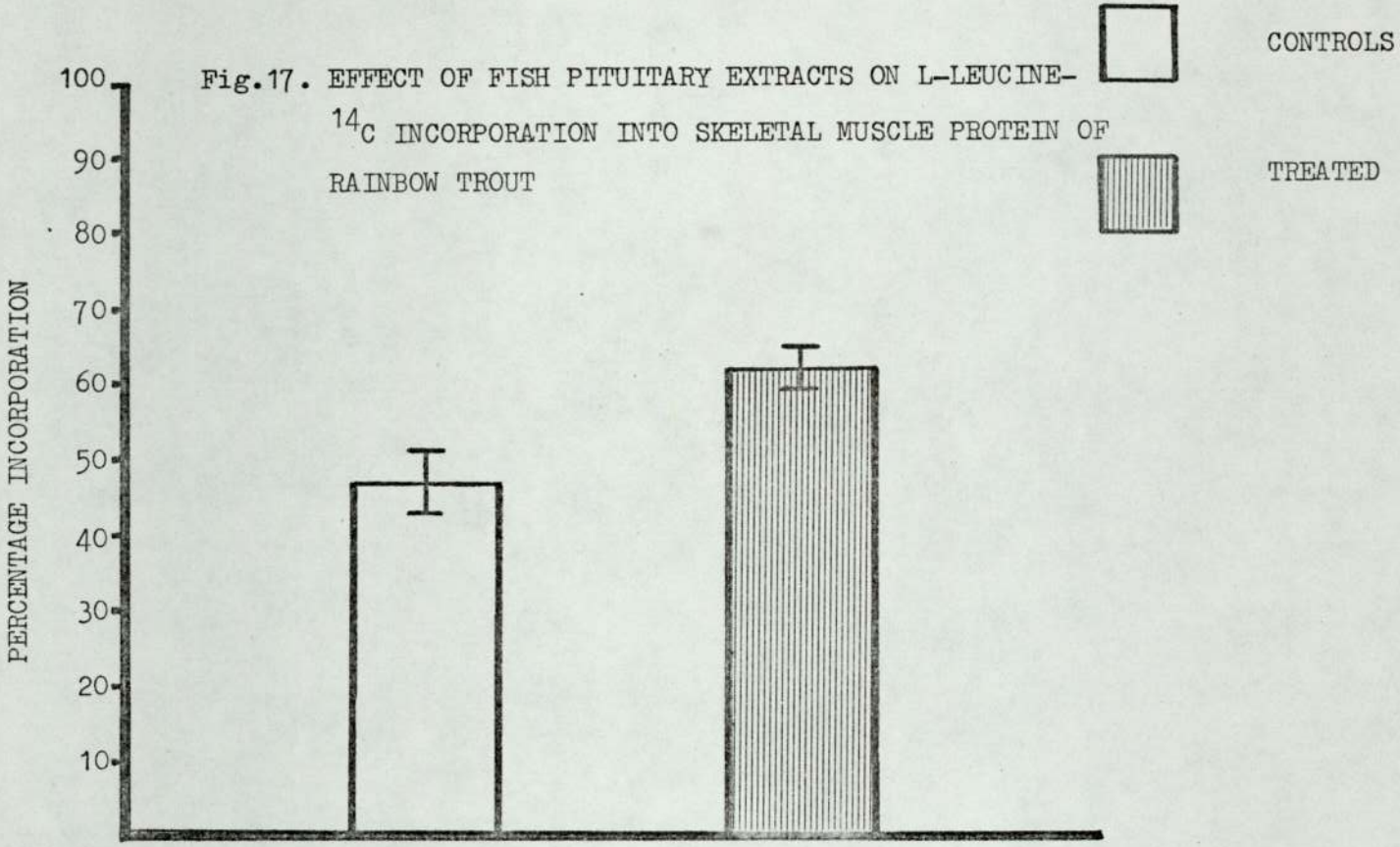


Fig.16. IN VIVO EFFECT OF SHARK GROWTH HORMONE ON INCORPORATION OF L-LEUCINE-14-C INTO SKELETAL MUSCLE OF RAINBOW TROUT



n = 3 ± S.E.M.

Fig.17. EFFECT OF FISH PITUITARY EXTRACTS ON L-LEUCINE-^{14}C INCORPORATION INTO SKELETAL MUSCLE PROTEIN OF RAINBOW TROUT



n = 4 $P < 0.05$ ± S.D.

TABLE 12. IN VIVO EFFECT OF MAMMALIAN AND FISH GROWTH HORMONE AND FISH PITUITARY EXTRACTS ON INCORPORATION OF L-LEUCINE-^{14}C INTO SKELETAL MUSCLE PROTEIN OF RAINBOW TROUT

Fish were injected intraperitoneally with GH or pituitary extracts. After 16 hours they were injected intraperitoneally with L-leucine-^{14}C (5 µci/100 gm body weight) and sacrificed 6 hours later. The values given for leucine-^{14}C incorporation are means ± S.E.M. The number of fish is shown in parenthesis.

	Leucine-^{14}C incorporation (% incorporation)		P
	CONTROLS	TREATED	
Porcine GH (0.25 mg/fish)	33.69% ±6.9 (6)	71.74% ±3.83 (6)	< 0.001
Porcine GH (0.1 mg/fish)	30.5% ±4.9 (3)	68.17% ±7.4 (3)	< 0.01
Shark GH (0.1 mg/fish)	56.82% ±12.01 (3)	62.15% ±7.3 (3)	
Rainbow Trout Pituitary Extracts (100 mg/fish)	47.29 ±4.09 (4)	62.06 ±2.59 (4)	< 0.05

2.3. EFFECT OF ANABOLIC STEROIDS AND DIETHYLSTILBOESTROL ON GROWTH AND PROTEIN METABOLISM IN RAINBOW TROUT.

2.3.1. MATERIALS AND METHODS

2.3.1.1. FISH

The fish used in these experiments were hatchery reared juvenile rainbow trout (Salmo gairdneri) purchased from Vortex Fish Farms, Donnington, Stow-on-the-Wold and Bibury Trout Farm, Gloucestershire. Each time about 500 fish fry were transported to the laboratory and kept in the stocking tanks for some time to grow them to a suitable size for experimental work. They were fed commercially available pelleted diet (Cooper Nutrition Products Ltd., Essex). The size of the pellet selected for feeding a particular size of fish was according to the recommendation made by the manufacturers. The fish were fed 2 - 3 times daily to satiation.

2.3.1.2. TANK SYSTEM

For growth trials, a system of 12 tanks was plumbed up on an open flow basis (Fig.18). The tanks (functional capacity 70 litres), purchased from W.C.B.Plastics Ltd., London were made of high density polythene. They were particularly selected because of their round shape and sloping bottoms. It was envisaged that sloping bottoms would facilitate the cleaning of the faecal matter. The tanks were mounted on a Bartangle framework. Each tank, as shown in Figure 18, overflowed from the bottom, via a narrow vertical standpipe (3/8" bore). Waste water, carrying faecal matter from the bottom of each tank flowed between the two standpipes and drained through the inner standpipe. Thus, the inner standpipe maintained a minimum level of water in the tank. This system of drainage of waste water rendered the tanks self cleaning. Each tank was supplied with water through a 3/8" inlet pipe arising from a ring main made of $1\frac{1}{2}$" P.V.C pipe connected to the header tank. Flow of water was adjusted by a membrane

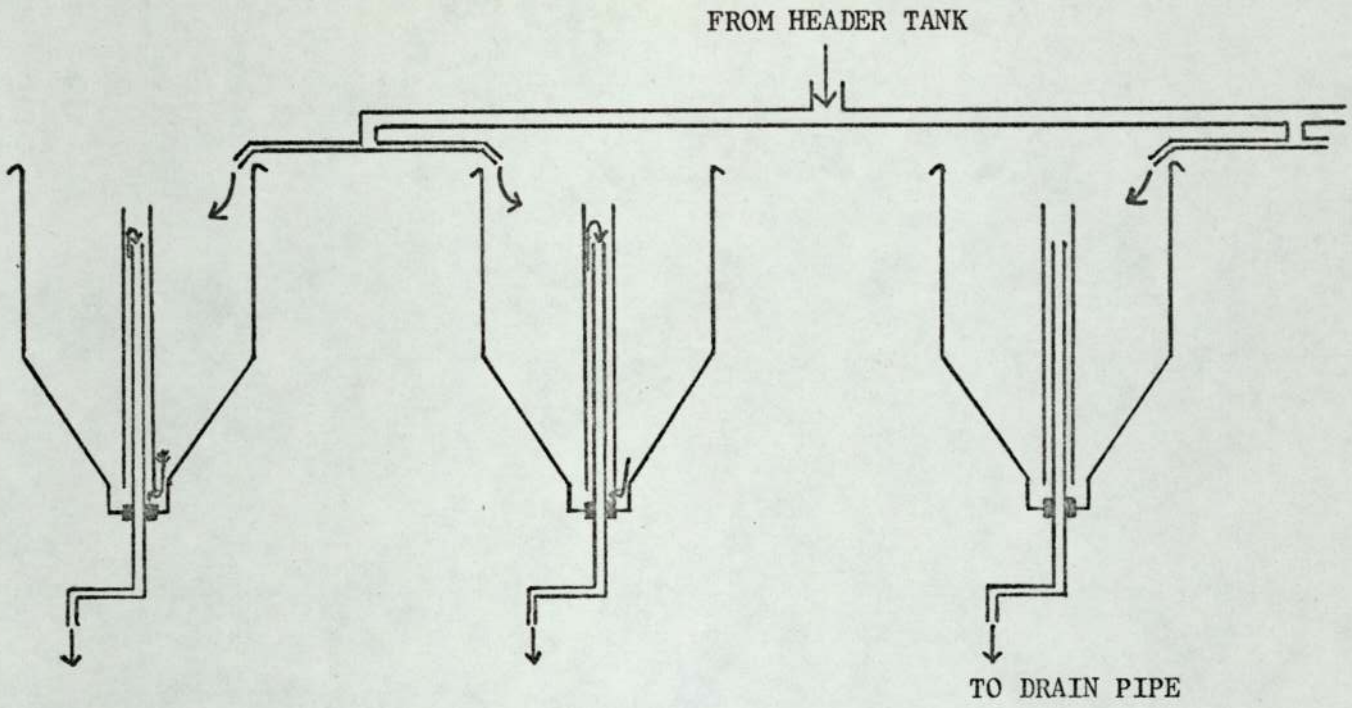


FIG. 18. TANK SYSTEM USED IN GROWTH EXPERIMENTS

PLATE 1. PART OF GROWTH EXPERIMENT RIG.

PLATE 2. NETLON COVERED TANK CONTAINING RAINBOW TROUT.

PLATE 3. INTERIOR OF A TANK SHOWING SLOPING BOTTOM FOR COLLECTION OF FAECES.

valve in the main pipe as well as by the taps connected to each inlet pipe. Prior to commencement of each experiment, fish were acclimatized for two weeks at ambient temperature in the experimental tanks. Flow of water in each tank was maintained at 1 litre per minute which ensured sufficient saturation of oxygen in water and kept the concentration of ammonia well below toxic levels.

2.3.1.3. HORMONAL TREATMENT

The chemical formulae of the compounds tested for their efficacy in promoting body growth in rainbow trout are shown in figure 19. These included:

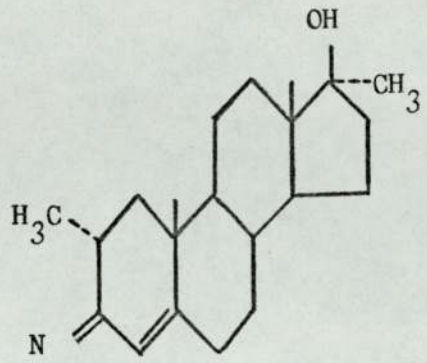
1. Dimethazine (2α,17α-dimethyl-17β-hydroxy-5α-androstan-3,3'-azine) a gift from Ormonoterapia Richter, Milan, Italy.
2. Norethandrolone (17α-ethyl-17β-hydroxy-19-norandrost-4-en-3-one), a gift from Searle,U.S.A.
3. Methenolone acetate (1-methyl-17β-hydroxy-5α-androst-1en-3one acetate) a gift from Schering, Berlin, Germany.
4. Diethylstilboestrol, purchased from Sigma Chemical Company, London.

These compounds were given with food at different levels of concentration to young rainbow trout for a certain period of time. The commercial trout feed, manufactured by Cooper Nutrition Products Ltd., was used as the basic stock in all diets. The specification of this feed, given by the manufacturers was as follows:

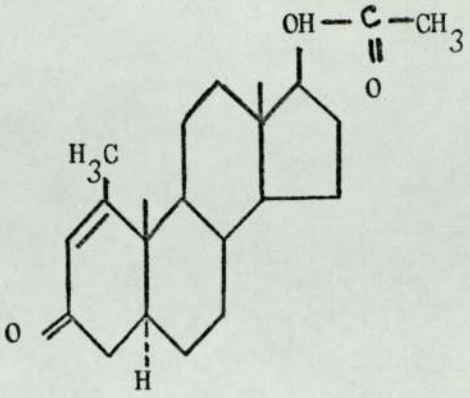
		Vitamin	I.U/Kg
Oil	45%		
Protein	40%	A	18000
Fibre	4.5%	D	2000
		E	30

The anabolic steroids and diethylstilboestrol were incorporated into the diet by the method described in section 2.1.3. 100 mg of the hormone were dissolved in 100 ml of ethanol. The concentration of this solution was so adjusted that an appropriate amount of the hormone was

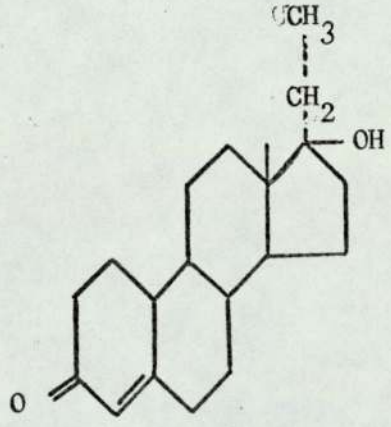
Fig.19. CHEMICAL FORMULAE OF COMPOUNDS TESTED



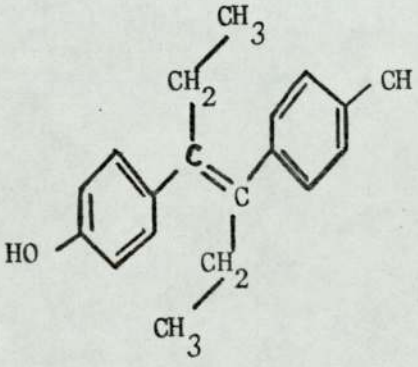
DIMETHAZINE



METHENOLONE ACETATE



NORETHANDROLONE



DIETHYLSTILBOESTROL

contained in 25 ml of ethanol. This was then sprayed on 1000 g of pelleted diet. After evaporation of the ethanol, the diets were stored at -20°C to avoid any possible degradation of the steroid. This method of incorporation of steroids into the diets was found to be satisfactory. Prior to application of this method, it was attempted to mix the hormones with food in a gelatine mixture, but it was not considered to be suitable because the powdered hormones did not make a homogeneous suspension in the gelatine solution.

A. DIMETHAZINE

Growth Experiments

120 rainbow trout of similar size (3.5 gms) were selected from the stocking tank and were split randomly into 4 groups of 30 fish each. Group 1 served as the control. The remaining groups were fed diets supplemented with dimethazine at concentrations of 5, 10 and 20 mg/kg dry food for a period of 48 days. The water temperature during this experimental period varied from 14.5 - 18°C. Mean water temperature during the first period (0 - 16 days) was 16.1 $\pm$ 1.5 whereas during the second (17 - 32 days) and third (33 - 48 days) period, it was 16.45 $\pm$ 1.3°C and 17.9 $\pm$ 1.7°C respectively. The fish were fed to satiation three times daily during week days, twice on Saturdays and once on Sundays. Photoperiod corresponded to natural day length during that time of the year. Fish were weighed individually at intervals of 16 days. Before weighing, all the fish of one group were taken out of the tank and kept in a plastic bin filled with water which was aerated continuosuly. Each fish was taken in a net and following the removal of excess water with a soft tissue paper, the weight was determined. Anaesthesia was not used throughout this study and handling of unanaesthetised fish did not appear to cause any adverse effects. At the end of the experiment, fish were sacrificed.

15 fish were randomly selected from each group and their liver and kidneys were weighed separately for determination of renosomatic and hepatosomatic indices. Pieces of skin, liver and kidney of fish from different groups were fixed in Bouin's fluid for histological examination. Skin, in all the specimens, was excised from the ventro-lateral side of the body wall near the pectoral fin.

In the light of results obtained from the above experiment, a second series of experiments was designed in which dimethazine was administered in lower doses of 5, 2.5 and 1 mg/kg dry food. The number of fish in each group was raised to 40. The initial average weight of the fish was 2.85 gms. The experiment lasted for 60 days during which time the water temperature varied from 12.0°C to 19.5°C. Mean water temperature during the first period (0 - 20 days) was 15.1 $\pm$ 2.0°C whereas during the second (21 - 40 days) and third (41 - 60 days) period, it was 18.1 $\pm$ 1.5 and 16.8 $\pm$ 1.8 respectively. Fish were fed to satiation twice daily during a 12 hour photoperiod. Feeding was stopped when the fish ceased to eat the food. Daily intake of food by each group of fish was noted. Weight and length measurements were made at intervals of 20 days. At the end of the experiment, 15 fish were selected at random from each group. After taking blood samples for haematocrit measurements, the fish were killed and livers and kidneys were excised and weighed separately to determine renosomatic and hepatosomatic indices. The viscera constituting the complete digestive tract and spleen were also weighed to get some idea of the effect of the hormone on weight gains in the eviscerated carcass. Fish carcasses were stored at -20°C for analysis of moisture and total nitrogen content of the skeletal muscle.

Leucine-^{14}C incorporation

30 rainbow trout of similar size (average weight 2 gm) were split into two groups of 15 fish each. One group was fed the control diet, while

the other received dimethazine supplemented diet (2.5 mg dimethazine/kg food). At the end of 20 days feeding, 5 fish were selected at random from each group and injected intraperitoneally with L-(U-^{14}C)-leucine (5 µci/100 gm body weight). Exactly 6 hours later, the fish were killed with a blow on the head and samples of skeletal muscle were stored at -20°C for determination of leucine-^{14}C into skeletal muscle protein. Measurements of radioactivity in the extracted protein from the muscle were carried out in the manner described in section 2.1.1.(A and B). The incorporation of leucine-^{14}C into skeletal muscle protein at 15.5°C water temperature was calculated as a percentage of the total sample activity (% incorporation).

B. NORETHANDROLONE

Growth Experiments

The experimental arrangements and environmental parameters for evluation of growth promoting effects of norethandrolone were identical to those for low doses of dimethazine except that this steroid was tested only at two concentrations of 5 and 2.5 mg/kg dry food. Both dimethazine and norethandrolone trials were run simultaneously and the same group of fish served as control for the two steroids. Fish were weighed and measured in length at intervals of 20 days. At the end of 60 days period, fish were killed and renosomatic index, hepatosomatic index, haematocrit (%),and visceral weight (% total body weight) were determined. Fish carcasses were stored at -20°C for analysis of moisture and total nitrogen in the skeletal muscle.

Leucine-^{14}C incorporation

Again the experimental arrangements and environmental parameters were identical to those in the corresponding experiment with dimethazine. Fish were fed norethandrolone supplemented diets (2.5 mg norethandrolone/kg food) for 20 days. The incorporation of intraperitoneally injected

l-leucine-^{14}C (5 µci/kg body weight) into skeletal muscle protein during a six-hour period was determined.

C. METHENOLONE AND DIETHYLSTILBOESTROL

75 rainbow trout (Salmo gairdneri), average weight 13.5 gms. were taken from the stock tank and were distributed randomly into three groups of 25 fish each. The first group served as the control, whereas the second and third groups were fed diets supplemented with methenolone (10 mg/kg food) and diethylstilboestrol (1.2 mg/kg food) respectively. The experiment lasted for 30 days during which water temperature varied from 12.0°C to 16°C. Mean water temperature during the first 15 days was higher (15.1 ± 1.8) than the second 15 days (13.8 ± 1.5). Fish were fed to satiation at least 2 times daily during a natural photoperiod Daily intake of food by each group was noted. Fish were weighed individually at biweekly intervals. At the end of the experiment 15 fish selected at random from each group were killed and samples of skeletal muscle were frozen at -20°C for analysis of moisture and total nitrogen.

2.3.1.4. ANALYTICAL TECHNIQUES

A. Moisture

Weighed samples of skeletal muscle were dried in aluminium dishes placed in a drying oven at 100°C till a constant weight was obtained. Moisture was interpreted as a percentage of the total sample weight.

B. Total Nitrogen

Total nitrogen was determined by the microkjeldahl procedure. A weighed sample of dried muscle was digested by concentrated sulphuric acid in 30 ml microkjeldahl flasks using a catalyst mixture of 32 parts potassium sulphate, 8 parts copper sulphate and 1 part powdered selenium. The distillation procedure was carried out in a Markham Distillation Apparatus. Ammonia released from the digested sample by the addition of 4 ml of 40% sodium hydroxide was carried over with the distillate. It was collected in 5 ml of saturated boric acid and titrated directly

against 0.01N hydrochloric acid using Tshiro's indicator (0.08% methyl red and 0.02% methylene blue dissolved in ethanol.)

C. Haematocrit

Since haematocrit values correlate with red cell count and haemoglobin values determined on the same fish (Snieszko, 1960), measurement of percentage haematocrit was carried out, in rainbow trout, to examine the effect of hormonal treatment on haematology. A sample of blood was withdrawn from the ductus cuvieri into a heparanized syringe. From the syringe the blood was collected directly into duplicate commercial heparinized capillary tubes. One end of each tube was sealed using a microburner and the tubes were centrifuged in a microhaematocrit centrifuge for five minutes. The packed cell volume was measured as a percentage of the total blood volume using a microhaematocrit reader.

2.3.1.5. HISTOLOGICAL TECHNIQUES

Pieces of skin, liver and kidney fixed in Bouin's fluid were dehydrated in gradually increasing concentration of alcohol, cleared in chloroform and embedded in paraffin wax (melting point 56°C). All specimens were sectioned at a thickness of 6μ and stained with eosin and Mayer's haematoxylin.

2.3.2. RESULTS

2.3.2.1. DIMETHAZINE

A. ACCELERATION OF GROWTH

Experiment 1

The weight data from the weighing of individual rainbow trout in the four groups treated with 0, 5, 10 and 20 mg dimethazine/kg food are presented in Table 13, Fig.20. It is clear that fish in the group which received 5 mg dimethazine/kg food grew at the fastest rate. After 48 days of feeding, the % increase in mean body weight in this group was 53% greater than that of the control group ($P<0.05$). Fish fed dimethazine at levels of 10 and 20 mg/kg food gained 27% and 3% more weight than the

control group. Daily increments of mean body weight during Period I (0 - 16 days), Period II (17 - 32 days) and Period III (33 - 48 days) are shown in Table 14, Fig.21. It is evident that higher doses (10 and 20 mg) of dimethazine resulted in an initial increase in daily increments of body weight but these were not significantly different from controls after 48 days. During Period I, the fish receiving 20 and 10 mg dimethazine/kg food were growing at a rate of 3.76% and 3.82% per day respectively, while in the control group daily gains were 3.53%. During Period II the daily weight gain in the group receiving 20 mg dimethazine/kg food dropped to 3.52% whereas the control group gained 3.73%. By the end of Period III, the inability of dimethazine, when given in high doses over a long period of time, to produce positive growth response in rainbow trout was obvious.

Experiment II

In the second experiment which was carried out to establish the optimum dose of dimethazine for rainbow trout, differences in percentage increase in mean weight of fish were evident after 20 days of feeding (Fig.22). As shown in Table 16, the weight gains in fish receiving dimethazine at levels of 5 and 2.5 mg/kg food were over 18% greater than in the fish receiving no steroid ($P<0.01$). The growth response was not so marked in the group fed 1 mg dimethazine/kg food. The anabolic effect of dimethazine was even more markedly noticeable at the end of 60 days. The two groups receiving dimethazine at concentrations of 5 and 2.5 mg/kg food gained 52% and 60% more weight than the control group. In these groups overall increases in mean weight were significantly greater ($P<0.01$) than among the control group. However, increase in the mean weight of fish in the group fed dimethazine at a level of 1 mg/kg food was not significantly different from that of the control group. Daily increments of mean body weight are shown in table 17. It is obvious from the data

that dimethazine at a concentration of 2.5 mg/kg food is the optimum dose of dimethazine for rainbow trout. In this experiment the effect of dimethazine on increase in body length was also examined. At the dosage levels tested, dimethazine did not produce any significant increase in body length during 60 days treatment (Table 18).

B. FOOD CONVERSION EFFICIENCY

The amount of food consumed and food conversion efficiency in various groups of fish treated with dimethazine is shown in table 19. The steroid when given at levels of 5, 2.5 and 1.0 mg/kg food did not cause any change in the consumption of food. However, the hormone produced an improvement in food conversion efficiency. As compared to the control group, the fish receiving 5 and 2.5 mg dimethazine/kg food showed 18% improvement in food conversion efficiency.

C. MUSCLE ANALYSIS

Since skeletal muscle constitutes the bulk of the carcass, the amount of moisture and total nitrogen in samples of skeletal muscle of fish fed dimethazine supplemented diets at concentrations of 0, 5, 2.5 and 1 mg/kg food was determined. The mean values of total nitrogen and moisture are shown in table 21. The steroid did not induce any significant change in these parameters, hence the quality of muscle in the fish grown on anabolic steroid appeared to remain unchanged. Similarly values for mean visceral weight (Table 21) also showed that increase in weight gain induced by dimethazine was not due to possible changes in the visceral mass.

D. LEUCINE-^{14}C INCORPORATION

As an index of protein synthesis, the *in vivo* incorporation of l-leucine-^{14}C into skeletal muscle protein was determined in fish fed dimethazine supplemented diet (2.5 mg dimethazine/kg food) for

20 days. The results are shown in Table 22. The percentage incorporation of leucine-^{14}C into muscle protein was significantly increased in the group treated with dimethazine ($P<0.01$). In this group the mean percentage incorporation levels were 78.34 ± 7.10 (S.D.) while the mean value for the control group was 56.2 ± 8.5.

E. EFFECT ON TISSUES

Liver. Neither in low nor in high doses, dimethazine produced any significant difference in the ratio of liver to body weights (hepatosomatic index, H.S.I). Values for HSI of various groups of fish treated with dimethazine are shown in Tables 15 and 20, and indicate that the anabolic effects of dimethazine in rainbow trout are divorced from hepatogrophic action. The histological examination of the livers of fish treated with higher doses of dimethazine (20,10 and 5 mg/kg food) for 48 days showed that the steroid had not caused any pathological change in the liver.

Kidney. A comparison of individual kidney weights with body weights (renosomatic index, RSI) in various groups of fish fed diets supplemented with dimethazine are given in tables 15 and 20. With the exception of the group which received 1 mg dimethazine/kg food, in all the other treated groups, the RSI were significantly greater than those of the control group ($P<0.05$). Histological examination of the kidneys of fish from groups fed diets supplemented with high doses of dimethazine (5, 10 and 20 mg/kg) did not show any hypertrophy of epithelial cells and of their nuclei of kidney tubules. However, there appeared to be an increase in the haemopoietic tissue in the kidney from the treated fish (Plate 4 and 5), which was probably responsible for increased renosomatic indices.

Skin. Dimethazine did not produce any histological changes in the skin. The thickness of the epidermis in the treated fish was not different from the control fish (Plate 6 and 7).

Haematocrit. The mean values for the haematocrit (%) of rainbow trout receiving dimethazine at concentrations of 5, 2.5 and 1 mg/kg food for 60 days is shown in table 20. Comparison of means indicated a significant increase ($P<0.05$) in percentage haematocrit only in the group treated with 5 mg dimethazine/kg food.

2.3.2.2. NORETHANDROLONE

A. ACCELERATION OF GROWTH

Since dimethazine produced anabolic response in rainbow trout only when it was given in minute quantities, another anabolic steroid, norethandrolone was tested at two dosage levels of 5 and 2.5 mg/kg food. Values for mean weights and percentage weight gains for fish fed this steroid with diet for 60 days are shown in Table 23 and Fig. 25. Increases in weight gains in the treated fish were significantly greater than those in the control fish ($P < 0.01$). Fish fed on norethandrolone at 5 and 2.5 mg/kg food levels for 60 days showed a net weight gain of 44% and 69% over the fish which received no steroid. Daily increments of mean body weights in the three groups of fish are shown in Table 24. In Period I (0 - 20 days), the group receiving 5 mg norethandrolone/kg food grew faster than the group which received steroid at 2.5 mg/kg food level. In Period II (21 - 40 days) and Period III (41 - 60 days), however, the later group showed maximum growth. Data given in Tables 23 and 24 show a drop in growth of fish in all groups during Period II. This was probably due to sudden variations in temperature which rose from $15.1 \pm 2.0^{\circ}C$ in Period I to 18.1 ± 1.5 in Period II. It is obvious from the results that, like dimethazine, norethandrolone produces maximum anabolic activity in rainbow trout only when given in minute quantities. The effect of the steroid on increases in body length (Table 25) was not as marked as on weight gains. Only in the group receiving norethandrolone at 2.5 mg/kg food level, values for mean body length were significantly greater than in the control group.

B. FOOD CONVERSION EFFICIENCY

Values for the amount of food consumed (% body weight per day) and food conversion efficiency are shown in table 26. There was not much difference in the amount of food consumed between various groups. Food conversion efficiency appeared to have improved considerably in the group fed norethandrolone supplemented diets.

C. MUSCLE ANALYSIS

Mean values for muscle nitrogen, moisture and visceral weights are shown in Table 28. Values for the treated group were not significantly different from those of the control. Like dimethazine, this anabolic steroid also did not appear to have affected the quality of the fish. The growth response was quantitative only.

D. LEUCINE-^{14}C INCORPORATION

Six hours percentage incorporation of leucine-^{14}C into skeletal muscle protein of rainbow trout fed norethandrolone at a concentration of 2.5 mg/kg food for 20 days was significantly greater than those of the control group ($P<0.01$). Mean percentage incorporation (Table 22, Fig.24) was 56.2 ± 8.5 (S.D) in the control group, while in the treated group the mean value was 77.06 ± 4.0.

E. EFFECT ON TISSUES

Values for HSI, RSI and haematocrit (%) of various groups of fish fed norethandrolone supplemented diets for 60 days are given in table 27. The steroid did not alter either hepatosomatic index or renosomatic index significantly in any of the treated groups. Thus, it appears that the action of norethandrolone in rainbow trout is free from hepatotrophic and renotrophic effects. The haematocrit values for one group (2.5 mg norethandrolone/kg food) showed significant increase over the control group ($P<0.05$).

Fig.20. CHANGES IN MEAN BODY WEIGHT OF RAINBOW TROUT FED DIMETHAZINE SUPPLEMENTED DIETS

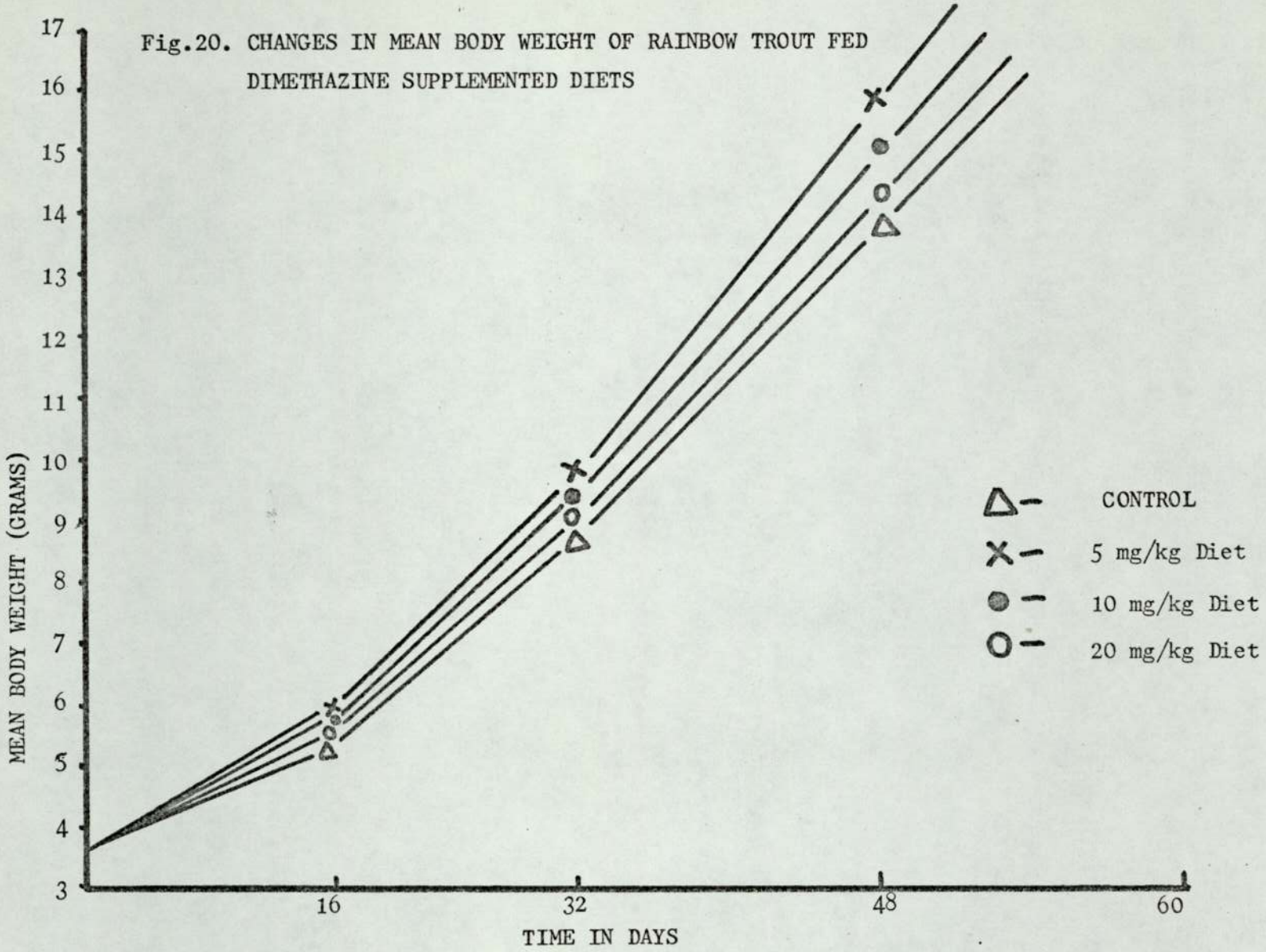


Fig.21. EFFECT OF FEEDING DIMETHAZINE-SUPPLEMENTED DIETS ON PERCENTAGE DAILY INCREMENT IN MEAN BODY WEIGHT OF RAINBOW TROUT

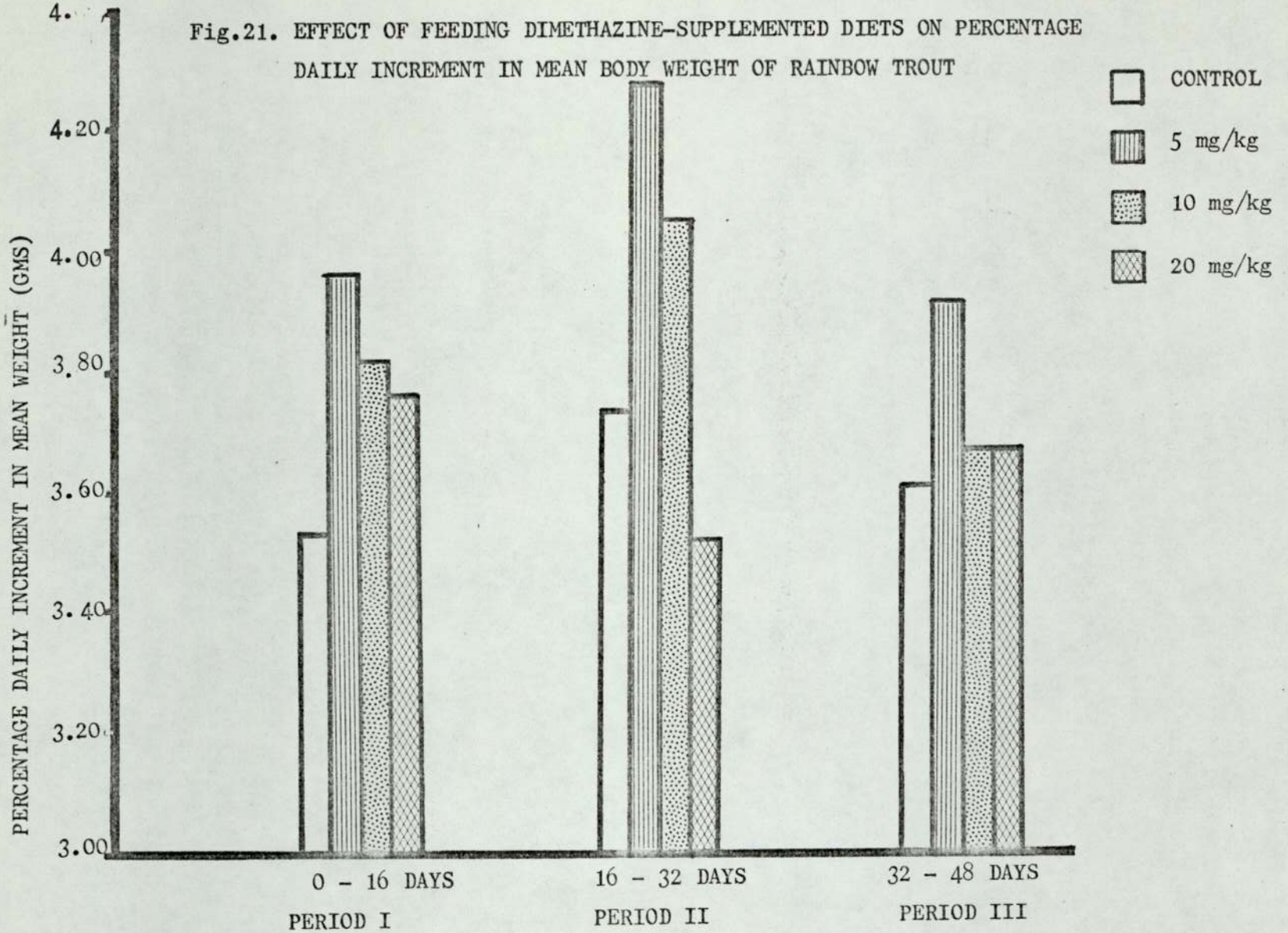


Fig.22. EFFECT OF FEEDING DIMETHAZINE-SUPPLEMENTED DIETS TO RAINBOW TROUT

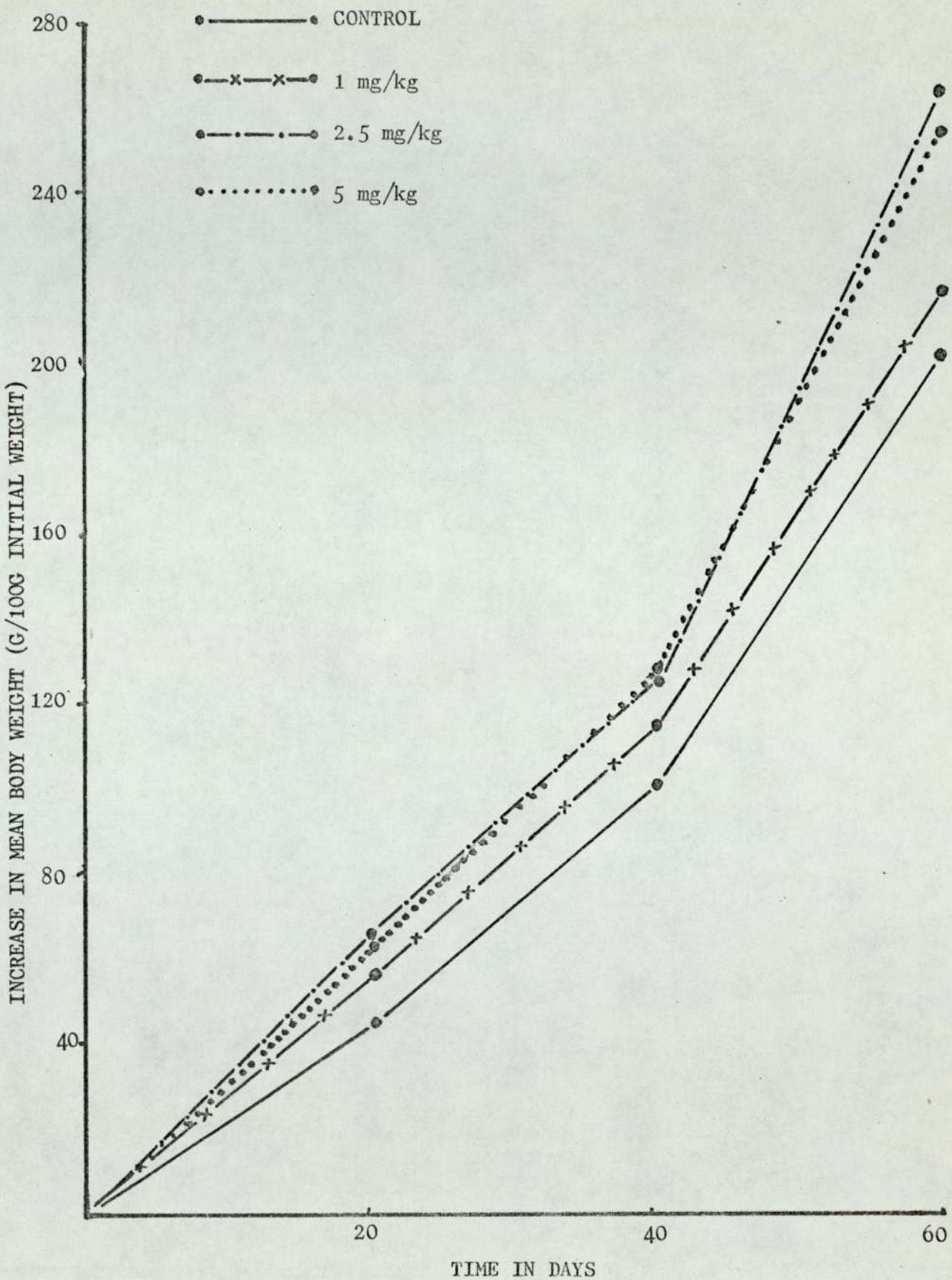


FIG.23. MEAN WEIGHTS (± S.D) OF RAINBOW TROUT FED DIMETHAZINE SUPPLEMENTED DIETS

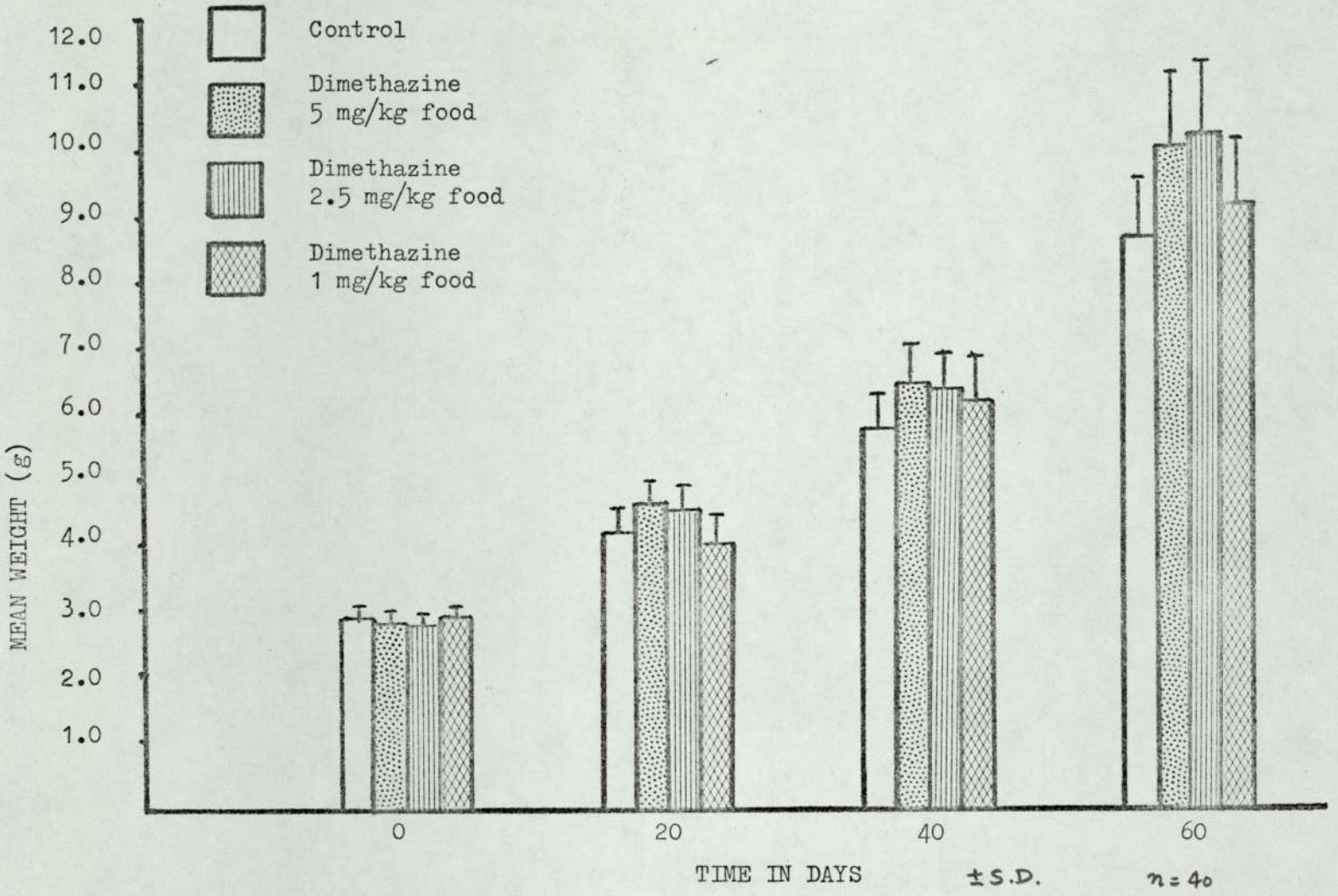
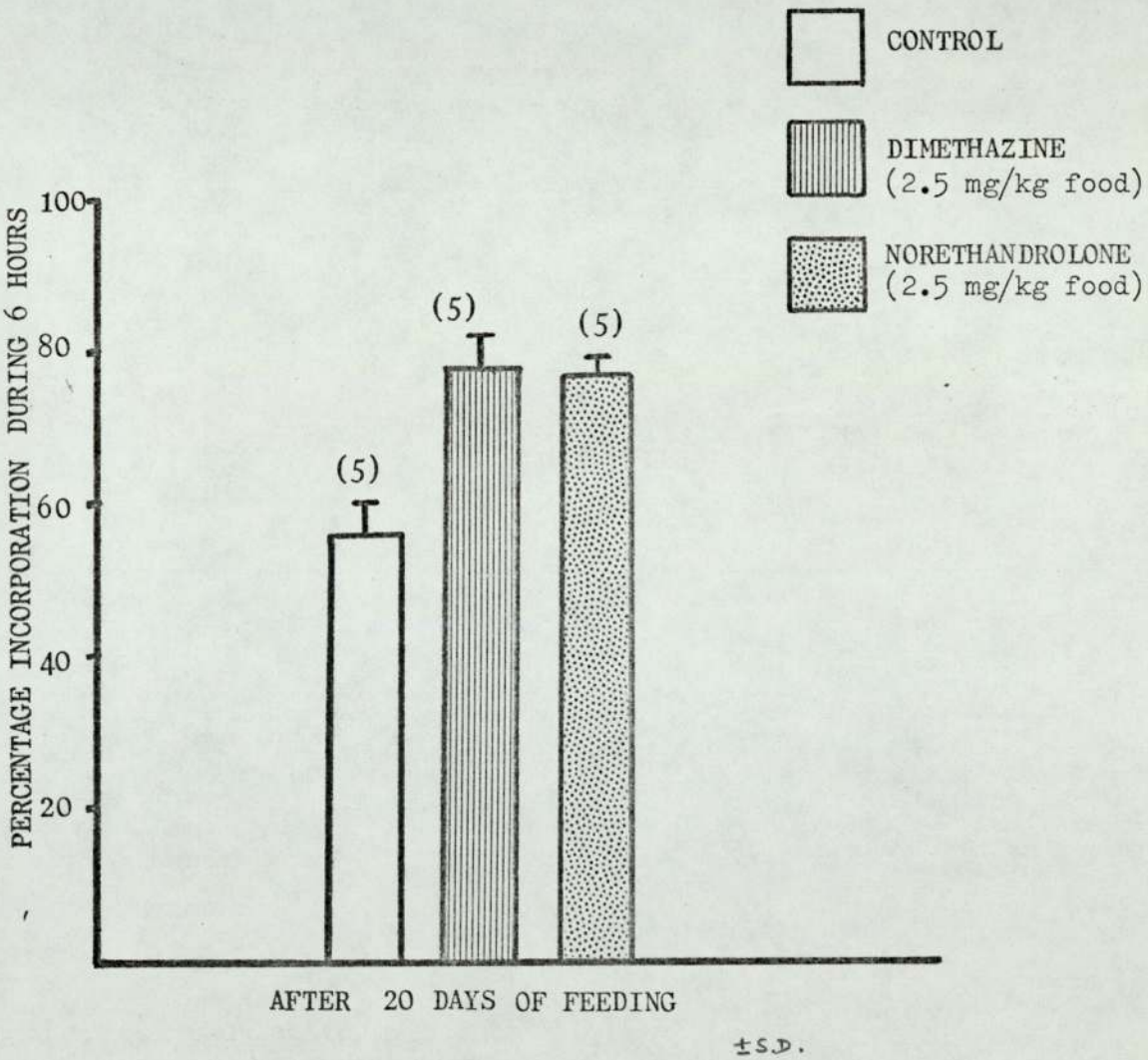


Fig.24. THE EFFECT OF FEEDING ANDROGENS ON PERCENTAGE LEUCINE-^{14}C INCORPORATION IN RAINBOW TROUT



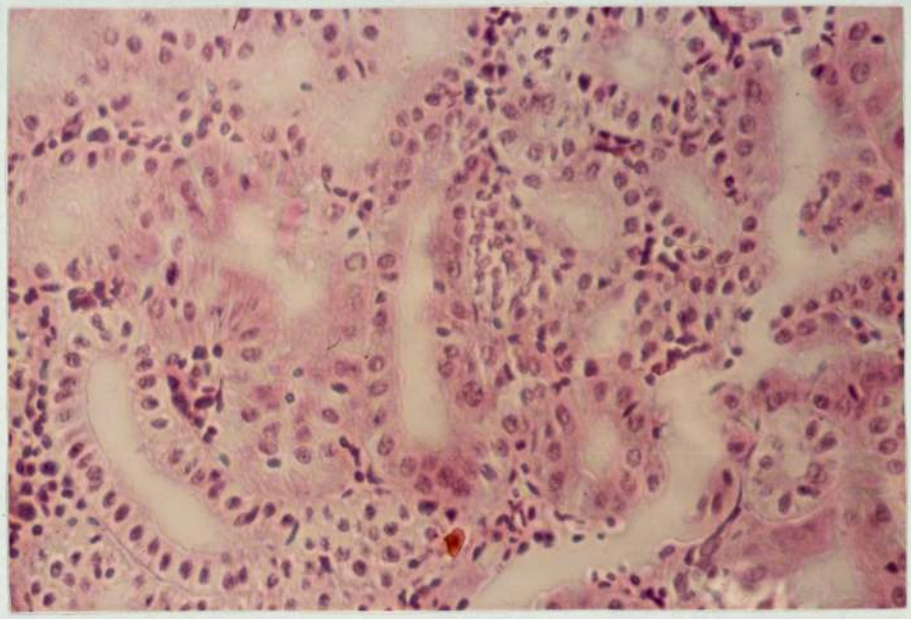


PLATE 4. SECTION OF KIDNEY OF AN UNTREATED RAINBOW TROUT. x 160

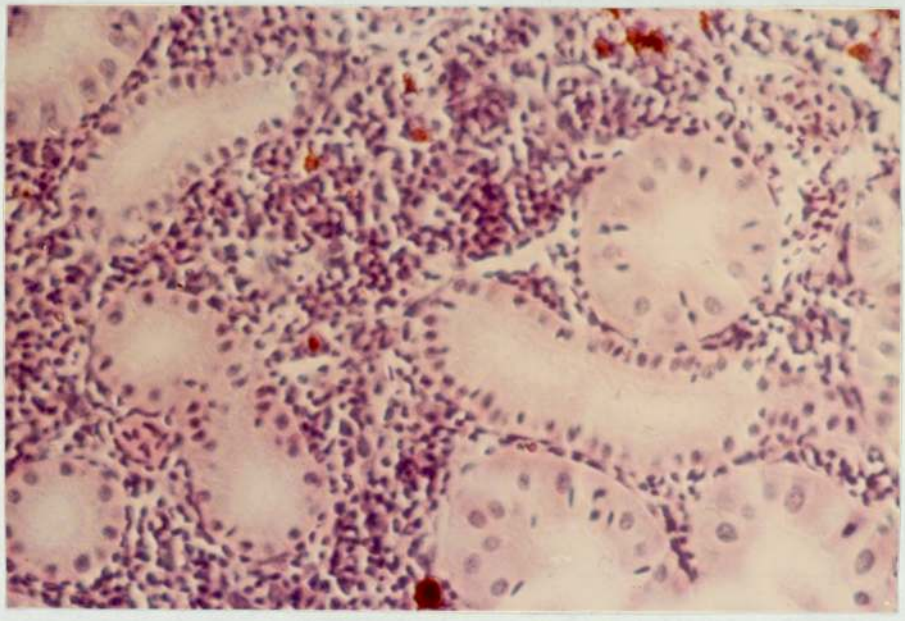


PLATE 5. SECTION OF KIDNEY OF A RAINBOW TROUT TREATED ORALLY WITH DIMETHAZINE (10 mg/kg FOOD) FOR 48 DAYS. x 160

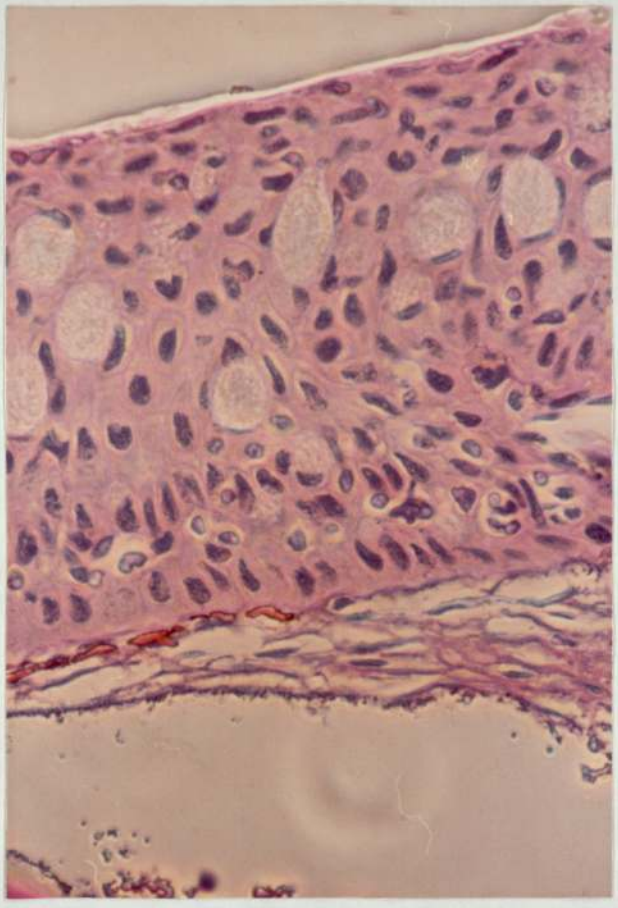


PLATE 6. SECTION OF EPIDERMIS OF AN UNTREATED RAINBOW TROUT. x 160

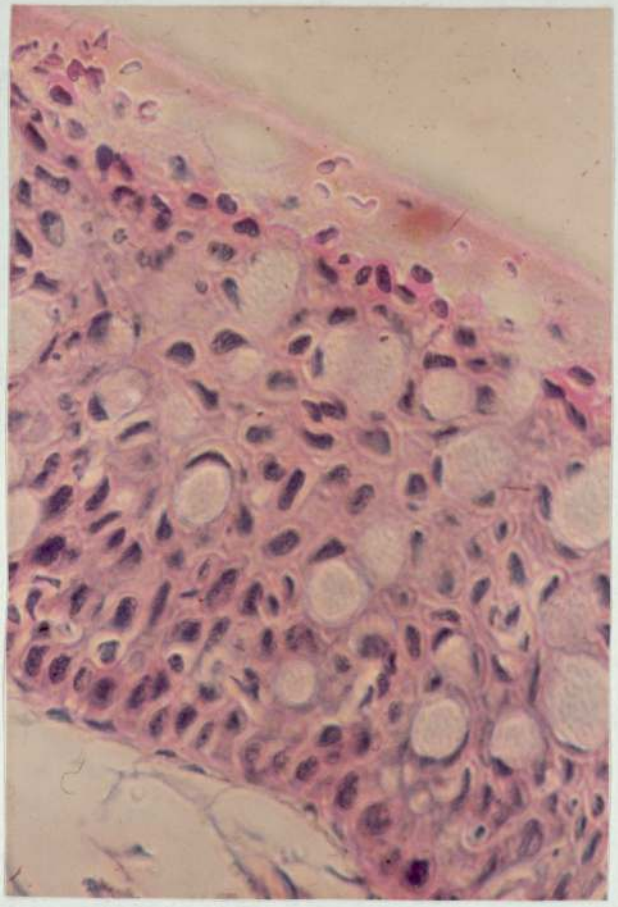


PLATE 7. SECTION OF EPIDERMIS OF A RAINBOW TROUT TREATED ORALLY WITH DIMETHAZINE (10 mg/kg FOOD) FOR 48 DAYS. x 160

Fig.25. EFFECT OF FEEDING NORETHANDROLONE-SUPPLEMENTED DIETS TO RAINBOW TROUT

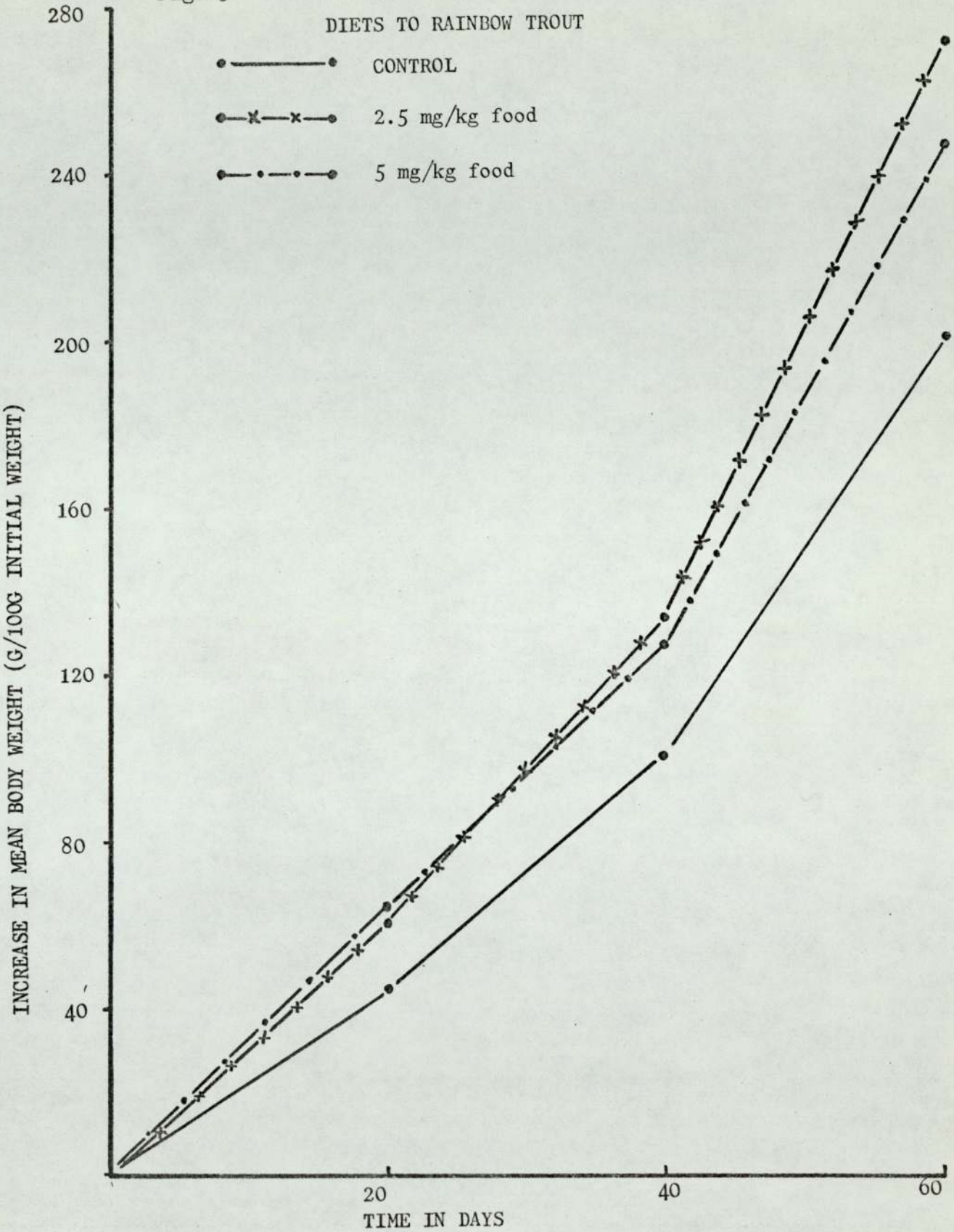


Fig.26. MEAN WEIGHTS (± S.D) OF RAINBOW TROUT FED NORETHANDROLONE SUPPLEMENTED DIETS

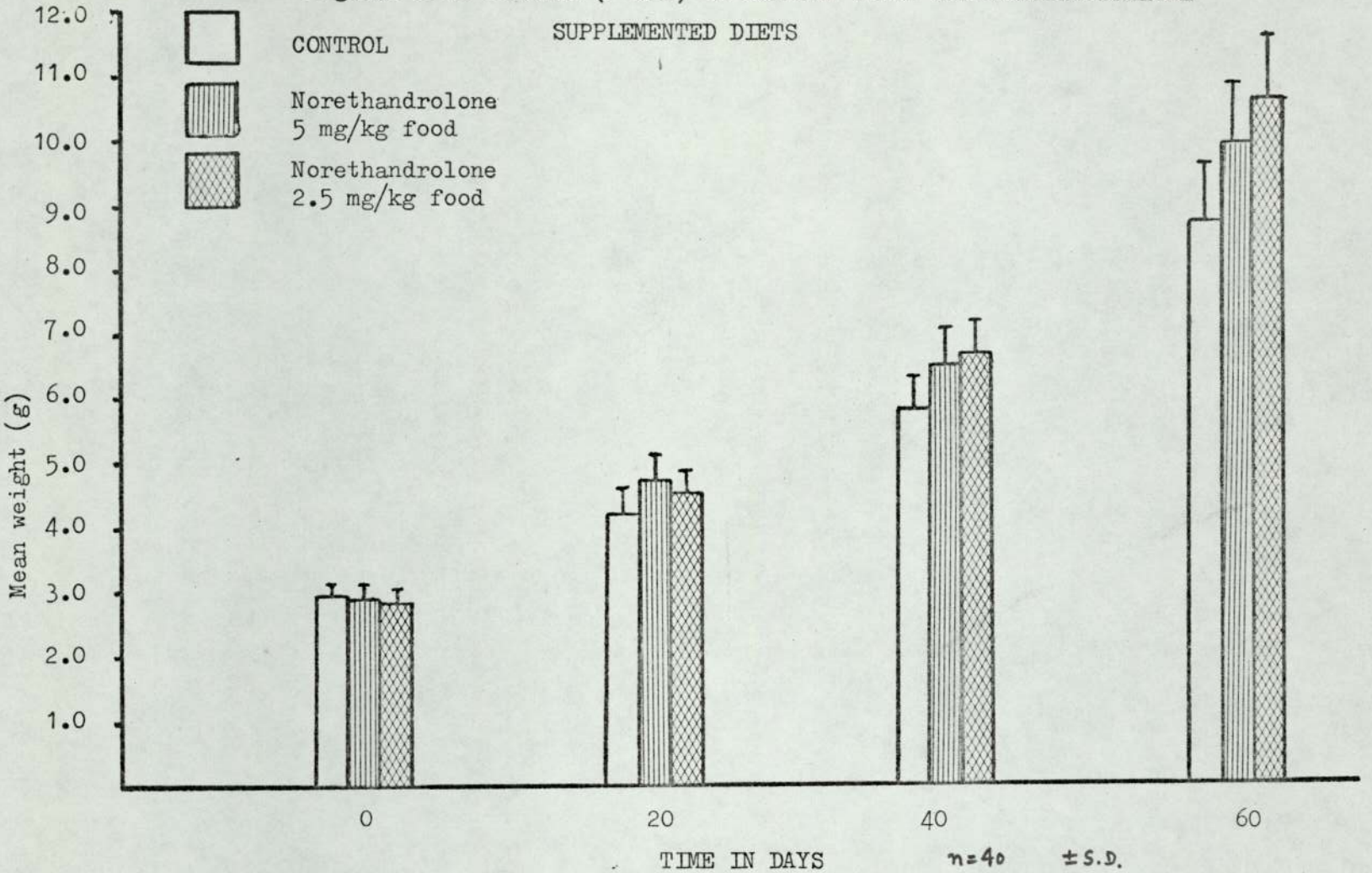


TABLE 13. CHANGES IN BODY WEIGHT OF RAINBOW TROUT FED DIMETHAZINE SUPPLEMENTED DIETS FOR 48 DAYS

VALUES GIVEN ARE MEANS (G) ± S.D. OF 30 FISH. PERCENT WEIGHT GAINS OVER CONTROLS ARE GIVEN IN PARENTHESIS

DURATION (DAYS)	CONCENTRATION OF DIMETHAZINE MG/KG DRY FOOD			
	0	5	10	20
0	3.52 ± 0.50	3.55 ± 0.46	3.57 ± 0.56	3.59 ± 0.50
16	5.50 ± 1.15	5.82 ± 0.90 (7.03%)	5.75 ± 1.30 (4.77%)	5.76 ± 1.02 (3.73%)
32	8.80 ± 2.56	9.79 ± 1.97* (25.25%)	9.49 ± 2.89 (15.85%)	9.01 ± 2.08 (0.55%)
48	13.90 ± 4.90	15.94 ± 4.14** (53.12%)	15.07 ± 5.59 (27.31%)	14.32 ± 4.10 (3.21%)

* $p < 0.1$ ** $p < 0.05$

Table 14. EFFECT OF ORAL ADMINISTRATION OF DIMETHAZINE ON DAILY INCREMENTS OF BODY WEIGHT OF RAINBOW TROUT

DOSE OF DIMETHAZINE (MG/KG DRY FOOD)	DAILY INCREMENT OF MEAN BODY WEIGHT (g/100g INITIAL WEIGHT)		
	PERIOD I (0 - 16 DAYS)	PERIOD II (17-32 DAYS)	PERIOD III (33-48 DAYS)
CONTROL (0 mg)	3.53	3.73	3.61
5 mg	3.96	4.27	3.91
10 mg	3.82	4.05	3.67
20 mg	3.76	3.52	3.67

Table 15. CHANGES IN RENOSOMATIC INDEX AND HEPATOSOMATIC INDEX OF RAINBOW TROUT FED DIMETHAZINE SUPPLEMENTED DIETS FOR 48 DAYS. VALUES GIVEN ARE MEANS ± S.D. OF 15 FISH

CONCENTRATION OF DIMETHAZINE (MG/KG DRY FOOD)	R.S.I.	P	H.S.I.	P
CONTROL (0 mg)	0.95 ± 0.11		1.85 ± 0.16	
5 mg	1.06 ± 0.16	<0.05	1.76 ± 0.43	Not significant
10 mg	1.07 ± 0.11	<0.01	1.76 ± 0.25	" "
20 mg	1.04 ± 0.12	<0.05	1.70 ± 0.30	" "

Table 16. CHANGES IN BODY WEIGHT OF RAINBOW TROUT FED DIMETHAZINE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEANS (GRAMS) ± S.D. OF 40 FISH. PERCENTAGE WEIGHT GAIN OVER CONTROLS IS GIVEN IN PARENTHESIS

DURATION (DAYS)	CONCENTRATION OF DIMETHAZINE MG/KG DRY FOOD			
	0	5	2.5	1
0	2.90 ± 0.32	2.86 ± 0.32	2.84 ± 0.26	2.91 ± 0.36
20	4.21 ± 0.78	4.67 ± 0.76** (18.11%)	4.66 ± 0.77** (18.91%)	4.57 ± 0.94 (11.87%)
40	5.83 ± 1.09	6.52 ± 1.28* (26.94%)	6.42 ± 1.23* (25.02%)	6.28 ± 1.49 (14.77)
60	8.78 ± 1.82	10.15 ± 2.33** (52.14)	10.32 ± 2.27** (60.63)	9.24 ± 2.17 (14.77)

*$p < 0.05$ **$p < 0.01$

Table 17. EFFECT OF ORAL ADMINISTRATION OF DIMETHAZINE ON DAILY INCREMENT OF BODY WEIGHT OF RAINBOW TROUT

DOSE OF DIMETHAZINE (mg/kg DRY FOOD)	DAILY INCREMENT OF MEAN BODY WEIGHT (G/100G INITIAL WEIGHT)		
	PERIOD I	PERIOD II	PERIOD III
	(0 - 20 days)	(21 - 40 days)	(41 - 60 days)
CONTROL (0 mg)	2.25	1.92	2.53
5 Mg	3.16	1.98	2.73
2.5 mg	3.20	1.88	3.03
1 mg	2.85	1.87	2.34

Table 18. CHANGES IN BODY LENGTH OF RAINBOW TROUT FED DIMETHAZINE SUPPLEMENTED DIETS
VALUES GIVEN ARE MEANS (Cm) ± S.D. OF 40 FISH

DURATION (DAYS)	CONCENTRATION OF DIMETHAZINE (Mg/Kg DRY FOOD)			
	0	5	2.5	1
0	6.76 ± 0.42	6.71 ± 0.41	6.72 ± 0.38	6.78 ± 0.40
20	7.54 ± 0.44	7.51 ± 0.39	7.46 ± 0.48	7.53 ± 0.39
40	8.12 ± 0.51	8.32 ± 0.56	8.29 ± 0.58	8.29 ± 0.59
60	9.18 ± 0.66	9.35 ± 0.76	9.38 ± 0.75	9.23 ± 0.86

Table 19. AMOUNT OF FOOD CONSUMED AND FOOD CONVERSION EFFICIENCY IN RAINBOW TROUT FED DIMETHAZINE SUPPLEMENTED DIETS

CONCENTRATION OF DIMETHAZINE MG/KG FOOD)	AMOUNT OF FOOD CONSUMED * (% BODY WEIGHT/DAY)			FOOD CONVERSION EFFICIENCY (G.WET WEIGHT/100G FOOD)			
	PERIOD I 0 - 20 days	PERIOD II 21-40 days	PERIOD III 41-60 days	PERIOD I 0-20days	PERIOD II 21-40days	PERIOD III 41-60days	TOTAL PERIOD (0-60 days)
CONTROL	4.39	4.09	4.67	51.37	46.95	54.12	51.35
5	4.76	3.77	4.40	66.42	52.48	63.13	60.75
2.5	4.92	3.70	4.69	65.0	51.01	64.73	60.93
1	4.55	3.77	4.27	62.64	49.56	55.06	55.16

* G dry diet/100 g Body weight at Beginning of each period (Cowey et al. 1973)

Table 20. CHANGES IN HAEMATOCRIT, RENOSOMATIC INDEX AND HEPATOSOMATIC INDEX OF RAINBOW TROUT FED DIMETHAZINE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEANS ± S.D. of 15 FISH

CONCENTRATION OF DIMETHAZINE (Mg/Kg DRY FOOD)	H.T (%)	R.S.I.	H.S.I.
CONTROL	30.4 ± 2.6	1.03 ± 0.11	1.63 ± 0.24
5	33.8 ± 3.7 **	1.27 ± 0.22 **	1.53 ± 0.21
2.5	33.0 ± 3.4	1.18 ± 0.23 **	1.58 ± 0.17
1	31.2 ± 3.7	1.07 ± 0.14	1.59 ± 0.19

* $P < 0.05$ ** $P < 0.01$

Table 21. CHANGES IN VISCERAL WEIGHT AND MUSCLE COMPOSITION OF RAINBOW TROUT FED DIMETHAZINE SUPPLEMENTED DIETS FOR 60 DAYS.

VALUES GIVEN ARE MEANS ± S.D. OF 15 FISH

CONCENTRATION OF DIMETHAZINE (Mg/Kg DRY FOOD)	VISCERA (% TOTAL BODY WEIGHT	% MUSCLE WET WEIGHT	
		MOISTURE	TOTAL NITROGEN
CONTROL	11.59 ± 2.25	76.15 ± 1.72	2.68 ± 0.04
5	10.61 ± 0.90	74.83 ± 2.10	2.71 ± 0.06
2.5	10.83 ± 1.74	75.36 ± 1.65	2.73 ± 0.06
1	11.34 ± 1.71	75.10 ± 2.23	2.71 ± 0.04

Table 22. EFFECT OF ANABOLIC STEROIDS, DIMETHAZINE AND NORETHANDROLONE ON L-LEUCINE-^{14}C INCORPORATION INTO SKELETAL MUSCLE PROTEIN OF RAINBOW TROUT.

Fish were fed steroid supplemented diets for 20 days. They were then injected with leucine-^{14}C (5 μci/100 gm body weight) intraperitoneally and sacrificed 6 hours later.
Values given for leucine incorporation are means ± S.D. of 5 fish

TREATMENT	LEUCINE-^{14}C INCORPORATION (% UPTAKE)	P
CONTROL	56.24 ± 8.51	
2.5 mg DIMETHAZINE/Kg FOOD	78.34 ± 7.10	<0.01
2.5 mg NORETHANDROLONE/ Kg FOOD	77.06 ± 4.04	<0.01

Table 23. CHANGES IN BODY WEIGHT OF RAINBOW TROUT FED NORETHANDROLONE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEAN (G) ± S.D. OF 40 FISH. PERCENTAGE WEIGHT GAIN OVER CONTROLS IS GIVEN IN PARENTHESES.

DURATION (DAYS)	CONCENTRATION OF NORETHANDROLONE. MG/KG DRY FOOD		
	0	5	2.5
0	2.90 ± 0.32	2.87 ± 0.38	2.86 ± 0.36
20	4.21 ± 0.78	4.72 ± 0.75 ** (19.28%)	4.51 ± 0.71 (12.52%)
40	5.83 ± 1.09	6.53 ± 1.15 ** (26.49%)	6.71 ± 1.07 ** (33.58%)
60	8.78 ± 1.82	9.97 ± 1.89 ** (44.63%)	10.66 ± 1.82 ** (69.97%)

* $P < 0.05$ ** $P < 0.01$

Table 24. EFFECT OF ORAL ADMINISTRATION OF NORETHANDROLONE ON DAILY INCREMENT OF BODY WEIGHT OF RAINBOW TROUT

DOSE OF NORETHANDROLONE (Mg/Kg DRY FOOD)	DAILY INCREMENT OF MEAN BODY WEIGHT (G/100 G INITIAL WEIGHT)		
	PERIOD I (0 - 20 days)	PERIOD II (21 - 40 days)	PERIOD III (41 - 60 days)
CONTROL	2.25	1.92	2.53
5 mg	3.22	1.91	2.63
2.5 mg	2.88	2.43	2.94

Table 25. CHANGES IN BODY LENGTH OF RAINBOW TROUT FED NORETHANDROLONE SUPPLEMENTED DIETS. VALUES GIVEN ARE MEANS (Cm) ± S.D. of 40 FISH

DURATION (DAYS)	CONCENTRATION OF NORETHANDROLONE (Mg/Kg DRY FOOD)		
	0	5	2.5
0	6.76 ± 0.42	6.74 ± 0.38	6.75 ± 0.39
20	7.54 ± 0.44	7.53 ± 0.36	7.44 ± 0.28
40	8.12 ± 0.51	8.31 ± 0.48	8.25 ± 0.50
60	9.18 ± 0.66	9.39 ± 0.77	9.70* ± 0.64

* $P < 0.001$

Table 26. AMOUNT OF FOOD CONSUMED AND FOOD CONVERSION EFFICIENCY IN RAINBOW TROUT FED NORETHANDROLONE SUPPLEMENTED DIETS

CONCENTRATION OF NORETHANDROLONE (Mg/Kg DRY FOOD	AMOUNT OF FOOD CONSUMED * (% BODY WEIGHT/DAY)			FOOD CONVERSION EFFICIENCY (G.WET WEIGHT/100 G.FOOD)			
	PERIOD I 0-20 days	PERIOD II 21-40 days	PERIOD III 41-60 days	PERIOD I 0-20 days	PERIOD II 21-40 days	PERIOD III 41-60 days	TOTAL PERIOD 0-60 DAYS
CONTROL	4.39	4.09	4.67	51.37	46.95	54.12	51.35
5	5.0	3.60	4.36	64.34	53.23	60.35	59.29
2.5	4.80	4.10	4.58	60.18	59.45	63.96	61.78

* G Dry diet/100 g body weight at beginning of each period

Table 27. CHANGES IN HAEMATOCRIT, RENOSOMATIC INDEX AND HEPATOSOMATIC INDEX OF RAINBOW TROUT FED NORETHANDROLONE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEANS ± S.D. OF 15 FISH

CONCENTRATION OF NORETHANDROLONE (Mg/Kg DRY FOOD)	H.T. (%)	R.S.I.	H.S.I.
CONTROL	30.4 ± 2.6	1.03 ± 0.11	1.63 ± 0.24
5	32.8 ± 3.6	1.08 ± 0.16	1.73 ± 0.26
2.5	32.8 ± 3.3 *	1.09 ± 0.19	1.66 ± 0.25

* $P < 0.05$

Table 28. CHANGES IN VISCERAL WEIGHT AND MUSCLE COMPOSITION OF RAINBOW TROUT FED NORETHANDROLONE SUPPLEMENTED DIETS FOR 60 DAYS. VALUES GIVEN ARE MEANS ± S.D. of 15 FISH

CONCENTRATION OF NORETHANDROLONE (Mg/Kg DRY FOOD)	VISCERA (% TOTAL BODY WEIGHT)	% MUSCLE WET WEIGHT	
		MOISTURE	TOTAL NITROGEN
CONTROL	11.59±2.25	76.15 ± 1.72	2.68 ± 0.04
5	11.54±1.56	75.55 ± 1.20	2.70 ± 0.05
2.5	11.61±1.64	74.86 ± 1.92	2.71 ± 0.07

2.3.2.3. METHENOLONE AND DIETHYLSTILBOESTROL

A. GROWTH TRIALS

Methenolone and diethylstilboestrol were tested at one dosage level only. The trial dose for methenolone was 10 mg/kg food, corresponding to the dose used clinically. DES was given at the concentration of 1.2 mg/kg food, the level suggested by Cowey et al. (1973). The weight data from the weighing of individual rainbow trout in the groups treated with methenolone and DES are shown in Tables 29 and 30 respectively. Both these compounds manifested growth depressing tendencies in rainbow trout. The adverse effect of DES on growth was more pronounced. Percentage weight gains in fish which received DES for 30 days were 18% less than those of the fish in the control group (Table 30, Fig.27) while the group fed methenolone gained 16% less weight (Table 29, Fig.27). Daily increments of mean body weight for fish fed DES and methenolone are shown in Table 32, Fig.28. Both DES and methenolone induced a decrease in daily increments of body weight during the first 15 days as well as the last 15 days. These hormones also impaired food conversion efficiencies (Table 32). As compared to methenolone , DES seemed to have affected food conversion efficiency more adversely.

B. MUSCLE ANALYSIS

Mean values for muscle nitrogen and moisture are given in Table 31. Values for fish treated with DES or methenolone were not significantly different from those of the control.

Fig.27. EFFECT OF METHENELONE AND DIETHYLSTILBOESTROL FEEDING ON GROWTH OF RAINBOW TROUT

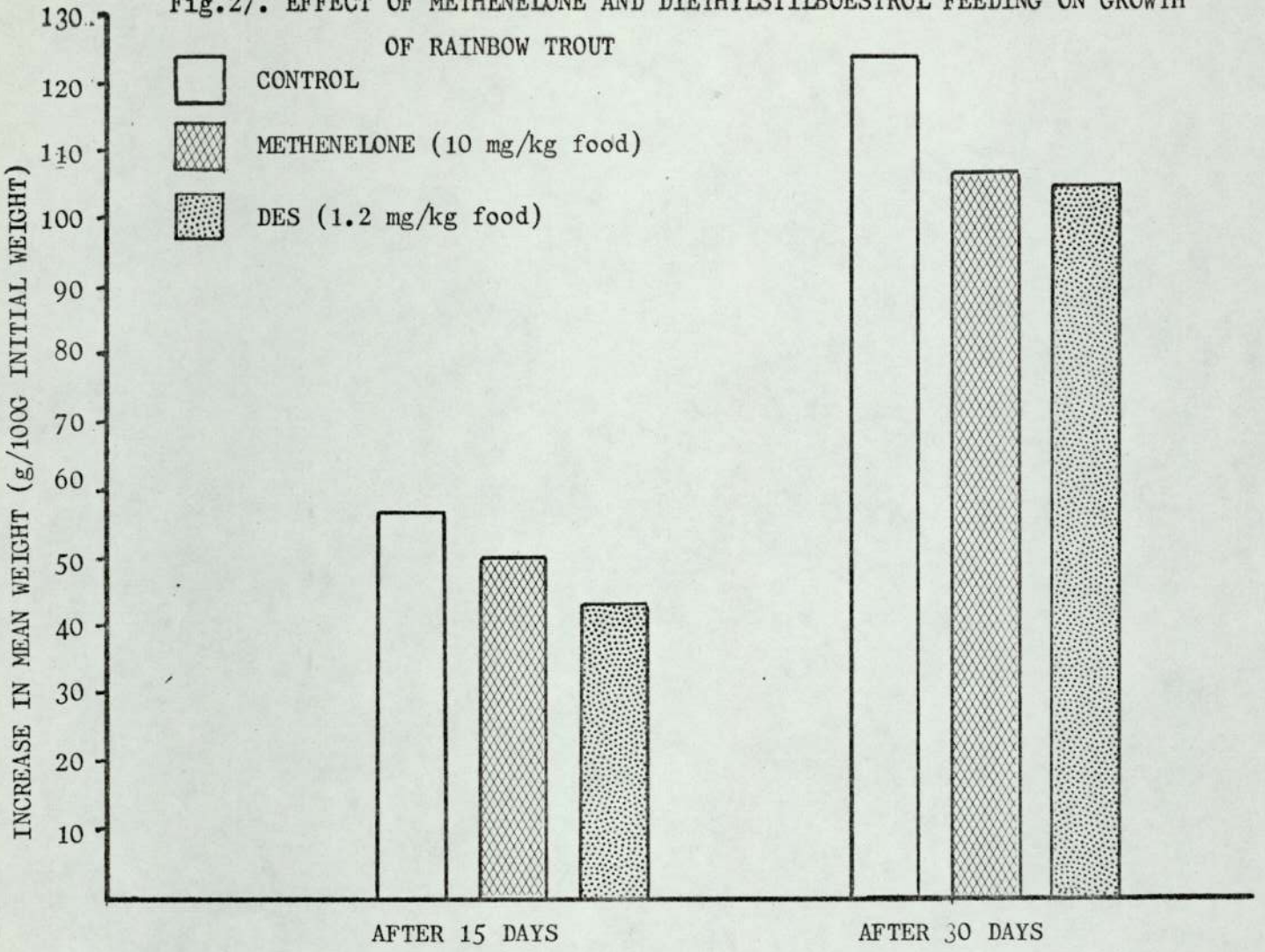


Fig.28. EFFECT OF FEEDING METHENELONE AND DIETHYLSTILBOESTROL SUPPLEMENTED DIETS ON PERCENTAGE DAILY INCREMENT IN MEAN BODY WEIGHT OF RAINBOW TROUT

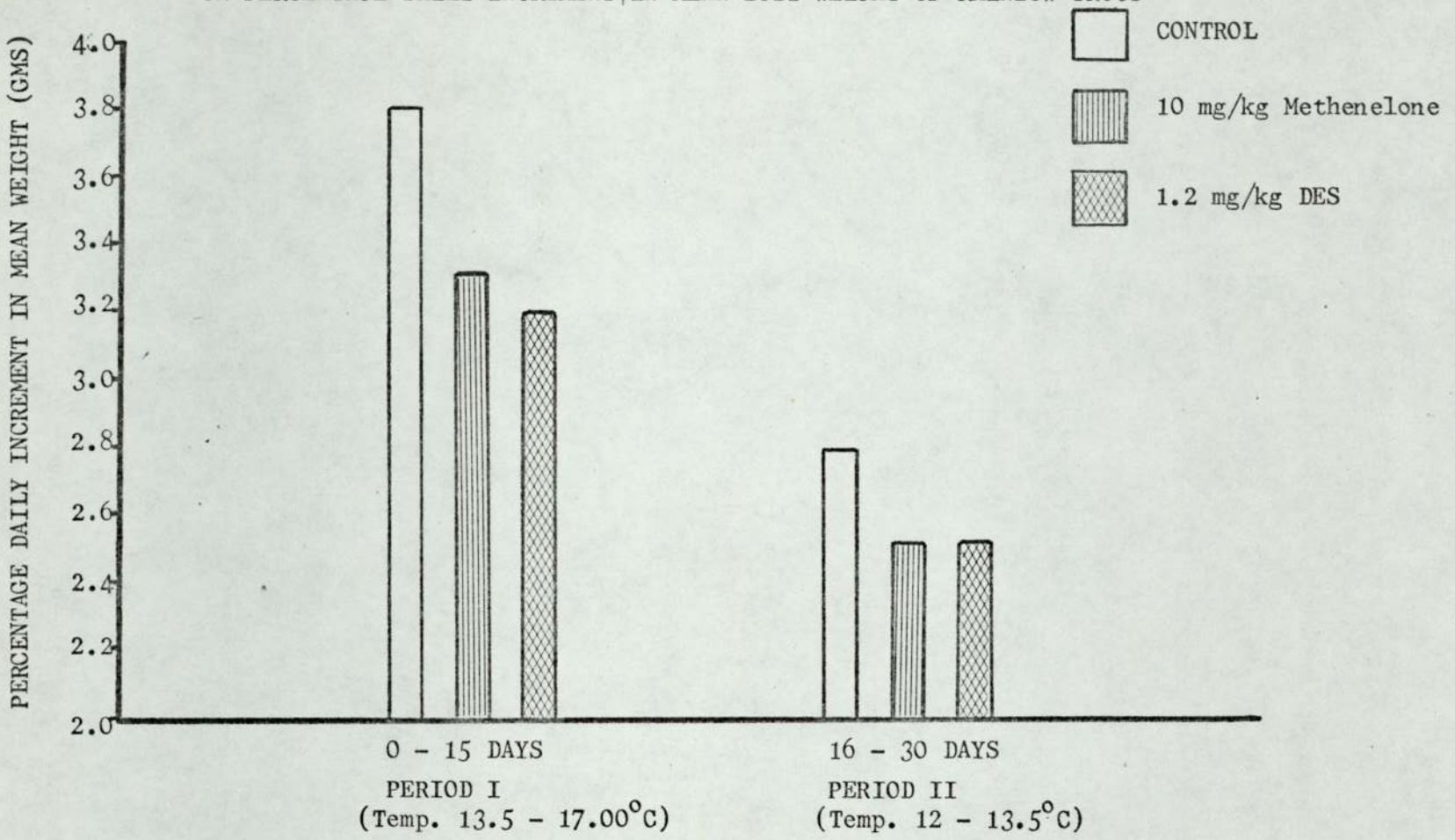


Table 29. CHANGES IN BODY WEIGHT OF RAINBOW TROUT FED METHENOLONE SUPPLEMENTED DIETS. VALUES GIVEN ARE MEANS (G) ± S.D. OF 25 FISH. DIFFERENCE IN PERCENTAGE WEIGHT GAIN OVER THE CONTROL GROUP IS SHOWN IN PARENTHESES

DIET	DURATION (DAYS)		
	0	15	30
CONTROL (0 mg Methenolone/ Kg Dry food)	13.48 ± 3.17	21.25 ± 4.47	30.21 ± 6.32
10 mg Methenolone 1 Kg Dry food	13.72 ± 2.84	20.59 ± 4.02 (-7.5%)	28.51 ± 5.35 (-16.3%)

Table 30. CHANGES IN BODY WEIGHT OF RAINBOW TROUT FED DIETHYLSTILBOESTROL SUPPLEMENTED DIETS. VALUES GIVEN ARE MEANS (G) ± S.D. of 25 FISH. DIFFERENCE IN PERCENTAGE WEIGHT GAIN OVER THE CONTROL GROUP IS SHOWN IN PARENTHESES.

DIET	DURATION (DAYS)		
	0	15	30
CONTROL (0 mg DES/Kg Dry food)	13.48 ± 3.17	21.25 ± 4.47	30.21 ± 6.32
1.2 mg DES/Kg Dry food)	13.12 ± 2.73	19.45 ± 4.61* (-9.4%)	27.00 ± 7.08* (-18.3%)

*P < 0.10

Table 31. MUSCLE COMPOSITION OF RAINBOW TROUT FED METHENOLONE OR DIETHYLSTILBOESTROL SUPPLEMENTED DIETS

TREATMENT AND DURATION	% WET WEIGHT	
	MOISTURE	TOTAL NITROGEN
CONTROL	75.55 ± 1.00	2.76 mg ± 0.05
10 mg METHELONONE/ Kg FOOD FOR 30 days	75.75 ± 0.93	2.77 mg ± 0.05
1.2 mg DES/Kg FOOD for 30 days	75.09 ± 0.92	2.70 mg ± 0.05

n = 15 ± S.D.

Table 32. DAILY INCREMENT OF BODY WEIGHT, AMOUNT OF FOOD CONSUMED AND FOOD CONVERSION EFFICIENCY IN RAINBOW TROUT FED METHELONONE AND DIETHYLSTILBOESTROL SUPPLEMENTED DIETS

DIET	DAILY INCREMENT OF BODY WEIGHT (%)		AMOUNT OF FOOD CONSUMED (% BODY WEIGHT/DAY		FOOD CONVERSION EFFICIENCY (g.wet weight/100g Food)	
	0 - 15 days	16 - 30 days	0 - 15 days	16 - 30 days	0 - 15 days	16 - 30 days
CONTROL	3.84	2.80	5.83	4.40	65.84	63.81
10 mg METHENOLONE/ Kg DRY FOOD	3.33	2.56	5.59	4.54	59.63	56.41
1.2 mg DES/Kg DRY FOOD	3.21	2.58	5.66	4.33	56.82	59.63

3. DISCUSSION

The effect of insulin, growth hormone, sex steroids and related substances on protein metabolism and growth in rainbow trout, noted in this study clearly indicates the significance of control of endocrine systems in the regulation of growth in cultured fishes. It was of particular interest to note that some of these hormones such as synthetic anabolic steroids when used as a supplement in the diet manifested pronounced proteo-anabolic activity and accelerated body growth.

EVALUATION OF INCORPORATION OF LEUCINE-^{14}C AS A PARAMETER OF PROTEIN SYNTHESIS

Since growth in fishes is associated with increase in body protein, the incorporation of leucine-^{14}C into skeletal muscle protein was used not only as an index of rate of protein synthesis but also as additional evidence (besides weight measurements) for somatic growth. Leucine was particularly selected because besides being an essential amino acid for rainbow trout (Shanks, Gahimer and Halver, 1962) it is known to be catabolized in mammals to a significant extent by extrahepatic tissues (Miller, 1962) and in particular by skeletal muscle (Manchester, 1965; Pain and Manchester, 1970; Neale, 1972; Odessey and Goldberg, 1972). Although incorporation of a labelled amino acid into protein indicates that protein is being synthesized, it may not always represent net protein synthesis, because this parameter is prone to various factors such as compartmentation, variations in precursor pool sizes, changes in breakdown rate and recycling and short term fluctuations in synthesis rates (Trenkle, 1974; Reeds, 1974; Henshaw, Hirsch, Morton and Hiatt, 1971; Goldberg, 1969 and Snipes, 1968). However, several of these factors are nullified by using percentage incorporation as an index (Jackim and LaRoche, 1973). This method tends to neutralize the effect

of changes in excretion rate, lack of uniformity in injection, differences in peritoneal absorption rates and variations in dilution due to differences in the ratio of length to body weight in fishes. Some workers have used specific activity of radioactive protein as an index (Das and Proser, 1967; Morris and Smith, 1967). In this study the incorporation of leucine-^{14}C into skeletal muscle protein of rainbow trout treated with insulin was interpreted both as percentage incorporation and as protein specific activity. When it was noted that experimental treatment, as in the case of insulin, could alter the concentration of leucine-^{14}C in the free amino acid pool as well as protein content of the muscle in rainbow trout, the percentage incorporation, being less prone to these changes was preferred to protein specific activity as an index of rate of protein synthesis in the subsequent experiments. A six-hour period of incorporation of leucine-^{14}C was selected in most of the experiments because it was expected that this would permit the label to penetrate all tissue barriers and at the same time minimize the effect of recycling of amino acids.

3.1. INSULIN

The physiological role of the endocrine pancreas in fishes is, at best, poorly understood (Chavin and Young, 1970; Epple, 1968,1969; Falkmer and Patent, 1972; Epple and Lewis, 1973) and the data on the influence of insulin on protein metabolism in Teleostei are limited to a few species and fragmentary. Nevertheless, there is some evidence for an important function of insulin in the regulation of protein synthesis (Tashima and Cahill, 1968; Ahmad and Matty 1975a,b; Ince and Thorpe, 1974). The work presented in this study involved: a. an evaluation of the effect of exogenous insulin administration on retention of protein in the skeletal muscle, incorporation of leucine-^{14}C into muscle protein and excretion of ammonia in ambient water, b. assessment of the regulation by insulin of protein synthesis during short term fasting and high- and low protein feeding and c. examination of the influence of hypoglycaemic sulphonylurea agents, glibenclamide and tolbutamide,on protein synthesis and growth in rainbow trout. The results obtained indicate an important role of insulin in protein metabolism in this species.

3.1.1. EFFECTS OF INSULIN

A. MUSCLE PROTEIN CONTENT

The results obtained with treatment of rainbow trout with insulin clearly indicate the influence of insulin on protein metabolism. The increase in muscle protein noted during the 36-hour period after insulin injection (3 and 5 I.U/mg) probably reflects an increased uptake of amino acids. This is consistent with earlier observations that insulin produced a marked decline in plasma amino acid nitrogen in Japanese eel, Anguilla japonica (Inui and Yokote, 1975b), European silver eel, A. anguilla (Ince and Thorpe, 1974) and Northern pike, Esox lucius (Thorpe and Ince, 1974). The dose of insulin used by these

workers was within the same range as used in the present study. A significant observation in this connection was made by Ince and Thorpe (1974) who noted that bovine insulin at 2 mg/kg dosage level lowered the plasma amino acid nitrogen but had no effect on blood glucose, while codfish insulin at the same dosage level affected both these parameters. This, perhaps, points towards a much wider role of insulin in fish metabolism. A less direct evidence of the influence of insulin on protein metabolism in fish has been presented in some recent studies. Palmer and Ryman (1972) showed that oral glucose loading caused a reduction in plasma amino acids, probably as a result of stimulation of insulin release. Alloxan induced hypoinsulinemia in the Japanese eel, Anguilla japonica produced an elevation of plasma amino acid nitrogen. Contrary to the results obtained in this study, administration of insulin into European yellow eel, Anguilla anguilla induced negligible protein retention in the muscle. However, the increase in liver protein was significant (Lewander, Dave, Johnsson-Sjöbeck, Larsson and Lidman, 1976)

B. INCORPORATION OF LEUCINE-^{14}C

The increase in muscle protein induced by insulin is substantiated by its effect on incorporation of leucine-^{14}C into skeletal muscle protein. A single injection of insulin increased both the percentage of incorporation of leucine-^{14}C into skeletal muscle protein and protein specific activity (Tables 4 and 5). A similar effect of insulin on the incorporation of isotopically labelled amino acids into skeletal muscle has been observed in other teleosts, Carassius auratus (Ahmad and Matty, 1975a), Fundulus heteroclitus (Jackim and LaRoche, 1973) and Esox lucius (Thorpe and Ince 1976). Contradictory to this are the findings on Opsanus tau (Tashima and Cahill, 1968) that insulin caused a decrease in the incorporation of glycine-^{14}C into liver protein, probably due to selective uptake of label by the muscle. In mammals, it is well est-

ablished that the stimulatory effect of insulin on protein synthesis in the skeletal muscle is accomplished in two steps: a. an increased transport of amino acids across the cell membranes and b. rapid incorporation of these amino acids into peptide chains (Sinex, 1968; Manchester, 1970a; Cahill et al, 1972: Lacy, 1973). However, the increased transport of amino acids may not always lead to an increase in the accumulation of amino acids in the inter- and intracellular free amino acid pools. Among the natural amino acids, the accumulation of glycine, proline and methionine in the muscle is enhanced by insulin, but with the possible exception of serine, threonine, histidine and alanine, none of the others was observable (Manchester, 1970a). This is in contrast to their incorporation into peptide chains which insulin enhances in every case. A similar pattern of the effect of insulin on leucine-^{14}C was observed in this study. Although insulin had a marked effect on the incorporation of leucine-^{14}C into muscle protein, there was no noticeable increase in the accumulation of free leucine in the free amino acid pool, as judged by the amount of radioactivity in the supernatant (Fig.5). Instead there appeared to be a trend towards a slight decline in the concentration. As pointed out by Cahill et al. (1972) this can be explained on the basis that being a branched-chain amino acid which is metabolised directly in the muscle (Manchester, 1965) leucine may have been actively accumulated and then subsequently oxidized. The observation made in this study that after insulin injection there was an increase in the incorporation of leucine-^{14}C into muscle protein and a slight decline in the concentration of the leucine in the free amino acid pool coincides precisely to an earlier finding made on the murrel, Ophicephalus striatus (Seshadri, 1959) that insulin caused an increase of protein bound amino acids and a decrease of free amino acids in the skeletal muscle.

C. AMMONIA EXCRETION

Analysis of ammonia excreted in ambient water by rainbow trout after insulin treatment was carried out to assess the effect of this hormone on protein and amino acid catabolism. Since ammonia is the main end product of nitrogen metabolism in fish and most of this is excreted extrarenally through gills (Smith, 1929; Wood, 1958; Fromm, 1963; Black, 1957) any change in ammonia excreted in ambient water after insulin administration should reflect the influence of the hormone on protein metabolism. A single injection of insulin (3 I.U/kg) produced a distinct decline in ammonia excreted by rainbow trout in ambient water at 14°C (Fig.10). This decrease in ammonia excretion is consistent with the increase in muscle protein content in rainbow trout noted after injection of the same dose of insulin. It may be suggested that the reduction in ammonia excretion caused by insulin resulted from a corresponding decrease in the breakdown of protein and deamination of amino acids in liver. The gills are also involved in the deamination processes (Forster and Goldstein, 1969). The view that in fishes insulin inhibits proteolysis in liver, as indicated by its action on ammonia excretion, is supported by a similar action of this hormone in mammals, in which it inhibits proteolysis both in liver (Mortimer, 1963; Exton et al. 1970) and muscle (Pozefsky et al. 1969).

3.1.2. PROTEIN SYNTHESIS DURING FASTING AND LOW- AND HIGH PROTEIN FEEDING.

The observation that incorporation of leucine-^{14}C decreased markedly during short term fasting, provides an indirect evidence in favour of the opinion that insulin plays a key role in the regulation of protein synthesis in some fishes (Table 6). It is generally believed that in mammals decreased levels of insulin during fasting (or protein restriction) act as a signal to both muscle and adipose tissue to release amino acids and fatty acids (Cahill, 1970, 1971, Cahill et al., 1966).

The elevated levels of free fatty acids resulting due to low levels of insulin during fasting suppress protein synthesis in skeletal and cardiac muscle and increase the oxidation of branched-chain amino acids (Buse and Buse, 1970; Buse et al., 1970). A similar situation seems to exist in fishes. A significant rise in free fatty acid levels in plasma and muscular tissues during the first 14 days of starvation has been reported in rainbow trout, Salmo gairdneri (Bilinski and Gardner, 1968). Decreased synthesis of protein in rainbow trout noted during fasting or low protein feeding in this study and also observed in killifish, Fundulus heteroclitus (Jackim and LaRoche, 1973) might have been due to a decrease in levels of insulin. Some support in favour of this view is found in the observation that plasma immunoreactive levels declined during a 5 days' fast in goldfish, Carassius auratus (Patent and Foa, 1971) and in response to protein restriction (Tashima and Cahill, 1968; Ahmad and Matty, 1975b). Contrary to the observations mentioned above, Cowey (1975) could not notice any difference in the incorporation of phenylalanine into muscle protein in response to high- and low protein feeding. With leucine and glutamic acid, low protein diets lead to greater enhancement of incorporation into muscle protein as compared to that in high protein diets. It is not possible to explain these observations on the basis of the view put forward here regarding the relationship of insulin and nutrients.

3.1.3. EFFECT OF GLIBENCLAMIDE AND TOLBUTAMIDE

Increased incorporation of leucine-^{14}C into muscle protein of rainbow trout after a single injection of glibenclamide (5 mg/kg body weight) observed in this study, appears to have occurred due to an increase in insulin levels as a result of stimulatory effect of glibenclamide on β-cells. A similar effect of tolbutamide-stimulated insulin release on the incorporation of leucine-^{14}C into muscle protein

has been shown in goldfish, Carassius auratus (Ahmad and Matty, 1975a). In mammals, acute insulin stimulating β-cytotrophic effect of sulphonylureas is well established (Breidahl et al. 1972) and there is no doubt that all sulphonylurea drugs produce a rise in plasma insulin as measured by either immunoreactive insulin or bioassay (Cerasi, Chowers, Luft and Widstrom, 1969; Chandalia, Hollobaugh, Pennington and Boshell, 1969; Pfeiffer, E.F., Pfeiffer, M., Distschuneit and Chang-su, 1959; Vallance-Owen, Joplin and Fraser, 1959). Palmer and Ryman (1972) and Köhler and Lippman (1963) found that tolbutamide did not produce hypoglycaemia effects in rainbow trout and goldfish respectively and concluded that the mechanism whereby sulphonylureas exert their hypoglycaemic action in mammals may be absent in fish. The observations made by these workers appear to reflect the independence of blood glucose from the actions of insulin in fish.

Since administration of glibenclamide had a stimulatory effect on protein synthesis in rainbow trout, therefore it was expected that this effect would also be reflected in the growth rate of rainbow trout fed glibenclamide and tolbutamide supplemented diets. However, these compounds produced deleterious effects on growth of rainbow trout. Glibenclamide not only depresssed weight gains but also impaired appetite and food conversion efficiency. It may be suggested that the negative effects of sulphonylureas on growth in fish might have been due to the antithyroid effects of these compounds as noticed in mammals (Brown and Solomon, 1958; McGaveck, Seeger, Hoar, Enzinger and Erk, 1957).

3.1.4. CONCLUSIONS

The results presented in this study suggest a key role of insulin as a proteo-anabolic hormone in rainbow trout. However, it is not possible to say with certainty that this is its primary function in this species because the effect of insulin on lipid metabolism has not been worked out yet and there are strong suggestions that at least in some fishes such as in Chondrichthyes (Patent, 1970) and goldfish (Minick and Chavin, 1972a) insulin might have as one of its primary functions the regulation of lipid metabolism. On the whole, the situation regarding the actual physiological function of insulin in fishes is far from being resolved. Unlike in mammals in which there is a general concensus that insulin is primarily concerned with the control of carbohydrate metabolism, there are conflicting views regarding its primary physiological function in fishes. Insulin may have the same action in fishes as in mammals but at least the relative importance of the hormone appears to be different in these groups (Fontaine, 1975).

Many investigators have suggested that insulin has a primary role in the regulation of carbohydrate metabolism and especially in the control of blood glucose (reviewed by Epple, 1969). The variations in the response of blood glucose to exogenous insulin in different species stems from the great variability in species sensitivity to mammalian insulin, the nutritional state of the animal, the habitat of the species and the purity and species specificity of the hormone.

The primary importance of insulin in glucose homeostasis appears to be questionable. Falkmer and Wilson (1967) suggested from their studies of the sculpin, Cottus scorpius, that insulin may be involved only to a small extent in blood sugar regulation of teleosts. Similarly Lewis and Epple (1972) failed to observe hyperglycemia in partially pancreatectomized eels and concluded that a strict homeostatic control

of glucose, as in mammals, is not present at least in many species. The reported blood sugar values of 0 mg% or almost 0 mg% in goldfish (Chavin and Young, 1970) and in normal carp (Yokote, 1970c) without any visible convulsions suggest that glucose may not be the main energy source in some fishes. This ability of some fish to survive extremely low blood glucose levels and the resistance of many fish to high doses of bovine insulin urges the necessity for re-evaluation of the importance of glucose homeostasis in fishes (Brinn, 1973). Tashima and Cahill (1968) found that a protein meal, but not an oral glucose load, increased the insulin levels in toadfish, Opsanus tau, and suggested that homeostatic mechanism for regulation of blood glucose concentration in fishes is independent of insulin levels. They suggested that the primary role of insulin in fishes, particularly in the carnivorous fishes is as proteo-anabolic hormone. A similar situation seems to exist in rainbow trout. The reported intolerance of rainbow trout to oral glucose load (Palmer and Ryman, 1972) and the actions of insulin on protein metabolism, as seen in the present study, clearly indicate a strong protein anabolic function of insulin in this fish. This view appears to have a phylogenetic precedent. According to Cahill et al. (1972) the protein anabolic effect of insulin appears to have come early in vertebrate evolution. Clark et al. (1968) showed that insulin had little effect on fat and carbohydrate metabolism in the rat foetus and likewise the primary stimulus for insulin release in the premature human infant (Grasso et al. 1968; Buchanan and Schwartz, 1967) or foetal monkey (Mintz et al., 1969) appeared to be amino acids and not glucose.

The role of insulin in lipid metabolism has been equally emphasised in some fishes. Minick and Chavin observed that in goldfish, Carassius auratus, several vertebrate insulins induced a decline in free fatty acids and phospholipids (1972a) while streptozotocin and alloxan in

addition to producing hypoglycemic effects also elevated FFA levels (1972b). As a result of these studies, the authors concluded that the insulin in goldfish has a predominant role in lipid metabolism. A similar role of insulin in Chondrichthyean fishes has been suggested by Patent (1970) who observed that in ratfish, Hydrolagus colliei and dogfish, Squalus acanthias, there was an abundance of lipids, in contrast to the sparse glycogen deposits in liver (70% and 80% of wet weight). From this, Patent (1970) implied that the concentration of body lipids in one organ must be of major physiological importance to the everyday metabolic needs of the animal. Insulin had negligible effect on tissue glycogen concentrations in these fish and therefore, Patent (1970) suggested that the hormones which primarily affect carbohydrate metabolism in mammals may instead be concerned with the control of lipid metabolism in chondrichthyes. Insulin might have an important role to play in Chondrichthyean fishes, but it does not seem to be the case in other species. Tashima and Cahill (1968) were unable to detect any alteration of FFA after injection of beef-pork insulin to toadfish, Opsanus tau. Similarly another lipid component, Cholesterol, has been shown to decline after partial pancreatectomy of eel, Anguilla rostrata (Lewis and Epple, 1972).

There appears to be some evidence to indicate that insulin plays a wide ranging role in controlling overall metabolism in some fishes, however, the primary role of insulin may vary between species due to different ways of living and dietary habits. Ince and Thorpe (1974) found that insulin produced a significant lowering of blood glucose, plasma cholesterol and amino acid nitrogen in the European silver eel, Anguilla anguilla. A similar wide ranging role of insulin was noted in the Northern pike, Esox lucius (Thorpe and Ince, 1974; Ince and Thorpe, 1975). Recently Lewander et al. (1976) have also suggested that insulin has rather extensive effects on the overall metabolism of yellow eel, A. anguilla.

These workers observed that in this species insulin had a marked effect on carbohydrate metabolism, qualitatively similar to those found in mammals, and exerted a strong lipolytic effect.

From the available information on the actions of insulin in fish, it is not possible to make a generalisation regarding its role on a particular aspect of metabolism in fish. The results presented in this study, however, suggest a strong proteoanabolic role of insulin in rainbow trout, which may be true for other carnivorous fishes also. Further work along the following lines may help to clarify the situation.

1. Using purified homologous hormone, investigation of the effect of insulin on the incorporation of isotopically labelled glucose and amino acids into various tissues under different experimental conditions.
2. The study of the effect of neutralising endogenous insulin by employing antibodies raised against species specific hormone on various aspects of metabolism.
3. Quantitation by radioimmunoassay of the response of Brockmann bodies to metabolite stimuli.
4. A detailed evaluation of anti-proteolytic effects of insulin both in liver and muscle. The investigation of the pattern of amino acids released from the skeletal muscle during experimentally induced hypo-insulinemia may be useful in evaluating the influence of insulin on catabolic processes.
5. The study of the effect of insulin on ammonia production by fish in ambient water at different temperatures may reflect any change in the response of fish during different states of metabolic activity.

3.2. GROWTH HORMONE AND PITUITARY EXTRACTS

The marked increase in the % incorporation of leucine-^{14}C into skeletal muscle protein of rainbow trout treated with porcine growth hormone, observed in this investigation is in keeping with the effects of mammalian growth hormone on protein metabolism in other fishes. Mammalian growth hormone increased muscle protein content in Oncorhynchus kisutch (Higgs et al. 1975), ratio of crude protein to ash in Salmo gairdneri (Enotomo, 1964b) and total nitrogen in Cottus scorpius (Matty, 1962). Shark growth hormone produced an upward trend in muscle protein synthesis in rainbow trout. It is not surprising to note the similarity in the biological activity of mammalian and shark growth hormones in promoting protein synthesis in rainbow trout, in view of the previous reports (Hayashida, 1973, 1972; Lewis et al. 1972) about the biological and immunochemical relationship between elasmobranchs and mammalian growth hormones. Like pituitary growth hormones of mammals, the growth hormone in the pituitary extracts of sharks (Squalus acanthias and Mustelus canis) and skate (Raja eglanteria) has the ability to stimulate the growth of tibial plate in hypophysectomized rats (Hayashida, 1973). Increase in the incorporation of leucine-^{14}C into skeletal muscle protein of rainbow trout after the injection of its own pituitary extracts noted in this study was perhaps due to the influence of growth hormone present in the pituitary extracts. It may be pointed out that, unlike elasmobranch growth hormone, the growth hormone of Teleostei does not stimulate growth in mammals nor does it show any immunochemical relatedness to mammalian growth hormone (Hayashida and Lagios, 1969; Hayashida, 1970).

To date, there is no information in literature regarding the effect of growth hormone on the incorporation of isotopically labelled amino acids into muscle protein of fish. The observation that growth hormone increased protein synthesis in rainbow trout is in agreement with the similar action of this hormone in mammals (Kostyo and Nutting,

1973; Trenkle, 1974; Engel and Kostyo, 1964; Knobil and Hotchkiss, 1964; Knobil, 1966; Kostyo, 1968; Pecile and Muller, 1968, 1972). It has been suggested that in mammals, the stimulatory effect of pituitary growth hormone on protein synthesis in the skeletal muscle is not mediated by insulin (Kostyo and Nutting, 1973). Whether or not this is also the case in fishes is not certain. Due to the difficulties encountered in estimating immunoreactive insulin in fish, it was not possible to measure plasma insulin levels in rainbow trout in response to growth hormone injections.

The available documentary evidence indicates that the general pattern of actions of growth hormone in fishes appears to be similar to that in mammals. Besides promoting protein synthesis, growth hormone stimulates lipolysis and glucose conservation in mammals (Fain, Kovacev and Scow, 1965; Russel, 1951). The hypophysectomy in Poecilia (Ball et al. 1965, 1966) and in Fundulus (Pickford, 1953a) is followed by deposition of glycogen in the liver, whereas injections of growth hormone have been shown to lead to a decrease in the ratio of lipid weight to dry weight in Oncorhynchus kisutch (Higgs et al., 1975).

The ability of growth hormone to manifest proteo-anabolic activity in rainbow trout, as noticed in this investigation is substantiated by the earlier findings that administration of growth hormone results in acceleration of overall body growth in fishes (Higgs et al., 1975; Chartier-Baraduc, 1959; Swift, 1954).

It is doubtful whether growth hormone will ever be used as a growth promoter in aquaculture due to the obvious difficulties in its administration to large numbers of fish.

3.3. ANABOLIC STEROIDS

The effectiveness of sex hormones and related substances as a supplement in the diet in promoting growth and food conversion efficiency in cultured fishes has an obvious significance in aquaculture. Most of the work in this field has been done with 17α-methyltestosterone which is a potent androgenic compound and its use in excessive doses can lead to the introduction of marked androgenic effects. In view of this, the anabolic steroids used in this study were chosen after a thorough scrutiny of the available information regarding their androgenic to anabolic index. Dimethazine, norethandrolone and methenolone have an advantage over methyltestosterone in the sense that in these compounds anabolic or mytrophic properties are to a fair degree dissociated from androgenic properties. Hence, it was not unreasonable to expect that incorporation of these steroids in the diet of rainbow trout at the dosage levels capable of evoking a positive growth response would not lead to the manifestation of androgenic effects.

The non-availability of isotopically labelled material did not permit the investigation of the chemical stability of these substances into fish food. However, Simpson et al. (1974) checked the stability of tritiated ethylestrenol incorporated into the fish food in the manner used in this study and found 85% undegraded steroid after one month.

3.3.1. ACCELERATION OF GROWTH

Of the three anabolic steroids tested for their efficacy in promoting growth and stimulating protein synthesis in rainbow trout, the addition of dimethazine and norethandrolone in the feeds, enhanced growth and protein synthesis and improve food conversion efficiency. Methenolone did not induce a positive growth response, probably because of the high dosage level (10 mg/kg food) at which it was administered.

The effect of dimethazine on growth in rainbow trout seems to be dependent on two factors: 1. dose and 2. duration of treatment. Besides these, the age of the animals and the water temperature might also have considerable influence on the response of fish to the administered hormone.

A. DOSAGE LEVEL

The results obtained in growth trials with dimethazine clearly indicate the dose dependent effect of dimethazine on acceleration of growth in rainbow trout. The administration of this hormone at concentrations above 5 mg/kg food did not induce a significant growth response (Table 14). At lower doses of 2.5 and 5 mg/kg food it produced up to 50% more weight gain as compared to controls. These results confirm earlier observations made in other reports. McBride and Fagerlund (1973) and Fagerlund and McBride(1975a) in studies with juvenile Pacific salmon, Oncorhynchus kisutch and O.tshawytscha, noted a distinct improvement in growth following oral feeding of 17α-methyltestosterone at concentrations of 10 and 1 mg/kg food. Recently Yamazaki (1976) has shown a similar effect of 17α-methyltestosterone at a concentration of 1 ppm in rainbow trout, Salmo gairdneri, goldfish, Carassius auratus, and kokanee, Oncorhynchus nerka. In all these studies, the administration of the hormone in higher doses resulted in suppression of growth. The optimum dose of dimethazine for rainbow trout appears to be 2.5 mg/kg food. At this concentration it proved to be most effective as a growth promoter. A similar figure (2.5 mg/fish) has been mentioned by Hirose and Hibaya(1968b) as optimum of 4-chlorotestosterone for rainbow trout.

Like dimethazine, norethandrolone also produced maximum growth response in rainbow trout at the lower dose level of 2.5 mg/kg food. Fish fed 2.5 mg norethandrolone/kg food for 60 days gained 69% more weight than the control group and 25% more than the group fed 5 mg dimethazine/kg food. These findings agree with those of Simpson et al. (1975) who demonstrated a significant increase in weight gains in rainbow trout and salmon parr treated with ethylestrenol, a steroid chemically very closely related to norethandrolone.

B. DURATION OF TREATMENT

The duration of time for which an anabolic steroid can effectively manifest a positive growth response in fish has great significance in fish cultivation. The results obtained with dimethazine and norethandrolone clearly indicate the limitations of large doses of the steroids in maintaining an increased growth rate for a long period of time. High doses of dimethazine (10 and 20 mg/kg food) resulted in an initial increase in daily increments of body weight but these were not significantly different from those of the control group at the end of 48 days (Fig. 21). A similar relation between dose of the steroid and duration of treatment was evident when dimethazine was fed to groups of fish at 5 and 2.5 mg / kg food levels for 60 days (Fig.22). During Period I (0 - 20 days) and Period II (21 - 40 days) the percentage increase in weight was almost equal in both the groups, but in Period III (41 - 60 days) the increase in percentage weight gains in the group receiving the lower level of dimethazine (2.5 mg) surpassed that of the group fed dimethazine at the higher level of 5 mg/kg food. The significance of duration of hormone administration was even more obvious in the case of norethandrolone. Fish which received 5 mg norethandrolone/kg food grew faster and showed a more rapid increase in percentage weight gains as compared to the group receiving 2.5 mg norethandrolone/kg food during Period I (0 - 20 days). However, during Period II (21 - 40 days) the growth rate in the lower dose group surpassed that of the higher dose group. At the end of Period III (41 - 60 days), the percentage weight gains in the lower dose group (2.5 mg/kg food) were 25% more than the higher dose group (5mg/kg food). A similar pattern was reflected in daily increments of body weight in fish fed norethandrolone at different levels (Table 23). These findings do not agree with those of Simpson et al. (1974) who observed that in rainbow trout treated with 12.5 mg ethylestrenol/kg food the percentage weight gains were greater than with

2.5 mg ethylestrenol/kg food. However, the effects of this compound on salmon parr were identical to the pattern reported in this study. The relatively deleterious nature of anabolic steroids in relation to growth, when given in large doses (as in the case of methenolone) and for longer periods of time, noted in this study is in agreement with similar observations made with 17α-methyltestosterone in guppy, Lebistes reticulatus (Clemens et al. 1966), goldfish, Carassius auratus and rainbow trout, Salmo gairdneri (Yamazaki, 1976) and Pacific salmon, Oncorhynchus kisutch and O. tshawytscha (McBride and Fagerlund, 1973).

C. TEMPERATURE

In the experiments conducted to examine the effect of dimethazine at 5, 2.5 and 1 mg/kg food levels for 60 days, there is an indication of the involvement of temperature as a regulatory factor in the response of rainbow trout to anabolic steroids. Water temperature during this experiment varied considerably and it seemed to have affected the response of fish to different dosage levels of the steroid. During Period II (21 - 40 days) water temperature suddenly rose from 15.1 $\pm$ 2.0°C to 18.1 $\pm$ 1.5°C and this appeared to have affected the growth of fish receiving the steroid more adversely than the fish receiving no steroid. Even among the groups receiving steroid, the adverse effect was not as marked in the group receiving 1 mg dimethazine/kg food as in the group fed 5 and 2.5 mg dimethazine/kg food. A similar response to variations in temperature was exhibited by fish fed norethandrolone. A direct evidence of the influence of temperature on response of fish to anabolic steroid has been shown by Fagerlund and McBride (1975b). These workers noted that in Pacific salmon receiving 0.2 mg and 1 mg 17α-methyltestosterone/kg food, the increase in weight gains over controls at 16.5°C was much greater than the corresponding increase in fish kept at 11.5°C. It can be argued that

the adverse response shown by rainbow trout fed on steroids to sudden variations in temperature in this study may be attributable to disturbance of physiological balance caused by the steroid which in turn affected the animal's capacity to adapt to an unfavourable change in external environment. Nevertheless, it is not unreasonable to assume that the effectiveness of anabolic steroids in accelerating growth even at low temperatures may be quite useful in trout or salmon hatchery management where water temperatures are low.

3.3.2. FOOD CONVERSION EFFICIENCY AND APPETITE

Besides acceleration of growth, another factor which is of considerable relevance to fish farming is the efficiency with which fish can convert food into flesh. Both dimethazine and norethandrolone improved food conversion efficiency at the dosage levels which were effective in stimulating growth (Tables 19 and 26). Similar improvements in utilization of food have been achieved in rainbow trout by use of 4-chloro-testosterone (Hirose and Hibiya, 1968b) and ethylestrenol (Simpson et al. 1974).

Although supplementation of 17α-methyl-testosterone in the diet of rainbow trout and goldfish resulted in increased consumption of food and appeared to have stimulated appetite and feeding behaviour (Yamazaki, 1976), no such response was noticeable in these studies. The daily consumption of food, calculated as percentage body weight in the fish fed dimethazine or norethandrolone supplemented diets was not different from that of the controls (Tables 19 and 26). Anabolic steroids are known to stimulate appetite (Kruskemper, 1968). The mechanism by which these hormones stimulate appetite in mammals may not be present in fishes. It may be pointed out that Yamazaki (1976) in his communication dealing with the application of hormones in fish culture mentioned an

increase in the amount of food consumed per fish in the group fed 17α-methyltestosterone. This may not necessarily mean an increase in appetite because the observed increase in consumption of food per fish could have been due to increased body mass as a result of steroid treatment and the ratio of weight of food consumed to body weight might have been similar both in treated and untreated fish as observed in these studies. Further evaluation of the influence of anabolic steroids on appetite and feeding behaviour in fish by employing "demand feeders" may yield significant information.

3.3.3. PROTEIN METABOLISM

The enhanced body growth and improved food conversion efficiency noticed as a result of oral administration of dimethazine and norethandrolone in growth trials was substantiated by the effect of these anabolic hormones on percentage incorporation of leucine-^{14}C into skeletal muscle protein. Oral administration of dimethazine or norethandrolone (2.5 mg/kg food) for 20 days in rainbow trout induced a marked increase in 6 hour leucine-^{14}C incorporation into skeletal muscle protein. These results agree with similar effects of anabolic steroids in mammals (Zafar et al. 1974; Daughady et al. 1975; Williams - Ashman and Redi, 1970; Metcalf and Gross, 1960; and Metcalf and Brioch, 1961). Feeding minute quantities of dimethazine (5, 2.5 and 1 mg/kg food) and norethandrolone (5 and 2.5 mg/kg food) for 60 days did not alter the moisture and total nitrogen content of the skeletal muscle. Thus, it appears that the mechanism by which anabolic steroids promote growth and protein metabolism in fish is entirely physiological in its action and depends on providing the normally operating anabolic processes with exogenous stimulus. This is of particular interest from the point of view of fish farming

because it indicates that acceleration of growth by application of anabolic steroids is not achieved at the expense of muscle quality. Information on the effect of anabolic steroids on the composition of muscle and other tissues is scanty. Only three observations have been reported which agree with the findings mentioned in this study. Administration of minute quantities of 17 α-methyltestosterone in juvenile Pacific salmon (Fagerlund and McBride, 1975a), ethylestrenol in rainbow trout (Simpson et al. 1974) and 4-chlorotestosterone in goldfish (Hirose and Hibiya, 1968a) did not alter the flesh characteristics.

3.3.4. SIDE EFFECTS

It is pertinent to fish farming that acceleration of growth in fish induced by anabolic steroids and related compounds should not be accompanied by tissue pathologies. Testosterone and its analogues possess hepatotrophic and renotrophic properties (Anstall, 1974, Kruskemper, 1968). It is evident from the reports dealing with the use of 17α-methyltestosterone as a stimulant of growth in fish that it can lead to the manifestation of marked androgenic effects. The steroids used in this study are claimed to have little, if any, androgenic effects in mammals (Kruskemper, 1968). Treatment of rainbow trout with dimethazine and norethandrolone for 60 days showed that these steroids were almost free of hepatotrophic and renotrophic properties at the dosage levels which induced significant weight gains. Neither northandrolone nor dimethazine altered hepatosomatic indices in rainbow trout fed steroid supplemented diets for 60 days. Dimethazine when given at high dosage levels of 5, 10 and 20 mg/kg food for 48 days did not produce any histological change in the liver. Other authors have also reported similar observations. Examination of liver did not reveal any hypertrophy in goldfish fed stanozolol

supplemented diets (Bulkley and Swilhart, 1973) or in rainbow trout fed ethylestrenol (Simpson et al. 1974). However, 4-chlorotestosterone increased hepatosomatic indices in goldfish and rainbow trout (Hirose and Hibaya, 1968,a,b) but it was noted to be mainly due to deposition of glycogen in the liver. The observation that dimethazine caused an increase in renosomatic indices confirms earlier observations made about some chemically related compounds in other reports. Bulkley and Swilhart (1973) in studies with goldfish, noted a distinct increase in ratio of kidney to body weights following four weeks of oral feeding of stanozolol. Similarly, occurrence of renal hypertrophy was observed in gonadectomized adult sockeye salmon, Oncorhynchus nerka (McBride and Overbeeke, 1971), in goldfish and rainbow trout treated with methyl-androstenediol and 4-chloro-testosterone (Hirose and Hibiya, 1968a, b) and in brown trout, Salmo trutta with testosterone and oestradiol (Ashby, 1957). Although the renosomatic indices of rainbow trout showed an increase as a result of dimethazine treatment, the histological examination of kidneys from fish fed 5, 10 and 20 mg dimethazine/kg food did not reveal any hypertrophy of the epithelial cells and of their muclei in kidney tubules. However, an increase in the renal haemopoietic tissues seemed to have occurred. This was probably responsible for alterations in the ratio of kidneys to body weights in the treated fish. An indirect evidence of sex steroids on haemopoetic organs of fish is found in the reports dealing with changes in haemopoietic organs with sexual maturity. Tamura and Honma (1970) found that the haemopoietic centre of the kidney, consisting of the head kidney and parenchymatous tissue of the body kidney was packed with immature basophils and lymphocytes at the stage of upstream migration in the goby, Leucopsarian petersi. After that a great decrease in the number of these cells occurred. A similar situation regarding the changes in the haemopoietic organs in relation to sexual maturity existed in flat-head goby, Luciogobius guttatus (Tamura and Honma, 1973, 1974). In migrating

and spawning Pacific salmon and rainbow trout, a relationship between changes in the haemopoietic tissues and hyperplasia of adrenocortical tissue was suggested by Robertson and Wexler (1960, 1962) and Robertson et al. (1961).

Unlike dimethazine, norethandrolone did not alter renosomatic indices and considering that its actions on growth are divorced from both hepatotrophic and renotrophic effects, makes it more attractive than dimethazine for possible use in fish farming.

Both norethandrolone and dimethazine appear to have a slight effect on haematocrit values. This is consistent with the similar effects of 17α-methyltestosterone observed in Fundulus heteroclitus by Slicher (1961) who injected male hypophysectomized killifish, Fundulus heteroclitus with methyltestosterone (2 μg/g weight-thrice weekly for one month and observed a marked increase in haematological values.

Histological examination of skin from rainbow trout fed high levels of dimethazine did not indicate any effect of this steroid on the epidermis or dermis. This is in contrast to the effect of 17α-methyltestosterone or 11-ketotestosterone in salmonids. 11-ketotestosterone increased the thickness of skin in both normal adult sockeye salmon, Oncorhynchus nerka (Idler et al. 1961) and in gonadectomized adults (McBride and Overbeeke, 1971). Similarly 17α-methyltestosterone induced a marked increase in the thickness of epidermis and dermis in various species of genus Oncorhynchus, (Yamazaki, 1972; McBride and Fagerlund, 1973). The discrepancy between these observations and the effect of dimethazine on skin in rainbow trout can probably be explained on the basis that 11-ketotestosterone is a naturally occurring androgen in this fish (Schreck, Flickinger and Hopwood, 1972) and 17α-methyltestosterone is chemically more closely related to naturally occurring androgens as compared to dimethazine. Hence, these two sex steroids may be more

effective in increasing thickness of skin,which is also brought about by endogenous androgens during spawning migration and gonadal maturation as pointed out by Stoklosowa(1966, 1970).

3.3.5. CONCLUSIONS

It is clear that the incorporation of some synthetic anabolic steroids such as dimethazine, 17α-methyltestosterone, norethandrolone and ethylestrenol into the diet can yield highly significant increases in growth rate and food conversion efficiency of both rainbow trout and salmon. High doses of these steroids when given for long durations can lead to the introduction of marked androgenic side effects. The results presented in this study show that oral administration of both dimethazine and norethandrolone can manifest pronounced proteo-anabolic activity in rainbow trout. It appears that the mechanism by which anabolic steroids promote growth is physiological in its action. This is of particular significance from the point of view of fish cultivation because it indicates that acceleration of growth by the application of anabolic steroids is not achieved at the expense of muscle quality. The effectiveness of some anabolic steroids to stimulate growth even at low water temperature may be quite useful in trout or salmon hatchery management where water temperatures are low. The investigation of the metabolism of these steroids and the rate at which these are excreted from the body during feeding on normal diets is required before their use in commercial fish diets could be initiated. Up to date only Simpson et al. (1974) have reported one experiment in which ingested tritiated ethylestrenol appeared to be quickly metabolized and excreted. There is a great need to search for compounds capable of increasing growth rate and food conversion efficiency and preventing premiscuous sexual maturation in the male in the cultured fishes. Recently it has been shown that incubation of fertilised eggs of Tilapia nilotica in water containing adrenosterone (5 mg/litre) for 3 months resulted in a significant enhancement of body growth accompanied by gonadal degradation (Katz, Abraham and Eckstein, 1976).

3.4. DIETHYLSTILBOESTROL

Diethylstilboestrol has been widely used in animal husbandry because of its ability to promote growth and improve food conversion efficiency without manifesting any renotrophic or hepatotrophic side effects. The future of this oestrogenic substance has been questioned because of its alleged carcinogenic side effects (Anonymous, 1972) and it seems unlikely that it will ever be used as a fish growth promoter.

The growth trial with DES presented in this study was conducted to resolve the conflicting situation which existed regarding its actions in fish. Ghuittino (1970) reported a slight depression of body growth in rainbow trout fingerlings fed DES supplemented diets at fairly high dosage levels (50 - 500 mg/kg food). Cowey et al. (1973) noted that DES when given in low doses (1.2 mg/kg dry food) accelerated the growth rate and improved food conversion efficiency in plaice, Pleuronectes platessa, and suggested that negative growth response to oral administration of DES noted in rainbow trout by Ghuittino could have been due to the use of excessive doses. In view of this argument, the growth trial presented in this study was conducted with DES using the dosage level (1.2 mg/kg food) considered by Cowey et al. as the optimum dose for fish. The results (Table 30) indicate a growth depressing effect of DES in rainbow trout fed diets supplemented with this oestrogenic compound for 30 days. This negative effect of DES on body growth was also reflected in decreased consumption of food and impaired food conversion efficiency (Table 32). These findings are in agreement with the catabolic effects of DES noted in goldfish, Carassius auratus (Hoar, 1958), Channel catfish, Ictalurus punctatus (Bulkley, 1973) and Coho parr (Fagerlund and McBride, 1975b). Oestradiol has also been seen to depress body growth in salmonids (Simpson, 1976) and from the available data on the effect of DES and oestradiol on body growth in fish, it can safely be assumed that oestrogens usually have a growth depressing action in fishes.

APPENDIX A

RADIOIMMUNOASSAY OF INSULIN

INTRODUCTION

The development of radioimmunoassay techniques has made it possible to measure the physiological levels of insulin in various body fluids in mammals. Marked immunological differences have been demonstrated between fish and mammalian insulins (Falkmer and Wilson, 1967; Smith, 1966;Wilson, 1966). These differences result in poor cross reactivity of teleost insulins with antibodies raised against mammalian insulin (Wilson and Dixon, 1961; Reid, Grant and Youngson, 1968; Patent and Foa, 1971) and make the measurement of insulin levels in fish with anti-mammalian insulin sera and mammalian insulin standards questionable. However, there are a few reports in literature which indicate that measurement of fish insulin by using antibody against mammalian insulin is not impossible. Wilson and Dixon (1961) found that insulin from Lota lota lacustris, Esox lucius, Lepomis gibbosus and Catostomus commersoni were neutralized by anti-bovine insulin sera. Tashima and Cahill (1968), Dixit, Morgan, Lindal and Lazarow (1969) and Watkins, Cooperstein, Dixit and Lazarow (1968) were able to measure insulin in fish with anti-mammalian insulin sera. Because of the great difficulties in obtaining pure fish insulin and specific antisera for radioimmunoassay, an attempt was made in this investigation to measure plasma insulin levels in rainbow trout with commercially available insulin assay kits.

MATERIALS AND METHODS

Two different types of insulin assay kits were used. First kit, obtained from The Radiochemical Centre, Amersham was based on double antibody method of Hales and Randle (1963a, b) while the second kit, Phadebas insulin test, Pharmacia (Great Britain) Ltd., London was based on solid phase immunoassay procedure described by Wide and Porath (1966).

The essential principle of assay is similar in both these techniques. A limited and fixed amount of antiinsulin antibody is cross-reacted with a mixture of the sample to be assayed and a constant amount of radioactivity labelled insulin (^{125}I-insulin). When the reaction is nearly complete, the antibody bound insulin is separated from the free insulin and radioactivity is measured. Since the available antigen-binding sites are limited, the insulin in the sample competes for the binding sites with the labelled insulin. The amount of labelled insulin bound to antibody will be inversely related to the amount of insulin present in the sample. The results obtained with unknown samples are compared with a standard curve prepared with samples of known insulin content.

In the double antibody method (Hales and Randle, 1963a, b; Morgan and Lazarow, 1963), the antibody bound insulin is separated from free insulin in the final reaction mixture by immunoprecipitation. In this process, the insulin-antibody complex is precipitated by reaction with a second antibody which is directed against the immunoglobulins of the first antibody. For example, the anti-insulin antibody raised in guinea pig is precipitated with antiguinea pig - γ - globulin. The precipitated antibody is separated by centrifugation or by microfiltration on filter discs of cellulose acetate. In the solid phase radioimmunoassay (Wide and Porath, 1966) the antibodies to insulin are covalently bound to Sephadex particles in the solid phase. In this case, the separation of antibody-bound insulin is easily accomplished by centrifugation.

I. Radioimmunoassay by Double-antibody Method

Reagents

The kit provided by the Radiochemical Centre, Amersham, included pre-precipitated anti-bovine insulin antibody and ^{125}I bovine insulin dissolved in a phosphate buffer (40 mmol, pH 7.4) containing

bovine serum albumin (0.51% w/v) and thiomersalate (0.6 mmol). Purified codfish insulin, a gift from the Diabetes Research Laboratory, The Wellcome Foundation Ltd., Kent, was used as standards. The following buffers were used in the assay:

Buffer A. a phosphate buffer (40 mmol, pH 7.4) containing bovine serum albumin (0.51% w/v) and sodium ethyl mercurithio-salicylate (thiomersalate) (0.6 mmol) was used to dilute the iodinated insulin.

Buffer B.was an isotonic phosphate buffer composed of buffer A plus 0.9% (w/v) NaCl. This buffer was used to dissolve the codfish insulin standards.

Buffer C. was high protein buffer consisting of buffer A and horse serum in equal proportions. It was used for washing the antibody precipitate. The horse serum used was No.5, Wellcome Reagents Ltd., Beckenham.

All the buffers were stored at 2 - 4°C.

The pre-precipitated antibody was reconstituted with deionized water (8 ml per vial as supplied). Each vial of ^{125}I-insulin contained 0.1 µg of iodinated insulin dissolved in 5 ml of buffer A. The working solution was prepared by adding 1 ml of the reagent to 7 ml of Buffer A. Thus, 0.1 ml of diluted mixture contained 250 picogram of iodinated insulin. By serial dilution of codfish insulin, standards containing 8, 6, 4, 2, 1 and 0.5 ng, insulin/ml were prepared.

Procedure

The assay was carried out in 3 ml pre-cleaned polystyrene tubes fitted with polythene stoppers. All the reactants were added in aliquots of 0.1 ml. At first, duplicate samples of unlabelled insulin (standards or unknown samples) were vortex mixed with insulin antibody and incubated in a refrigerator at 4°C for 8 hours. Blank (having no insulin antibody) and zero tubes (containing buffer B in place of unlabelled insulin) were also treated in a similar manner. Following this initial incubation, 100 µl of ^{125}I-insulin (working solution) was

added into each tube. All the tubes were vortex mixed and incubated for another 18 hours at 4°C. At this stage, 3 samples of iodinated insulin were added to empty tubes and kept at 4°C for measurement of total radioactivity added into each assay tube. After 18 hours incubation, the immunoreaction was assumed to be complete and the anti-body bound insulin was separated from free insulin by centrifugation for one hour at 2000g. The supernatant was decanted off and the precipitate remaining in the tube was washed with 1 ml of Buffer C at 4°C by vortex mixing. The tubes were again centrifuged and supernatant decanted off. Each tube was washed 3 times with Buffer C. At the end of the final washing and centrifugation, the precipitate in the tubes was allowed to dry at room temperature for 6 hours. The radioactivity of the precipitate was counted for 10 minutes using a gamma scintillation spectrophotometer 500 (ICN Tracerlab Division, Belgium). The counts were corrected for background.

II. Solid Phase Radioimmunoassay.

Reagents

Phadebas insulin assay kit contained guinea-pig anti-porcine insulin coupled to Sephadex, ^{125}I-insulin and a buffer (pH 7.4) for reconstituting lyophilized ^{125}I-insulin and diluting Sephadex antiinsulin complex. Codfish insulin was used as standards as in the previous assay.

Procedure

The details of the procedure were similar to those described for the double antibody method. The Sephadex-anti-insulin complex and ^{125}I-insulin were mixed with insulin standards or unknown samples. This mixture was incubated at room temperature for 12 hours. The solid particles were kept in suspension by slow rotation of tubes during incubation. At the end of the incubation insulin bound to Sephadex-

anti-insulin complex was separated from free insulin by centrifugation for 2 minutes at 2000g. The solid particles were washed three times with 0.9% saline. After final washing and centrifugation procedure, the supernatant was withdrawn carefully with an aspirator and radioactivity in each tube was determined.

RESULTS AND DISCUSSION

In both the assay techniques used, neither cod insulin or insulin in trout plasma samples cross-reacted with antibody raised against porcine or bovine insulin. In the zero tubes which contained antibody and labelled insulin there was 48% binding between ^{125}I-insulin and anti-porcine insulin antibody in the solid phase immunoassay and 50% binding between ^{125}I-bovine insulin and anti-bovine insulin antibody in the double antibody procedure. The amount of labelled insulin bound to the antibody did not alter in the samples i.e. even in the presence of cod insulin or rainbow trout insulin. This observation clearly demonstrated that cod or rainbow trout insulin did not have any affinity with antisera raised against mammalian insulins. These results confirm earlier findings of Patent and Foa (1971) and Wilson and Dixon (1961) who showed that cod-fish insulin did not crossreact with anti-mammalian insulin antibody.

It is clear from this investigation that measurement of fish insulin by radioimmunoassay techniques is very difficult by employing non-specific antisera and hormone. Further investigation by using species specific antisera and insulin (labelled as well as standard) may yield valuable information regarding the peripheral effects of insulin in fishes.

APPENDIX B

EFFECTS OF GOLD THIOGLUCOSE IN RAINBOW TROUT

INTRODUCTION

It is well established that in mammals parenteral administration of gold thioglucose causes necrosis of the ventromedial hypothalamus (satiety centre) and is followed by a condition of hyperphagia and obesity (Marshall, Barnett and Mayer, 1955). The damage to the hypothalamic cells occurs as a result of deposition of gold thioglucose, which takes place due to selective affinity of these cells to glucose moiety of gold thioglucose (Mayer and Marshall, 1956). It has been pointed out by Debons, Krimsky, From and Pattinian (1974) that gold thioglucose damage to the ventromedial hypothalamus is related to the presence in this region of specific glucoreceptor cells and further, that these cells are sites where signals pertaining to glucose utilisation by the organism are received and trasmitted to the neural apparatus which adjusts food intake to caloric requirements.

In fishes, there is little information on the hypothalamic control of intake of food or on the effects of gold thioglucose. A preliminary investigation of the influence of parenteral administration of gold thioglucose on increase in body weight and plasma glucose levels in rainbow trout was undertaken. It was of particular interest to examine the effects of gold thioglucose in fish because there is some evidence regarding the involvement of insulin in sensitivity of ventromedial hypothalamus to gold thioglucose in mice (Debons, Krimsky and From, 1970). Hyperglycemia produced as a result of alloxan or anti-insulin antibody administration reduces the sensitivity of the satiety centre to damage by gold thioglucose (Debons, Krimsky, Likuski, From and Cloutier, 1968; Debons, Krimsky, From and Cloutier, 1969). Intrahypothalamic injection of insulin restores the sensitivity of satiety

centre to gold thioglucose in diabetic mice (Debons et al. 1970).

MATERIALS AND METHODS

60 rainbow trout, weighing 2 - 2.5 gm were split into three groups of 20 fish each. One group served as controls, while the other two groups were injected intraperitoneally with gold thioglucose (8% aqueous solution) at the dosage level of 1.0 and 1.25 mg/gm body weight. After administration of gold thioglucose, fish were closely watched for 48 hours. The treated fish developed a dark colour which changed to the normal state within 36 hours. All the fish in the group which was given gold thioglucose at a concentration of 1.25 mg/gm body weight died within 2 days. However, rainbow trout in the low dose group survived and resumed normal feeding after three days.

Fish were fed to satiation twice daily during a natural photoperiod. The temperature during the 60 days experimental period varied from 15°C to 18.5°C. Fish were weighed individually at intervals of 30 days.

Blood for glucose assay was obtained from ductus cuvieri. Glucose was determined in 10 µl plasma samples by means of a Beckman Glucose Analyzer.

At the end of the experiment, small pieces of brain, including hypothalamus were preserved in Bouin's fluid for histological examination.

RESULTS AND DISCUSSION

The weight data from weighing of individual rainbow trout in the groups treated with 0 and 1.0 mg gold thioglucose/gm body weight is shown in Table A. It is clear that fish in the treated group grew faster than the control fish. The increase in weight gain in the treated fish was significantly greater than that in the control fish ($p < 0.05$). After 60 days, the percent increase in the mean body weight of fish injected with gold thioglucose was 75% greater than that of the control group.

Plasma samples had been unfrozen for two days before assay of glucose. However, estimation of glucose level in these samples showed an increase in glucose levels in the gold thio glucose treated group ($P < 0.01$). Mean values for plasma glucose were 31.7 ± 8.5 mg/ml ($\pm$S.D) in the treated group and 19.1 ± 7.2 mg/ml in the control group.

Histological examination of part of the brain together with the hypothalamus did not show any identifiable effect of gold thioglucose.

The results presented here do not appear to be sufficient for making any firm conclusion regarding the action of gold thioglucose in rainbow trout. Further investigation of the effects of gold thioglucose at 1.0 and 0.85 mg/gm body weight level for a longer period of time may yield useful information.

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TABLE A. Changes in mean body weight of rainbow trout injected with gold thioglucose. Values given are means ± S.D. of 20 fish. Percent weight gain over controls is given in parentheses.

Treatment	Duration		
	0	30 days	60 days
Control	2.21 ± 0.24	4.14 ± 0.97	8.48 ± 2.10
Gold thioglucose/ 1 mg/gm body weight	2.22 ± 0.22	4.20 ± 1.44 (2%)	10.16* ± 2.53 (74%

*P < 0.05

4. REFERENCES

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