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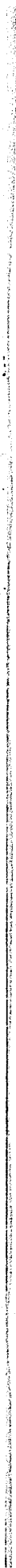
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Diabetogenous symptoms in pellet fed hatchery-
reared Atlantic salmon smolts (Salmo salar)

by

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A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science,
to the School of Graduate Studies, University of
Ottawa.

February 1978.

Candidate

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ABSTRACT

The profitable rearing of the Atlantic salmon (Salmo salar L.) requires artificial diets high in carbohydrates or fats or both in order to avoid the high cost of protein feed; this practice is monitored by growth criteria such as animal length and body weight. The present study investigates the physiological state of hatchery-reared salmon smolts raised under cage culture conditions and fed a commercial pellet food of elevated carbohydrate content.

Our results show metabolic disturbances that strongly suggest a diabetogenous situation: hyperglycemia, hypertriacylglycerolemia, some glucosuria and occasional ketonuria.

Histopathological examination confirms the initial suggestion: significant reduction of the beta to alpha cell ratio in the islets of Langerhans, frequent alteration of the disposition of insular cells, hydropic changes in the B cells such as degranulation, vacuolization and nucleic pyknosis, evidence of hypertrophy and hyperplasia of B cells and pronounced neoformation of islet tissue. In addition, hyalinization of both the exocrine and the endocrine pancreas was observed as well as fibrosis, lipomatosis and microangiopathy of the exocrine acinar tissue and an increase in intrapancreatic nerve cell number.

In conclusion, the smolts fed the pellet diet displayed a syndrome reminiscent of mammalian diabetes; a comparison is drawn between diabetes mellitus and the observed form of salmon diabetes.

The study emphasizes the need to involve physiological criteria in the assessment of the suitability of artificial diets especially in the rearing of healthy salmon intended for restocking of natural environments.

RESUME

La rentabilité de l'élevage du saumon de l'Atlantique exige un régime alimentaire artificiel riche en hydrates de carbone ou en lipides ou dans les deux afin d'éviter le coût élevé des protéines; cette pratique est contrôlée au moyen des critères de croissance tels longueur et poids de l'animal. La présente recherche examine l'état physiologique de smolts de pisciculture élevés en captivité en cages marine et alimentés d'une diète commerciale à grain sec à haute teneur en hydrates de carbone.

Nous avons observé un dérangement du métabolisme qui suggère fortement un état diabétique: hyperglycémie, hypertriglycéridémie, quelques cas de glycosurie et une cétonurie occasionnelle.

L'examen histopathologique confirme les observations initiales: réduction significative du rapport bêta-alpha dans les îlots de Langerhans, altération fréquente de l'architecture des cellules insulaires, changements hydropiques dans les cellules B tels que dégranulation, vacuolisation et pyknose nucléaire, hypertrophie et hyperplasie des cellules B et néoformation marquée dans les îlots. On observe de plus une hyalinisation du tissu pancréatique exocrine et endocrine, une fibrose, une lipomatose et une micrangiopathie du tissu acinaire exocrine ainsi qu'une augmentation des cellules ganglionnaires intrapancréatiques.

En résumé, les smolts nourris avec une diète à grain sec exhibent un syndrome qui rappelle le diabète du mammifère; une comparaison est établie entre le diabète sucré et le "diabète" du smolt.

L'étude souligne l'importance des critères physiologiques dans l'évaluation du rendement de diètes artificielles spécialement dans l'élevage de saumons bien en santé et destinés au repeuplement des eaux.

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PREFACE

The necessity to increase the amount of animal protein available for human consumption has led to the current world wide interest in the captive rearing of commercially valuable fish species. The practice is gaining much popularity largely due to the availability of artificial diets consisting mainly of low cost fats and carbohydrates which supplement the caloric value of the diet once the expensive protein requirements of the fish have been met. However, past evaluations on the suitability of artificial diets in fish have greatly over emphasized growth as an index of dietary efficiency (Brelt et al., 1969; Saunders and Henderson, 1974). Inadvertently these studies have unfortunately neglected to assess the overall physiological condition of hatchery-reared fish.

The fisheries departments of the government of Canada and of other countries are seriously involved in the pisciculture of the Atlantic salmon and invest large sums of money towards this goal. Surely, whether the aims of culture practices are for direct cropping or for release with intent to stock wild fish populations, the fish at all times should be maintained in a fit physiological state. Indeed, a cropped fish must not only be edible but must be as nutritious and palatable as wild animals in order to gain access into the world market. Perhaps more importantly, fish destined to be released for stocking purposes must retain their full adaptive potential and health if they are to survive the challenges of life in the wild. Hence, the present study centers on the physiological condition of

hatchery-reared salmon, a field of research which definitely demands more attention.

In order to accomplish this task it was decided, as part of a larger study on diet-growth relationships, to investigate if hatchery-reared salmon smolts which were raised under cage culture conditions in Passamaquoddy Bay, New Brunswick, and fed a widely used commercial pellet food diet, displayed any physiological upsets.

INTRODUCTION

Teleost metabolism is believed to be adapted to diets normally low in available carbohydrate and high in protein (Love, 1970; Palmer and Ryman, 1972). More specifically the Atlantic salmon is held to have a limited capacity to digest and assimilate levels of carbohydrate beyond approximately 9% of digestible carbohydrate (Strand, 1958). In fact, Strand has shown that excess carbohydrate in the diet was not an available energy source unless the fish were simultaneously treated with exogenous epinephrine. Similarly, Tunison (1940) and Phillips et al. (1948 and 1966) have demonstrated that the speckled trout, Salvelinus fontinalis, was physiologically unable to handle high dietary levels of carbohydrates and that consequently carbohydrates were only limited sources of energy. In both of these salmonids, the authors have reported that a high level of dietary carbohydrate resulted in excessive hepatic glycogen deposition leading to hepatomegaly; the condition was associated with marked increases in blood glucose levels. The above anomalies of carbohydrate metabolism in hatchery-reared salmonids are implied to signify that in these fish there possibly exists a deficiency in circulating insulin whereby insulin-assisted tissue glucose uptake failed to capitalize on the carbohydrate energy source made available. More recently, Wendt and Saunders (1973) have recognized the existence of a hyperglycemic syndrome in hatchery-reared pellet fed salmon smolt attributable to some fault in the hatchery regime such as temperature-photoperiod conditions, advanced smoltification, high carbohydrate diet and feeding

regimen and consequently have considered as a possible outcome the establishment of a form of diabetes. For these reasons the physiological parameters judged to be most likely affected by the commercial pellet food diet (Table 1) were those related to carbohydrate metabolism.

Spontaneous diabetes mellitus (Sekoke disease) was diagnosed in farm raised Japanese carp, Cyprinus carpio, accidentally fed a rancid silk worm pupae diet (Yokote, 1970 a and b; Nakamura et al., 1970 and 1971). The metabolic aspects of the disease were symptomatic of mammalian diabetes mellitus. The carp were skinny in appearance and showed moderate hyperglycemia, glycosuria, infrequent ketonuria, decreased glucose tolerance and some resistance to insulin. The histopathology of Sekoke disease also closely resembled that observed in mammalian diabetes. The B cells of the islets of Langerhans were reduced in number and exhibited hydropic changes (cytoplasmic vacuolization, cytoplasmic glycogen infiltration and pyknotic nuclei); degranulation, obscure cell contour, nuclear hypertrophy and scarcity of mitotic division were also clearly evident. Clear cells, regarded as precursors to B cells, showed hyperplasia as evidenced by the increased number and the formation of clumps adjacent to capillary walls in the central region of the islets; an arrangement similar to that of B cells. The exocrine pancreas showed fatty degeneration and acinar cells around blood vessels were inflamed. At the level of the kidneys the glomerular capillaries were dilated, the

Table 1. Composition and estimated calorific value of diets fed to smolts during cage culture experiment in salt water.

Components	Pellet diet ^a			Herring diet ^b		
	wet %	dry %	% calories	wet %	dry %	% calories
protein	57.0	62.0	64	16.4	49.7	35
carbohydrate	13.5	14.7	15	1.0	3.0	2
fat	8.0	8.7	21	13.0	39.4	63
ash	13.0	14.1		2.6	7.9	
water	8.0	---		67.0	---	
fibre	0.5	0.5		---	---	
Kcal/gm ^c	4.6	4.9		4.7	6.7	

(a) From pellet food analysis certified by manufacturer for Ewos Salmon Grower F 159 6P.

(b) Estimate based on fat content of herring from Passamaquoddy Bay area (Leim, 1957;

Stoddard 1967 and 1968) and other components approximated (Vinogradov, 1953;

Black, 1958; Love, 1970).

(c) Calorific values of components as estimated for animals in general; 4 (protein), 9 (fat), and 4.0 (carbohydrate) Kcal/g.

arterioles showed hyalinization, the tubules were swollen, the tubular cells showed hydropic changes and the interstitial space contained an increasing amount of lymphoid tissue. In the liver the parenchymal cells were atrophied, fat and glycogen stores were depleted and a cirrhosis marked by the presence of much cellular disintegration was seen. The retina of the eye, the muscular, the adrenal cortex and the pituitary tissues were also affected in a manner symptomatic of diabetes. Furthermore, Yokote has apparently recently detected diabetes in a group of reared salmonoid fish, the ayu-fish, Plecoglossus altivelis (Renold et al., 1973). Certainly this preamble of pathological diabetogenous symptoms, induced in the carp by means of a faulty diet, provides ample evidence to entertain and pursue the thesis that the rearing of salmon under conditions where the animals have little opportunity for physical activity and are fed a high caloric density diet of elevated carbohydrate content may induce pathological conditions resembling diabetes mellitus.

Often in animals, diabetes has been associated with a high carbohydrate dietary component (Campbell, 1970). Such was the case for man where it was found that in flourishing socioeconomic societies such as the Western world, a reduced amount of exercise coupled to a high dietary intake of carbohydrates led to a greater incidence of diabetes mellitus (Tsuji, 1970). Similarly, Stauffacher et al. (1971) showed that the incidence of diabetes in certain rodents such as the spiny mouse, Acomys cahirinus, and the sand rat, Psammomys obesus, was detectable as

spontaneous diabetes only when the animals were reared under laboratory conditions where they received a diet of high caloric density. Likewise, teleosts when reared on high carbohydrate diets have displayed various physiological defects of metabolism believed to be related to diabetes. For example in the speckled trout, Salvelinus fontinalis, a high level of dietary carbohydrates increased the level of blood glucose (Phillips et al., 1958). In the rainbow trout, Salmo gairdneri, an elevated dietary component of carbohydrate induced a several fold increase in hepatic glycogen and an 8 to 9 fold increase in muscle glycogen, and in these fish, the liver to muscle glycogen ratio was much reduced and resembled that observed in starving fish (Hochachka and Sinclair, 1962). Moreover, under a similar feeding regimen, Palmer and Ryman (1972) found a high degree of mortality in rainbow trout primarily due to pathological glycogen deposition in the liver; the fish were also noted to be hyperglycemic. In the goldfish, Carassius auratus, long term feeding of a high carbohydrate diet resulted in excessive hepatic glycogen accumulation, abnormal fatty deposition, general protein depletion and frank hepatomegaly paralleled by hyperglycemia (Palmer and Ryman, 1972). Inappropriate hyperglycemia in teleosts, implying low glucose tolerance, is held to be analogous to the intolerance symptomatic of diabetes mellitus in humans (Falkmer, 1961; Palmer and Ryman, 1972).

In the past, artificial diets have been shown to have a direct effect on the pancreatic tissue of teleosts. Indeed

teleostean endocrine pancreatic tissue is known to have a high dependency on protein metabolism (Epple, 1969). A diet combining high fat and high carbohydrate coupled with a lack of muscular activity while in captivity resulted in a decrease in the number of islets of Langerhans of the rainbow trout, Salmo irideus (Hess, 1935). In these trout cellular degeneration of the islet tissue with nuclear pyknosis and fatty infiltration of the pancreatic tissue were held to be somewhat akin to a prediabetic condition or an incipient diabetes (Ashley, 1972). In Cyprinoidea, hyperphagia was responsible for pathological enlargement of the adipose tissue and pancreatic degeneration (Ple'hn, 1938). Salmonids maintained under an artificial environment showed an increased lipid content which translated into fatty degeneration of the liver and the pancreas (Wood et al., 1957). Fatty degeneration of hepatic and pancreatic parenchymal tissues of the chinook salmon, Oncorhynchus tshawytscha, was related to low protein diets and it was postulated that in the absence of specialized adipose tissue both the liver and the pancreas may act as major fat storage sites (Donaldson, 1942; Robertson and Wexter, 1960). In the coho salmon, Oncorhynchus kisutch, a high carbohydrate diet promotes hepatic fatty acid synthesis as seen by marked increases in lipogenic enzyme activities of the liver surpassing those found within mesenteric adipose tissue (Huangsheng, 1977). Consequently, it is foreseen that diets high in carbohydrate or both carbohydrate and fat may lead to fatty infiltration of the liver and the pancreas to the point that normal function is

disrupted. Also, it has been shown that the islet tissue of the sculpin, Cottus quadricornis, relied heavily on protein calories as an energy source for cellular maintenance and growth (Epple, 1969). Further, the protein content of artificially reared salmonids was found to be lower than in wild fish (Wood et al., 1957). Hence, it is suggested that the commercial pellet food diet, as employed in this study, somehow interferes with a normal protein metabolism whereby the protein requirement of the islet tissue may not be met and consequently the endocrine function of this tissue may be adversely affected. From the above it might be predicted that teleosts are incapable of sustaining the proper level of endocrine pancreatic function while coping with excessive dietary caloric intake and it might be inferred that a carbohydrate supplemented diet with reduced protein content might induce a state of hypoinsulinemia thus reflecting a pseudo-diabetic condition.

Furthermore, there exist several physiological factors in teleosts which render the dietary induction of diabetogenous symptoms feasible. For instance, the administration of a glucose load to several teleost species rapidly induced a more marked and persistent hyperglycemic state and this was taken as an indication that teleosts have a lower glucose tolerance than do mammals (Falkmer, 1961; Tashima and Cahill, 1968; Epple, 1969; Yokote, 1970 b; Khanna and Rekhari, 1972; Palmer and Ryman, 1972; Wardle, 1972; Khanna and Gill, 1973; Bhatt, 1974; Ince and Thorpe, 1974; Thorpe and Ince, 1974). In a few species an hyperglycemic plateau was observed

in the glucose tolerance curve while in most species recovery was slow and often took as long as 24 to 48 hours (Table 2). Ultimately, it was perceived that a delayed response to endogenous release of insulin in teleosts, possibly due to a lower metabolic rate in poikilotherms, favors a rapid and sharp rise in blood glucose following carbohydrate feeding and force feeding (Plisetskaya and Kuz'mina, 1971; Khanna and Gill, 1972). In turn, these upsets may induce a persistent hyperglycemic state in teleosts fed over prolonged periods of time with diets of elevated carbohydrate content.

The above is supported by the fact that the islet B cells of teleosts appear to be hypersensitive in their response to stimulation. Indeed, in both the chinook salmon, Oncorhynchus tshawytscha, and the rainbow trout, Salmo gairdneri, hyperphagia was accompanied by severe B cell degranulation and the appearance of numerous mitotic processes (Hess, 1935; Donaldson, 1942). In the stinging catfish, Heteropneustes fossilis (Khanna and Rekhari, 1972), in the snake head, Channa punctatus (Khanna and Gill, 1973) and in the spotted catfish, Clarias batrachus (Bhatt, 1974), glucose loading resulted in diabetic-like hydropic B cell degeneration characterized by loss of specific granules, cytoplasmic vacuolization and pyknosis of nuclei leading to acute B cell exhaustion; in the snake head the internal cellular configuration of the islets of Langerhans was also significantly altered. Administration of exogenous hydrocortisone to the shorthorn

Table 2. Glucose tolerance test in teleosts and in man.

Species and normal blood or plasma glucose	Glucose load (1) and route of administration and normal glucose range.	Peak hyperglycemia developed and particularities of the glucose tolerance curve.	Authors
<u>Cottus scorpius</u> (shorthorn sculpin) 9 mg % (fasted)	0.5 gm/kg. body weight. (intra-muscular). 0 mg % to 19.8 mg %	126 mg %, slow decent, recovery after 24 hours	Falkner, 1961.
<u>Opsanus tau</u> (toadfish) 40 mg %	0.5 gm/kg. body weight. (intra-venous). 0 mg % to 50 mg %	150 mg %, plateau, recovery after 24 hours.	Tashima and Cahill Jr., 1968. Moule and Nace, 1963.
<u>Cyprinus carpio</u> (European carp) 41 mg % (fasted)	0.5 gm/kg. body weight. (oral) 25 mg % to 54 mg %	127 mg %, recovery after 6 hours	Yokote, 1970 b.

Table 2. Glucose tolerance test in teleosts and in man.

Species and normal blood or plasma glucose	Glucose load ⁽¹⁾ and route of administration and normal glucose range.	Peak hyperglycemia developed and particularities of the glucose tolerance curve.	Authors
<u>Cyprinus carpio</u> (European carp)	0.5 gm/kg. body weight. (oral)	140 mg %, slightly above normal after 6 hours.	Yokote, 1970 b.
78 mg % (fasted)	42 mg % <u>diabetic</u> 194 mg % range		
<u>Heteropneustes fossilis</u> (stinging catfish)	1 gm/kg. body weight. (intra-muscular)	188 mg %, plateau, recovery after 72 hours.	Khanna and Rekhar, 1972.
84 mg %			

Table 2. Glucose tolerance test in teleosts and in man.

Species and normal blood or plasma glucose	Glucose load ⁽¹⁾ and route of administration and normal glucose range.	Peak hyperglycemia developed and particularities of the glucose tolerance curve.	Authors
<u>Pleuronectes platessa</u> (plaice) 20 mg % (fasted)	0.7 to 0.9 gm/kg. body weight (into bloodstream) 15 mg % to 25 mg %	60 mg %, level still elevated after 6 hours.	Wardle, 1972.
<u>Channa punctatus</u> (snake head) 59 mg % (fasted)	0.5 gm/kg. body weight. (intra-muscular) 32 mg % to 56 mg %	167 mg %, recovery after 48 hours	Khanna and Gill, 1972 and 1973.
<u>Carassius auratus</u> (goldfish) 23 mg %	0.75 gm/kg. body weight. 18.9 mg % to 38.1 mg %	181 mg %, recovery after 4 hours.	Khanna and Gill, 1973. Chavin and Young, 1970 and 1973.

Table 2. Glucose tolerance test in teleosts and in man.

Species and normal blood or plasma glucose	Glucose load ⁽¹⁾ and route of administration and normal glucose range	Peak hyperglycemia developed and particularities of the glucose tolerance curve.	Authors
<u>Clarias batrachus</u> (Teysmann's spotted catfish)	1 gm/kg. body weight. (intra-muscular)	298 mg %, recovery after 48 hours	Bhatt, 1974.
61 mg %	48 mg % to 72 mg %		
<u>Anguilla anguilla</u> (European eel)	0.5 gm/kg. body weight. (via intestine)	70 mg %, plateau, recovery after 24 hours.	Ince and Thorpe, 1974.
50 mg % (fasted)			
<u>Esox lucius</u> (Northern pike)	0.5 gm/kg. body weight. (intra-arterial)	300 mg %, recovery after 48 hours	Thorpe and Ince, 1974.
50 mg % (fasted)	25.2 mg % to 59 mg % (Dec. - Jan.)		

Table 2. Glucose tolerance test in teleosts and in man.

Species and normal blood or plasma glucose	Glucose load ⁽¹⁾ and route of administration and normal glucose range	Peak hyperglycemia developed and particularities of the glucose tolerance curve.	Authors
<u>Homo sapiens</u> (man, non-diabetics) 90 mg % (fasted)	Ingestion of 50 gm of glucose.	140 mg %, normal levels within 3 hours.	Guyton, 1971.
<u>Homo sapiens</u> (man, diabetics) 140 mg % (fasted)	Ingestion of 50 gm of glucose	200 mg %, near full recovery after 6 hours.	Guyton, 1971.

(1) The administration of a glucose load of 0.5 gm/kg. body weight to teleosts is comparable to the dosages used to determine the presence of diabetes mellitus in humans (Yokote, 1970 b).

sculpin, Cottus scorpius (Falkmer, 1961), and to the European carp, Cyprinus carpio (Yokote, 1970 d), caused necrotic B cell changes symptomatic of mammalian diabetes mellitus. Hence, it is believed that long term utilization of a high carbohydrate diet will result in acute B cell changes which eventually may be succeeded by a frank diabetic state.

Finally, the ability of teleost insulin to effectively regulate blood glucose appears sluggish at best. In fact, in teleosts, the hypoglycemic response to exogenous insulin is highly variable in spite of the fact that the serum insulin levels following fasting or force feeding in fish are similar to those reported in man (Table 3). More precisely, the shorthorn sculpin, Cottus scorpius, showed resistance to insulin (Falkmer, 1961) and although the degree of resistance was somewhat lower in the toadfish, Opsanus tau, insulin activity gave results similar to those found in humans in a state of decompensated diabetes, namely, high activity on rat adipose tissue and little on muscle (Cahill et al., 1964; Tashima and Cahill, 1968). Besides this apparent natural resistance to endogenous insulin, several studies have commented on the presence of other factors contributing towards inefficient glucose regulation in teleosts. Moule and Yip (1973) found that in the brown bullhead, Ictalurus nebulosus, proinsulin synthesis and its subsequent conversion to insulin were insensitive to glycemia whereas in mammals glycemia does induce the appropriate synthesis and release of insulin. The same authors quoted similar findings for the cod, Gadus callarias, and similar results were obtained in the

Table 3. Changes in circulating insulin levels in teleosts and in mammals following hyperphagia or the ingestion of a high carbohydrate meal.

Species	Fasted level (μ U/ml)	Response to force feeding or to a high carbohydrate meal	Authors
<u>Opsanus tau</u> (toadfish)	19±6 (serum)	61±11	Tashima and Cahill Jr., 1968.
<u>Carassius auratus</u> (goldfish)	36±9 (serum)	doubles	Patent and Foa, 1971.
<u>Mus norvegicus</u> (Wistar rat)	31 (plasma)	71.7	Montoya and Herrera, 1974.
<u>Homo sapiens</u> (man)	10.20 (blood)	150	Gorman, 1973

anglerfish, Lophius piscatorius (Trakatellis and Schwartz, 1970). Further, Ince and Thorpe (1977) demonstrated that unlike in mammals, dosages of glucose above 100 mg/kg. of body weight failed to stimulate an appropriate insulin secretion in the European eel, Anguilla anguilla. Last of all during the first portion of a prolonged fasting or migratory starvation, teleosts are believed to sustain a powerful and potent gluconeogenic activity enabling them not only to resist a fall in blood glucose (Fontaine and Hatey, 1953; Wendt and Ericson, 1972) but to actually increase their state of glycemia (Navasimhan et al., 1971; Butler, 1968) to the point where excessive glucose can act as an anti-insulinogenic agent on endogenous insulin release (Palayer, 1962; Thorpe and Ince, 1974; Ince and Thorpe, 1976). From the above it is concluded that due to the specific nature of teleostean carbohydrate metabolism, teleosts are not geared to handle an elevated carbohydrate intake.

The major criterion used as a basis for the diagnosis of diabetes is inappropriate hyperglycemia in the fasting condition. (Gorman, 1973; Krah1, 1974; Montoya and Herrera, 1974). The hyperglycemic syndrome of diabetes in turn implies, though certainly does not mean, concomitant low plasma insulin and high plasma glucagon (Montoya and Herrera, 1974; Topping and Targ, 1975; Alford et al., 1977). In short, metabolism reflecting a state of hypoinsulinemia and hyperglucagonemia entails the appearance of a series of biochemical disorders which together lend support to the

diagnosis of diabetes. A deficiency in circulating insulin results in a failure in glucose uptake by insulin-assisted tissues whereby a hyperglycemic state develops. There also ensues a failure of glycogenesis and subsequent impairment of the ability to store carbohydrate. Further, a failure in activity of lipoprotein lipase leads to a build up of plasma triacylglycerol levels and also effectively blocks lipogenesis. The absence of de novo synthesis of fatty acids along with a fall in amino acid insulin-assisted cellular uptake whereby protein synthesis decreases, result in emaciation of the body. On the other hand, an excess of vascular glucagon suppresses glycolysis and promotes gluconeogenesis and in both instances, these metabolic disorders contribute heavily towards a rising hyperglycemia. An oversecretion of glucagon also results in excessive lipolysis entailing mobilization of fatty acids from adipose tissue resulting in increases in levels of plasma triacylglycerol and eventual depletion of the body fat stores. The drastic mobilization of fatty acids soon leads to a build up of acetyl Co A and depressed activity of the tricarboxylic acid cycle further aggravates increases in hepatic levels of acetyl Co A. As a consequence of abnormally high levels of acetyl Co A, pyruvate is preferentially converted into oxaloacetate which becomes abundant and serves as substrate for gluconeogenesis. Also, the excess acetyl Co A may be metabolized into cholesterol and ketone bodies (Coll - Garcia et al., 1971). Eventually the ketones may accumulate in the blood giving rise to frank ketonemia accompanied by

ketonuria and keto-acidosis.

In addition to the metabolic disorders already described, numerous changes also occur at the level of morphology and histology of the pancreas. In humans, Warren and Le Compte (1952), Maclean et al. (1955), Le Compte (1960), Ogilvie (1964), Warren et al. (1966), and Gepts (1972) have contributed greatly towards the establishment of major histopathological quantitative changes in diabetics. The beta to alpha cell ratio of diabetics was found to be distinctively lower than in non-diabetics and indicated a relative increase in A cells due to some reduction in B cells. The relative proportion of B cells to A cells of severely affected islets may vary as much as to become the inverse of normality.

The qualitative changes noted to occur within the pancreas of diabetics are firmly established (Gepts, 1964; Ogilvie, 1964 and Okamoto et al., 1971) and have been amply reviewed (Warren and Le Compte, 1952; Warren et al., 1966; Volk and Lazarus, 1970 and Gepts, 1972).

The most common and most typical pancreatic lesion in diabetes mellitus, reported to be present in 41% of diabetic cases, was that of hyalinization of the pancreatic islets. Such affected islets showed deposition of the glycoprotein hyaline along the capillaries and into the intercellular spaces; whole islets may be afflicted. Islet neoformation was reported to occur in at least 32-34% of the cases and was consequently held to be an important change (Warren and Le Compte, 1952). However, the regeneration was not considered effective

since the B cell population gradually decreased with the progress of the disease despite islet neoformation. The third most striking insular change, occurring in 23% of cases, was that of fibrosis where the islet tissue appeared to be engulfed and invaded and eventually replaced by fibrous tissue scars. Next in importance came hypertrophy of islet tissue with an incidence of 8%. Hypertrophy may be visualized either in islets of normal shape where B cells and their hyperchromatic nuclei were enlarged or took place in irregular shaped islets where B cells formed long tortuous cords of columnar cells reflecting to a degree a reversion to a duct like epithelium. Fifth in importance were cellular hydropic changes or otherwise called atrophy of B cells, with a low incidence of 3%. This disorder, often observed in spontaneous diabetes of mammals, was better described as degranulation, vacuolization and glycogenization of B cells implying cellular breakdown due to overstimulation. A sixth change of equivalent importance, was the occurrence of pyknotic nuclei with shrunken cells and nuclei. On an individual basis it was acknowledged that there may be much variability in the incidence of the above-mentioned anomalies. In general it was found that 28-33% of the diabetic cases showed normality of islet tissue with respect to qualitative islet tissue changes.

Changes involving the pancreas as a whole were also an important aspect of the pathogenesis of diabetes mellitus. In 71% of the diabetic cases, fibrosis of exocrine pancreatic

tissue was prominent. This was often associated with fibrosis of the islets and was represented by the appearance of diffused proliferations of fibrous tissue within the exocrine tissue which eventually came to increase pancreatic lobulation and surround individual acini. The probable cause was believed to be one of arteriosclerosis entailing tissue ischemia with resultant pancreatic atrophy. Second in importance with a high incidence of 63% came lipomatosis. This fatty infiltration of exocrine tissue was often associated with fibrosis and atrophy of parenchymal exocrine tissue. A last common anomaly was that of arterio-arteriosclerosis with an incidence of 7%.

MATERIALS AND METHODS

1. Experimental animals.

A. Origin.

All Atlantic salmon (Salmo salar L.) used in this study were obtained from the Cobequid hatchery, Nova Scotia. They originated from parent wild stock salmon from Philip River, Nova Scotia. The fry were maintained on a pellet diet (Ewos salmon grower) at the hatchery until they had attained the parr stage.

B. Animals for characterization and topography of pancreatic tissue.

The pancreatic exocrine and endocrine tissue of the northern Atlantic salmon was investigated in a control group of parr procured directly from Cobequid hatchery. The fish were shipped by air in plastic bags containing cold fresh water and MS 222 anaesthetic (1:20,000) to the Department of Biology, University of Ottawa. The parr were held in insulated fiber glass tanks¹ provided with dechlorinated Ottawa tap-water. The water was continuously recirculated through a charcoal-oyster shell filter and a cooling unit which maintained the water at a temperature of $14 \pm 0.1^{\circ}\text{C}$. The fish were fed ad libitum three times daily using Ewos salmon grower. Accumulated feces were removed each day and every

¹ Frigid Units Incorporated (Living Stream), 3224 Sylvania Avenue, Toledo, Ohio, 43613

second day half of the water was changed to prevent accumulation of dissolved ammonia and to reduce the incidence of bacterial and fungal infections. Photoperiod was not rigorously controlled but approximated 12 hours of light alternating with 12 hours of darkness. The parr were adapted under these conditions for at least one month prior to use.

C. Animals from feeding experiment.

In December 1972, the Canadian Fisheries Research Board Biological Station situated in Saint Andrews, New Brunswick, received one thousand parr from Cobequid hatchery, Nova Scotia. At that time, the 19 month old parr had a mean weight of 30.7 gm. and a mean length of 14.2 cm. The parr wintered in fresh water holding tanks under laboratory conditions similar to those described previously.

In early spring, the fish were tagged using the salmon flag tag developed at the Biological Station (Saunders and Allen, 1967; Saunders, 1968; Jakobsson, 1970). A tag consisted of a small oblong piece of blueish-gray plastic bearing a code on one side and the name of the station on the other. The tag was secured to a nylon thread by means of a standard monofilament suture. The nylon thread was then secured at the mid point of another nylon thread again by means of a suture. The ends of this latter nylon thread served as tag ties. Two 20 gauge hypodermic needles were inserted through the dorsal muscles just below the dorsal fin of anaesthetized fish. The tag ties were passed into the bore-hole of the needles which were then removed. The nylon threads, which now transpierced the dorsal myotome,

were firmly tied.

On June 5, 1973, the fish which could now be classified as smolts, were divided into two groups and placed in Passamaquoddy Bay for sea water cage culture. At this time, the 25 month old smolts weighed approximately 80 gm. and were about 20 cm. long. One group (pellet fed) was supplied with a standard pellet food diet. The other group (herring fed) received fresh whole mashed Atlantic herring (Table 1) captured from Passamaquoddy Bay. The herring diet was supplemented with pellet food from July 27 until August 13 and from October 6 until October 16 to avoid the thiaminase problem of a pure herring diet (Saunders and Henderson, 1974). On October 17, crushed lobster shells were added to the herring diet. The lobster shell supplement was replaced by shrimp meal on October 23. The pellet fed group received pigmented pellet food from October 17. The lobster shell, shrimp meal and pigmented pellet food were used to induce a more natural flesh color.

On November 21, the cage culture experiment was concluded. The pellet fed smolts had a mean weight of 278.2 gm. and a mean length of 28.9 cm. The herring fed smolts in turn had grown to a mean weight of 321.7 gm. and a mean length of 29.9 cm. Both groups were brought into the laboratory at the station where they were placed in separate circular tanks containing aerated bay salt water flowing in a circular motion and each group was maintained on its own diet until December 5. After a fasting period of 2 days, the smolts of each group were sampled at random for the present study.

2. Experimental Protocol

Since most of the parameters considered throughout this study are subject to rapid changes (Chavin, 1973) all determinations and observations were from fasted fish. This procedure permits a proper assessment of an incidence of inappropriate hyperglycemia which is a prime factor characterizing and establishing the presence of a diabetic state.

Quantitative measures of plasma glucose (N=49), plasma triacylglycerol (N=48), plasma beta-hydroxybutyric acid (N=45), hepatic glycogen (N=47) and semi-quantitative measures of urine glucose, urine ketones and urine pH (N=30) were determined from the pellet fed smolts. As a rule glycemia in teleosts depends largely on the phylogenetic position and the mode of life of the species and hence one encounters wide interspecies differences in mean glycemic levels. Further, within species, the level of glycemia will be influenced by the particular state of activity and to the degree of sensitivity of the fish to external stimuli (Plisestskaya and Kuz'mina, 1971; Thorpe and Ince, 1974). In the light of this information and in the absence of a standard representative blood glucose level for salmon smolts of the size and shape encountered in this study, the glycemic levels of 50 herring fed smolts whose dietary carbohydrate component did not exceed 3% dry weight and which were maintained under identical experimental conditions as the pellet fed smolts, were determined in order to establish a normal plasma glucose range for these animals. Consequently, hyperglycemia was

defined in a clinical sense in relation to that glycemic range approximating a near normal distribution in the herring fed smolts. On this basis the pellet fed smolts were divided into two subgroups, namely, the normoglycemic pellet fed smolts and the hyperglycemic pellet fed smolts. These two subgroups were then compared in relation to the remainder of the parameters analyzed. This permitted a more accurate account of the true metabolic state of these fishes and made it possible to relate the latter to morphological and histological changes at the level of the pancreas. There being very little information available on the status of ketone bodies in teleosts and since the herring diet contained a high fat content (39.4% dry weight, Table 1), the plasma beta-hydroxybutyric acid levels of 47 herring fed smolts was determined in order to better appreciate and discuss the findings observed in the pellet fed smolts. Glucosuria, ketonuria and urine pH were determined in both the pellet fed smolts and the herring fed smolts to further explore the dissimilarities between these two groups (Table 4). Fork-length and weight growth data provided by the hatchery were analyzed in both the pellet fed and the herring fed groups of salmon smolts to denote any instances of abnormal growth.

Pancreatic exocrine and endocrine tissue were studied under light microscopy and electron microscopy in a control group of 12 parr. The pancreatic tissue of 7 normoglycemic and 6 hyperglycemic pellet fed smolts was

investigated in order to denote the presence of any histopathological changes that might have been incurred under the high carbohydrate feeding regimen. The following quantitative determinations were computed in both the parr and the pellet fed smolts: islet diameter size, beta to alpha cell ratio and the A cell to B cell percentage population as a measure of changes in the number of A cells and B cells per islet (table 5). Sections of pancreatic tissue were closely examined in order to depict the incidence of the following qualitative changes: hyalinization, fibrosis, islet neof ormation, hypertrophy, hydropic changes, pyknotic nuclei, lipomatosis and microangiopathy.

3. Analytical Procedures.

A. Collection of urine, plasma and liver.

a. Urine.

The pellet fed and herring fed smolts were stunned by a sharp blow on the head and a urine sample was obtained by pressing gently along each side of the abdomen of the fish. The urine samples were collected in 5 ml. disposable plastic beakers. Hema Combistix strips were used to test fresh urine samples for glucosuria and acidosis and Ketostix strips for ketonuria.

b. Plasma

Stunned fish were placed into a small "V" shaped trough. A blood sample was obtained by cardiac puncture using a 2 ml. disposable plastic syringe equipped with a

one inch 23 gauge hypodermic needle. The barrel and needle core were previously heparinized with ammonium heparin (Ammonium heparin, A.H. Thomas, Philadelphia). This technique consistently provided 1.5 ml. of blood. The blood sample was immediately centrifuged at 500 g for 10 minutes. The blood plasma was removed using Pasteur pipettes and placed in pre-labelled one dram vials which were kept on ice until frozen and stored at -20°C .

c. Liver.

Once the fish had been cut open along the mid ventral line, liver tissue pieces were excised and placed in pre-numbered disposable metallic weighing trays. They were frozen immediately in a dry ice-acetone bath. Sampling was completed within 3 minutes after initial handling of the fish in order to minimize glycogen depletion (Geddes and Rapson, 1973).

B. Analysis of plasma glucose.

Deproteinization of plasma was carried out according to an ultra-micro modification of the Somogyi (1930) 1/40 micro technique. The zinc filtrate thus obtained is free from enzyme inhibitors, and also from competitors such as non-fermentable reducing substances which are co-precipitated with the plasma protein yielding a filtrate which leads to the determination of true plasma glucose values.

The procedure used for the determination of plasma glucose was a micro-macro modification of the method reported in Sigma technical bulletin No. 510 (1972). Glucose was then

oxidized in the presence of oxygen and water by means of glucose oxidase to form gluconic acid and peroxide. The formed peroxide was then used to oxidize colorless 0- dianisidine by means of peroxidase to form coloured oxidized 0- dianisidine. The procedure was adapted for a 0.02 ml aliquot of deproteinized plasma. A reaction time of 30 minutes and an incubation temperature of 37°C were selected (Saifer et al., 1958). The reaction was stopped by using 7.8 N sulphuric acid to lower the pH below 0.5 units. This step eliminated any turbidity which may have formed during the reaction period and also stabilized the colorimetric readings for at least 12 hours and permitted some flexibility in the reading time (Saifer et al., 1958; Washko and Rice, 1961).

Unknown plasma samples were determined from a standard curve. Glucose standards were made from a 1,000 mg % stock glucose standard prepared from dry glucose. As this method is specific for beta-D-glucose, the stock standard was not used until 2 hours after preparation to ensure that mutarotation had reached equilibrium. The standards were made in a solution of 0.3% benzoic acid neutralized with Tris buffer to a pH of 7.3. Unbuffered benzoic acid has a pH of 3 which interferes with the reaction by giving lower readings (Washko and Rice, 1961). Standards of 40 mg %, 120 mg % and 300 mg % all peaked at 540 nanometers and consequently this wavelength setting was used to read all values. The procedure gave stoichiometric readings over a range of glucose standards from 25 mg % to 400 mg %.

Readings were done on a Junior Coleman II A spectrophotometer model 6/20 by blanking at 70% transmittance. Reproducibility was very satisfactory ($\pm 2\%$ transmittance) and observed variations were within the inherent error associated with the spectrophotometer. The colour reagent, 0-dianisidine, and the enzyme reagents were freshly prepared prior to each set of determinations and utilized in a combined form thus eliminating a pipetting stage. Harleco serum was used as control.

C. Analysis of plasma triacylglycerol.

Blood plasma triacylglycerol levels were measured by means of a Turner fluorometer model III using the method developed by Phillips (1969) for Turner Associates Incorporated¹. The technique consists of precipitating the proteins with isopropanol and treating the supernatant with a zeolite mixture to remove phospholipids, glucose, bilirubin and other interfering substances. The unknown plasma samples and triolein standards were saponified with alcoholic KOH to give glycerol. The glycerol was then oxidized with sodium metaperiodate in sulfuric acid to produce formaldehyde. The formaldehyde was condensed with acetylacetone and ammonia to yield the fluorescent 3,5-diacetyl-1,4-dihydrolutidine. Unsaponified plasma blanks were also run to account for incomplete removal of free glycerol and other interfering substances in the plasma.

¹ G.K. Turner Associate Incorporated, 2524 Pulgas Avenue, Palo Alto, California, 94303, U.S.A.

Blood plasma triacylglycerol levels were determined from a linear standard curve between 0 to 250 mg % of triolein. The fluorescence intensity of the reagent blank was subtracted from each standard to obtain the net fluorescence. In the case of unknown samples, the corresponding fluorescence intensity of the unknown serum blank was subtracted from it to obtain the net fluorescence. After zeroing the fluorometer, calibration with sensitivity set at 30X was performed so that the reagent blank read 5 divisions on the scale and the highest standard read 80 divisions. All readings were taken using Turner filter No. 110-812 (405) as primary filter and Turner filter No. 110-817 (8) as secondary filter. Samples exceeding 80 divisions in readings were diluted by a factor of two along with their plasma blank with deionized water and reread. This method of dealing with values higher than 250 mg % was valid for all samples below 500 mg %, higher concentrations had to be further diluted so as to fall on scale.

D. Analysis of plasma beta-hydroxybutyric acid.

The demonstration of an increase in plasma levels of a single ketone body is judged to imply increases in the plasma level of other ketone bodies (Page and Culver, 1962). Consequently, there being only enough plasma left to assay for one of the ketone bodies, it was decided to measure beta-hydroxybutyric acid plasma levels since it is a more stable form.

Deproteinization of plasma samples consisted in mixing an equal volume of ice cold 30% perchloric acid, waiting for 10 minutes, then centrifuging at 3,000 g. for

10 minutes. The supernatant was collected and its volume measured. Universal indicator in the amount of 0.0005 ml. was added and the protein-free supernatant was neutralized with 1M KOH - 3M K₂CO₃ in an ice water bath. The potassium perchlorate precipitate was removed by centrifuging at 3,000 g. for 10 minutes and the supernatant was used for the determinations.

Measurements were done using a Unicam model SP 1800 ultraviolet spectrophotometer. The method used was that of Williamson et al. (1962) modified for dealing with the slightly smaller sample size of 1 ml. The procedure involves oxidation of D-(-)-beta-hydroxybutyric acid to acetoacetic acid at pH 8.5 using Rhodopseudomonas spheroides D-(-)-beta-hydroxybutyric dehydrogenase procured from Sigma. The reaction was carried out in the presence of hydrazine which reacts with the acetoacetic acid to form hydrazone and thus allows the reaction to proceed quantitatively in the direction of acetoacetic acid. Under the above conditions, at least 98% of beta-hydroxybutyric acid is oxidized while diphosphopyridine nucleotide is reduced bringing about the stoichiometric formation of an equivalent amount of DPNH. The increase of absorbance at 340 nanometer due to the formation of DPNH is a measure of the reaction. In the modified version a two fold concentrated hydrazine hydrate was added to tris buffer before each set of determinations. This step eliminates an additional pipetting stage whereby 0.5 ml. of the combined solution was utilized per assay.

Thus, the cuvette contained a final volume of 1.625 ml. Determinations were obtained from a linear standard curve ranging from 0.1 mg. % to 20 mg. %. A reaction time of 50 minutes was employed for standard curve and unknown assays.

E. Analysis of liver glycogen

Glycogen was precipitated according to a modified version of Good et al. (1933). Alkaline hydrolysis in potassium hydroxide was prolonged in a water bath at 100°C until a homogeneous fluid was formed (Bartley and Dean, 1968). To ensure maximum extraction of glycogen, the modification consisted in precipitating the glycogen by adding 0.1 ml of 2% sodium sulfate for every 200 mg. of liver tissue followed by absolute alcohol containing 0.1% lithium chloride to make a final concentration of alcohol from 65 to 70%. This treatment permits the precipitation of that glycogen portion which is normally resistant to alkali hydrolysis by co-precipitating the glycogen along with the sulfate which mechanically brings down the glycogen (Osterberg, 1929-30). Because of the partial solubility of glycogen in alcohol, only one precipitation in 65 to 70% alcohol was done. By this procedure less than 2% of the glycogen is lost (Walaas and Walaas, 1950). The precipitate was washed once with 0.5 ml. of 65% alcohol in order to remove non-fermentable reducing substances (Walaas and Walaas, 1950). Acid hydrolysis was carried out at 100°C for 3 hours in a water bath with 0.4 ml. of 1N H₂SO₄ used for every 200 mg. of tissue. The hydrolysate was

neutralized with 1N NaOH using a drop of bromthymol blue as indicator. Glucose content was determined using the previously described glucose oxidase/peroxidase procedure. The calculation of glycogen content was obtained in the following manner:

$$\frac{(\text{absorbance of unknown sample})}{(\text{absorbance of glucose standard})} \times \frac{(\text{concentration of glucose standard})}{(\text{in mg. in amount of standard solution used})} \times \frac{(\text{dilution factor})}{(100)} \times \frac{(100)}{(\text{mg. of tissue in sample size})} \times (0.90) = \frac{\text{mg. glycogen per 100 mg. wet weight of liver tissue.}}{}$$

The value 0.90 is used to correct for incomplete hydrolysis and changes in molecular weight thus converting glucose to anhydrous glycogen (Roe and Dailey, 1966: Carroll et al., 1956).

F. Estimation of urine glucose, ketone and pH.

Urine glucose was measured using Hema Combistix reagent strips. The intensity of colour development varies from light to medium to dark and encompasses a range in glucosuria from 0.25 mg.% to 0.5 mg.%. Urine pH was also measured by means of this clinical reagent strip.

Urine ketones were estimated using Ketostix reagent strips. The colour intensity which develops varies from small to moderate to large indicating a range of ketonuria from 5 mg.% to 10 mg.% of acetoacetic acid. The test reagent strip is less sensitive to acetone and does not react

with beta-hydroxybutyric acid.

G. Growth patterns of pellet fed and herring fed smolts.

Growth data consisting of measurements of weights and lengths of the fish at the parr stage in fresh water prior to experimentation on cage culture and of the fish as smolts at various period of times during sea water cage culture was made available for utilization in this study by the authorities of Saint Andrews Biological Station. From the data, a figure showing the weight growth pattern and the length growth pattern over time was constructed for the pellet fed and the herring fed smolts.

4. Collection of Pancreatic Tissue and Histological Procedures.

A. Tissue collection.

The parr were killed by anaesthetic euthanasia in tertiary amyl alcohol, after which the anterior portion of the digestive tract was exposed by making a mid ventral incision from the region of the heart to the level of the descending intestine. The area under investigation extended from the transverse septum to the ascending intestine. For orientation purposes the area was divided arbitrarily into 5 distinct exploratory sites lettered from A to E and encompassing various sections of the gut track where pancreatic tissue was likely to be found (Fig. 8) After reflecting the stomach posteriorly, 2 to 3 or more tissue

pieces were excised from each of the different exploratory sites and placed in small numbered petri dishes, containing appropriate fixative. The tissue pieces were trimmed down to approximately 4 mm cubes for light microscopy and 1 mm cubes for electron microscopy and washed in fixative and then immediately placed in pre-labelled 1 dram vials containing fresh fixative. An identical sampling procedure was observed for pellet fed smolts from cage culture in sea water.

B. Fixation, embedding, sectioning and staining.

a. Light microscopy.

Pancreatic tissue pieces were fixed in either Helly's fluid or Bouin's fluid without acetic acid for 24 hours (Lillie, 1965). Helly fixed tissue was washed in running tap water for 12 hours and subsequently dehydrated to 70% ethanol. Bouin fixed tissue was treated with 50% alcohol for 6 hours and then with 70% alcohol until most of the picric acid had been extracted. All of the tissue pieces were then routinely dehydrated. The major portion of the tissue pieces were embedded in paraplast-plus via xylene. A few tissue pieces were embedded in epon 812 via propylene oxide (Luft, 1961). Paraplast-plus embedded tissue blocks were sectioned between 4-6 microns on a Spencer microtome model 820 using razor blades inserted into a special razor blade holder. Epon-embedded tissue was sectioned between 0.5-2 microns using a Sorval Porter-Blum ultra-microtome model MT-1 and glass knives. Albumen fixative was used to affix the tissue sections to microscopic slides.

Paraplast-embedded tissue sections were stained by one of the following procedures: a modified Gomori's chrome alum hematoxylin phloxine (Gomori, 1941; Bell, 1946) where phloxine counterstain is replaced by a mixture of xylidine de Ponceau (Ponceau 2R, C.I. No. 16150) and acid fuchsin (C.I. No 42685) followed by the application of yellowish light green S.F. (C.I. NO 42095) for granular differentiation of the various cell types within the islets of Langerhans (Bencosme, 1952; Lazarus, 1958); a modified aldehyde fuchsin by Gomori (1950) followed by Ehrlich's hematoxylin and counterstained with Masson's trichrome stain as modified by Lazarus (1958), also used for granular distinction of islet cell types; permanganate-iron alum-phosphotungstic acid hematoxylin for critical staining of A-cell granules (Levene, 1964); and phloxine-azure- hematoxylin for general cytoplasmic islet cell differentiation (Maldonado and San José, 1967).

Epoxy-embedded tissue sections fixed for light microscopy were pre-treated according to Munger's specifications (1961) which enabled the application of aldehyde thionine or aldehyde fuchsin followed by Ehrlich's hematoxylin and Masson's trichrome stain as modified by Lazarus (1958). Epoxy-embedded tissue sections fixed for electron microscopy were stained directly with methylene blue-azure II-basic fuchsin (Humphrey and Pittman, 1974) for general cellular differentiation, needed for orientation purposes while thin sectioning.

b. Electron microscopy.

Tissue collected for electron microscopy was fixed for 2 hours in 5% glutaraldehyde made in 0.1 M phosphate buffer (pH 7.4). Excess fixative was then removed by washing twice in phosphate buffer. The glutaraldehyde-fixed tissue was transferred to a 2% osmium tetroxide solution for 2 hours after which it was washed in buffer and dehydrated through a graded series of ethanol. The tissue was embedded in epon 812 via propylene oxide according to Luft (1961). Thin sections for electron microscopy as well as 0.5-2 micron sections for thin sectioning orientation were obtained using a Sorval Porter-Blum ultra-microtome model MT-1 and glass knives.

Electron microscopic sections mounted on grids were contrasted by staining for 15 minutes in 5% uranyl acetate made in 50% ethanol (Watson, 1958) then in a combination of lead citrate and sodium citrate in alkaline milieu for 2 minutes (Reynold, 1963).

C. Examination of sections.

All of the paraplast-embedded tissue blocks representing all of the five exploratory sites from each of the 12 parr, from 7 normoglycemic pellet fed smolts and from 6 hyperglycemic pellet fed smolts were entirely sectioned, mounted in sequence, stained and examined. Response to various staining procedures was studied and the procedure producing the best cellular differentiation was adopted in staining the majority of the sections. With the aid of an ocular micrometer the size of

all encountered islets of Langerhans on one section per block for only 4 parr but for the 13 smolts, was calculated by measuring both the wide and narrow planes of the islets and taking the average, from which a size frequency curve was constructed. Using the same islets the mean beta to alpha cell ratio was determined for each fish and the mean A and B cell population per islet for each fish was calculated. The differential cell counts were performed using a hand tally. Counts involving a difference greater than 5% were redone. Quantitative measurements excluded immature islets which were predominantly populated by B cells and clear cells. Both parr and smolt insular tissue and exocrine pancreatic tissue were closely scrutinized to denote incidences of qualitative changes.

Most of the epoxy embedded tissue blocks were sectioned to points of high interest where serial sections were produced for examination under light and electron microscopy. Light microscopic classification of islet A and B cell types was confirmed through granular distinction of cell types on serial sections under an A.E.I. model EM 6B electron microscope. Ultrastructural characteristics aiding in distinguishing these cell types were reported.

D. Photographic techniques.

Selected sections from light microscopic studies were photographed using a Carl Zeiss photomicrographic camera model C-35 and an Ikophot-M light exposure meter. Black and white photographs were taken on Ilford Roll Pan

F 135-20 films. Coloured pictures were obtained using Kodak Ektachrome type B 135-20 films. Electronographs were taken on standard Kodak electron image plates KP 635 19.

5. Statistical Procedures.

For the purpose of this study a probability of $P < 0.05$ was taken as significant. All sample distributions were analyzed using "Goodness of fit for continuous distribution" test to find out whether or not a normal distribution was approximated (Steel and Torrie, 1960). Distributions not being normal, the nonparametric Wall-Wolfowitz runs test was used to establish the existence of any difference between the two differently fed groups of smolts for any one particular parameter (Siegel, 1956). The nonparametric median chi square test was used to find out if the values of one group (i.e. pellet fed, pellet fed and hyperglycemic, pellet fed and normoglycemic, herring fed) were greater than those of another group for any one particular parameter (Siegel, 1956).

RESULTS

1. Metabolic Parameters.

A. Glycemia.

The plasma glucose distribution amongst the pellet fed smolts was significantly different from that of the herring fed smolts (Wald-Wolfowitz runs test, $p < 0.040$). That is, the distribution of plasma glucose levels in the herring fed group approximated normality whereas in the pellet fed group (Fig. 1) the distribution was bimodal. For this reason the nonparametric median chi square test was used to determine that the mean plasma glucose level in the pellet fed smolts ($169 \pm 11 \text{ mg.}\%$ ¹; $N=49$) was significantly ($p < 0.0005$) higher than that of the herring fed smolts ($106 \pm 5 \text{ mg.}\%$; $N=50$). Within the pellet fed group the first peak displayed a plateau of elevated glucose levels and the distribution was extensively skewed to the right. The second peak was represented by a few very high plasma glucose levels. Based upon the plasma glucose distribution of the herring fed smolts and the first clear peak in the pellet fed group (Fig. 1) a state of hyperglycemia is defined here as those glucose values which exceed $150 \text{ mg.}\%$. On this basis, 53% of the pellet fed smolts and 10% of the herring fed smolts were judged to be hyperglycemic (Fig. 2).

B. Triacylglycerol.

The mean plasma triacylglycerol level for the

¹ $\bar{X} \pm S\bar{x}$, for all reported values.

Figure 1. Distribution of plasma glucose in
Atlantic salmon smolts fed either a commercial pellet food
or a low carbohydrate whole mashed herring diet for 6
months.

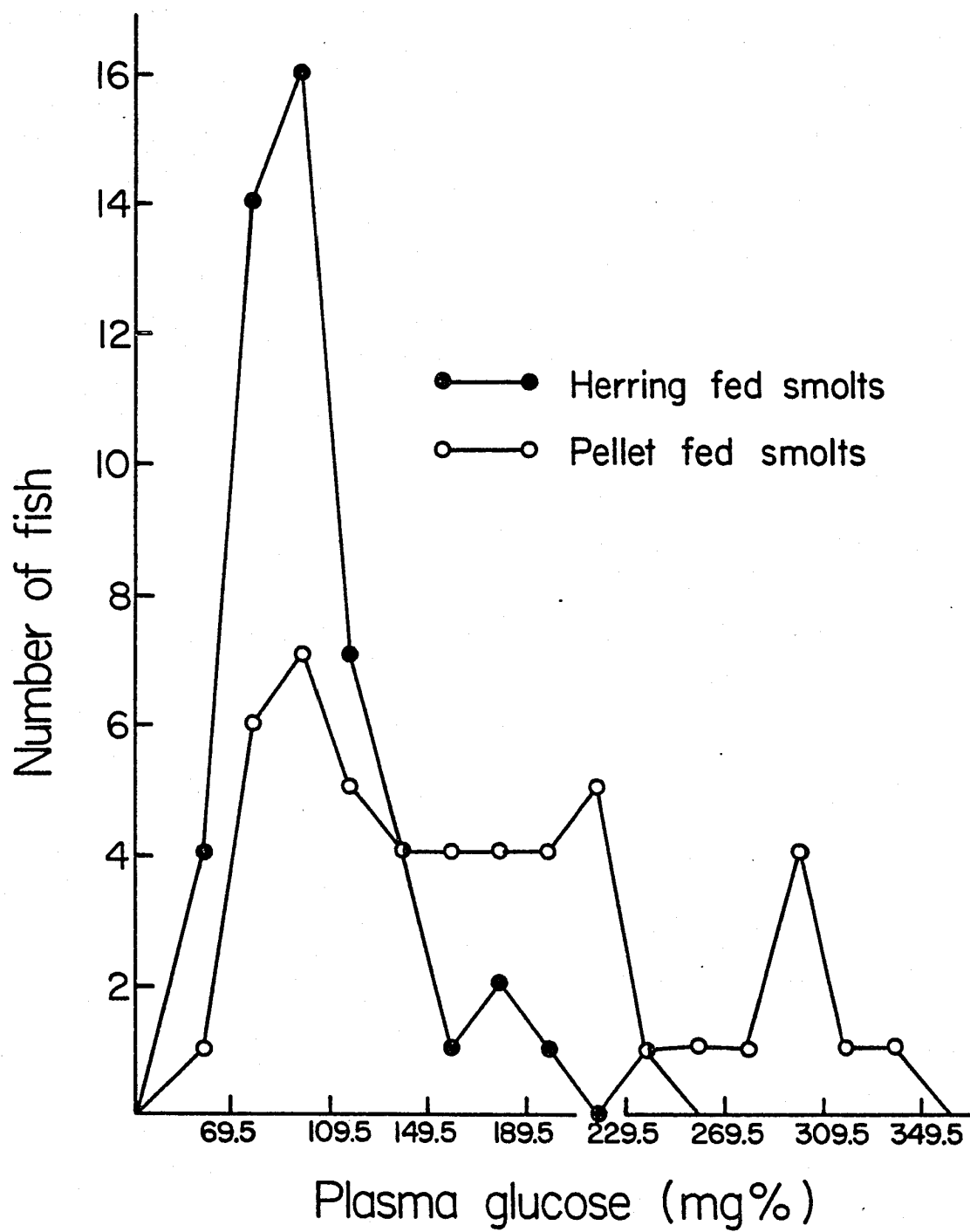
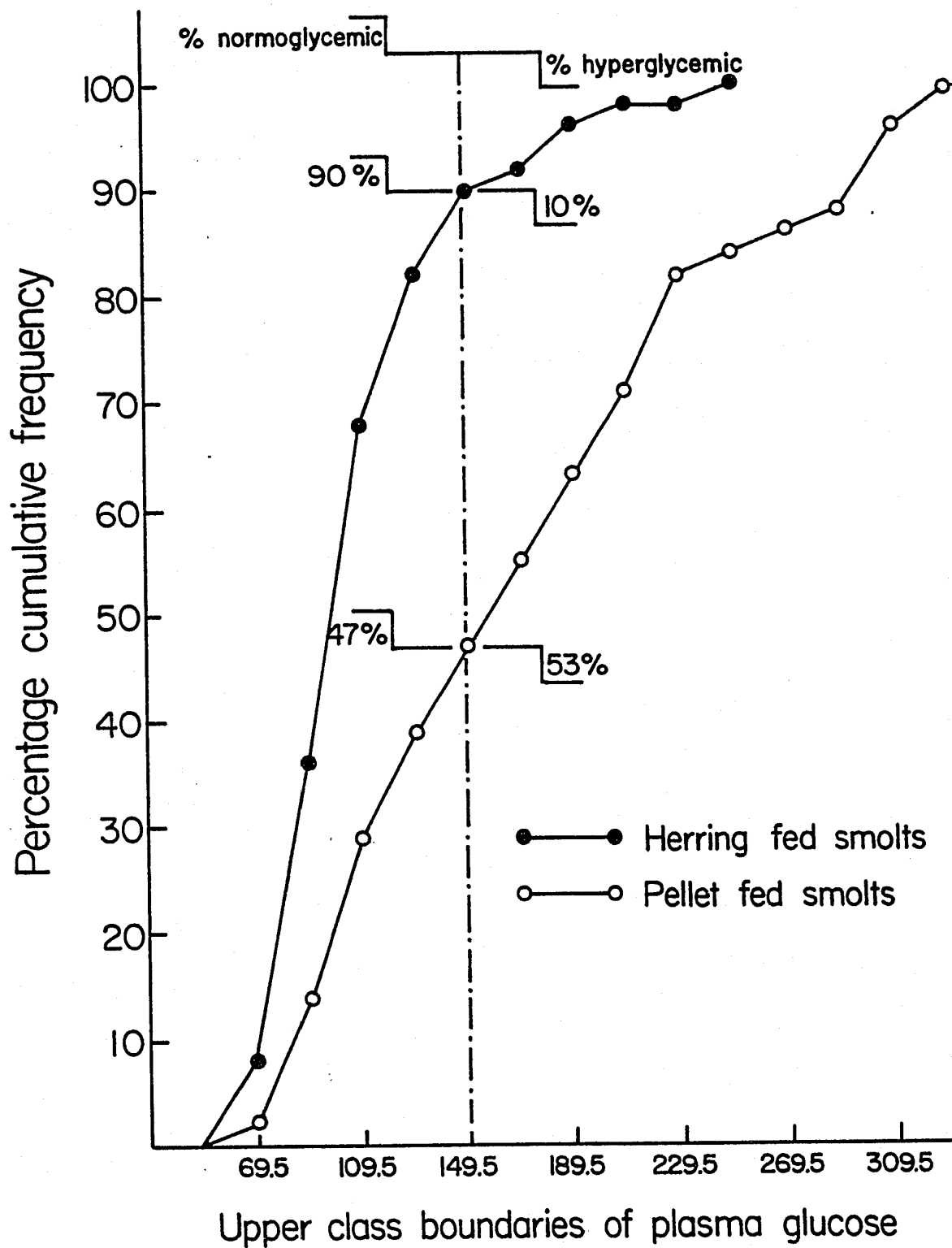


Figure 2. Ogives of plasma glucose levels for pellet fed and herring fed Atlantic salmon smolts to indicate the incidence of hyperglycemia. The data was taken from Fig. 1.



pellet fed group was 243 ± 14 mg.% (N=48). The plasma triacylglycerol distribution was not normal (Fig. 3) and when the triacylglycerol levels of the pellet fed smolts were divided into two groups, normoglycemic pellet fed fish and hyperglycemic pellet fed fish, it was found that the mean plasma triacylglycerol of the normoglycemic pellet fed smolts (183 ± 13 mg.%; N=22) was lower than that of the hyperglycemic fish (295 ± 19 mg.%; N=26). This difference was significant at the 0.005 level (median chi square test). Both distributions were bimodal (Fig. 3). Whereas all of the first peaks were approximately below 250 mg.% of plasma triacylglycerol, all of the second peaks were above. A percentage tally based on the modal distribution showed that 77% of the normoglycemic salmon had plasma triacylglycerol levels below 250 mg.%. This picture was reversed in the hyperglycemic salmon as 65% of the fish had plasma triacylglycerol levels above 250 mg.%.

C. Beta-hydroxybutyric acid.

The mean plasma beta-hydroxybutyric acid level of the pellet fed group (0.37 ± 0.04 mg.%; N=45) was significantly ($P < 0.005$) higher than that of the herring fed smolts (0.26 ± 0.04 mg.%; N=46) but the mean level of this metabolite was higher in the normoglycemic pellet fed group (0.47 ± 0.07 mg.%; N=20) than in the hyperglycemic pellet fed group (0.29 ± 0.05 mg.%; N=25). This latter difference was also significant ($P < 0.025$). Further, it can be seen in Fig. 4 that while the distribution of beta-hydroxybutyric acid is similar in the herring fed and hyperglycemic pellet fed

Figure 3. Plasma triacylglycerol levels of pellet fed fish divided into hyperglycemic and normoglycemic groups.

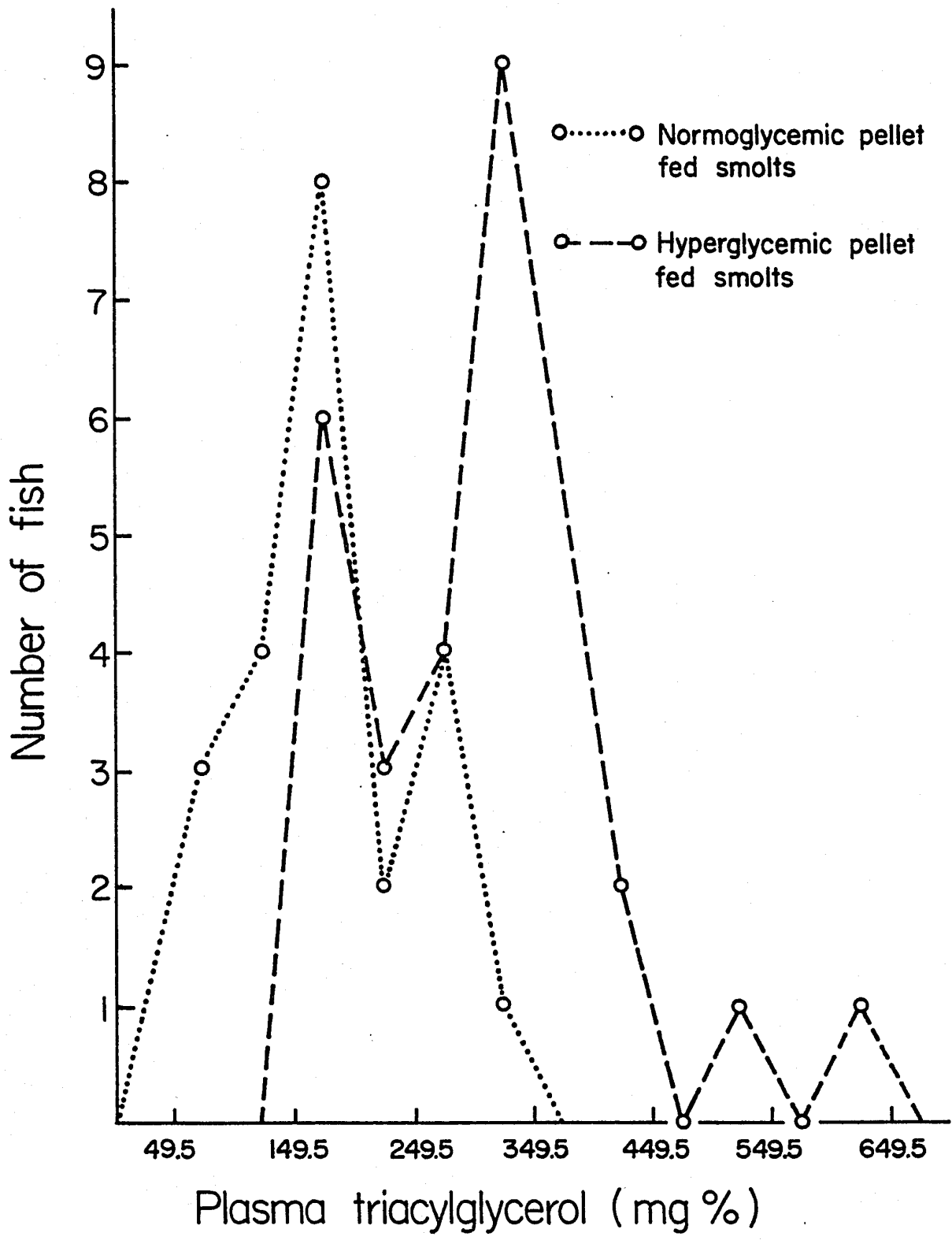
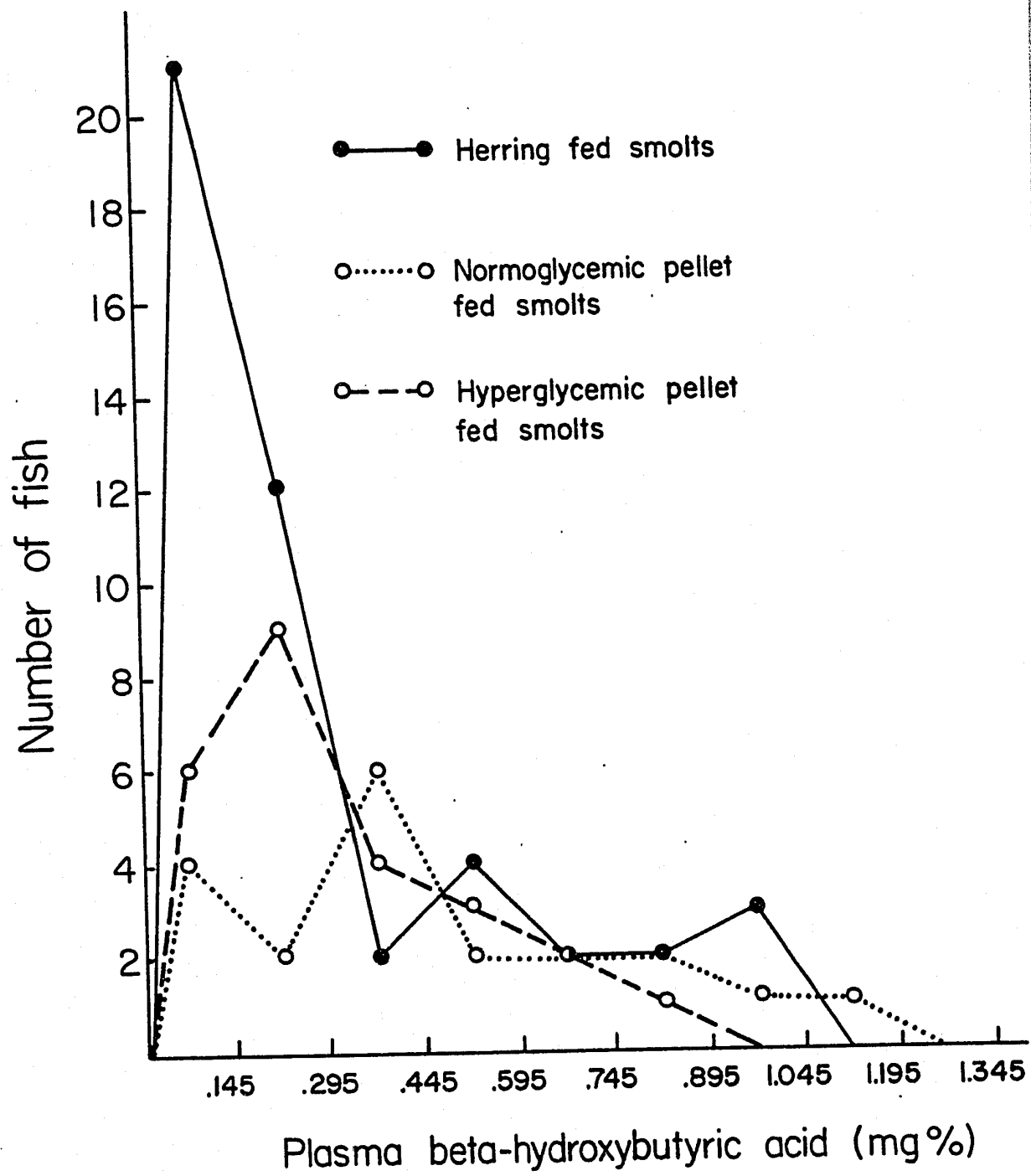


Figure 4. Plasma beta-hydroxybutyric acid of herring fed, hyperglycemic pellet fed and normoglycemic pellet fed Atlantic salmon after two days of fasting.



groups that of the normoglycemic pellet fed group is quite different. That is, a much greater proportion of the pellet fed normoglycemic fish had high plasma beta-hydroxybutyric acid.

D. Hepatic glycogen content.

The mean liver glycogen for the pellet fed fish was 1.12 ± 0.06 mg./100 mg. wet weight of liver (N=47). The liver glycogen distribution was again not normal. The mean liver glycogen of the normoglycemic pellet fed smolts (1.21 ± 0.12 mg./100 mg. wet weight of liver; N=22) was not significantly different than that of the hyperglycemic pellet fed smolts (1.12 ± 0.08 mg./100 mg. wet weight of liver; N=25) and their distributions were nearly identical (Fig. 5).

E. Urinalysis.

The results of the analysis of the urine collected from 30 pellet fed salmon and 11 herring fed salmon are summarized in Table 4. Whereas the fasted herring fed smolts never showed glucosuria, three of the pellet fed group did. Ketone bodies were not detected in the urine of the fasted herring fed smolts and the urine pH was found to be 8.5. On the other hand 40% of the fasted pellet fed smolts did have at least low levels of ketonuria accompanied by urinary acidosis, pH 6.6. But the incidences of glucosuria, ketonuria and urine acidosis were alike in the normoglycemic and the hyperglycemic pellet fed Atlantic salmon smolts.

Figure 5. Liver glycogen content of hyperglycemic and normoglycemic pellet fed Atlantic salmon smolts fasted for two days and maintained in bay salt water at 5°C.

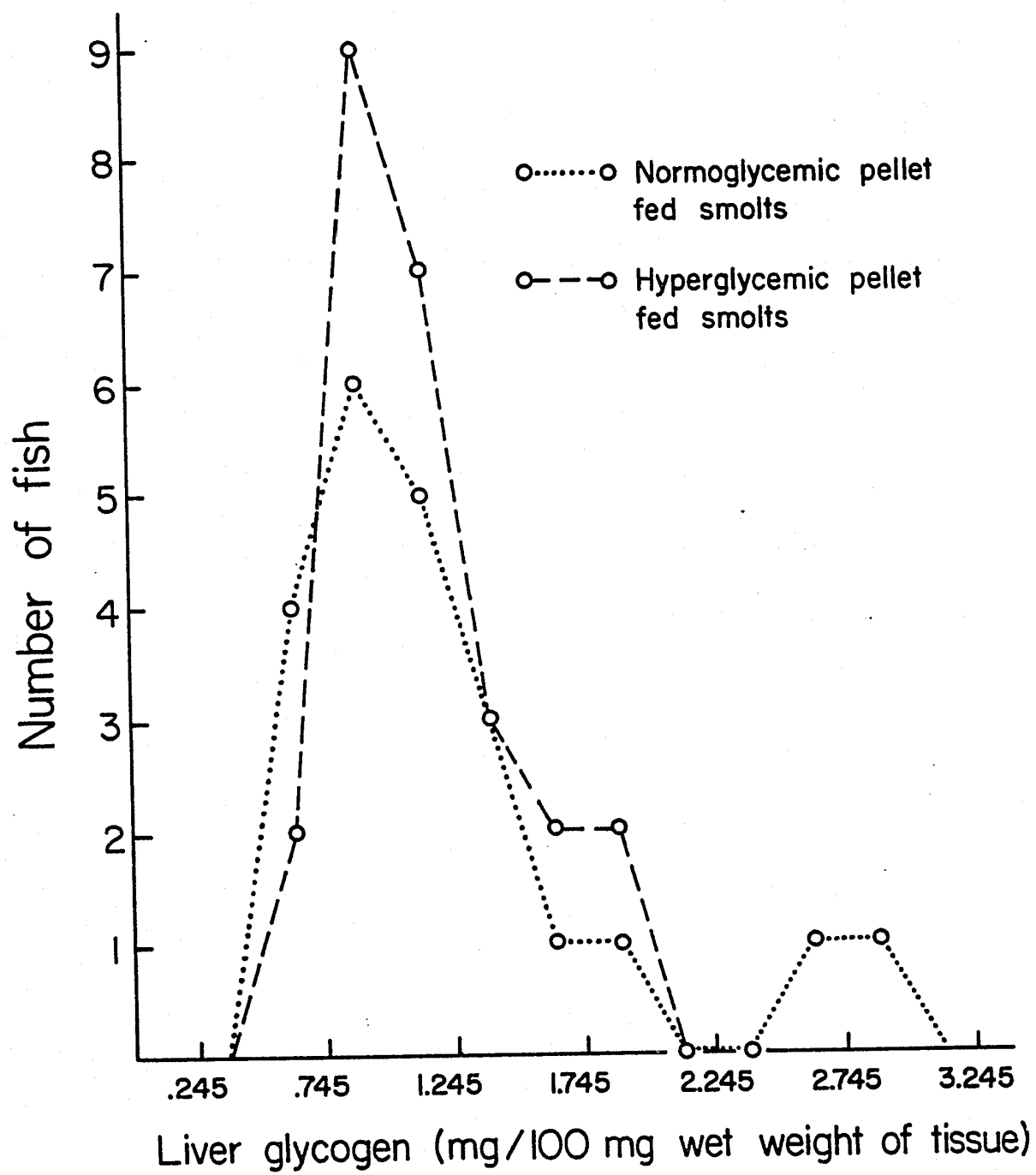


Table 4.

Urine analysis for glucose and pH using Hema Combistix reagent strips and for ketone bodies using Ketostix reagent strips in Atlantic salmon smolts fed two different diets for 6 months then fasted for 2 days.

Groups Parameters	Herring fed N=11	Combined pellet fed N=30	Normoglycemic pellet fed N=15	Hyperglycemic pellet fed N=15
Glucosuria				
Negative	11	27	14	13
Light	0	2	1	1
Medium	0	1	0	1
Dark	0	0	0	0
% positive	0%	10%	7%	14%
Ketonuria				
Negative	11	18	9	9
Small	0	12	6	6
Moderate	0	0	0	0
Large	0	0	0	0
% positive	0%	40%	40%	40%
pH				
Acidic (6.6)	0	12	6	6
Alkaline (8.5)	11	18	9	9

F. Growth Data.

The mean weight and length of the pellet fed smolts were 298 ± 11 gm. and 29.9 ± 0.3 cm. respectively and did not differ significantly from those of the herring fed smolts, 336 ± 16 gm. and 30.1 ± 0.6 cm. The mean weight of the normoglycemic pellet fed smolts (294 ± 16 ; $N=21$) was only slightly lower than that of the hyperglycemic pellet fed smolts (303 ± 17 ; $N=26$). Their mean lengths were also similar (30.1 ± 0.5 c. and 30.2 ± 0.5 cm. respectively). Hence, at the end of the cage culture experiment all groups were of approximately the same length (Fig. 6). However, over the course of the cage culture experiment, the pellet fed group did not grow as fast in weight as the herring fed group thereby resulting in a 38 gm. difference in weight between the means of these two groups (Fig. 7).

2. Pancreatic Tissue Parameters.

A. Exocrine

a. Compact and diffuse nature of acinar tissue.

The exocrine pancreas of Atlantic salmon was of the mixed type, that is, composed of two distinct forms of distribution of tissue, namely pancreas compactum and pancreas diffusum. The compact tissue consisted of either narrow elongate solid masses of tissue distributed along the hepatic artery and the portal vein or more bulbous masses which completely surrounded various structures such as the pancreatic duct and the cystic duct, portions of the common bile duct, the extrahepatic duct and even partially

Figure 6. Lengths of Atlantic salmon groups used during this investigation. Throughout the course of the cage culture experiment, the herring fed smolts are compared to the pellet fed smolts, whereas at the end, the pellet fed group is divided into normoglycemic pellet fed fish and hyperglycemic pellet fed fish. All measurements were done on 24 hour fasted fish.

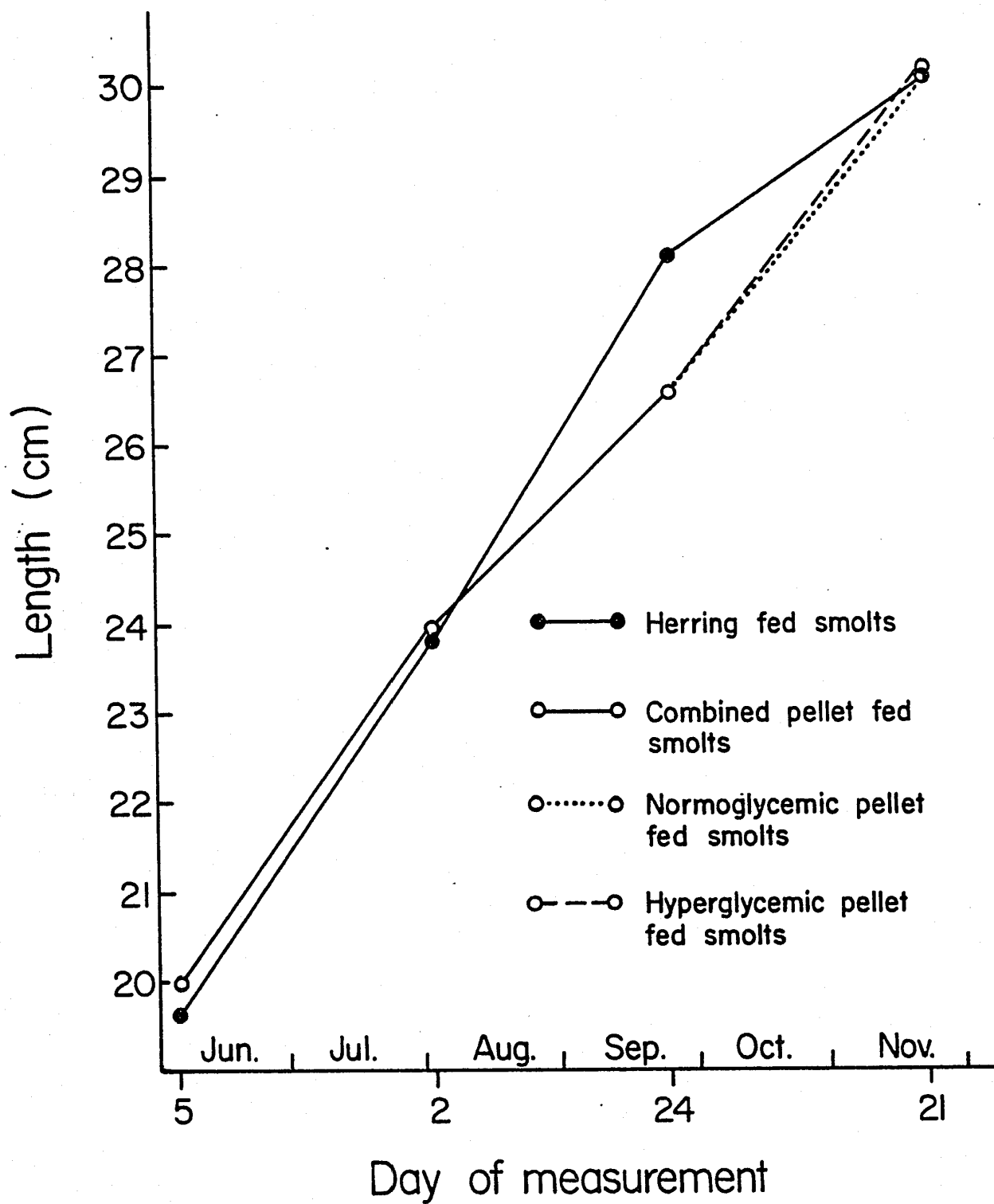
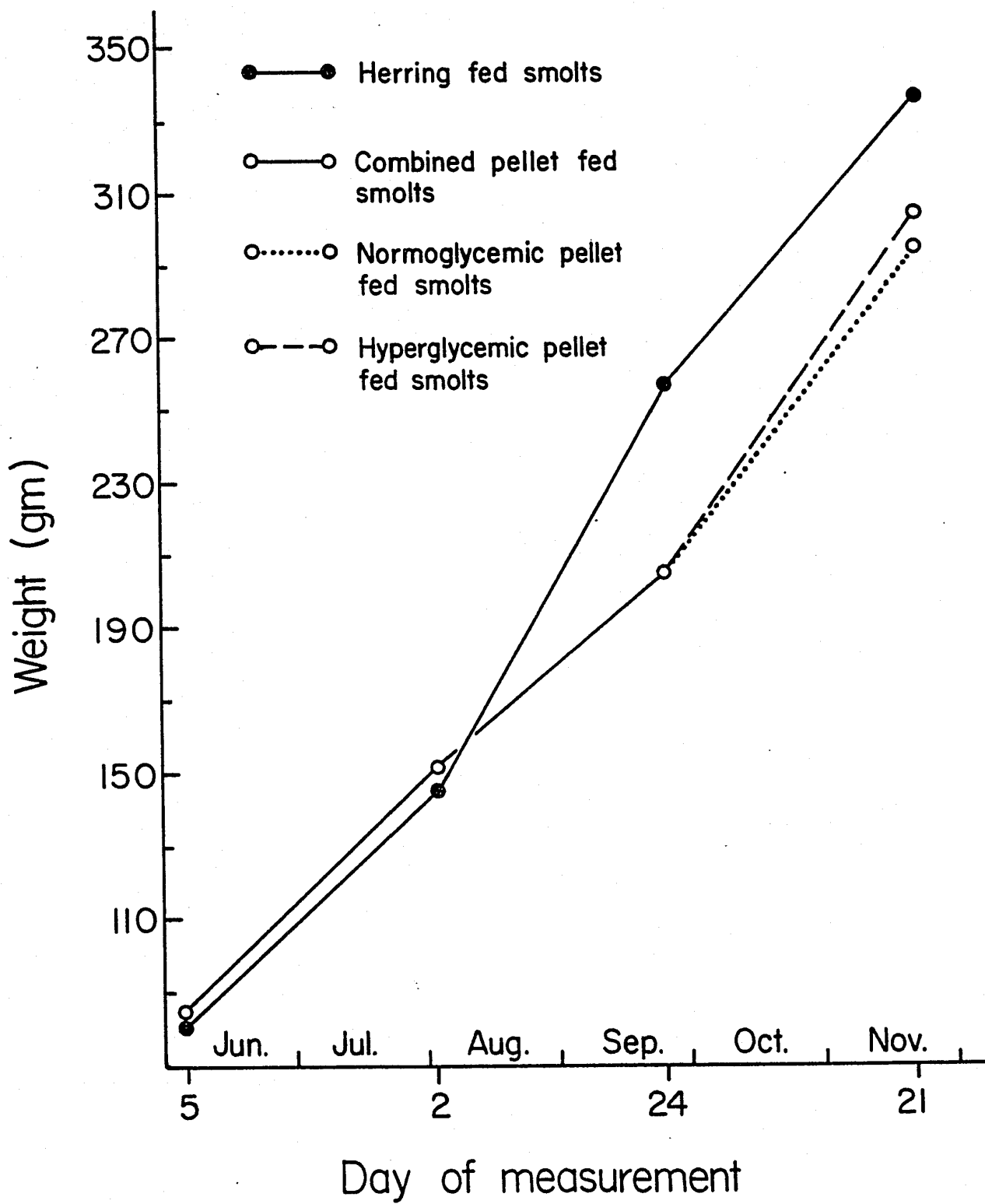


Figure 7. Weightsof Atlantic salmon groups used during this investigation. Throughout the course of the cage culture experiment, the herring fed smolts are compared to the pellet fed smolts, whereas at the end, the pellet fed group is divided into normoglycemic pellet fed fish and hyperglycemic pellet fed fish. All measurements were done on 24 hour fasted fish.



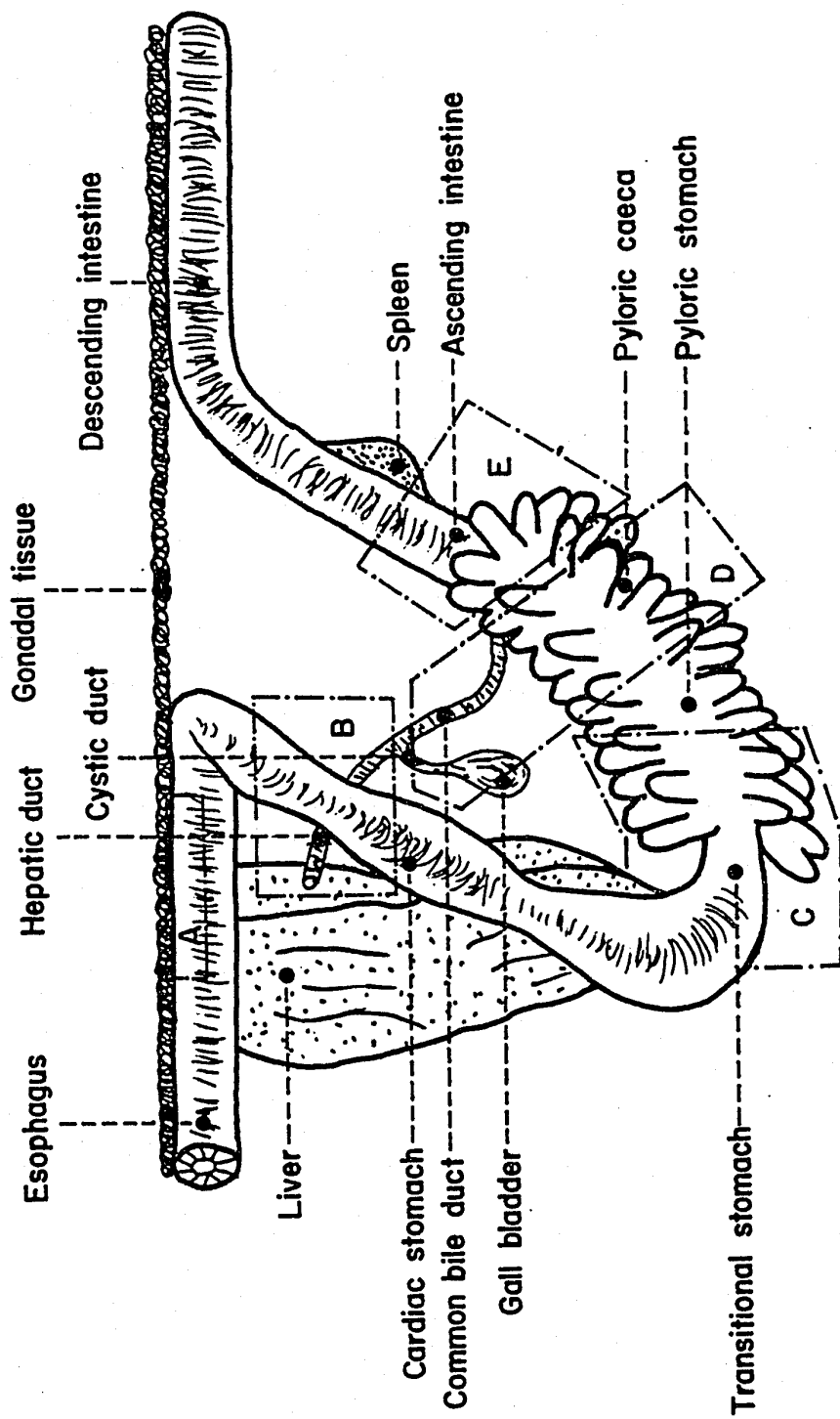
surrounded the intrahepatic duct (Fig. 8); within the liver, the compact tissue around the intrahepatic duct was embedded right in the outer connective tissue layer of the duct. Often large clusters of adipose tissue represented by signet cells, boarded the outskirts of the compact zones of tissue and occupied the space between the focal points of high density areas of compact tissue. Now and then the acinar cells were highly interspersed with numerous adipose cells. The diffuse tissue consisted of elongated strands of tissue where the exocrine component was reduced to a few layers of acinar cells lying over a dense central area of adipose tissue. This form of distribution of exocrine tissue was encountered around the esophagus and alongside the gonads and the spleen but was most evident between the pyloric caecae (Fig. 8).

The typical acinar cells (Fig. 17 and 21) converged towards a lumen and had numerous well defined zymogen granules in their apical portion. The cytoplasm took up hematoxylin readily and the granules were both aldehyde fuchsin and acid fuchsin positive depending on the staining procedure followed.

b. Main pancreatic ductular system and
anastomosing ductular system.

A large pancreatic duct was found in the vicinity of the junction between the pyloric stomach and the ascending intestine. It received two large tributaries as it extended towards the digestive tract. In turn, the

Figure 8. Schematic diagram showing the exploratory sites for the investigation of pancreatic tissue in the Atlantic salmon, Salmo salar.



tributaries branched into series of smaller ducts distributed within the substance of the compact tissue and the diffuse tissue (Fig. 13).

Aside from the above described main pancreatic ductular system, numerous small ductules composed of low to flat cuboidal epithelial cells branched off from the main pancreatic ductular system and anastomosed into one another. Occasionally these ductules contained endocrine cells, particularly B cells at their outer boundary and often the ductules were continuous with nests of islet cells or buds of islet tissue wherein B cells and clear cells could be identified (Fig. 13 and 19). At times the ductules traversed a peripheral region of immature islets of Langerhans predominantly composed of B and clear cells. Frequently, small islets of Langerhans were found distributed along the course of a ductule or were contiguous to ductules. These arrangements of ductules and endocrine tissue were taken as evidence of islet neoformation from ductule epithelial cells.

c. Innervation.

The exocrine and the endocrine pancreas contained numerous nervous tissue elements. Longitudinal sections of segments of the larger pancreatic ducts exposed a thick connective tissue layer which frequently harboured large solitary nerve cells (Fig. 13 and 23). Only rarely were small groups of nerve cells observed. On one occasion a large intrapancreatic ganglion was seen (Fig. 25). Within the islets of Langerhans numerous unmyelinated nerve

fibers coursed between the endocrine cells. Two distinct types of nerve terminals in the form of large boutons, were seen either between endothelial cells and endocrine cells or abutting onto an endocrine cell (Fig. 9 and 10). Innervated islet tissue cells were partially or heavily degranulated suggesting a secretory role for this nervous tissue.

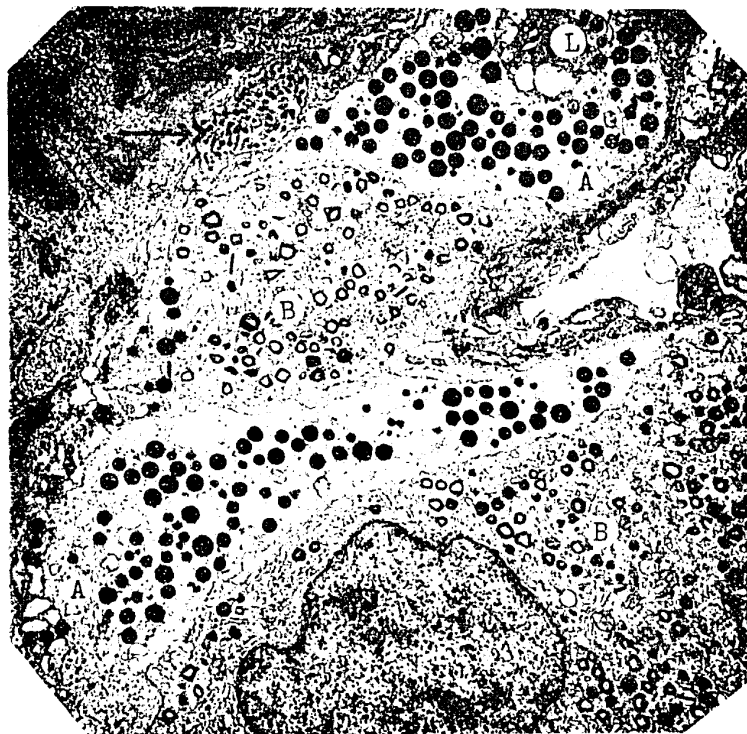
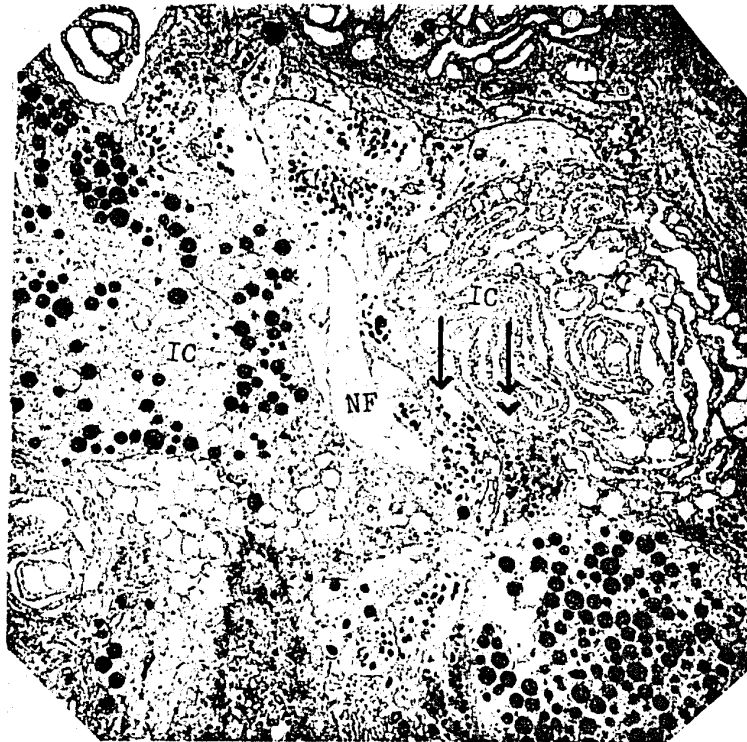
B. Endocrine.

a. Islet tissue size, shape and distribution.

The endocrine tissue of Salmo salar consisted of intrapancreatic islets of Langerhans of variable size and shape. Aside from immature islet forms which have been previously described and very small spherical islets which are always located adjacent to anastomosing ductules, larger spherical islets were usually found in close proximity to branches of the tributaries of the pancreatic duct which on occasion penetrated the inner peripheral region of these islets. The largest islet forms, found exclusively in the compact exocrine tissue, were not associated with pancreatic ducts. They were either irregular in shape and solitary or consisted of large aggregations of islet tissue where several large islets were linked together by means of islet tissue bridges. The highest density as well as diversity of islets of Langerhans was located within the compact exocrine tissue surrounding the main pancreatic duct and lining the hepatic artery and the portal vein. The diffuse tissue of the pyloric caecal region and the neighbouring adjoining regions

Figure 9. Electronograph illustrating several nerve fibers (NF) and numerous nerve terminals of two distinctive types, one (arrow) more electron lucent than the other (double-headed arrow), containing an abundance of synaptic secretory vesicles and abutting on partially or totally degranulated islet cells (IC). Double fixation, glutaraldehyde and osmium tetroxide, then stained in uranyl acetate followed by a combination of lead and sodium nitrate. X 7,574.

Figure 10. Electronograph showing marked characteristic differences between (A) cell and (B) cell secretory granules. Alpha secretory vesicles contain mostly cores of high electron opacity whereas beta secretory vesicles display a preponderance of polymorphous crystalline cores with empty centers. Note the presence of a more electron opaque type of nerve terminal (double-headed arrow) abutting on the top most A cell which contains a lysosome-like (L) organelle. Double fixation, glutaraldehyde and osmium tetroxide, then stained in uranyl acetate followed by a combination of lead and sodium nitrate. X 7,574.



of the transitional stomach and ascending intestine contained many smaller islets (Fig. 8). Around the esophagus and near to the gonads, little islet tissue was found.

- b. Islet cell types, staining properties, cellular configuration and prominent features.

The application of classical staining techniques to sections for light microscopy revealed the presence of only 3 cell types; i.e. A or alpha, B or beta and C or clear cells (Fig. 14). The A cells were characterized by granules which were selectively stained by phosphotungstic acid hematoxylin or xyloidine de Ponceau or acid fuchsin after treatment with the mordant iron alum. These cells occupied mainly the peripheral region of the islets. A few of these cells possessed long cytoplasmic processes projecting towards capillaries. Under the electron microscope the alpha secretory granules which measured from 210-480 m μ , consisted of a central spherical core of high electron density and a surrounding halo either translucent or of medium electron density, and were enclosed by a limiting membrane of wavy outline (Fig. 10). The B cells were aldehyde fuchsin, aldehyde thionin and faintly Gomori chrome alum hematoxylin positive. They were characteristically located around capillaries and formed anastomosing groups which ran tortuously within a more central region of the islets to form a network throughout the islets (Fig. 13).
Light microscopic identification of B cells

was confirmed on serial sections at the electron microscopic level where beta secretory granules, measuring from 210-510 m μ , were highly polymorphic and contained an abundance of crystalloid cores forming such geometric figures as bars, triangles, tetrahedrons and pentagons (Fig. 10). Clear cells took on a faint rosy background stain with aldehyde fuchsin or were strongly positive to Gomori's chrome alum hematoxylin (Fig. 14 and 15). They were found everywhere between the A and B cell groups throughout the islet and penetrated the peripheral A cell layer to a considerable extent.

c. Percentage cell population and beta to alpha cell ratio.

The average percentage cell population per islet for the parr was 24% for A cells, 48% for B cells and 28% for clear cells (N=66 islets). The mean beta to alpha cell ratio in the parr was 2.12; a total of 104 islets of 4 fish were considered.

3. Effect of Diet on Pancreatic Tissue Parameters.

A. Quantitative changes.

a. Diameter of islets.

The mean diameter of islets of Langerhans in 7 normoglycemic pellet fed smolts, 147.2 ± 9.7 (N=105), did not differ significantly from that in 6 hyperglycemic pellet fed smolts, 151.0 ± 11.3 (N=115); the mean diameter size of both the normoglycemic and hyperglycemic smolts was, however, greater than that found in 4 parr, 93.8 ± 5.2 (N=123). The

islet diameter distributions of the normoglycemic smolts, the hyperglycemic smolts and the parr were comparatively similar to one another (Fig. 11) with the exception that in parr all islets were below 320 μ in diameter.

- b. The average number of A cells and B cells per islet, the relative A cell and B cell percentage distribution per islet and the beta to alpha cell ratio.

In comparison to one another, the hyperglycemic salmon had 13% fewer B cells per islet and 35% more A cells per islet than did the normoglycemic salmon. Further, out of the 115 islets investigated in hyperglycemic fish, 23% of these showed a superior A cell population; this situation was never encountered amongst the normoglycemic fish where islet B cell population was always the highest.

The relative percentage of A cells and B cells per islet in the parr was respectively 35% to 65% which was not considered different than in the normoglycemic pellet fed smolts, 37% to 63%. Both, however, differed greatly from the hyperglycemic pellet fed smolts which showed 48% for A cells and 52% for B cells (Table 5).

The mean beta to alpha cell ratio of the normoglycemic pellet fed smolts (2.18) did not differ significantly from that of the parr (2.12). However, the mean beta to alpha cell ratio of the hyperglycemic pellet fed smolts (1.57) was 18% lower than that of the normoglycemic smolts and this difference was significant at the $P < 0.005$ level (median chi square test).

Figure 11. Distribution of islet diameter size in Atlantic salmon parr and in normoglycemic and hyperglycemic smolts fed a commercial pellet food for 6 months.

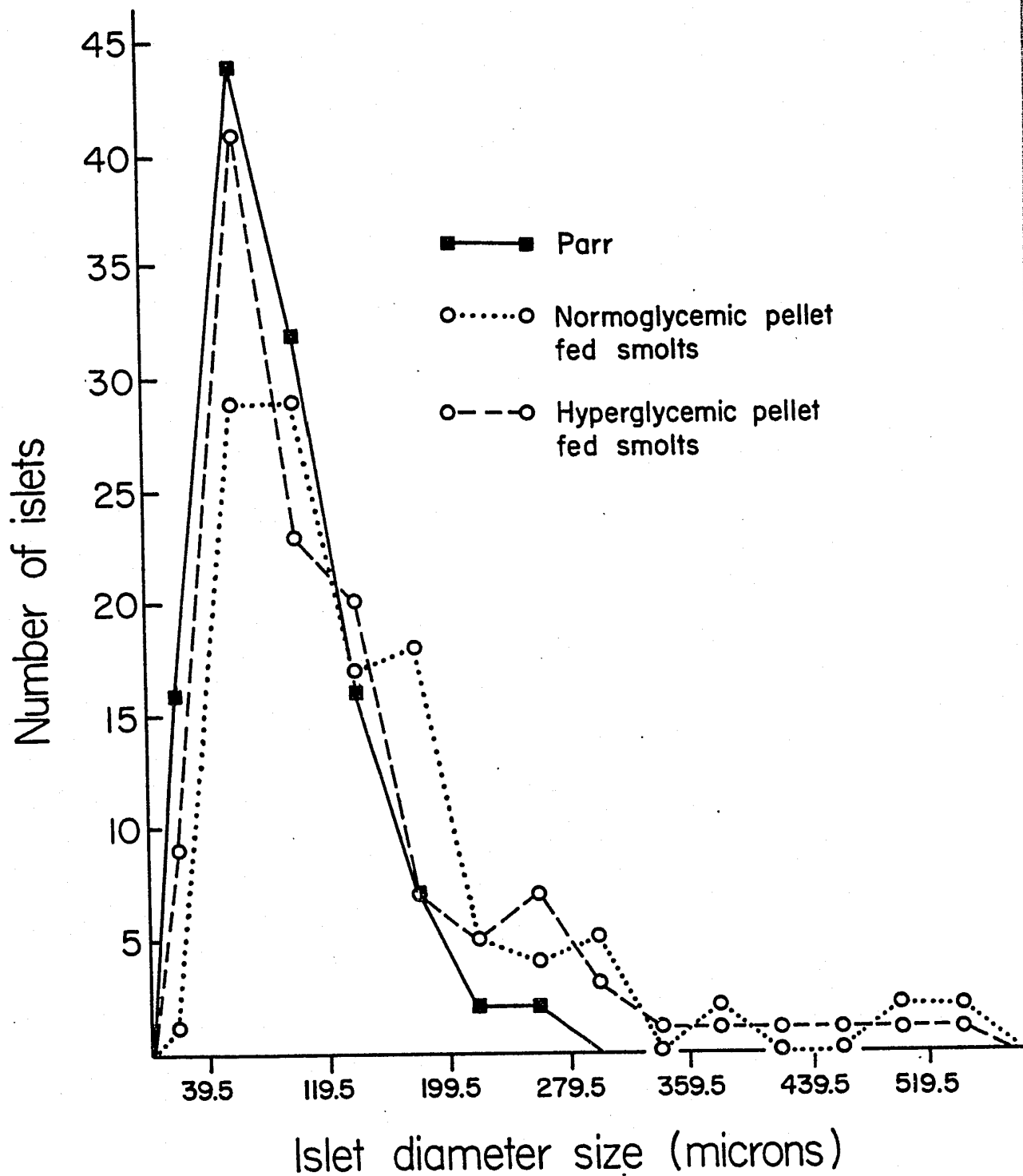


Table 5.

Summary of quantitative islet tissue measurements for Atlantic salmon as parr in fresh water and as smolts under cage culture in bay salt water while fed a commercial pellet food diet

Group	Parr N=4	Normoglycemic smolts N=7	Hyperglycemic smolts N=6
Parameters			
Number of islets and cells counted			
islets	104	105	115
A cells	2,253	5,131	7,567
B cells	4,278	8,713	8,295
Average number of cells per islets			
A cells	22	49	66
B cells	41	83	72
Relative percentage A cell and B cell population per islet			
A cells	35%	37%	48%
B cells	65%	63%	52%
Mean beta to alpha cell ratio ^a			
	2.12	2.18	1.57

a) Calculated from individual islets.

B. Qualitative changes.

a. Normoglycemic pellet fed smolts.

The histological examination of the pancreatic tissue in 7 normoglycemic smolts revealed that in two cases where plasma triacylglycerol were low, 98 and 128 mg.%, the pancreatic tissue appeared normal and very much like the control parr (Fig. 12, 13 and 14). In the remaining 5 normoglycemic cases where plasma triacylglycerol levels were above 244 mg.%, the most important and readily noticeable change afflicting a few of the islets was that of hyperplasia and hypertrophy (Fig. 15 and 16). In these islets the B cells were larger, contained larger nuclei and were more numerous than in normal islets. On occasion cords of B cells were observed. Due to an increase in number of B cells, the A cells of these islets looked compressed and deformed as if crowded by the B cells (Fig. 15). Hyperplasia and hypertrophy was observed to be more pronounced and of more frequent occurrence in the smolt exhibiting the highest plasma triacylglycerol level of 344 mg.%. In 3 cases there was an infrequent and moderate hyalinization of islet tissue where collagenous like material was seen lining the capillaries. The exocrine pancreatic tissue appeared normal in the normoglycemic pellet fed smolts (Fig. 21) showing histopathological islet tissue changes when compared to the control parr.

b. Hyperglycemic pellet fed smolts.

The endocrine tissue of hyperglycemic pellet fed smolts showed several pathological signs. The most prominent

Figure 12. Normal islet tissue of normoglycemic pellet fed Atlantic salmon smolts. The B cells of light grey cytoplasm form more central insular cell groups (open circles) which are surrounded by the more peripheral A cells containing red granules (arrows). The clear cells have a dark grey cytoplasm (arrow heads) and are intermingled with the A cells. Helly's fluid. Gomori's chrome alum hematoxylin counterstained with ponceau-acid fuchsin and light green. X 300.

Figure 13. Normal pancreatic tissue of normoglycemic pellet fed Atlantic salmon smolts. The B cells containing granulation are distinctively stained by aldehyde fuchsin and form more central groups of cells anastomosing with each other to form seemingly a network throughout the islet. The cytoplasm of the A cells contains red granules whereas the agranular cytoplasm of the clear cells takes on a faint background stain of aldehyde fuchsin. In the upper right quadrant note the longitudinal sections of pancreatic ducts exposing the connective tissue layer within which large nerve cells (thin arrow) are embedded. In the lower left portion, cross sections of branches of pancreatic duct tributaries (large pentagon) are seen to further branch and give rise to a ductule (small pentagon) from which emanates a bud of islet tissue (thick arrow) suggestive of islet neoformation. Helly's fixative. Aldehyde fuchsin followed by Ehrlich's hematoxylin then counterstained with ponceau-orange G and light green. X 300.

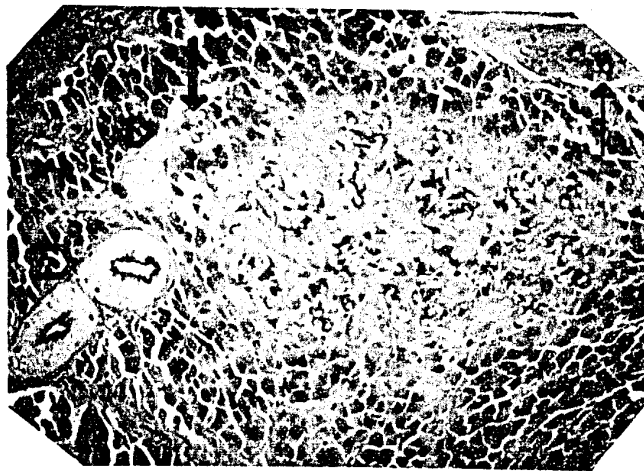
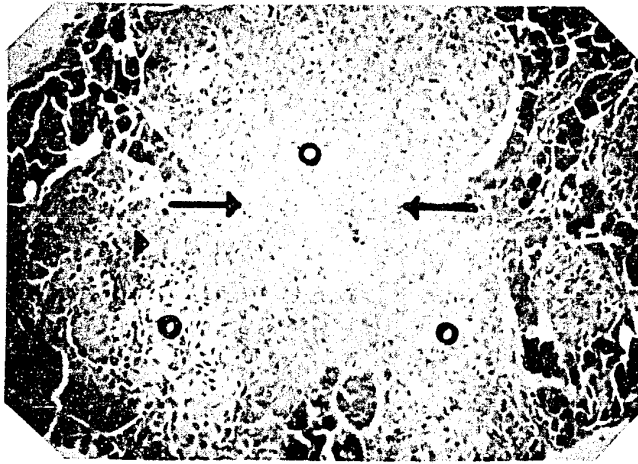


Figure 14. Enlargement of a portion of figure 12 showing clearly the three cell types. The (B) cells with light grey cytoplasm form groups which surround the capillaries. The more solitary (A) cells possess coarse red granules and are intermingled with dark grey staining clear cells (C) around the B cell groups. The red blood cells (arrows) are vividly stained reddish-orange. Helly's fluid. Gomori's chrome alum hematoxylin counterstained with ponceau-acid fuchsin and light green. X 1,200.

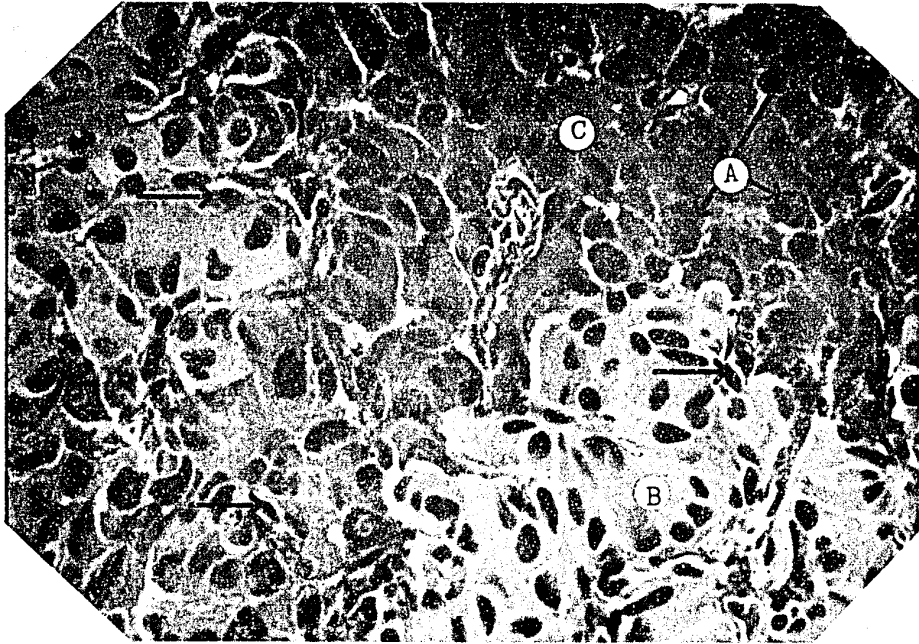
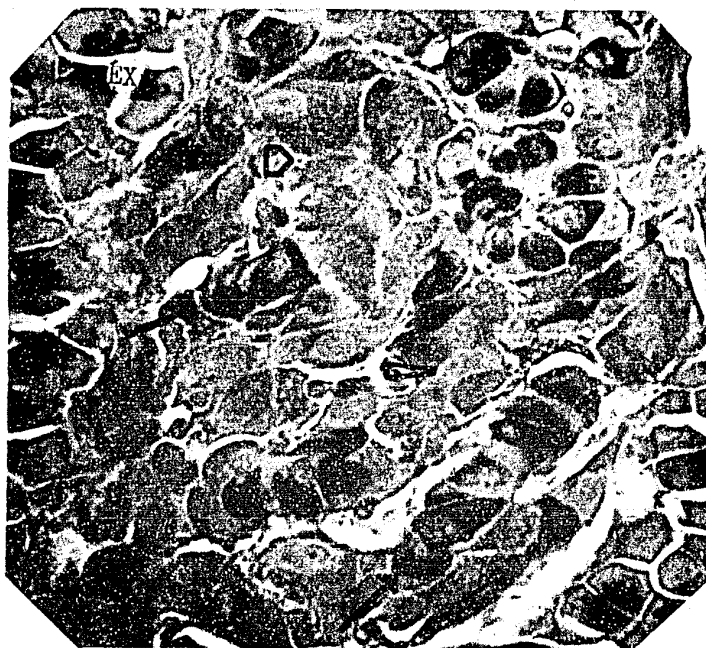


Figure 15. Hyperplasia of B cells in normoglycemic pellet fed Atlantic salmon smolts. The islet exhibits an overwhelming majority of B cells where in a few cases large nuclei and a greater abundance of heavily granulated cytoplasm (horizontal arrows) are evident features. Coincidentally B cell atrophy is also visible where there is a reduction in the size of the nucleus and scanty granulated cytoplasm (vertical arrows) which is suggestive of overexertion and possibly leads to cellular exhaustion. In general A cells appear compressed and deformed as if crowded (pentagons) by the increasing B cell population. The clear cells (spear head) are much fewer in number and retain a faint background stain of aldehyde fuchsin. A portion of the exocrine pancreas (EX) is also shown. Helly's fluid. Aldehyde fuchsin followed by Ehrlich's hematoxylin then counterstained in ponceau-orange G and light green. X 1,200.

Figure 16. Hypertrophy of B cells in normoglycemic pellet fed Atlantic salmon smolts. The B cells form cords of cells (arrows) and are less numerous whereas the A cell population (pentagon) is greater and the clear cell population (spear head) barely apparent. Red blood cells (arrow-head) are vividly stained yellowish-orange. Portions of the exocrine pancreas (EX) are also shown. Helly's fluid. Aldehyde fuchsin followed by Ehrlich's hematoxylin then counterstained in ponceau-orange G and light green. X 1,200.



change seen in all cases and affecting a great number of islets was a definite alteration of the internal cellular configuration of the islets of Langerhans (Fig. 17). The B cells were reduced in number and group formation was no longer obvious. The A cells were more numerous and were found to be evenly distributed with clear cells at the periphery of the islets and frequently they penetrated the B cell areas. The clear cells no longer formed intermediate zones of endocrine cells between the B cell groups and the more peripheral A cells. Aside from being intermingled with A cells, they appeared to be present in greater number and invaded the B cell groups to a far greater extent. As a result of this increasing A cell population together with a decreasing B cell, the A to B cell percentage distribution of many of the islets of Langerhans was reversed from about 38% for B cells to 62% for A cells (Fig. 17) to that seen in normoglycemic fish (Fig. 13). On many occasions B cells showed vacuolization and degranulation suggestive of hydropic changes, cellular degeneration and exhaustion (Fig. 18). In general neoplasia of islet tissue was more apparent in the hyperglycemic pellet fed smolts where there was a remarkable increase in the number of very small immature duct associated islets of Langerhans (Fig. 19). In all cases a few of the islets were affected by a more pronounced hyalinization than that seen in the normoglycemic smolts (Fig. 24). Occasionally islets severely damaged by hyalinization were seen where cellular differentiation could no longer be established and where the vast majority of the nuclei were pyknotic (Fig. 24). In only

Figure 17. Alteration of the internal cellular configuration of the islets of Langerhans in hyperglycemic pellet fed smolts. The B cells are drastically reduced in number and no longer form independent groups as seen in Figures 12, 13 & 14; they may be even located in part within the periphery of the islets (arrow). There is a noticeably remarkable increase in the A cell population whereby A cells become interspersed among B cells (arrow head) and appear more evenly intermingled with clear cells. Clear cells appear greater in number and they also have invaded the B cell regions. In this particular hyperglycemic fish the beta to alpha cell ratio was only 0.89 (EX = exocrine pancreas). Helly's fluid. Aldehyde fuchsin followed by Ehrlich's hematoxylin then counterstained with ponceau-orange G and light green. X 300.

Figure 18. Hydropic changes of the islet tissue in hyperglycemic pellet fed smolts. Cell contours are no longer discernible and the granulated cytoplasmic areas of B cells are studded with numerous small vacuoles (arrow) depicting cellular degeneration and exhaustion. Reddish cytoplasmic areas of A cells are poorly distinguished (pentagon). Red blood cells (spear head) are vividly stained yellowish-orange. Note globular aggregation of fat (arrow head) bordering the periphery of the islet. In this fish a plasma glucose level of 191 mg.% and a plasma triacylglycerol level of 307 mg.% was found. Helly's fluid. Aldehyde fuchsin followed by Ehrlich's hematoxylin then counterstained with ponceau-orange G and light green. X 1,200.

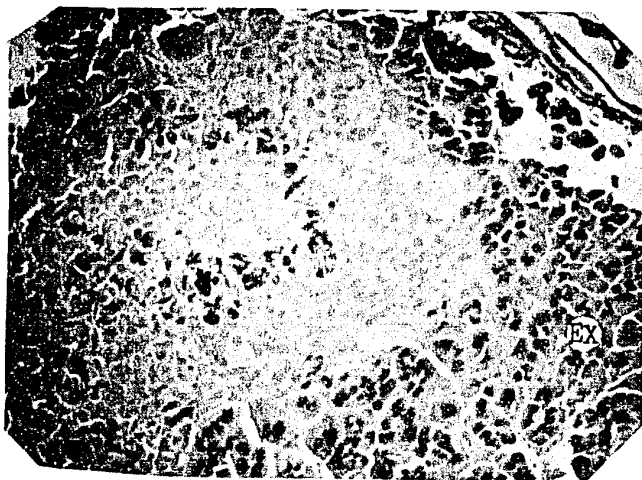
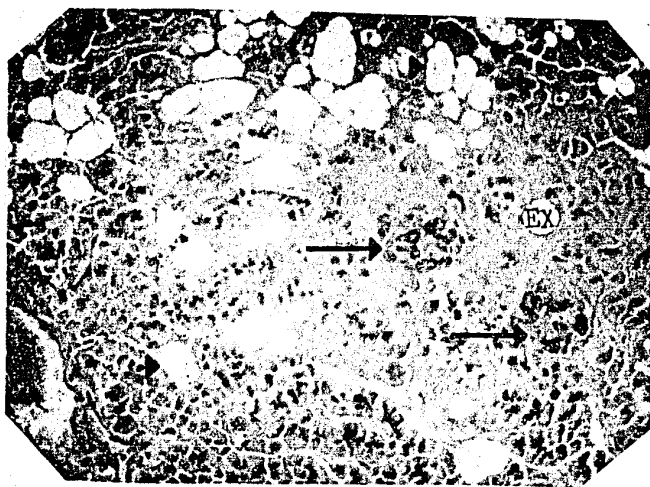
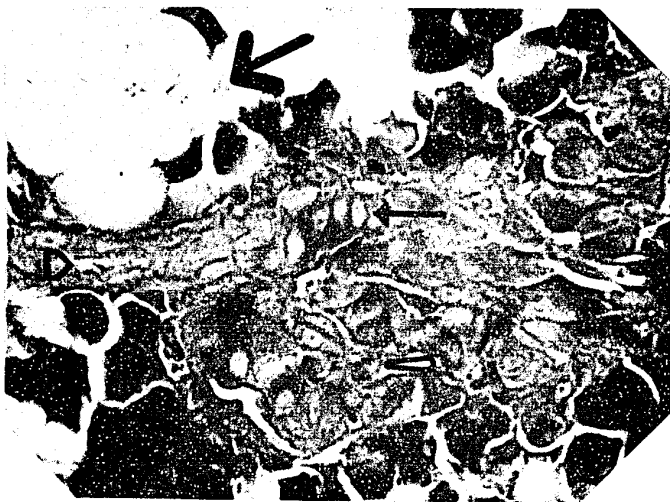


Figure 19. Elevated incidence of islet tissue regeneration in hyperglycemic pellet fed smolts. A small ductule (pentagon) belonging to an anastomosing ductular system, contains a cord of B cell elements (small arrow) at its outer periphery and is contiguous to a nest of islet tissue (spear head) mostly represented by B cells. Note the presence of a large lipomatotic inclusion (large arrow). Helly's fluid. Aldehyde fuchsin followed by Ehrlich's hematoxylin then counterstained with ponceau-orange G and light green. X 1,200.

Figure 20. Lipomatosis of compact pancreatic exocrine tissue in hyperglycemic pellet fed smolts. Fat droplets invade the acinar tissue and coalesce to form huge irregular inclusions of fats (arrow heads) which is associated with atrophy of the acinar tissue. Note the distinctively low zymogen granule content of the exocrine parenchyma cells as compared to that observed in Figures 17 & 21. Two small islets of Langerhans (arrows) are found within the exocrine tissue (EX). Helly's fluid. Aldehyde fuchsin followed by Ehrlich's hematoxylin then counterstained with ponceau-orange G and light green. X 300.



two cases and at that in only a very few islets, B cell hyperplasia and hypertrophy was evident as described in the normoglycemic group.

The exocrine pancreas of the hyperglycemic pellet fed smolts displayed various evident histopathological changes. Certain areas of exocrine tissue were afflicted by hyalinization where strands of collagenous like tissue lined the smaller blood vessels and tended to partition the exocrine tissue (Fig. 23). Hyalinization of exocrine tissue frequently paralleled hyalinization of islet tissue (Fig. 23). Often collagenous like tissue contained a vast amount of elastic fibers indicative of fibrosis and on rare occasions entire portions of exocrine tissue were degenerated and replaced by fibrous tissue scars wherein acinar cells were no longer distinguishable (Fig. 22). The islets within these necrotic areas were usually also degenerate with pyknotic insular cells (Fig. 24). Also, at these sites, microangiopathy of arteriolar vessels was evident with the walls of the blood vessels being laden with an excessive amount of elastic fibers (Fig. 22 and 23). Lipomatosis of exocrine tissue was quite noticeable where spherical fat droplets of varying size infiltrated the exocrine tissue (Fig. 20). Finally, a much greater number of nerve cells were sighted within the pancreatic ductular connective tissue layer of the hyperglycemic smolts (Fig. 13&23) and a few mitotic figures were encountered. In one of these fish several intrapancreatic ganglia were found and a few of these were heavily infiltrated by capsular connective tissue suggestive of fibrosis in that some of the cell bodies appeared degenerated whereas others were greatly hypertrophied (Fig. 26).

Figure 21. Normal compact pancreatic exocrine tissue (EX) in normoglycemic fasted pellet fed smolts. Exocrine cells form acini around lumens and their apical portion contains an abundance of zymogen granules. A portion of a cross section of an arteriole is seen on the left upper side (pentagon). The mid-top area shows a peripheral region of an islet of Langerhans (IL) wherein A cells stand out as red granulated cells (arrow head) in contrast to the agranular clear cells which are only lightly stained by aldehyde fuchsin (spear head). Helly's fluid. Aldehyde fuchsin followed by Ehrlich's hematoxylin then counterstained with Masson's trichrome. X 1,200.

Figure 22. Fibrosis, hyalinization and microangiopathy of compact pancreatic exocrine tissue in hyperglycemic pellet fed smolts. Most of the parenchymal tissue has been replaced by greenish hyaline-like deposits and aldehyde fuchsin fibrillar elastic-like elements whereby the tissue appears heavily scarred. Here and there isolated zymogen granules are found and a diagonal streak of zymogen granules and acinar cell cytoplasm (arrows) are the only evidence of the presence of exocrine tissue. The walls of the arterioles are unusually thick (pentagons) and heavily laden with elastic connective tissue. Helly's fluid. Aldehyde fuchsin followed by Ehrlich's hematoxylin then counterstained with ponceau-orange G and light green. X 1,200.

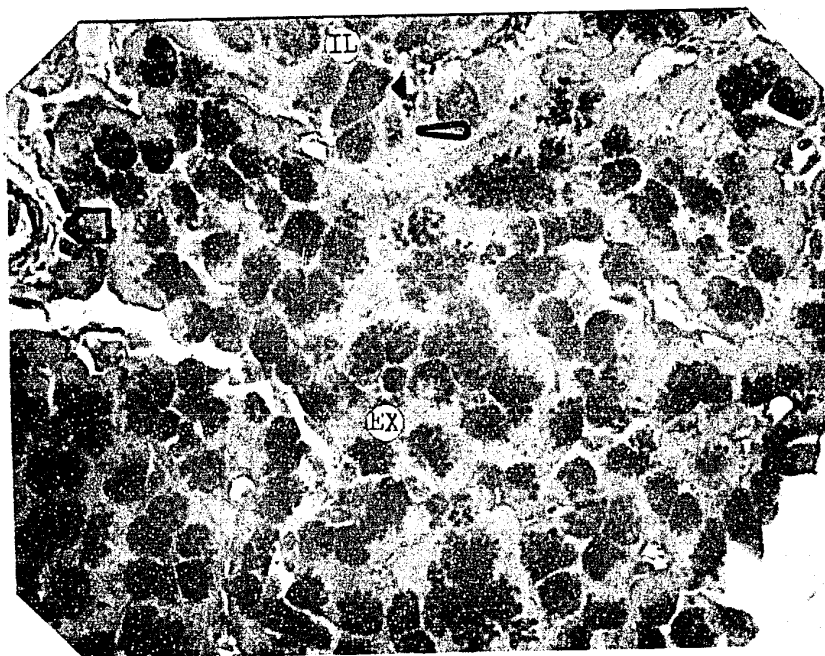


Figure 23; Hyalinization of compact exocrine tissue (EX) accompanied by hyalinization of islet tissue in hyperglycemic pellet fed salmon smolts. Excessive amounts of light green hyaline deposits primarily around the arteriole are seen to also invade the acinar tissue in the form of strands lining and surrounding capillaries. The acinar tissue is atrophied where nuclei are hyperchromatic and zymogen granules are indistinguishable as compared to Figure 17; the tissue is also affected by lipomatosis (arrow heads). The islet of Langerhans (IL) is also afflicted; cellular differentiation is no longer apparent and most nuclei are pyknotic. The plasma glucose and triacylglycerol levels of this fish were respectively 228 mg.% and 222 mg.%. Note the large nerve cell (arrow) located within the connective tissue layer of a pancreatic duct. Helly's fluid. Aldehyde fuchsin followed by Ehrlich's hematoxylin then counterstained with ponceau-orange G and light green. X 300.

Figure 24. Enlargement showing hyalinization of the islet tissue as well as pyknosis of insular tissue in hyperglycemic pellet fed salmon smolts. Strands of greenish hyaline-like material has invaded the islet of Langerhans (IL) mostly along capillaries. There remains only a few cells which may be distinguished as B cells with scanty aldehyde fuchsin cytoplasm and hyperchromatic nuclei (arrows) suggestive of atrophy and degeneration. Most endocrine cells possess pyknotic nuclei and appear exhausted. In this case the exocrine tissue (EX) is also atrophied; zymogen granules are lacking, acinar cells show vacuolization (arrow heads) and possess either hyperchromatic or pyknotic nuclei and lipmatosis (pentagons) is also evident. Most of the islets of this fish were severely affected; plasma glucose was elevated to 178 mg.% and plasma triacylglycerol reached 556 mg.%. Helly's fluid. Aldehyde fuchsin followed by Ehrlich's hematoxylin then counterstained with ponceau-orange G and light green. X 1,200.

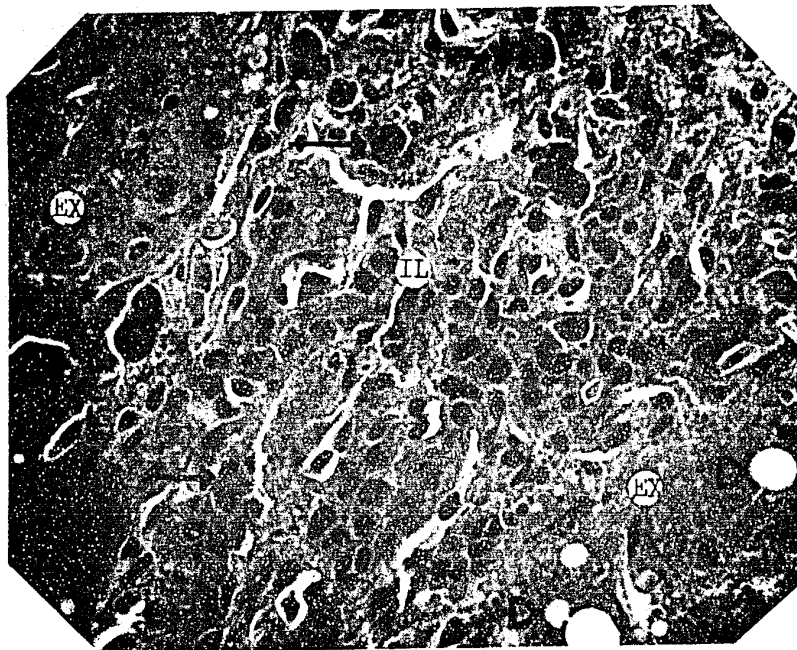
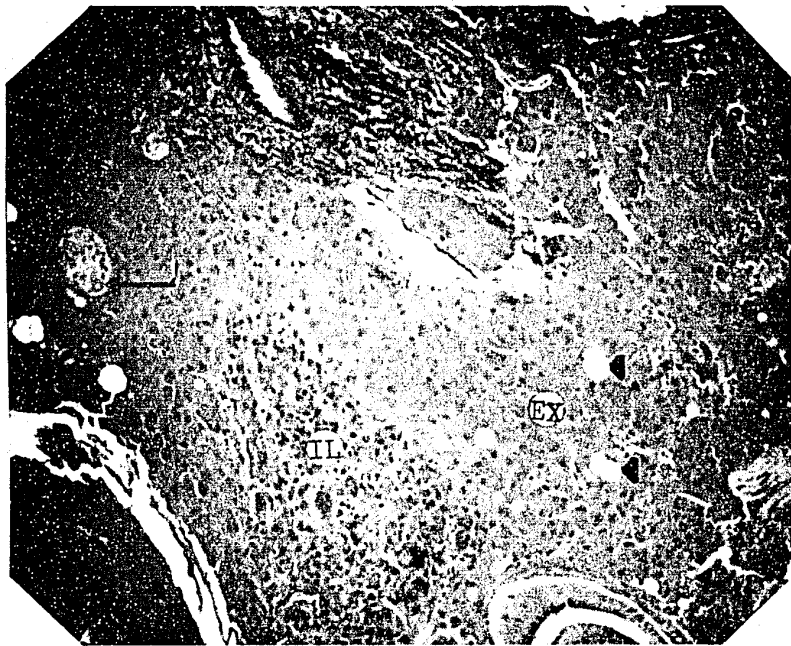
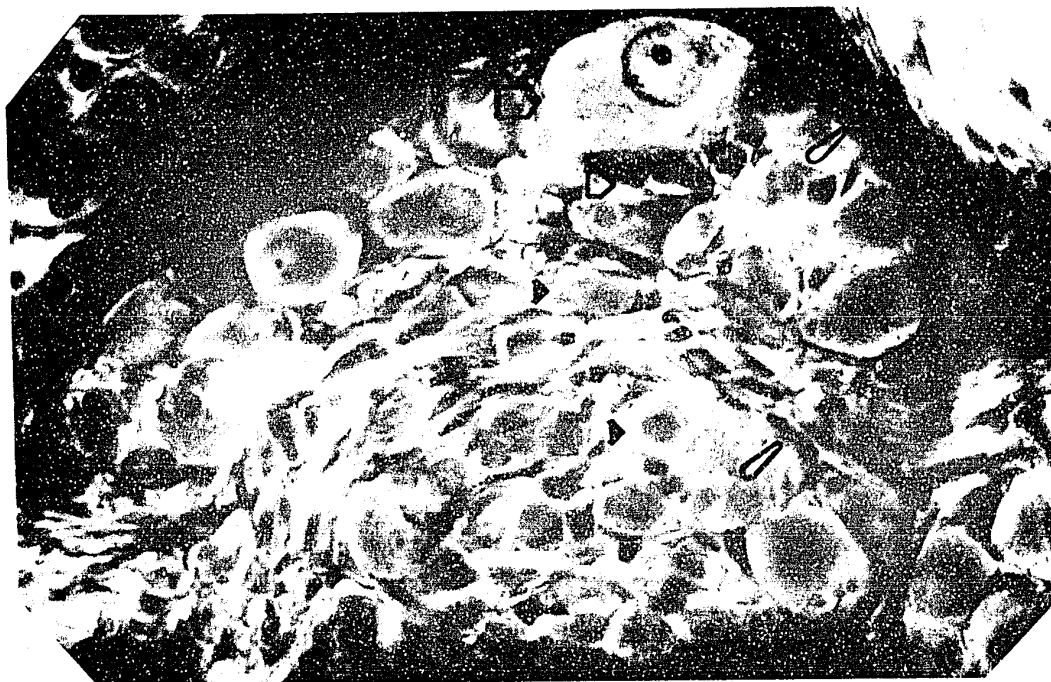
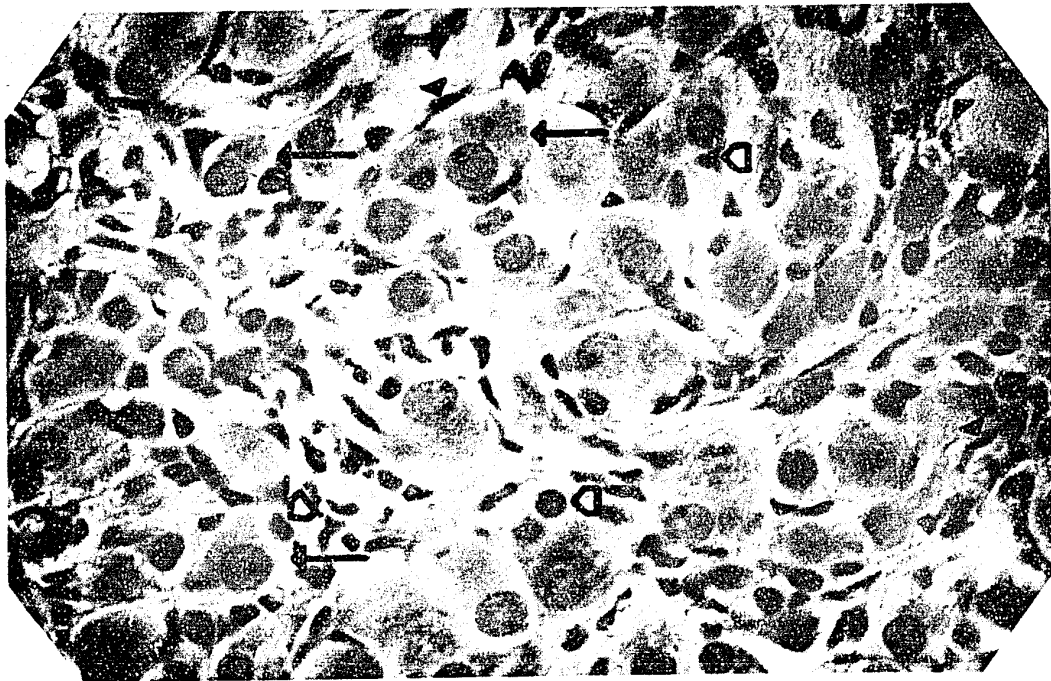


Figure 25. Normal intrapancreatic ganglion in pellet fed Atlantic salmon. Numerous cell bodies (arrows) are found to contain eccentric nuclei and conspicuous nucleoli. A few nerve cells are seen to possess capsular satellite cells (pentagons). Spindle like cells (arrow heads) are also obvious. Bouin's fixative. Aldehyde fuchsin followed by Ehrlich's hematoxylin then counterstained with Masson's trichrome. X 1,200.

Figure 26. Hypertrophy and cellular exhaustion of intrapancreatic ganglia in hyperglycemic pellet fed Atlantic salmon smolts. A large hypertrophied nerve cell (large pentagon) is seen at the top of the micrograph surrounded by a few satellite cells (small pentagon). In the mid bottom portion several cell bodies are small and possess scanty cytoplasm (arrow heads) which is suggestive of cellular exhaustion. Capsular connective tissue (spear heads) invades the substance of the ganglion and tends to augment lobulation of the already highly lobulated ganglion which is coherent to an incidence of fibrosis. Bouin's fixative. Aldehyde fuchsin followed by Ehrlich's hematoxylin then counterstained with Masson's trichrome. X 1,200.



DISCUSSION

The overall physiological condition of the pellet fed salmon smolts suggested a pathological state. These fish showed a 53% incidence of inappropriate hyperglycemia under fasted condition (Fig. 2). Whilst the argument might be made that the pellet fed smolts were normoglycemic it seems unlikely. In fact 67% of the blood glucose values of the pellet fed smolts were above the normal salmonid blood glucose range of 46 mg.% to 123 mg.% (Chavin and Young, 1970). Further, a literature survey on acknowledged hyperglycemic states in teleosts (Tables 1 and 6) indicated that in a majority of species the hyperglycemic peaks reached were actually lower than the 150 mg.% selected to denote hyperglycemia in this study. In the opinion of this author, the criterion of 150 mg.% is a rather conservative figure.

Having concluded that the pellet fed Atlantic salmon smolts displayed hyperglycemia it was thought to look for other derangements, both metabolic and histological, to see if the impaired glucose control was accompanied by secondary etiological diabetic factors which would support the hypothesis that hatchery-rearing could induce diabetogenous symptoms. Also, these findings would confirm that the observed hyperglycemia was not simply a transitory phenomenon resulting from the high degree of sensitivity of teleosts to external stimuli (Chavin, 1973).

In so doing, it was found that both the normoglycemic and the hyperglycemic pellet fed smolts exhibited elevated

Table 6. Normal glycemic range and mean glucose level in several teleost species and representative hyperglycemic states following various induction methods.

Species	Mean glucose level and normal glycemic range	Induction of a hyperglycemic state	Authors
<u>Opsanus tau</u> (Toadfish)	19 mg. % 0 mg. % -----50 mg. %	Alloxan treatment, 400 mg./kg. of body weight. p. * 130 mg. %	Nace, 1956.
<u>Ictalurus nebulosus</u> (Brown bullhead)	59 mg. % 10 mg. % -----120 mg. %	Injection of distilled water p. 120 mg. % Alloxan treatment, 460 mg./kg. of body weight. p. 179 mg. %	Murrel & Nace, 1959.
<u>Opsanus tau</u> (Toadfish)	19 mg. % 0 mg. % -----120 mg. %	Alloxan treatment, 700 mg./kg. of body weight. p. 150 mg. %	Moule and Nace, 1963.

Table 6. Normal glycemic range and mean glucose level in several teleost species and representative hyperglycemic states following various induction methods.

Species	Mean glucose level and normal glycemic range	Induction of a hyperglycemic state	Authors
<u>Anguilla rostrata</u> (American eel)	77.8±5.6 mg. %	Injection of a saline solution 129.8±12.0 mg. %	Butler, 1968.
<u>Carassius auratus</u> (Goldfish)	28.5±9.6 mg. % 18.9 mg. % -----38.1 mg. %	Saline injection, 0.58% Na Cl p. 81.3 mg. %	Chavin and Young, 1970.
<u>Channa punctatus</u> (Snake head)	44.2±2.4 mg. % 32 mg. % -----56 mg. %	Sham operation p. 81 mg. % Isletectomy p. 150 mg. % Force fed isletectomized p. 170 mg. %	Khanna and Gill, 1972.

Table 6. Normal glycemic range and mean glucose level in several teleost species and representative hyperglycemic states following various induction methods.

	Mean glucose level and normal glycemic range	Induction of a hyperglycemic state	Authors
<u>Pleuronectes platessa</u> (Plaice)	15 mg.% -----25 mg.%	Captured by trawl p. 120 mg.%	Wardle, 1972.
<u>Salmo salar</u> (Atlantic salmon, hatchery parr)	42.5±3.0 mg.%	Forced exercise for 15 minutes. p. 80.6±26.7 mg.%	Wendt and Ericson, 1972.
<u>Salmo salar</u> (Atlantic salmon, wild smolts during downstream migration)	60.6±13.8 mg.%	Captured in a net at the shut of a barrage. 135.9±11.7 mg.% Caught overnight in a cage and sampled the following day. 158.6± 8.5 mg.% Held for 2 days before release 176.1±37.2 mg.%	Wendt and Ericson, 1972. (for mean plasma glucose) Drouin, 1973, unpublished.

Table 6. Normal glycemic range and mean glucose level in several teleost species and representative hyperglycemic states following various induction methods.

Species	Mean glucose level and normal glycemic range	Induction of a hyperglycemic state	Authors
<u>Anguilla anguilla</u> (European eel)	77.8±5.6 mg. %	Injection of a saline solution 129.8±12 mg. %	Butler, 1968.
<u>Esox lucius</u> (Northern pike)	46.9 mg. % 25.2 mg. % -----59.9 mg. %	Administration of adrenalin, 0.05 mg./kg. of body weight. p. 140 mg. % Administration of noradrenalin, 1 mg./kg. of body weight p. 158 mg. % Administration of bovine glucagon, 1 mg./kg. of body weight p. 125 mg. %	Thorpe and Ince, 1974.

Table 6. Normal glycemic range and mean glucose level in several teleost species and representative hyperglycemic states following various induction methods.

Species	Mean glucose level and normal glycemic range	Induction of a hyperglycemic state	Authors
<u>Salmo salar</u> (Atlantic salmon, hatchery parr-smolt stage)	25 mg. % -----50 mg. %	Undetermined spontaneous hyperglycemia	Wendt and Saunders, 1973.

* p. denotes peak hyperglycemia reached.

levels of plasma triacylglycerol (Fig. 3). Based upon the first clear peaks in each group hypertriacylglycerolemia was then defined in a clinical sense as encompassing those values exceeding 249 mg.%. Consequently, it was observed that no fewer than 46% of the pellet fed smolts showed hypertriacylglycerolemia. Of the two groups of pellet fed fish, 23% of the normoglycemic fish had hypertriacylglycerolemic values and 65% of the hyperglycemic fish showed hypertriacylglycerolemia which in many cases paralleled the hyperglycemia (Appendix). However, in the eel-pout, Zoarces viviparus, intraspecies variation of plasma triacylglycerol levels may arise due to sex or a particular physiological state such as intra-ovarian brooding or spermatogenesis (Pekkarinen and Kristofferson, 1975). Hence, although the smolts were not sexed, it is possible that the bimodality in plasma triacylglycerol distribution in both the normoglycemic and the hyperglycemic pellet fed groups (Fig. 3) may reflect sexual differences.

Based on the status of plasma triacylglycerol levels, it becomes apparent that both the normoglycemic and the hyperglycemic pellet fed smolts may have been affected by the prolonged feeding regimen of a commercial pellet diet. Indeed, both of the pellet fed groups showed an incidence of 40% of ketonuria and urinary acidosis and a scarce glucosuria whereas these symptoms were totally absent in the herring fed group. Also, in spite of the fact that both the herring fed and the pellet fed groups were always fed to satiety, the weight data indicates that the pellet fed salmon showed

slower growth (Fig. 7). However, this may simply indicate different caloric intake by the two groups (Table 1).

Research at the level of the histology of the pancreatic tissue was pursued in order to ascertain if the biochemical changes denoted in the pellet fed groups could be correlated with the histology of the exocrine and endocrine pancreas.

Within the hyperglycemic pellet fed smolts the 53% occurrence of hyperglycemia along with a 65% incidence of hypertriacylglycerolemia, a 40% incidence of ketonuria and urinary acidosis and a moderate but infrequent glucosuria, were accompanied by major anomalies at the level of both the islets of Langerhans and the pancreatic exocrine tissue.

Within the endocrine tissue, a pronounced change in the relative percentage of A cell (48%) to B cell (52%) population per islet, represented a 13% decrease in the number of B cells per islet and 35% increase in the number of A cells per islet. Accordingly, the mean beta to alpha cell ratio was significantly reduced by 18% (Table 5). Also, 23% of the investigated islets showed a superior A cell population. Consequently, the internal cellular disposition of the islets was altered such that; the fewer B cells no longer formed distinctive groups and the A cells were more evenly intermingled with the clear cells and often penetrated the B cell areas (Fig. 17). On frequent occasions B cells showed vacuolization and degranulation suggestive of exhaustion (Fig. 18). Islet hyalinization was also common and more

advanced than that observed in the normoglycemic pellet fed fish. In severely affected islets the cells were shrunken with pyknotic nuclei and cellular differentiation became impossible (Fig. 24). B cell hyperplasia and hypertrophy were discernible but to a lesser extent than in the normoglycemic fish. Islet neoformation was pronounced (Fig. 19).

At the level of the exocrine pancreas large areas of tissue and enclosed islet tissue were concomitantly afflicted by hyalinization (Fig. 23). Occasionally regions of necrotic exocrine tissue were encountered where acinar cellular atrophy and degeneration resulted from extensive fibrosis (Fig. 22). Microangiopathies were frequently evident and lipomatosis was quite noticeable (Fig. 20, 22 and 23). Major changes were also observed within the innervation of pancreatic tissue. In general an abundance of solitary nerve cells were sighted and in one case intrapancreatic ganglia were observed to be gravely afflicted by fibrosis wherein the nerve cells exhibited both hypertrophy and atrophy suggestive of over-excitation leading to exhaustion (Fig. 26).

It is apparent then that the changes observed were not responses to non specific environmental stresses and that the physiological condition of the hyperglycemic pellet fed smolts closely resembled a mammalian-like diabetogenous state. Concurrently, the physiological condition of the normoglycemic pellet fed smolts may be attributable to a

prediabetogenous state. This is suggested by a 23% incidence of hypertriacylglycerolemia, a 40% incidence of urine ketosis and a single case of glucosuria. It is further substantiated by B cell hyperplasia (Fig. 15) and hypertrophy (Fig. 16) and an occasional moderate hyalinization within the islets of Langerhans of the normoglycemic pellet fed smolts.

Many aspects of the symptoms exhibited by the pellet fed fish may be considered to parallel mammalian diabetes mellitus. The pellet fed smolts showed a marked incidence of inappropriate hyperglycemia under a fasted condition which is the ultimate symptom establishing the presence of a diabetic state in mammals (Gorman, 1973; Krah1, 1974; Montoya and Herrera, 1974). Furthermore, the glycemic distribution was bimodal which is a characteristic of the glucose tolerance distribution of diabetic humans (Bennett and Miller, 1970; Rushforth, 1971). Also, the hyperglycemia appears related to a state of hypoinsulinemia and hyperglucagonemia and this would agree with the findings of Montoya and Herrera (1974) and those of Topping and Targ (1975) for streptozotocin diabetic rats. They also concur with those of Alford et al. (1977) for non-ketotic, non-obese human diabetics. The suspected hyperglucagonemia may indicate the presence of a genetic factor contributing to the development of the diabetes (Unger and Falloona, 1974).

However, unlike mammals, blood glucose may not be the ultimate carbohydrate to monitor in teleosts in order to establish a glycemic level and consequently better understand

carbohydrate metabolism. For instance, Simpson (1926), Falkmer (1961) and Moule and Nace (1963) have commented on the presence of some protein-sugar compound or a polysaccharide in the blood of teleosts. This "masked carbohydrate" may account for more than one third of the total blood carbohydrate reducing substances. In fact, this component may even exceed blood glucose values in response to factors which normally affect the blood glucose level. Supporting the existence of an important carbohydrate substrate other than glucose, are the reports of a lower minimal range of 0 mg.% of glucose in the shorthorn sculpin, Cottus scorpius, (Falkmer, 1961) and in the toadfish, Opsanus tau, (Moule and Nace, 1963). A further evidence is the induction of a blood glucose of 0 mg.% in the European carp, Cyprinus carpio, without hypoglycemic convulsions (Yokote, 1970 c).

Moreover, there are indications in teleosts that the role of glucose in carbohydrate metabolism may differ somewhat from that observed in mammals. For example, in the anglerfish, Lophius piscatorius, (Trakatellis and Schwartz, 1970) as well as in the cod, Gadus callarias and in the brown bullhead, Ictalurus nebulosus, (Moule and Yip, 1973) glucose fails to promote proinsulin synthesis and its subsequent conversion to insulin. In the European eel, Anguilla anguilla, elevated blood glucose levels did not stimulate an appropriate insulin release (Ince and Thorpe, 1977) and excess blood glucose acted as an anti-insulinogenic agent during the first phase of a prolonged starvation (Palayer, 1962). A similar anti-insulinogenic effect of glucose was reported for the northern

pike, Esox lucius (Thorpe and Ince, 1974; Ince and Thorpe, 1976). Furthermore, teleosts are noted for their ability to resist a fall in glycemia during the first phase of a prolonged starvation (Fontaine and Hatey, 1953; Wendt and Ericson, 1972) where a potent gluconeogenesis may actually increase the state of glycemia (Butler, 1968). Hence, there exists major physiological differences between teleosts and mammals pertaining to carbohydrate metabolism and for these reasons the salmon diabetogenic hyperglycemia may resemble but not necessarily parallel that which is observed in mammalian diabetes mellitus.

Some aspects of the hypertriacylglycerolemia in the pellet fed smolts may be held to reflect a mammalian diabetic condition. A state of hyperglycemia accompanied by one of hypertriacylglycerolemia as encountered in the salmon smolts, are cardinal symptoms of mammalian diabetes (Sailer, 1973; Epple, 1977). In the smolts the variability of the relationships between the degree of hyperglycemia and the magnitude of hypertriacylglycerolemia where some levels paralleled one another whereas others did not, prevented the establishment of a significant correlation between the hyperglycemia reached and the hypertriacylglycerolemia developed (Appendix). These findings are identical with those of Bierman (1971) for humans. Further, in certain cases of obese onset diabetes, it is feasible that the hypertriacylglycerolemia may actually precede the onset of diabetes and this could possibly account for the hypertriacylglycerolemia observed within the normoglycemic pellet fed smolts (Coll - Garcia et al., 1971). However, to the exclusion of one fish, all of the normoglycemic pellet

fed smolts with hypertriacylglycerolemia had blood glucose levels above 125 mg.% and consequently these may well be hyperglycemic. As previously indicated the upper limit of a normal blood glucose range for salmonids is reported at 123 mg.% by Chavin and Young (1970) and further, Wendt and Saunders (1973) considered 122 mg.% as hyperglycemic for salmon at the parr-smolt stage.

On the other hand, the hypertriacylglycerolemia of the pellet fed smolts may not be clearly and undeniably associated with the mammal-like diabetic state. In teleosts it still remains to be shown unequivocally that triacylglycerols are an important transport phase of fatty acids. Also, it has yet to be investigated if a rise in plasma triacylglycerol under a diabetogenous state is due to a failure in lipoprotein lipase activity as is the case in mammalian diabetes (Topping and Targ, 1975). Furthermore, although Bierman (1971) considered a 40-50% increase in plasma triacylglycerol as sufficient to depict a hypertriacylglycerolemic state in diabetic humans (this in fact supports the criterion of 250 mg.% selected to denote instances of hypertriacylglycerolemia in the smolts) it remains that plasma triacylglycerol levels in teleosts in general appear much greater than those observed in this study. For instance, in the eel pout, Zoarces viviparus, females are reported to have a normal level of 1,500 mg.% whereas in males a level of 718 mg.% is encountered; during ovarian-brooding female levels rose to 5,000 mg.% and during spermatogenesis those of the males reached 2,400 mg.%. In the brown trout, Salmo trutta, the same authors reported a

summer level of 930 mg.% in females, which declines to 664 mg.% in the winter during artificial spawning (Pekkarinen and Kristofferson, 1975); measurements however, included free glycerol. Finally, in the European eel, Anguilla anguilla, Larsson and Lewander (1972) determined a fasted level of 467 mg.% and in a later publication Larsson (1973) observed a higher level of 610 mg.%; tripalmitin was used as standard. For the above reasons the association of hypertriacylglycerolemia and hyperglycemia must be regarded with reservations.

In contrast to what may be observed in most diabetic mammals, the diabetogenous condition of the pellet fed smolts was not marked by an elevation of plasma levels of the ketone body beta-hydroxybutyric acid, but yet, instances of ketonuria and urinary acidosis were present. In fact, under fasted condition, the upper limit of the range of plasma beta-hydroxybutyric acid in the normoglycemic and hyperglycemic pellet fed groups and in the herring fed control group was similar to that found in the rainbow trout, Salmo gairdneri, and in the sockeye salmon, Oncorhynchus nerka, under normal conditions (Jonas and Bilinski, 1965). However, the first mode in the bimodal distribution of beta-hydroxybutyric acid of the herring fed group is below 0.3 mg.% (Fig. 4). The first major peak in the distribution of beta-hydroxybutyric acid in both the normoglycemic and the hyperglycemic pellet fed smolts was also lower than 0.3 mg.%. In the rainbow trout and in the sockeye salmon the normal basal range of acetoacetate was above 0.3 mg.% and indicated higher concentrations for beta-

hydroxybutyric acid (Jonas and Bilinski, 1965). The present values then denote an incidence of hypoketonemia amongst all of the salmon groups. This hypoketonemia is noted to be extensive within the herring fed and the hyperglycemic fed groups whereas it is moderate within the normoglycemic pellet fed group. The above results are congruent with the idea that the Atlantic salmon possess the necessary metabolic machinery to handle an excess of ketone bodies and avoid the accumulation of blood ketone bodies. Hence, it is postulated that the salmon can utilize ketone bodies with greater efficiency than mammals and that the efficiency is possibly adaptable. This is supported by the fact that in this study none of the fasted salmon exhibited increases in plasma beta-hydroxybutyric acid typical of those found in fasted mammals. Further, starvation in rainbow trout is not associated with pronounced changes in plasma levels of ketone bodies (Jonas and Bilinski, 1965). Of course this hypothesis remains to be tested. That teleosts can show increased plasma ketone levels has been reported by Jonas and Bilinski (1965). These authors found that rainbow trout underwent a pronounced change in response to stress. Also, during the spawning stage, a slight rise of blood ketones was noted to occur in the rainbow trout and in the sockeye salmon. The discrepancy between blood and urine levels of ketone bodies in the pellet fed group is unresolved. Unfortunately the urine ketosis of Sekoke carp was not verified by parallel determination of ketonemic levels. It must be

mentioned, however, that in certain mammals as well, diabetes mellitus is not accompanied by ketogenesis (Stauffacher, 1971).

The growth data provided by Dr. R.L. Saunders at St. Andrews hatchery was analyzed for two reasons. First, Krahl (1974) includes amongst the major abnormalities of diabetes mellitus the failure of growth and secondly Topping and Targ (1975) have shown a profile of continuous decrease in weight in streptozotocin diabetic rat while the controls showed continuous increase in weight. They suspect that the changes in weight in part reflect a defect in protein synthesis in chronic diabetes. In the present study the weight growth pattern of the pellet fed smolts shows some attenuation during the month of August and slower growth thereafter (Fig. 7). This may suggest a defect in protein synthesis. However, the results are not conclusive.

The quantitative histopathological changes noted to take place within the islet tissue of the hyperglycemic pellet fed smolts are akin to those observed in mammalian diabetes mellitus. They are also a strong indication attesting to the presence of a form of diabetes in pellet fed hatchery-reared smolts. A significantly decreased mean beta to alpha cell ratio in these smolts concurs with the findings of Maclean et al. (1955), Ogilvie (1964) and Volk and Lazarus (1970) who showed a significant reduction of the average beta to alpha cell ratio in human diabetics. The change in the relative percentage of A cell to B cell population from 35% for A cells and 65% for B cells in the parr control group, to 48% for A cells and 52% for B cells in the hyperglycemic pellet fed smolts is

seen as a major upset. Indeed, with exception of the bluefin tuna, Thunnus thynnus (Planas and Garcia, 1964), the percentage A cell population relative to that of B cells of most teleosts varies from 15% to 40% (Table 7). This indicates that the 48% reached in the hyperglycemic pellet fed smolts is substantially different from normal. This etiological aspect suggest that hyperglucagonemia may be an important symptom in salmon diabetes. Although this is not generally a prevailing condition in mammalian diabetes, as stated earlier, it does occur in non-ketonic, non-obese human diabetics (Alford et al., 1977) and is an important feature in some human genetic-related diabetes (Unger and Falloona, 1974). In contrast to diabetic mammals, the hyperglycemic pellet fed smolts fail to show a reduction in islet size.

Most of the qualitative histological changes described within the pancreatic tissue of the hyperglycemic pellet fed smolts are qualitatively similar to changes seen in human diabetics (Bell, 1946, 1947 and 1952; Gepts, 1964; Okamoto et al., 1971). These changes also lend strong support to the idea of a form of diabetes existing in the hyperglycemic pellet fed smolts. The frequent and obvious alterations in the internal cellular disposition of the islets of these smolts is undoubtedly related to the hyperglycemic state. Indeed, such changes have been reported to occur in the snake head, Channa punctatus, consequent to glucose loading (Khanna and Gill, 1973). In humans and in many mammals the insular cells do not occupy a specific internal

Table 7. Islet cells of teleosts and percentage islet cell populations.

Species	Classical cell types			Clear cells	Other cell types	Authors
	A	B	D			
<u>Salmo salar</u>			not detected			
(Atlantic salmon)	24%	48%	at L.M. level	28%	5th and 6th	Drouin, unpublished
<u>Platycephalus indicus</u>						
(Sand gurnard)	20%	60%	20%	---	---	Fujita, 1968.
<u>Pagrosomus major</u>						
(Porgy)	"	"	"	---	---	" "
<u>Mylio macrocephalus</u>						
(Pargos)	"	"	"	---	---	" "
<u>Ictalurus nebulosus</u>						
(Brown bullhead)	40%	45%	---	15%	---	Bencosme et al., 1965.
<u>Ictalurus punctatus</u>						
(Channel catfish)	15%	42.5%	42.5%	absent	---	Brinn, Jr., 1971.
<u>Gadus callarias</u>						
(Baltic cod)	20%	30%	30%	---	4th, 20%	Thomas, 1970.

Table 7. Islet cells of teleosts and percentage islet cell populations.

Species	Classical cell types			Clear cells	Other cell types	Authors
	A	B	D			
<u>Cottus scorpius</u>						
(Shorthorn sculpin)	25%	25%	25%	25%	---	Falkmer, 1961.
<u>Cyprinus carpio</u>						
(European carp)	40%	50%	5-9%	---	---	Nakamura and Yokote, 1971.
<u>Carassius carassius longsdorfi</u>						
(Crucian carp)	25%	50%	15%	10%, D-like	4th and 5th	Kobayashi and Takahashi, 1970.
<u>Opsanus tau</u>						
(Toad fish)	50%	50%	---	50% along	---	McCormick, 1924-5;
	along with clear cells			with A cells		Nace, 1956; Like et al., 1964.
<u>Thunnus thynnus</u>	64%	36%	large	Brockmann body		Planas and Garcia, 1964.
(Bluefin tuna)	80%	20%	small			

Table 7. Islet cells of teleosts and percentage islet cell populations.

Species	Classical cell types			Clear cells	Other cell types	Authors
	A	B	D			
<u>Neomaenis griseus</u>						
(Gray snapper)	present	50%	present	---	4th	Bowie, 1924-5.
<u>Elops machinata</u>						
(Ten pounder)	present	30-50%	present	present	---	Biuw, 1972.
<u>Elops saurus</u>						
(Giant herring)	"	"	"	"	"	"
<u>Megalops cyprinoides</u>						
(Ox-eye herring)	"	"	"	"	"	"
<u>Chana punctatus</u>		most	more numerous	B-like		Khanna and Singh, 1971;
(Snake head)	fewer than D	numerous	than A	cells	---	Khanna & Gill, 1973.
<u>Salvelinus leucomaenis pluvius</u>		most				Honma and Tamura,
(Japanese char)	meager in #	numerous	numerous	absent	amphiphils	1968.

Table 7. Islet cells of teleosts and percentage islet cell populations.

Species	Classical cell types			Clear cells	Other cell types	Authors
	A	B	D			
<u>Scorpaena scropha</u>						
(Large-scaled scorpion fish)	present	present	low frequency	present	---	Mosca, 1957; Boquist and Patent, 1971.
<u>Conger japonicus</u>						
(Congo eel)	present	present	sparse	absent	4th	Kobayashi and Takahashi, 1974.
<u>Seriola quinqueradiata</u>	low	most		slightly more		
(Yellowtail)	frequency	numerous	absent	numerous than A.	---	Watari et al., 1970.

----- = not identified.

disposition within the islets. The B cell degranulation of the hyperglycemic pellet fed smolts as well as the other hydropic changes such as cytoplasmic vacuolization and nucleic pyknosis are distinctive features associated with spontaneous diabetes in Sekoke carp, Cyprinus carpio (Yokote, 1970 a). These changes are also found in several teleosts following the administration of a glucose load where they were observed to lead to acute B cell exhaustion (Khanna and Rekkari, 1972; Khanna and Gill, 1973; Bhatt, 1974). Hence, these changes are held to be related to a state of hyperglycemia and indicate that the B cells of the islet tissue are involved in the salmon form of diabetes. These results concur with those found in human diabetics where such changes were held to reflect frank diabetes (Bell, 1947). Further, these changes were often observed in spontaneous diabetes of mammals (Warren and Le Compte, 1952). The hyalinization of both the endocrine and the exocrine pancreas and the fibrosis, the microangiopathy of the arterioles and the lipomatosis of the pancreatic acinar tissue are considered as prominent histopathological changes in the hyperglycemic pellet fed smolts. These observations are congruent with those found in established diabetes of humans (Lazarus and Volk, 1962; Ogilvie, 1964; Warren et al., 1966).

The islet neoformation noted to occur on a larger scale in the hyperglycemic pellet fed smolts concurs with similar findings associated with human diabetes (Warren and Le Compte, 1952; Volk and Lazarus, 1970). Such neoplasia of islet tissue was also reported in the chinese hamster, Cricetulus griseus

following experimental diabetes as quoted by Ostberg et al. (1975). Unfortunately Yokote (1970 a) has not indicated whether or not an incidence of islet regeneration was prevalent in his diabetic carps. However, instances of islet neoformation in the Atlantic salmon suggesting a course of events from ductular epithelial cells to ductular endocrine cells, to ductular clusters of endocrine cells, to nests and buds of islet cells are concomitant with similar observations noted to occur in the gray snapper, Neomaenis griseus (Bowie, 1924-25) as well as in the German carp, Cyprinus carpio (Titlbach, 1968).

At present there is no direct evidence in vertebrates for the neogenesis of islet tissue from a transdifferentiation of pancreatic ductular epithelial cells into pancreatic endocrine cells. But in higher vertebrates there is some indirect evidence in favor of this hypothesis. For example, islet neoformation from pancreatic ductules was reported in the embryos of the white leghorn chick (Prsybylski, 1967) rat and mouse (Pictet and Ruller, 1972) and in human neonates and adults, endocrine cells were localized amongst the epithelial ductular cells and patients with endocrine tumors are observed to show the various phases of islet neoformation encountered in the Atlantic salmon (Deconinck et al., 1971 and 1972; Pictet and Ruller, 1972). Furthermore, in the lower vertebrates there are numerous accounts in favour of the hypothesis: indirect evidence is present in the toad, Bufo bufo (Epple, 1966), in the amphibian Ichthyophis kohtaoensis (Welsch and

Storch, 1972), the snake, Elaphe climacophora (Watari et al., 1970) the salamander, Ambystoma tigrinum, as well as in the newt, Diemictylus viridescens (Sato et al., 1966). Stages of islet neoformation similar to those encountered in the Atlantic salmon were also reported in the following holocephalans; the rabbit fish, Chimaera monstrosa (Fujita, 1964) and the ratfish, Hydrolagus colliei (Patent and Epple, 1967). In the Atlantic hagfish, Myxine glutinosa, endocrine cell formations representative of each of the steps described in the Atlantic salmon were reported by Ostberg et al. (1975). It appears then that a greater incidence of islet neoformation in the hyperglycemic pellet fed smolts is an important change and one which may be associated with the diabetic condition possibly reflecting a greater need for insulin.

In the hyperglycemic pellet fed smolts, the remarkable increase in the incidence of sightings of intrapancreatic nerve cells accompanied by infrequent mitotic activity is interpreted as evidence for nerve cell hyperplasia. Also, the fibrosis afflicting intrapancreatic ganglia wherein some nerve cells hypertrophy while others atrophy suggest over-excitation leading to exhaustion. Together these manifestations are held to represent a need by the smolts to exercise a greater autonomic nervous system control over pancreatic endocrine secretion under a diabetogenous state in response to an inappropriate hyperglycemia. That these changes are hyperglycemic related is supported by the findings of Brinn (1973) who showed that glucose loading in the carp lead

to ultrastructural changes in an insular ganglionic cell thought to be associated with insulin secretion. Further, in teleostei there exists ample evidence favoring the hypothesis that the autonomic nervous system may play an important role in regulating pancreatic hormone secretion. As in the salmon, the endocrine glandular cells of the following species were reported to be richly innervated: the yellowtail, Seriola quinqueradiata (Watari et al., 1970); the scorpion fish, Scorpaena scropha (Boquist and Patent, 1971), the carp, Cyprinus carpio (Nakamura and Yokote, 1971), the American eel, Anguilla rostrata, as well as several Ictularidae (Brinn, 1973) and the Crucian carp, Carassius carassius longsdorfi (Kudo and Takahashi, 1973). In all of the species unmyelinated nerve fibers were seen within the substance of the islet tissue where they terminated close to endocrine cells. So far, the best evidence for a direct nervous regulation on pancreatic endocrine cells is described in the swordtail, Xiphophorus helleri, by Klein (1971). The author describes the findings of an active neuro-junctional site within a nervous terminal where synaptic vesicles aggregate in the vicinity of an endocrine cell and material of high electron density accumulates against the axon membrane, in the intercellular space and on the glandular cell. In the long-finned tuna, Germo alalunga (Planas et al., 1968), nervous tissue was reported to be present within the walls of excretory pancreatic ducts and this agrees with the findings of this study. In the amphibian, Bufo arenarum, ganglionic cells were found within the exocrine pancreas and in the

toad, Bufo bufo, a large ganglion was located in close proximity to the exocrine pancreatic tissue (Epple, 1966). These findings in lower vertebrates are similar to those described in the Atlantic salmon and lend further support to an autonomic nervous system intervention on pancreatic hormone release. According to Brinn (1973) vagal stimulation in mammals elicits insulin release while glucagon release is associated with sympathetic influence. Although nerve terminals in teleost remain to be classified as either adrenergic or cholinergic, the presence of two distinctive types of synaptic terminals in the salmon (Fig. 9) favors the implication of both parasympathetic and sympathetic pathways with functions similar to that found in mammals. That the autonomic nervous system may play a key role in setting a proper balance of pancreatic endocrine release is supported by the fact that in the spiny mice Acomys cahirinus, diabetes is believed to be related to the fact that there are no autonomic nerve endings in close proximity of the endocrine pancreatic cells (Renold et al., 1973). Surely the hyperplasia of solitary nerve cells and the fibrosis of intrapancreatic ganglia are important histopathological changes that are undoubtedly related to the diabetic condition of the hyperglycemic pellet fed smolts.

The marked B cell hyperplasia and hypertrophy encountered within the islet tissue of the normoglycemic pellet fed smolts coupled with mild islet tissue hyalinization is interpreted as suggestive of a prediabetic state. In some of the islets of these fish the extent of the B cell hyperplasia was so

pronounced that the A cells appeared deformed as if crowded by the B cells (Fig. 15). Also, some of the islets of these fish showed a superior beta to alpha cell ratio in comparison to normal islet tissue. The above results are held to signify that although there was no impairment of glucose control in these fish there was nonetheless a greater demand for insulin. The presence of an incidence of hypertriacylglycerolemia in this group of smolts perhaps reflects a problem in glucose utilization and consequently this could substantiate a need for greater amounts of insulin. A study by Robertson and Wexler (1960) has indicated that in the Pacific salmon, Oncorhynchus nerka, a greater demand for energy during migration and while spawning did result in hypertrophy of the islet tissue. This supports the interpretation of hyperplasia and hypertrophy as a greater need for insulin in order to maintain normal glucose utilization in the normoglycemic pellet fed smolts. Moreover, Ogilvie (1964) has shown that in obese human patients which are prone to develop diabetes, an increase sugar tolerance and an excessive beta to alpha ratio are prime factors preceeding the onset of diabetes. Also, in humans, a non advanced state of hyalinization was interpreted as a sure sign of ensuing diabetes and consequently looked upon as a cause of diabetes and not a result (Bell, 1959). These findings typically prediabetic symptoms in humans are similar to those observed in the normoglycemic but hypertriacylglycerolemic pellet fed smolts and hence support the idea that these fish are also prediabetic.

In conclusion, the overall aspects of the symptoms encountered amongst the pellet fed smolts are indeed very similar to those found in mammalian diabetes mellitus: bimodality of glucose distribution, hyperglycemia under a fasted condition, glucosuria, hypertriacylglycerolemia, evidence for hypoinsulinemia and hyperglucagonemia, decreased beta to alpha cell ratio, B cell hydropic changes, islet tissue neoplasia, hyalinization of pancreatic endocrine and exocrine tissue, fibrosis, microangiopathy and lipomatosis of exocrine acinar tissue and a prediabetic phase characterized by B cell hyperplasia and hypertrophy as well as an early hyalinization. The only major difference is the absence of hyperketonemia which does not appear to develop in salmonids.

Several factors may be involved in predisposing hatchery smolts to develop diabetes: a pronounced growth rate in comparison to parr (R.L. Saunders, personal communication) where body growth may exceed pancreatic endocrine tissue growth, excessive caloric intake, improper balance in the ratio of the diet components (that is, reduced protein, elevated carbohydrate and low fat) and confinement where there is reduced activity.

In conjunction with these probable factors there are at least two indices which suggest that the pellet fed smolts may be showing symptoms of spontaneous diabetes. Firstly, in the early phase of the evolution of the disease B cell hyperplasia and hypertrophy may be taken as evidence for hyperinsulinemia; this may reflect an hereditary predisposition to glucose intolerance. Secondly, in hyperglycemic smolts, glucagon would appear to participate as an insulin antagonistic hormone (35%

increase in number of A cells per islet) and this could be substantiated by the frequent encounter of neoformation of islet tissue. In fact, the above are attributes of spontaneous diabetes in animals (Stauffacher et al., 1971; Renold et al., 1974).

In this study, the investigation of pancreatic tissue in salmon parr revealed that the distributions of exocrine pancreatic tissue (compact and diffuse) were similar, if not identical, to those found in the rainbow trout, Salmo gairdneri irideus, where elements of compact tissue were located in the vicinity of a large pancreatic duct and where diffuse tissue was localized over the surface of fatty tissue surrounding the caecal appendages (Hess, 1935). The findings of compact tissue along the portal vessels agrees with observations made of the European eel, Anguilla anguilla (Kukla, 1958), the spotted catfish, Clarias batrachus, the stinging catfish, Heteropneustes fossilis, (Khanna, 1963) and the American eel, Anguilla rostrata, (Brinn and Epple, 1972; Brinn, 1973). However, in these species the exocrine tissue was reported to be entirely compact. As in the Atlantic salmon, diffuse tissue was reported to be located between the pyloric caeca in the anchovy, Engraulis tilara, as well as in the sable fish, Hilsa ilisha, (Khanna, 1961), in the chinook salmon, Oncorhynchus tshawytscha, (quoted from Seshadri, 1961) and in the char, Salvelinus leucomaenis pluvius (Honma and Tamura, 1968), but these fish typified solely diffuse exocrine pancreatic tissue. The occurrence of pancreatic tissue around the bile ducts in the Atlantic salmon agrees with earlier

observations on C. batrachus as well as H. fossilis (Khanna, 1963) and the Nile fish, Clarias lazera, (Al-Gauhari, (1960). Hence, the sites of localization of pancreatic tissue in the Atlantic salmon were found to be typical of teleostean pancreatic tissue.

Like the Atlantic salmon, several teleosts have entirely intrapancreatic islets of Langerhans and lack the Brockmann bodies which are extrapancreatic islet tissue masses (Hess, 1935; Kukla, 1958, Khanna, 1961 and 1963; Sato et al., 1966; Kobayashi and Takahashi, 1974). Hence, while Brockmann bodies are certainly of frequent occurrence within the teleost group they are definitely not found in all species. One peculiar aspect of salmon islet tissue, not previously reported in other teleosts, is the presence of large aggregations of islet tissue in pellet fed fish. In diabetic humans Warren and Le Compte (1952) have described as a form of hyperplasia and hypertrophy the presence of projections of endocrine tissue amongst the acini and the fusion of islets of Langerhans. Perhaps islet aggregates in hatchery reared salmon are an early manifestation of endocrine tissue derangement in response to being fed a commercial pellet food of elevated carbohydrate content. The fact that such aggregates are not described in studies on the histogenesis of pancreatic tissue in teleost (Smallwood and Derrickson, 1933; Belsare, 1974) and the failure to find these within the pancreatic tissue of the herring fed smolts favors the idea of morphological changes due to diet.

The localization of A cell types within the periphery of the islets of Langerhans in Salmo salar is noted to be a

feature of common occurrence in several teleosts (Hess, 1935; Weinreb and Bilstad, 1955; Falkmer, 1961; Honma and Tamura, 1968; Thomas, 1970; Hirano and Honma, 1971; Khanna and Singh, 1971; Khanna and Gill, 1973; Klein and Lange, 1972 and 1974 a; Thomas, 1975). Furthermore, as in a few of the A cells of the salmon, the A cells of the dab, Limanda limanda (Thomas, 1975), the swordtail, Xiphophorus helleri (Klein and Lange, 1972 and 1974 a) and the cod, Gadus callarias (Thomas, 1970), were characterized by the presence of long cytoplasmic processes. The presence of a core of high electron opacity within the secretory vesicles of the A cells of the salmon is a distinctive ultrastructural aspect common to most teleostean A cells (Falkmer, 1966; Sato et al., 1966; Watari et al., 1970; Boquist and Patent, 1971; Nakamura and Yokote, 1971; Brinn, 1971 and 1973; Klein and Lange, 1972; Thomas, 1975).

In the current study, the consistent presence of B cells within the more central region of the islets in the Atlantic salmon is concomitant with similar observations made in the majority of teleosts (Weinreb and Bilstad, 1955; Mosca, 1957; Falkmer, 1961; Planas and Garcia, 1964; Bencosme et al., 1965; Honma and Tamura, 1968; Planas et al., 1968; Titlbach, 1968; Kobayashi and Takahashi, 1970; Gabe and Mutoja, 1971; Khanna and Gill, 1973; Klein and Lange, 1974 b; Thomas 1975). Also, the described fine structure of the beta secretory granules of the B cells of the Atlantic salmon concurs with observations previously made on the salmon by Théret and Palayer (1967), and the presence of crystalline cores was reported for most other teleosts species.

The insight gained on histological staining procedures for teleostean islet tissue merits discussion. The application of Bouin fixative without acetic acid proved to be unsatisfactory. The A cells as well as the clear cells of the islets of Langerhans were not preserved or else appeared highly vacuolated. Difficulties with proper fixation of peripheral A cells and clear cells in the shorthorn sculpin, Cottus scorpius, have also been reported by Falkmer (1961) when formalin or alcoholic fixative were used. The utilization of Helly's fluid as a fixative was very satisfactory. But sections had to be submitted to the mordant iron alum for at least one hour in order that the classical acid A cell stains would satisfactorily color the alpha granules. The application of Gomori's chrome alum hematoxylin gave results opposite of expectations. The otherwise aldehyde fuchsin positive B cells were now of a very light grey color whereas the weakly aldehyde fuchsin clear cells stained dark grey. This may well indicate that the clear cells are akin in some way to B cells in the Atlantic salmon. The failure to demonstrate a D cell element at the light microscopic level in the Atlantic salmon concurs with similar findings for the rainbow trout, Salmo gairdneri irideus (Hess, 1935; Weinreb and Bilstad, 1955). However, at the electron microscopic level a D cell element was identified and it is remarked that most of the time only cytoplasmic areas were encountered. Hence, the D cell of the Atlantic salmon is very small and like that of other teleosts it was elongate and of low frequency (Grosso, 1950; Honma and Tamura, 1968; Thomas, 1975).

SUMMARY

1. Hatchery-reared Atlantic salmon smolts raised under cage culture conditions and fed a widely used commercial pellet food of elevated carbohydrate content developed a high incidence of hyperglycemia and occasionally showed glucosuria under a fasted state.
2. A considerable number of the hyperglycemic pellet fed Atlantic salmon smolts had elevated plasma triacylglycerol levels whereas a low percentage of the normoglycemic pellet fed Atlantic salmon smolts were similarly affected.
3. Under fasted conditions there were no apparent increase in plasma levels of the ketone body beta-hydroxybutyric acid in either the herring fed group or the pellet fed group of Atlantic salmon smolts. Indeed, the majority of the plasma levels were below normal among the herring fed fish and, to a lesser extent among the hyperglycemic pellet fed fish as well. The normoglycemic pellet fed fish tended towards normal levels.
4. Infrequent urine keto-acidosis was encountered only in the pellet fed group of Atlantic salmon smolts where the incidence was equally divided between the normoglycemic and the hyperglycemic fish.
5. The beta to alpha cell ratio of the islets of Langerhans of the hyperglycemic pellet fed Atlantic salmon smolts was significantly reduced. This was due to a decrease in the B cell population per islet as well as a substantial increase in the A cell population per islet. This was

judged to indicate that states of hypoinsulinemia and hyperglucagonemia were present.

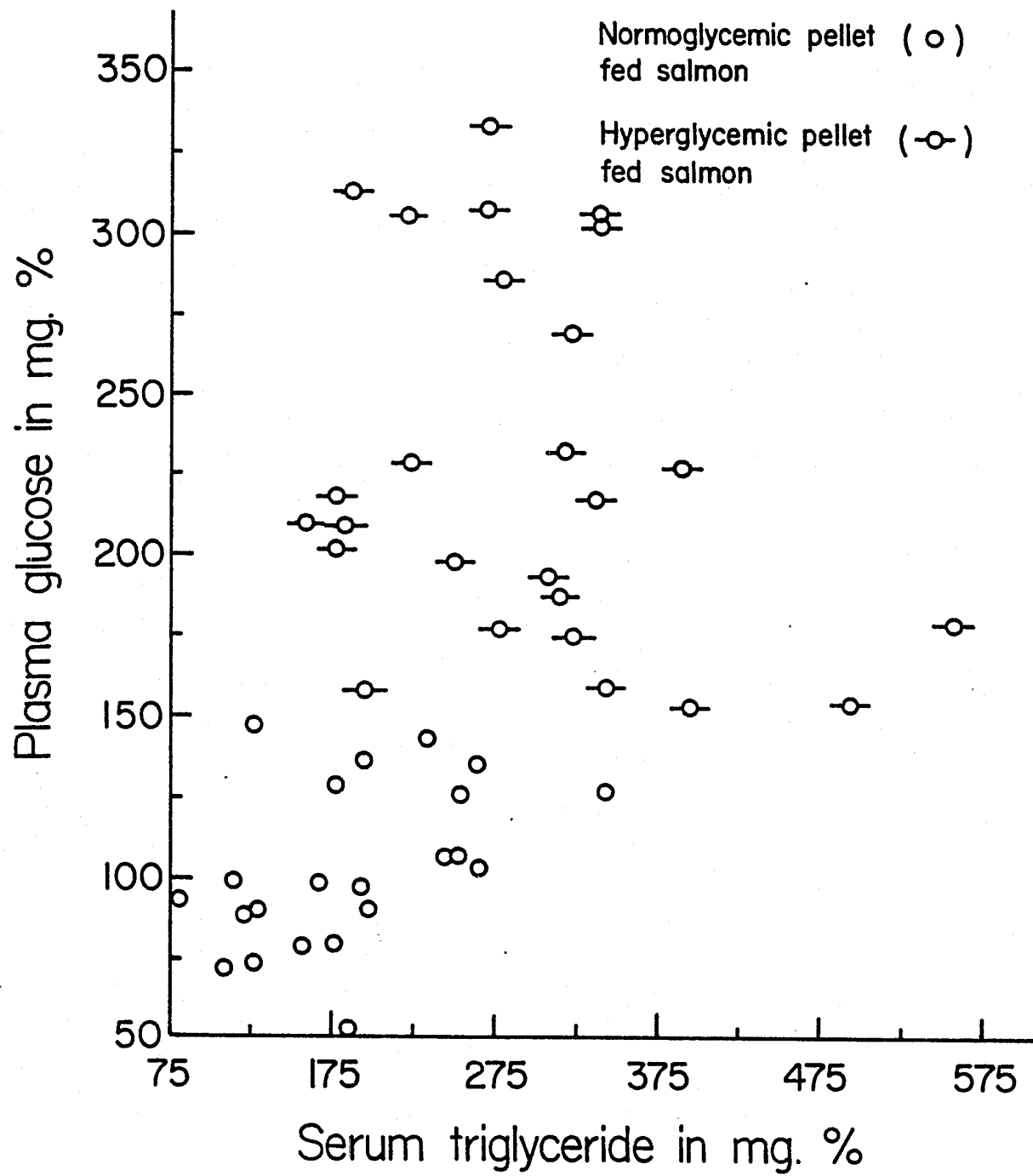
6. In many of the islets of Langerhans of the hyperglycemic pellet fed Atlantic salmon smolts the internal cellular configuration was altered. In general, affected B cells showed such hydropic changes as degranulation, vacuolization, hyperchromatic nuclei, pyknosis and cellular atrophy, instances of B cell hyperplasia and hypertrophy were evident. Hyalinization of islet tissue was prominent. A marked neoformation of islet tissue from pancreatic ductules was observed in all cases. The exocrine pancreatic tissue of these fishes exhibited hyalinization, fibrosis, microangiopathy, lipomatosis and acinar cellular atrophy. There was also an increase sighting of intrapancreatic nerve cells and in one case intrapancreatic ganglia were affected by fibrosis.
7. The B cells of the islets of Langerhans of the normoglycemic pellet fed fish which had elevated plasma triacylglycerol levels, frequently showed hyperplasia and hypertrophy. Mild instances of islet tissue hyalinization were also encountered.
8. The pancreatic tissue of the Atlantic salmon was found to consist of compact and diffuse exocrine elements within which the islets of Langerhans were distributed. The islet themselves showed A or alpha, B or beta and C or clear cells at the light microscopic level.

SYNOPSIS

The physiological condition of the Atlantic salmon smolts (Salmo salar) fed a commercial pellet food diet of high caloric density indicated the presence of spontaneous diabetes. The hyperglycemic syndrome appeared in salmon smolts undergoing accelerated growth induced by qualitatively inappropriate food. Also, the smolts were raised in confined quarters where there was little opportunity for physical activity, another important environmental component thought to lead to the development of spontaneous diabetes. The metabolic aspect of secondary etiological factors includes hypertriacylglycerolemia and an infrequent glucosuria under fasted condition; keto-acidosis does not appear to be a typical condition in fishes. The early stage of the development of the disease is marked by a pronounced hyperplasia and hypertrophy of islet tissue B cells implying a state of hyperinsulinemia which is suggestive of the presence of a form of insulin disorder. The frank diabetic state involves islet tissue changes reflecting a state of hypoinsulinemia accompanied by one of hyperglucagonemia. The major histopathological anomalies affecting both the islets of Langerhans and the pancreatic exocrine tissue are similar to the major changes associated with diabetes mellitus of humans. The Atlantic salmon, may serve as model for a study of the pathogenesis of human diabetes mellitus. Finally, the physiological condition of artificially raised fish should be considered as an important factor when assessing the efficiency of diets.

APPENDIX

Figure 27. Correlation between plasma levels of glucose and plasma levels of triacylglycerol for the pellet fed smolts. The values correlated significantly in the pellet fed group ($r_s=+.51$, Spearman rank correlation; $p < 0.0005$), however, correlation was not significant amongst the normoglycemic and the hyperglycemic pellet fed groups.



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