# CHAPTER ONE

## INTRODUCTION

Salivary glands are present in many groups of animals, both in invertebrates and vertebrates. The invertebrates' salivary glands have quite a different evolutionary origin than that of the vertebrate ones and, therefore, these have not been considered here in this brief review of structure and function of the salivary glands.

In lower vertebrates like fish and amphibians, salivary glands are not well developed. The reptiles possess glands of serous and mucous types. In some snakes, there are 'poison glands', which are serous in nature and appear to be homologous with the parotid gland. The birds also have poorly developed salivary glands.

In mammals, salivary glands are well-developed, their structure varying with the way of life and feeding habits of the animals. There are three pairs of major salivary glands in the mammals and, according to their position, they are called as 'submandibular', 'sublingual' and 'parotid'. These glands lie at some distance from the oral mucosa with which they communicate through extraglandular ducts. Minor glands lie in the submucosa itself and open in the mouth cavity through numerous short excretory ducts. In some cases, minor glands are also well developed to such an extent so as to be considered major glands, as in rabbit (Cragie, 1948; Krause, 1984), the Virginian opposum, Didelphis (Schakelford and Wilborn, 1968; Pinkstaff, 1975) and the Australian brush tail opposum,

Trichosurus vulpecula (Young and van Lennep, 1978). In some cases, sublingual glands may be absent or inconspicuous. In Cynopterus sphinx sphinx and Taphozous kacchensis (Patil, 1984), these are inconspicuous; and in the armadillo Doasypus, these are totally absent (Schakelford, 1963).

All major salivary glands are compound, i.e. their secretory endpieces are drained by a branched duct system. Generally, the glands are lobulated, the parenchyma consists of descrete lobules of closely packed secretory endpieces and intralobular ducts are intralobular connective tissue septa. The lobules are gathered together into more or less distinct lobes, each related to a major branch of the excretory duct tree. As would be expected, the arrangement of blood vessels and nerves conforms to this architecture. The shape of the resultant organ varies considerably, being determined largely by the pressure exerted by the surrounding structures. The parotid gland, lying superficially, tends to be flat and diffuse, spreading out between facial planes. The deeper mandibular and sublingual tend to be more compact, so that in contrast to the parotid, the main excretory duct originates within the gland rather than outside of it.

In recent years, a large amount of experimental, ultrastructural and biochemical research has been carried out on laboratory animals such as rabbit, cat, dog, mouse, and above all, the rat; and a great many histological, physiological and functional data on salivary glands are described in a number of

reviews, articles and books (Andrew, 1964; Kurtz, 1964; Sreebny and Meyer, 1964; Pigman and Gottschalk, 1966; Schneyer and Schneyer, 1967; Jamieson, 1972; Selinger et al., 1974; Chretien, 1977; Young and van Lennep, 1977; 1978; Barka, 1980; Gresik, 1980; and Pinkstaff, 1980).

A) SALIVARY GLANDS OF RAT :

a) Anatomy :

i) Submandibular Glands :- These lie on the either sides of midline and are most conspicuous in structure. They are almost in contact with the midventral line and extend from the level of the hyoid to just proximal end of the mandibulum.

ii) Sublingual Glands :- These structures are closely attached to the anterolateral surface of the mandibular glands and their main excretory ducts share a common enveloping connective tissue sheath. The sublingual gland is somewhat lighter in colour than the submandibular gland and can easily be separated from the sublingual glands.

iii) Parotid Glands :- These are most conspicuous from the lateral view. The glands are diffused extending dorsolaterally behind the ear and caudally to the shoulder and clavicle-lying over their anterior border, are the large and conspicuous exorbital lacrimal gland. The main parotid duct arises behind the exorbital lacrimal gland.

b) Microscopic Anatomy :

i) Submandibular Gland :- The submandibular gland has well defined capsule and a fairly predominant duct system. The most proximal unit of the submandibular gland secretory system is the acinus, usually composed of large polygonal cells, grouped around a small lumen, these cells are called as 'acinar cells'. The acinar cells are surrounded by myoepithelial cells resting upon the basement membrane of acinar cells. The lumen of the acinus opens into a duct system called as 'intercalated duct'. Intercalated ducts consist of a cuboidal or flattened epithelium surrounding a narrow lumen. Intercalated ducts are also lined by myoepithelial cells, the contraction of myoepithelial cells serves to expel the saliva formed from acinus into intercalated duct and from intercalated duct to the next portion (Leeson, 1956; Tandler, 1965; Tamarin, 1966; Cutler and Chaudhary, 1973; Garret and Parsons, 1973). The intercalated duct soon widens to become the striated duct, lined by columnar epithelial cells. In rats, the most proximal portion of the striated duct is further distinguished by the presence of numerous granules and it is convoluted, often separately designated as the convoluted granular tubule (Flon and Gerstner, 1968; Flon et al., 1970; Materazzi, 1967; Materazzi and Vitaioli, 1969; Cutler and Chaudhary, 1973). The cells of convoluted granular tubules are pyramidal in shape (Tamarin and Sreebny, 1965) with basally situated nuclei. The granular tubules extend to form striated ducts. The cells of striated duct are usually simple columar and striated with

centrally situated nuclei. Striated ducts in turn lead into a lesser number of small excretory ducts. Finally, the small excretory ducts merge into one main excretory duct that leads from main glandular mass to the oral cavity, where it terminates (Leeson, 1967).

ii) Sublingual Glands :- These salivary glands are uncapsulated, they differ from submandibular in the fact that they are pale in colour and a majority of their alveoli are of mucous type. Acinar cells are mucous in nature and surrounded by serous demilunes. Intercalated, striated and intralobular ducts are poorly developed.

iii) Parotid Glands :- The gland is enclosed in a well defined fibrous connective tissue capsule. The cells are pyramidal in shape. The duct system is well developed, consisting of intercalated duct, striated ducts and excretory duct.

B) SECRETION AND ITS CONTROL :

Secretion and its products are more or less complex molecules. They are synthesized and accumulated in the cytoplasm and latter extruded to the outside of the cell after proper stimulus. Secretion of saliva from the salivary glands is under nervous as well as hormonal control.

a) Nervous Control :

In all the animals, nerves from both the divisions of the autonomic nervous system, parasympathetic and sympathetic,

can be found going to all the three great salivary glands. Initiation and maintenance of the secretion by the salivary glands is almost exclusively dependent on the parasympathetic and sympathetic nerves. Parasympathetic stimulus is a stronger stimulus for secretion and its fibres are distributed to all the salivary glands. These nerves are cholinergic and their released transmitter, acetylcholine, either directly stimulates the receptors on the secretory cells or stimulates the intrinsic cholinergic nerves that release the additional quanta of acetylcholine. Each acinar cell has five to ten axons, converging on its receptors. These receptors are bound to the cell membrane and may be identical with membrane-bound enzyme, guanylate cyclase, which manufactures cyclic GMP within the cell. The electrical response of the membrane to acetylcholine is rapid depolarization. Parasympathetic nerve stimulation not only starts and maintains secretion within the acinar cells and intercalated ducts but also activates transport events in duct cells that change the initial secretion into the final saliva.

Sympathetic nerves pass to the salivary acini and, on stimulation, evoke secretory response. These nerves release catecholamines (norepinephrine, epinephrine and dopamine) that affect two receptors on the secretory cell membrane, namely the ($\alpha$) and ($\beta$) andregenic receptors. Sympathetic changes in salivary secretion seems to result mainly from the activation of $\beta$-andregenic receptors.

The dual autonomic regulation of these glands is unusual

because the parasympathetic and sympathetic system, both stimulate secretory, metabolic, trophic, muscular and circulatory functions in similar directions.

b) Hormonal Control of Ducts and Acini :

Since Professor Lacassagne's discovery of sex dimorphism in mouse submandibular gland (Lacassagne, 1940), numerous investigations of the hormonal control of this gland have been made. These have established that, principally, three hormones have an influence on the submandibular gland secretion; androgenic, thyroid and adrenal (Grad and Leblond, 1949; Arvy and Gabe, 1950; Shafer and Muhler, 1955; Raynaud, 1960, 1964). At the same extreme, the purely mucous sublingual gland appear to be relatively free of the hormonal control (Baker and Abrams, 1955). Baker and Abrams also showed that thirtyfive days after hypophysectomy, the absolute weight of the parotid gland, and the ratio of parotid weight - body weight are reduced significantly over those of non-hypophysectomised rats.

i) Influence of the Adrenal Gland :- In castrated adrenalectomised rats, the submandibular gland underwent a loss of weight. The gland exhibited complete atrophy of the secretory tubules. The cells of the tubules no longer showed signs of secretory activity, they were low cuboidal with eosinophilic striations at the basal aspect, but no granules at the apical aspect. These results suggest a specific adrenal action on the submandibular gland (Raynaud, 1964). In adrenalectomised animals, small doses of androstene-dione has

little action on the tubules, both in males and females. This observation led to the thought that the gland acted either through an intermediatry or in co-operation with another gland.

ii) Influence of the Thyroid Gland :- The influence of the thyroid on the development and secretion of the submandibular gland in the rat has been shown ( Leblond and Grad 1948; Grad and Leblond, 1949; Sreebny et al., 1957; Ranand, 1964; Humphreys and Michael 1987). Thyroid destruction entails a loss of weight of the submandibular gland. In females, there is a complete regression of secretory tubules. They are lined by low cuboidal cell and completely devoid of granules, but in males, destruction of thyroid does not induce comparable atrophic changes in the secretory tubules (Raynaud, 1964). In thyroidectomised castrated rats, thyroxine alone cannot restore the normal secretory activity of the tubular segement. The joint action of testosterone is necessary (Leblond and Grad, 1948; Grad and Leblond, 1949; Sreebny et al., 1957).

iii) Influence of the Hypophysis :- Baker and Abrams (1955) showed the influence of hypophysis over salivary glands. They showed that hypophysial factor plays an important role in maintaining the parotid and submandibular gland acini. Baker and Abrams showed that thirtyfive days after hypophysectomy, parotid gland weight reduced significantly. Concurrently, acinar cells became smaller and contained fewer and smaller zymogenic granules. Considerable variations occurred in the

histology of the acinar portion of the submandibular gland after hypophysectomy (Yoshimura, 1956; Argonz and de Corral Saleta, 1960; Bixler et al., 1957, 1959). The acid mucopolysaccharides of the cells were depleted. Similarly, diffuse staining with PAS was reduced. PAS +ve granules were usually reduced.

iv) Influence of Hormones over the Convoluted Granular Ducts :- Numerous reports have appeared related to the effects of altered endocrine function on the convoluted granular ducts. A tremendous work has been performed on the effect of castration, testosterone administration and castration followed by testosterone on male mouse and rat submandibular glands and much of this work was reviewed by Chretien (1977). She has reported a decrease in the height of the granular duct cells from 27 to 16 µm within one month following the castration, and width of cells at the basal region decreased from 16 to 8 µm. Chretien (1977) also reported reversal of these changes after an injection of testosterone propionate. Apart from this, the convoluted granular tubules have been considered to be the site of synthesis of a number of polypeptides, whose synthesis is again hormone dependent. Amylase is secreted by granular ducts in mouse and hamster (Chretien and Zajdela, 1965; Smith, et al., 1971; Smith and Frommer, 1972 a,b). The granular ducts have also been considered as a source of salivary kallikrenin (Orstavik et al., 1975) and non specific proteases (Bhoola et al., 1973; Ekfors and Hopsu-Havu, 1971; Junqueira et al.,

1949; Lagunoff et al., 1962; Riekkinen and Niemi, 1968; Shafer et al., 1959; Sreebny and Meyer, 1964). It has also been suggested that the role of granular ducts is a source of renin which is known to be present in high concentrations in submandibular glands of rodents (Bhoola et al., 1973; Bing and Faarup, 1965; Bing et al., 1967; Gutman et al., 1973) and salivary gland hormones, Nerve Growth Factor (NGF) (Ellison, 1967; Goldstein and Burdman, 1965; Hendrey and Iversen, 1973; Schwab et al., 1976), Epidermal Growth Factor (EGF) (Cohen, 1962; Cohen and Elliot, 1963; Turkington et al., 1971; Ladda et al., 1979), Mesodermal Growth Factor (Weimar and Haraguchi, 1975; Liske and Reber, 1976) found by RIA and non-fluorescent staining the highest concentration of non-suppressible insulin like activity and glucagon like immunoreactive substances were showed in the submandibular gland of several species (Silverman and Dunbar, 1974; Lawrence et al., 1975; 1976 a,b, 1977; Bathena, 1977; Pisanty et al., 1975; Dunbar et al., 1977; Smith et al., 1979) carried out number of experiments in which rats were subjected, 2-4 weeks after the removal of the submandibular glands, to arginine or epinephrine infusion, fasting, insulin-induced hypoglycemia or oral glucose load and came to the conclusion that the submandibular glucagon does not contribute to plasma levels of glucagon, nor does it significantly affect the handling of the carbohydrate metabolism.

v) Influence of Insulin on Salivary Gland :- Sporadic

clinical observations have since long been implicated the salivary glands in diabetes but yet their role in glucose metabolism remains unknown.

Several investigators worked to find out the effect of insulin on carbohydrate metabolism and secretory functions of the salivary glands. Palla et al. (1967) carried out a comparative study of amylase biosynthesis in the rat pancreas and parotid gland in experimentally induced diabetes rats. Liu and Lin (1969 a,b) showed the relationship between the growth of salivary glands, insulin and growth hormone. Szymczyk et al. (1971) showed the effect of alloxan diabetes on the content of sialic acids and the activity of Uridyl transferases in the rat salivary glands. Murakami (1974) studied several enzymes for carbohydrate metabolism in rat submandibular gland in response to experimental diabetes and insulin treatment. After Palla et al. (1967), Zebrowski and Brimmer (1978) also studied amylase levels in parotid glands of experimentally-induced diabetic rats, in addition to parotid glands, they have also studied submandibular glands. Pillai et al. (1989) studied proteolytic enzymes like trypsin and amylase in all three major salivary glands in alloxan induced diabetic rats. The peroxidase activity in the diabetic salivary gland was studied by Anderson and Shapiro in 1979 and in 1980. They have also studied the effect of alloxan diabetes and insulin on protein synthesis, DNA and RNA. Anderson and Johnson (1981) studied only parotid gland and saliva during diabetic condition.

According to them, the weight of the parotid gland remained unaffected but the amylase level is reduced and the peroxidase activity was significantly increased both in parotid and saliva. They have also showed histochemically accumulation of lipid within the acinar cells. Anderson (1983) again studied parotid glands of alloxan-treated rats. He showed reduction in parotid gland weight, RNA and protein and amylase activity (40 %). In contrast, he showed increase in peroxidase activity (54 %). Jaffa et al. (1984) studied kallikrinin levels in diabetic rats, they found significant reduction in this enzyme in the submandibular gland. Masu Nobuo (1986) showed that the amount of collagen in the submandibular glands of streptozotocin-treated rats is lower than that of the controls. Pillai and Nadar (1987) studied lysosomal enzymes in diabetic rat salivary glands, where they found significant increase in the levels of β-glucoronidase and acid phosphatase in all the salivary glands. The effect of alloxan diabetes on the morphology and histology of murine salivary glands was studied by Dixon (1987). Decomargo et al. (1981) treated post-pubertal male mice with alloxan and showed changes in the ultrastructure of the submandibular gland tubules consistent with feminization changes.

The above review clearly indicates that the insulin is a required hormone for growth and secretion of submandibular and parotid gland. A few reports also indicate dependency of the sublingual on insulin. A number of investigators studied salivary glands in the diabetic conditions, keeping in front of

them two views :

1) effect on carbohydrate metabolism in salivary glands;

2) effect on secretory activity of salivary glands.

But yet no one has studied the effects of acute metabolic disorders like changes in lipid metabolism and ketosis of diabetic mellitus on salivary glands.

C) EFFECTS OF INSULIN WHICH ARE INDEPENDENT OF GLUCOSE METABOLISM :

a) Protein Synthesis :

Insulin not only affects the carbohydrate metabolism in various tissues but it carries a number of other functions too, insulin is an important protein anabolic hormone, its deficiency leads to negative nitrogen balance by reducing peripheral protein synthesis and by diverting amino acids for neoglucogenesis. According to Wool and Cavicchi ( 1967), insulin changes the translation of genetic information into protein synthesis at the ribosomes in a short time, since actinomycin D does not influence this effect of insulin, whereas puromycin and cyclo-heximide do. This shows that insulin has a direct effect on the ribosomes to increase the translation of messenger RNA, thus forming new proteins. Insulin causes active transport of many of the amino acids into the cells. Among the amino acids most strongly transported are valine, leucine, isoleucine, tyrosine and phenylalanine, over a longer period of time. Insulin also increase the rate of transcription of RNA in the

cell nuclei, thus forming increased quantities of RNA. Insulin also inhibits the catabolism of proteins, thus decreasing the rate of amino acids released from the cells (Guyton, 1986; page no. 928).

b) Antilipolytic Effect of Insulin :

The antilipolytic effect of insulin is of special importance to the consideration of the metabolic disorders of diabetes mellitus. Insulin inhibits the release of glycol and free fatty acids by adipose tissue of the rat. Triglyceride lipase determines the rate of lipolysis and is inhibited by insulin (Foresch et al., 1965; Jungas, 1966; Mahler, 1964; Raghupathy et al., 1975; Pushpendran and Eapen, 1974).

c) Ketosis :

During insulin deficiency, glucose is no longer a major substrate for cellular metabolism; free fatty acids and ketones serve as main fuel (An-quoc et al., 1984; Renner et al., 1984; Chen et al., 1987; De Fronzo et al., 1987; Severson et al., 1987). Lipase liberates free fatty acids from deposits of triglycerides from liver and adipose tissue. The free fatty acids release is no longer adjusted to the actual needs and ketone bodies are formed from free fatty acids and their presence for longer time in blood is called as 'ketosis' (Guyton, 1986; page no. 78; Jirgen et al., 1972; Wilson et al., 1987).

d) Lipoprotein Metabolism :

Large portions of fatty acids are also utilised within

the liver itself to synthesize triglycerides. Most of these, are then released from liver cells to the blood in the form of lipoproteins, the hydrophobic lipids (triglycerides and cholesterol esters) are transported in blood plasma in the core of lipoprotein particles, the surface of which is coated with polypeptides (apolipoprotein) and hydrophilic lipids (phospholipids and cholesterol) (Brewer, 1981; Jackson et al., 1976). To cover energy requirement in different tissues, fatty acids are transported either in non-esterified form bound to plasma or in esterified form as triglycerides, transported in chylomicrons and very low density lipoprotein (VLDL) secreted from liver (Michael et al., 1983; Cupp et al., 1987). The factors known to influence the rate of secretion of VLDL particles are the plasma insulin (Olefsky et al., 1974) and the plasma free fatty acid (Kissebah et al., 1976). The factors regulating the transport of triglycerides are distributed in the blood and diurnal variation of lipoprotein lipase in different tissues. In adipose tissue, lipoprotein lipase activity has been found to be increased about 3 to 6 hours after repeated meals (Lithell et al., 1978 a,b; PaganoMirani et al., 1983; Pykalisto et al., 1975) and insulin seems to be an important hormonal regulator for the regulation of the lipoprotein lipase activity (Sadur and Eckel, 1982; Yki-Jarvinen et al., 1984; Mochaziki et al., 1985) showed that following the streptozotocin induced diabetes rats' hearts contained lower lipoprotein lipase activity than normal. Lipoprotein lipase activity also changed during pregnancy and lactation (Champigny

et al., 1987).

The salient features of this review on salivary glands shows that :

a) Salivary glands of rats are under the control of various endocrine glands like thyroid, pituitary, testes, adrenal, pancreas, etc. Secretion of these glands is influenced by the presence of hormones secreted by these glands.

b) In the study of control of endocrine pancreas in salivary glands, attention was given only to the carbohydrate metabolism and secretory activities of submandibular and parotid glands only. Some reports described the effect of insulin on protein, DNA and RNA metabolism of submandibular and parotid glands. Why the sublingual gland is neglected by several workers in this study is not understood except Pillai and Nadar,(1987; Pillai et al., 1989).

D) THE REASONS THAT LED TO THE UNDERTAKING OF THE PRESENT INVESTIGATION :

a) During diabetic condition, not only glucose metabolism is disturbed, but there are some severe metabolic changes in the body, like ketosis, which are sometimes fatal. There are also changes in lipid, lipoprotein and protein metabolism.

b) The effects of these metabolic changes in different organs in diabetic conditions were studied by several workers in

many insulin sensitive tissues (Randle et al., 1964, 1966; Thompson and Williamson, 1975; Elkles and Hambley, 1977; Felber et al., 1977; Behera and Patnaik, 1981; Sharma et al., 1981; Robert et al., 1982; Knauer et al., 1982; Nelson et al., 1982; Nomura et al., 1984; Mayfield et al., 1985; Sochor et al., 1985; Aguis et al., 1985; Cheshchevik, 1985; Serrano et al., 1985). But none has attempted to study the effects of above mentioned metabolic disorders occurred due to diabetic condition in the salivary glands. Pillai and Nadar (1987) studied lysosomal enzymes like β-glucuronidase and acid phosphotase in diabetic salivary glands. They showed an increase in the enzyme activities in diabetic salivary glands and related to the formation of ketone bodies due to diabetes. Similar changes were also shown by Chari et al. (1983) in blood cells. Mohanam and Bose (1983) also showed an increase in the lysosomal enzyme activities in the connective tissue of diabetic rats.

In the present investigation, it was decided to study the changes in the enzyme activities like lipase and esterase.

We have selected lipase and esterase because of the following reasons :

1) Lipase is involved in lipid metabolism. One form of lipase is lipoprotein lipase, whose activity is regulated by insulin. It is at a higher level in the presence of insulin, while the other forms of lipase are involved in lipolytic activity

even in the absence of insulin and they are present in the plasma and pancreatic lipase ~~is present~~ is secreted in the duodenum, whereas lipoprotein lipase is a tissue specific enzyme.

2) There is an elevation of lipid peroxides in plasma and also in liver (Pritchard et al., 1986). It was intended to know whether there is a lipid peroxidation of the membrane of salivary gland cells during diabetes, due to which there might be an increase in the lysosomal enzymes. Previously we have shown an increase in β-glucuronidase and acid phosphatase in diabetic salivary glands, which are lysosomal enzymes, esterase is also a lysosomal enzyme, we want to find out effects of induced diabetes on esterase activity to confirm the formation peroxides and damage to the salivary gland membranes.

E) PLAN OF PROPOSED WORK :

Keeping in view the above mentioned reasons and the amount of work done on the different organs and salivary glands in diabetes mellitus, it was decided to study enzymes like lipase and esterase of salivary glands of albino rats (Rattus norvegicus) in diabetic condition.

A) Choice of the Animal

While selecting the subject for research the care was taken to select such an animal which is commonly used in the

study of diabetes. Male albino rats were selected for the study to avoid hormonal fluctuations of females. Animals were collected from Hindustan Antibiotics, Pune. They were reared in the animal house of Zoology Department, Shivaji University, Kolhapur for 10 days.

b) Choice of the Techniques

i) Diabetes is induced with the help of alloxan monohydrate, of course it can be very well induced with the help of streptozotocin, glucose loading and removal of pancreas. Though streptozotocin is commonly used, at the time of experimentation it could not be made available. For glucose loading time is required that is why we have avoided that technique, because of short of time for this course. The removal of pancrease is not adopted to avoid changes that would take place in glucagon concentration.

ii) As this is M.Phil dessertation we could not go for isolation, kinetics and histochemical studies of lipase and esterase, only their biossays were carried out. These studies will be included in the next plan.

C) Critical evaluation of the observations

i) The results obtained in the present investigations were analysed critically using Standard Error and Students' 't' test. They were tubulated and represented with the help of histograms and line graphs.

ii) The changes in the enzyme activities were studied in relation to glucose concentration in blood, irrespective of normal and diabetic condition and in progressing diabetes, ~~because~~ In alloxan induced diabetes there was gradual increase in the blood glucose levels. The changes were studied during fasting and feeding, because organs studied were salivary glands which are in a resting stage during tasting and their secretion was activated in during feeding.

iii) The results obtained were discussed with the help of available literature.

F) PRESENTATION OF THE THESIS

It was decided to divide the present thesis into five chapters, the first being on the introduction, it gives brief review of salivary glands in general, their secretion and control, the reasons that led to the present investigation and plan of proposed work.

The second chapter describes the material and methods employed for the present investigations. The third chapter describes the effect of alloxan diabetes on glucose level of blood, body weight and esterase bioassays in salivary glands, at different intervals after the induction of diabetes during fasting, and fasting and then feeding.

The fourth chapter includes effects of induced diabetes on lipase activity in rats at different time intervals

after the induction of diabetis during fasting and twelve hours of fasting but one half hour feeding thereafter.

The fifth chapter concludes the whole work in the form of summary and concluding remarks.

A detailed bibliography is given at the end of the thesis.