SCHOOL OF POSTGRADUATE STUDIES
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CERTIFICATION

THIS IS TO CERTIFY THAT THE DISSERTATION -

Influence of Genetic and some Dietary Factors on Glucose Tolerance in Normal and Alloxan-Induced Diabetic Rats (*Rattus norvegicus*)

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UNIVERSITY OF LAGOS
DISSERTATION APPROVED
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ON.....DAY OF.....19.....

INFLUENCE OF GENETIC AND SOME DIETARY FACTORS ON GLUCOSE TOLERANCE IN NORMAL AND ALLOXAN–INDUCED DIABETIC RATS (RATTUS NORVEGICUS)

BY

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B.Sc. Biology (Unilag), 1984.

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF PHILOSOPHY (BIOLOGY) IN THE AREA OF GENETICS, UNIVERSITY OF LAGOS

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DEDICATION

To my first degree relatives consisting of my parents: Mr & Mrs. S. A. Taiwo, and my sibling most especially Mr. Debo Taiwo.

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I am greatly indebted to my supervisor, Dr. P.G.C. Odeigah. His unrelenting encouragement, valuable suggestions and constructive criticisms constituted an important driving force in this study.

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Above all I give glory and thanks to God Almighty for giving me the life and strength to finish this research project.

A winner never quits and
a quitter never wins.
He who fights and runs away
lives to fight another day.

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disease as a major public health problem has been stressed in several reports (Cahill, 1979; Stout, 1979; Yudkin; 1986). The complicated pathophysiology of diabetes has also been discussed in various aspects by Soskin (1941) and Young (1941). Apart from the classical symptoms such as glycosuria, polyuria and emaciation, other important symptoms include polyphagia (excessive appetite) and polydipsia (excessive thirst). However, diabetes can be completely asymptomatic, or it can appear as an isolated disorder of any organ or system. Fulminant ketoacidosis (elevated concentration of ketone bodies in the body tissues and fluids) which is fatal unless immediately treated, may be the first sign (Cahill, 1979).

Often diabetes is manifested by one of the long-term complications which are many and severe. For example, the presenting event may be of a myocardial infarction in a young man, an unexpectedly large newborn, pruritus vulvae in the female, and recurrent skin infections. Other pathologic states such as foot ulcer, retinopathy (non-inflamatory disease of the retina), or proteinuria (protein in urine), or many other phenomena which at first glance appear unrelated may provide a clue to the diagnosis of diabetes (Cahill,1979). Diabetes mellitus is protean in its manifestations and this variability is central to its diagnosis (Cahill, 1979; Yudkin, 1986).

In Yudkin's account, it was pointed out that people with diabetes are at risk of certain infections when their blood glucose control is poor. There may be carbuncles or

boils and a particularly dangerous infection can spread as a necrosing process through skin and fiscia (necrosing fasciitis). Changes also take place in the capillaries of the retina and the renal glomerulus in longstanding diabetics. The damage is produced by the cumulative effects of elevation of blood glucose, so that 80% of diabetics have some retinopathy 20 years after diagnosis. Generally, over 1 percent of all diabetics go blind every year from retinopathy. Much of this is, however, preventable (Yudkin, 1986).

Vascular disease (arterial, arteriolar and capillary) is the largest and most intractable problem in clinical diabetes (Baird and Strong, 1974). In a group of 370 male diabetics in an industrial population maintained for 10 years, the death rate of diabetics was 2.6 times higher than that of the matched controls, and the excess mortality was highest in the group under 45 years of age (Pell and D' Alonzo, 1970). The most striking difference was seen in the incidence of coronary heart disease which caused the deaths of nearly three times as many diabetics as non-diabetics. Other workers have shown a high frequency of abnormal oral and intravenous glucose tolerance and hyperglycaemia in subjects with Ischaemic Heart disease (Kingsbury, 1966; Wahlberg and Thomasson, 1968; Falsetti et al, 1970; Sloans et al, 1970). Other organs or systems such as the kidney and nervous system are also affected in diabetes (Baird and Strong, 1974; Yudkin, 1986). Even the skin and the small intestine are not spared.

Diabetes has been found in all human societies where any consistent search has been made. The same is true of animals, particularly those living in association with man, whether as domestic or laboratory animals. Thus diabetes has been described in mice, rats, cats, dogs, pigs, horses, cattle, sheep and goats in addition to foxes (Fox, 1923), monkeys (Sokoloverorva, 1960; Hamilton and Brobeck, 1963), and hippopotamuses (Hayashi, 1967). Diabetes, particularly in domestic animals, has been comprehensively reviewed by Brunk (1968).

Whereas the familial occurrence of diabetes has been described in many animals, the precise genetic basis has only been established in a few (Renold, 1968). Quite significant is the fact that the genetic basis for the occurrence of hyperglycaemia and/or obesity may be a single gene mutation as in the case of mice (Renold, 1968). It may also be a complex and possibly variable polygenic system as in the case of the Chinese hamsters (Butler, 1967). Indeed, in Acomys cahirinus (spiny mice) or Psammomys obesus (sound rats) the inherited feature of the abnormal metabolic trait is simply derived from the apparent generalized predisposition of these species to the development of diabetes and/or obesity. It is quite likely that the availability of sufficient information on a sufficient number of generations of these animals would lead to conclusions similar to those for the mice or Chinese hamsters. These animals can be used as animal models of human diabetes mellitus which is also known to have important genetic component.

Apart from diabetes that arises spontaneously in animals as a result of gene mutation, diabetes can also be produced experimentally by administration of a compound known as alloxan (Lukens, 1948; Howell and Taylor, 1967). This type of experimental diabetes was first produced by Dunn et al (1943) when they discovered that alloxan caused necrosis of the pancreatic islets. Analysis of the literature clearly showed that the majority of works on diabetes using animal models were carried out using alloxan - diabetic rats. This is due to the fact that unlike in genetically diabetic rats where animal models usually arise from spontaneous gene mutation and selective breeding over a long period of time (Renold,1968), experimental diabetes can easily be induced in animals by alloxan administration. Moreover, diabetes of a desired grade of severity can easily be obtained by giving a particular does of the compound (Lukens, 1948). Thus, alloxan - induced diabetic rats were used as animal model of human diabetes in this study.

According to Baird and Strong (1974), two main types of human diabetes have long been recognised, and it is now clear that the level of plasma insulin correlates well with the clinical picture and the type of treatment required in each type of diabetes. The two main types are the juvenile - onset (type 1) diabetes mellitus and the maturity - onset (type 2) diabetes mellitus. Based on necessity for insulin therapy, juvenile - onset and maturity - onset diabetes are commonly and preferably referred to as insulin -dependent diabetes mellitus (IDDM) and noninsulin - dependent diabetes mellitus (NIDDM) respectively (Baird and Strong, 1974; Cahill, 1979).

Insulin - dependent diabetes mellitus (IDDM) usually develops during the first 40 years of life in patients of normal or less than normal weight. The majority develop severe symptoms of diabetes acutely over a period of several weeks or months. If treatment with insulin is delayed fatal ketoacidosis rapidly develops. The noninsulin - dependent diabetes mellitus (NIDDM) usually appears in middle-aged or elderly patients who are often obese and in whom hyperglycaemia can usually be controlled by dietary means alone or, if not, by an oral hypoglycaemic compound. Insulin is detectable in the plasma of nearly all patients in this category, and they are therefore less prone to develop ketosis (ketoacidosis). In this sense the disease is less severe than in the insulin - dependent type; however, the complications associated with long-term diabetes occur in both types (Baird and Strong, 1974; Cahill, 1979).

It is now clear that many environmental factors may contribute to the development of diabetes in a genetically predisposed subject (Barnett et al; 1981; Horton, 1983). According to Horton (1983), the environmental factors that cause predisposition to noninsulin - dependent diabetes mellitus (NIDDM) include diet, obesity, physical inactivity, various forms of stress, hormonal imbalance, drugs, toxins and ageing. The degree of carbohydrate intolerance depends on the interaction between these environmental and genetic factors. Certain HLA (human lymphocyte antigen) associations have been implicated for causing predisposition to insulin-dependent diabetes mellitus (IDDM). The HLA region which is

on the short arm of chromosome six consists of several genes, many of which also play important role in immune response. These have been broadly classified into the HLA class I, II and III genes. The class II region encodes the major susceptibility to insulin-dependent diabetes mellitus (IDDM). The possible predisposing environmental factors to this type of diabetes include viruses, drugs and chemicals (Barnett and Todd, 1990). Therefore, effort to prevent or treat diabetes (NIDDM or IDDM) should be aimed primarily towards eliminating the predisposing environmental factors. Also, the genetic component needs to be more clearly defined for better therapeutic approach to diabetes.

It is well known that dietary treatment is a primary therapy in NIDDM and is a vital injunctive treatment to IDDM (Gill, 1990). The observation that diet is the fundamental element of therapy in most cases of diabetes is perhaps the only uncontroversial conclusion of the University Group Diabetes Program, which cast some doubt on the value of at least two drugs widely used in the treatment of diabetes (Mann, 1980). Based on the importance of diet in diabetes and the doubt surrounding the efficacy of some anti-diabetic drugs, it will be interesting to consider the glycaemic effects and, therefore, the possible therapeutic significance of many local food stuffs in diabetes.

The influence of a high carbohydrate diet on glucose tolerance and its possible role in the pathogenesis of NIDDM have already been the subject of extensive investigations. Many years ago, Himsworth (1935) demonstrated that very low

carbohydrate diets (50g a day) caused impaired glucose tolerance in normal volunteers, and that very high carbohydrate diets (500g a day) have an opposite effect. Brunzell et al (1971) similarly reported improved glucose tolerance with high carbohydrate feeding in mild diabetes whereas Grey and Kipnis (1971) found a 50 percent decrease in fasting blood glucose concentrations and no change in glucose tolerance. These studies suggest that over-eating has a positive effect on glucose disposal efficiency in nondiabetic subjects.

Gain in body weight which may lead to obesity is an important long term consequence of overfeeding. The work of Sims et al (1973) on experimentally induced obesity is important in this respect. Their research was conducted to determine if the metabolic abnormalities commonly associated with long - standing, spontaneous obesity would develop if lean men with no personal or family history of obesity become obese by overeating. In groups of subjects who gained approximately 25 percent above their original weight and whose adipose tissue mass doubled (by increasing adipose cell size), fasting and glucose - stimulated plasma insulin concentrations were increased. Oral and intravenous glucose tolerance were decreased, but did not become abnormal. Moreover, insulin resistance in both muscle and adipose tissue was revealed by fore-arm perfusion and in vitro incubation techniques (Horton et al, 1975).

Thus when subjects were at their initial, lean body weight the higher carbohydrate diet seemed to be associated

with increased basal and insulin - stimulated rates of glucose metabolism. After weight gain, the responses to insulin were significantly decreased indicating the development of insulin resistance. Thus, high carbohydrate diets per se (ignoring the effects of the resulting obesity) seem capable of increasing insulin sensitivity leading to improved plasma glucose response.

It is however important to note that the studies which imply that high carbohydrate feeding may increase insulin sensitivity and, therefore, improve glucose metabolism were conducted in nondiabetic subjects; the results might be different in diabetics. This is because it is possible that high carbohydrate diets may be deleterious in that group (Coulston et al, 1983). Also, analysis of the existing reports indicating increased insulin senstivity resulting from high carbohydrate diet (Himsworth, 1935; Brunzell et al 1971) revealed that a large part of the improvement in glucose tolerance took place when dietary carbohydrate was increased from low (less than 10%) to moderate (30-40%) of daily caloric intake. Further, significant improvement did not occur until the carbohydrate intake was increased to approximately 60-70% daily caloric intake. Moreover, the crucial issue is not whether high carbohydrate diets improve insulin sensitivity, but whether plasma glucose concentration will be reduced in diabetics fed with high carbohydrate diets. This is important because diabetes is a disorder whose best known characteristic is elevation of blood glucose (WHO, 1985).

There are reports of significant deterioration in glucose tolerance resulting from high carbohydrate feeding in certain diabetic patients (Reaven and Olefsky, 1974; Ginsberg et al, 1976). Simply increasing dietary carbohydrate intake by 12-15% can lead to significant elevations of postprandial (after meal) glucose concentrations in patients with "chemical" diabetes and even normal subjects. Since these studies were performed with liquid formula diets, it is important to consider what happens when such patients are eating solid food. It was noted that increasing dietary carbohydrate from approximately 40% to 55% did not lead to deterioration in diabetic condition in a 20-week out-patient study (Weinser et al, 1974). However, approximately half of the 18 patients studied had a fasting plasma glucose concentration (FPGC) of less than 125 mg/dl (nondiabetic level). The two patients with FPGC in excess of 200 mg/dl (diabetic) demonstrated a two - to three - fold increase in 24 - hour urine glucose excretion on the 55 percent carbohydrate diet.

In 1979, the Food and Nutrition Committee of the American Diabetes Association published dietary recommendations advocating a high carbohydrate diet for diabetics. This was followed in 1980 by advice from the special Report Committee of the Canadian Diabetes Association that the diets of all diabetic patients should be 45 percent carbohydrate or more. Later, concern was expressed that official liberalisation of a previous carbohydrate restriction might be used as a license to consume those carbohydrate foods which would

compromise good diabetic control (Reaven, 1980; 1981). This matter has been well debated (Jarret, 1981a; 1981b). One outcome has been to re-emphasise the possible therapeutic value of fibre supplement in diabetic diets (William et al,1980). This is based on the observation that diets with high fibre content improve glucose tolerance in diabetic subjects (Mann, 1980).

A major impediment to the therapeutic use of fibre in diabetic diets is, however, the requirement that they should be intimately mixed with the food, to simulate a situation analogous to that found in unprocessed foods (Jenkins et al, 1979; Williams et al, 1980; Cohen et al, 1980). At present the clinical use of purified fibre supplement is therefore limited both by this requirement and by the unpalatability of the viscous fibre materials. Only two products, an experimental guar crispbread (Jenkins et al 1978; Jenkins et al, 1980) and granulate (Aro et al, 1981) have been found to be palatable and effective but neither is produced commercially. Since diet still remains the fundamental aspect of diabetic therapy (Mann, 1980; Jenkins et al, 1982; Gill,1990), it is necessary to investigate some other common dietary substances for their effects on glucose tolerance. This will bring to attention, the possible therapeutic significance of such dietary substances in diabetes mellitus. The dietary substances which have hitherto received little or no attention in this respect include common salt (NaCl) and common varieties of pepper (Capsicum annuum L. fam. Solanaceae).

Much of the previous works on common salt (Nacl) were on its cardiovascular effects while those on pepper particularly the cluster peppers (C. annuum var. fasciculatum Sturt) have been focused on its pharmacodynamics and toxicology. Extensive data from epidemiological, clinical and animal experimental studies have indicated a causal relationship between salt consumption and blood pressure (Dahl, 1972; Luft et al, 1977; Lenel et al, 1948; Meneely et al, 1953). Furthermore, elevation of plasma cholesterol by chronic excess salt feeding in rats and dogs has been suggested as one possible biochemical basis for a link between atherosclerosis and hypertension in man (Dahl, 1960). The pharmacodynamics and toxicologic effects of pepper on intestinal absorptive cells due to its constituent pungent principle called capsaicin has also been studied (Lille and Ramrez, 1935; Sirsatanic and Khanolkar, 1960; Nopanitaya and Nye, 1974; Monsereensor, 1980).

A detailed discussion on the structure, uses and systematics of C. annuum can be found in Purseglove (1968). Essentially, C . annuum is a variable herb, or sub-shrub which is sometimes woody at the base. It is much branched, erect and 0.5 - 1.5m high. The fruit is by far the most important part of the plant based on its taxonomic, dietary and medicinal significance. The fruit is an indehiscent many-seeded berry that is variable in size, shape, colour; and degree of pungency. It is green or purplish, ripening to red, orange, yellow, brown, cream, or purplish.

The considerable taxonomic importance of the fruit is attested to by its use in the separation of the species (C. annuum) into seven botanical varieties. Out of these, three varieties were chosen for this study because of their prevalence and common use for cooking in Nigeria (Plate 1). These are C. annuum var. fasciculatum Sturt, C. annuum var abbreviatum Fingerh and C. annuum var. grossum (L.) Sendt. Brief taxonomic descriptions of these varieties by Purseglove (1968) are presented below:

(i) C. annuum var. fasciculatum Sturt - Fruits are clustered, erect, slender, about 7.5cm long and very pungent. As the fruits are not borne singly it is probable that these are forms of C. fruitescens.

(ii) C annuum var. abbreviatum Fingerh - Fruits are generally ovate, wrinkled, 5cm long or less.

(iii) C. annuum var. grossum (L) Sendt - Fruits are large with basal depression, inflated, red or yellow, flesh thick and mild.

The dietary importance of peppers, according to Purseglove (1968), include mainly their use in cooking in various ways or being eaten raw in salads. The pungent property of peppers contributes most significantly to their dietary importance. In Nigeria, particularly in the southern part, it is believed that taking the pungent pepper promotes good health and longevity (Personal survey). This is probably why the pepper soup made mainly with C. annuum var. fasciculatum is still very popular. Sweet peppers (C. annuum var.

grossum) are often stuffed with meat and are also pickled. The dried fruits are ground to produce powdered paprika, which is used as a condiment and in cooking. It is also a constituent of Hungarian goulash. Chilli peppers (C. annuum) are used for culinary purposes and for seasonings. Chillies are the hot ingredient of curry powder, which is made by grinding roasted dried chillies with tumeric, coriander, cumin and other spices. Chillies are extensively used in Central America and are constituents of dishes such as tamales and chile con carne. Pepper sauce, such as fabasco, is made by pickling the bulb in strong vinegar or brine. Extracts of chillies are used in the manufacture of ginger beer and other beverages. The medicinal significance of Chilli pepper is indicated by the use of its fruit as an antibacterial substance. It is also a remedy for back pains, rheumatism and swollen feet in Hawaii while constituting an important ingredient in Central African medicine (Watt and Breyer-Brandwijk, 1962).

Reports from some short-term studies have recently suggested that dietary substances such as salt and pepper (Thorburn et al, 1986; Onokpite, 1987a), and even natural palm-wine (Onokpite, 1987b; 1987c) may affect glucose homeostasis in the nondiabetic state. Also pertinent is the possibility that the degree of intake of these substances, particularly that of salt (Odeigah and Obieze, 1986), may vary as a result of genetically - controlled differences in taste recognition thresholds. delete ?

More studies are, however, still needed on the influence of genetic and common dietary factors, that are yet to receive considerable attention, on glucose tolerance in nondiabetic as well as diabetic states.

Thus, the present study was undertaken to determine whether an 8-year period of isolation can cause genetic differences in the pattern of glucose tolerance in SPD rats. Also, the comparative effects of some common dietary elements (usually taken with carbohydrate meals) on oral glucose tolerance (OGT) in normal and alloxan-induced diabetic rats will also be determined. These common dietary substances include: C. annuum var. fasciculatum (cluster peppers), C. annuum var. abbreviatum (Wrinkled peppers), C. annuum var. grossum (sweet peppers), and common salt (NaCl) as previously mentioned. Considerable attention will be focused on the therapeutic implications of these dietary substances in human diabetes mellitus. The possible role of genetic factors in the differences in incidence of diabetes in human populations will also be evaluated.

It is hoped that the outcome of this work will reveal some common dietary substances that can decrease glycaemic response in experimental rats and possibly in man. The basic principle of management of diabetes has generally been restriction or avoidance of sugar and sugary foods (Gill, 1990) coupled with insulin therapy for type 1 diabetes, and hypoglycaemic drug (sulphonurea compounds) therapy for type 2 (Baird and Strong, 1974; Cahill, 1979; Gill, 1990). To date, however, these conventional therapeutic methods have not provided the

total solution to the problems of diabetics. For instance, there exists the issue of non-compliance with the restriction or avoidance of sugar and sugary foods by diabetics (Gill, 1990). Not long ago, evidence that insulin stimulates development of atherosclerosis was brought forward (Stout, 1979). Moreover, a high risk of hypoglycaemia (abnormally low blood glucose) frequently attends the use of insulin and sulphonurea compounds. Chlorpropamide, the most widely available sulphonurea drug in the tropics frequently causes severe hypoglycaemia sometimes with permanent brain damage or death (Gill, 1990).

The use of common food substances that possess hypoglycaemic activity may compliment the conventional methods of diabetic management. Such therapeutic strategy will definitely reduce the hypoglycaemic drug and insulin demand by the diabetics. This will therefore significantly alleviate the problems and risks associated with the use of these conventional agents in the management of diabetes. Moreover, many tropical countries like Nigeria now face economic problems which directly affect the provision of health care. Thus, relatively simple therapeutic improvements will alleviate such economic problems and, as a result, lead to a significant reduction in diabetic morbidity and mortality.

2.0 MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Chemicals

(i) Sera - Pak Glucose reagent kit (Miles Laboratories limited, Slough, England).

(ii) Glucose Monohydrate (Merk, Darmstadt, W.Germany)

(iii) Alloxan (2,4,5,6 - Tetraoxypyrimidine)

(iv) Sodium Chloride (Reagent grade)

(v) Anaesthetic Ether.

2.1.2 Glassware

(i) Measuring Cylinders

(ii) Pasteur Pipettes with Rubber Teats

(iii) Conical Flasks

(iv) Microhaematocrit Tubes

(v) Microlitre Pipettes

(vi) Test Tubes

2.1.3 Equipment and Instruments

(i) Centrifuge

(ii) Spectrophotometer (Spectronic 20)

(iii) Salter and Metler Weighing Balances

(iv) Dissecting Set

(v) Ratogram (Restraint Device: Locally Manufactured)

(vi) Seal-Ease (Tube Sealer) and Holder (Glay Adam, New Jersey).

(vii) Cannula (Intramedic Polyethylene tubing i.d. 0.34", 0.d. 0.5")

(viii) Microcannula (Portex Intravenous Cannula 2FG 0.d. 0.63mm Green Luer 200/300/010)

(ix) Thermometer

(x) Syringes and Needles

(xi) Surgical Blade and Holder

(xii) Stitching Needle and Thread

2.1.4 Miscellaneous Materials

(i) Sprague - Dawley (SPD) Rats and Cages

(ii) Animal Feeds (Pfizer, Ikeja)

(iii) Fruits of Three Varieties of Chilli Peppers (Plate 1). viz: Cluster Peppers (C.annuum var. fasciculatum) Wrinkled Peppers (C.annuum var. abbreviatum) and Sweet Peppers (C.annuum var. grossum).

2.2 METHODS

2.2.1 Animal Husbandry

Adult rats of both sexes weighing 150-200g were obtained from the Laboratory Animal Centre (LAC) of the College of Medicine, University of Lagos (CMUL). The animals were caged (males and females separately) in a group of 3-5 rats per

cage. The temperature was 27±2°C. Rat pellets (from Pfizer) and tap water were made available ad libitum. The cages were thoroughtly cleaned and the rats examined. On this regime, the animals remained uniformly healthy and active throughout the period of study.

2.2.2 Preliminary Work to Establish the Appropriate Doses of Pepper, Salt and Alloxan.

Personal survey was undertaken to know the pepper varieties most commonly consumed in Nigeria. The three pepper varieties mentioned in section 2.1.4.iii above were found to be the most widely used. Suitable dose of these pepper extracts was then established to be 15.0mg/100g body weight (b.wt) by giving varying doses and then observing the general condition of animals after the dosing -Lower doses than 15.0 mg/100g b.wt. of extract had no glycaemic effects while higher doses caused violent acrobatic jumps by the rats. The diabetogenic dose of alloxan was found to vary from 4.0 - 8.0mg/100g b.wt according to the literature (Lukens,1948). Experimental trials to induce diabetes with alloxan in the laboratory however indicated that the appropriate dose for this study was 4.0mg/100g. b.wt. This dose was the least but significantly diabetogenic dose as assessed by 24-hour fasting plasma glucose concentration (FPGC) which was 203mg/100ml on the fifth day (D5), taking the day of alloxan administration as D1. Salt was administered as normal (physiologic) saline (9.0g%) at a dose of 9.0mg/100g b.wt. (Onokpite, 1987). Alloxan was given intravenously (Luken, 1948) while the

dietary substances (salt and peppers) were orally administered since they are normally consumed through the mouth.

2.2.3 Preparation and Administration of Alloxan Solution

Experimental (alloxan) diabetes discovered by Dunn et al (1943) was induced by intravenous (jugular vein) administration of 4.0mg/100g body weight (b.wt.) alloxan (see Appendix 2) as 0.8g% solution. This drug is known to be diabetogenic in animals due to its pancreatic beta cytotoxicity (Dunn et al, 1943; Lukens, 1948). Each healthy non-fasted adult rat was anaesthetized using ether fumes, weighed, and laid supine on a dissecting board. The anterior aspect of the neck was shaved and a longitudnal skin incision, 1.5 cm long, made on the mid-line of the shaved area. By gentle dissection, the jugular vein was exposed and punctured using a 23-gauge sterile steel needle. A single lumen flexible polyethylene cannula (Portex intravenous cannula, 2FG 0.d. 0.63mm Green Luer 200/300/010) was inserted and manipulated carefully towards the heart. Freshly - prepared alloxan solution was then infused. The day of alloxan administration was regarded as the first day or D1. Glucose Tolerance Test (GTT) was carried out on D5 after fasting, the animals for 24 hours. Also in the nondiabetic group, glucose tolerance was assessed after fasting the animals for 24 hours.

2.2.4 Extraction, Preparation and Administration of Dietary Substances

2.2.4.1 Extraction of Pepper

This was done by the Soxhlet Method using the Soxhlet Extractor (Fig.1). The apparatus is essentially a modified distillation set-up whereby water vapour condenses and the hot distilled water fall in drops on the specimen for extraction. The specimen is normally put inside a filter paper thimble positioned directly below the condenser. The fruits of the three pepper varieties (Plate 1) being the part normally consumed were obtained in large quantities from Agege local market in Lagos for drying and homogenization. 5g of the well dried, homogenized pepper powder was put into a filter paper thimble and inserted into the soxhlet apparatus for extraction.

2.2.4.2 Preparation of Pepper and Salt Solutions

2.2.4.2.1 Pepper: The initial concentration of the extract was usually higher than the desired concentration of 15.0mg/ml. By dilution with distilled water, the initial concentration was adjusted to 15.0mg/ml using the following formular:

$$\frac{V_1}{C_1} = \frac{V_2}{C_2}$$

where C_1 = original concentration (mg/ml); V_1 = original volume (ml).
C_2 = required concentration (mg/ml); and V_2 = required volume (ml).

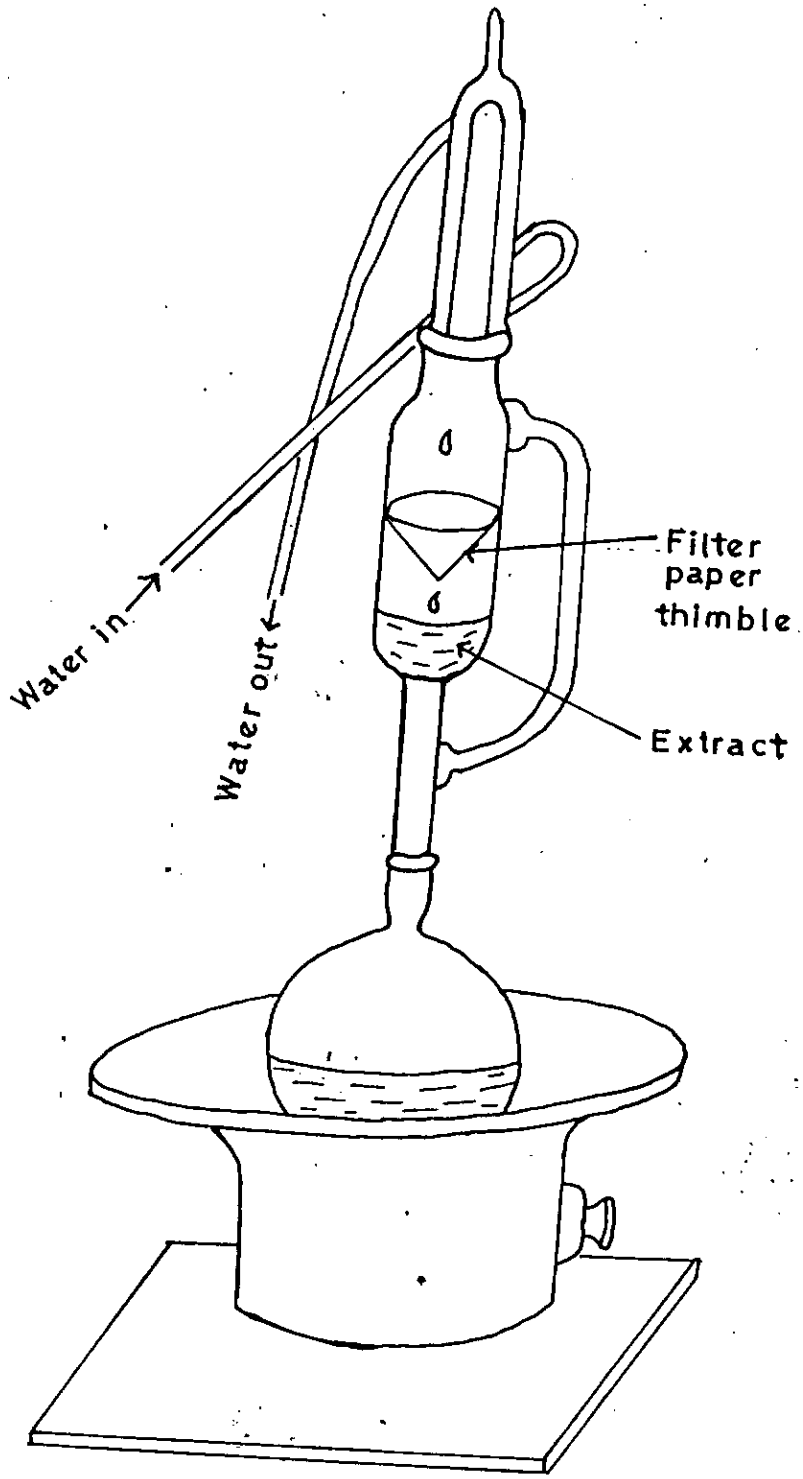

Fig. 1. The Soxhlet Extractor $\times\frac{1}{5}$

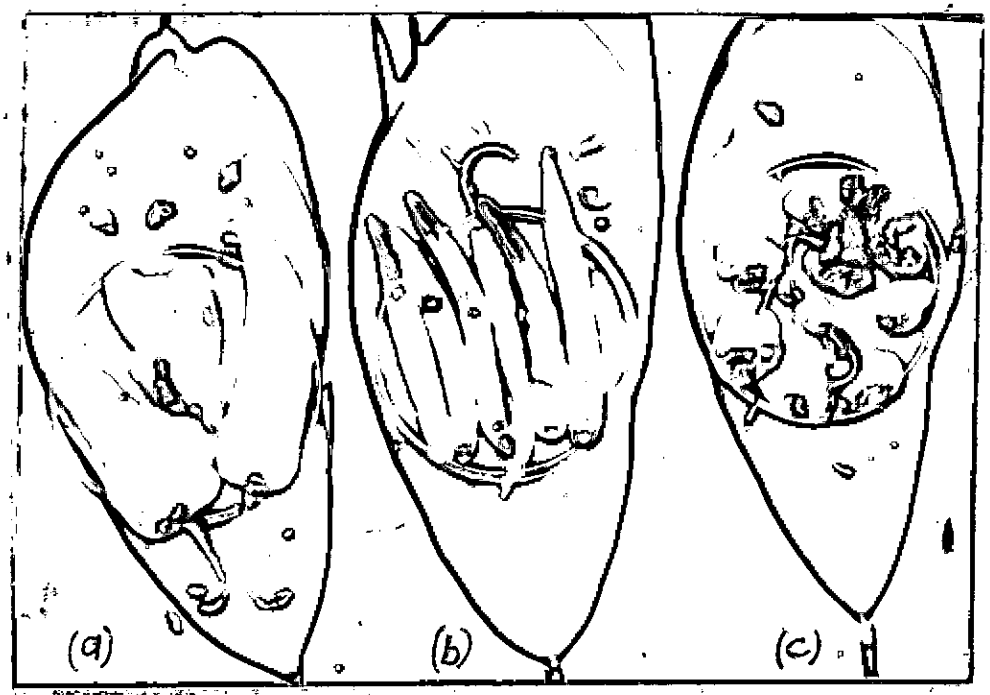

Plate 1. The three varieties of chillie peppers used in the study:
(a) Capsicum annuum var. grossum (sweet peppers);
(b) C. annuum var. fasciculatum (cluster peppers);
(c) C. annuum var. abbreviatum (wrinkled peppers).

volume (ml). Note that V_2 should finally be adjusted to V_1.

2.2.4.2.2 Salt: Since the total volume of solution of each dietary substance required on each day of experiments is less than 25.0ml, 2.25g of salt (reagent grade) was put in a measuring cylinder and distilled water was added to make up the volume of the solution to 25.0 ml level. The solution was then shaken well to completely dissolve the salt. It is important to note that 2.25g, of salt in 25.0ml solution is equivalent to 0.9g% (normal saline) which is the required salt concentration for the experiments.

2.2.4.3 Administration of Salt and Pepper Solutions

The solutions prepared above were combined with glucose and administered orally for Glucose Tolerance Test (GTT). Since 30g% glucose solution is normally required for the test (Junod et al, 1969; Onokpite, 1987), 7.5g glucose was weighed into a measuring cylinder. Normal saline or pepper extract was then gradually added and the solution shaken thoroughly and intermittently until a true solution was formed and the 25.0 ml level of the measuring cylinder was reached. It should be noted that 7.5g glucose in 25.0 ml solution is equivalent to the required 30g% glucose for the GTT. The procedure for the GTT is detailed in the section below.

2.2.5 Glucose Tolerance Test (GTT)

Glucose tolerance was analysed by oral glucose tolerance

test (OGTT) using standard procedure (Junod et al, 1969; Onokpite, 1987). The test was performed on the fifth day (D5) taking the day of alloxan administration as the first day (D1). This was based on the fact that in animals, particularly in rats, alloxan-diabetes is characterised by a triphasic alteration of plasma glucose levels (Lukens, 1948) and that on D5, the third and permanent hyperglycaemic phase of alloxan diabetes would have set in and stabilized (Lukens, 1948; Beach et al, 1956). After fasting the animals for 24 hours, OGTT was carried out by oro-gastric intubation at 0900h. This was accomplished under light ether anaesthesia, using a single-lumen polyethylene cannula (intramedic polyethylene tubing i.d 0.34", o.d 0.5"). The Cannula was manipulated into the oesophagus until about 12.0 cm length has gone in. A glucose load (30g%) was then infused at a volume of 1.0ml/100g b.wt. Prior to intubation and under anaesthesia, the tail was cut using a sterile surgical blade to obtain 125 µl of blood sample into heparinized capillary tubes. These were then centrifuged at 3,000 r.pm for 10 minutes to separate the plasma. Subsequent blood collections were made at 30-minutes interval for 2 hours. The last blood sample was collected an hour later at 180 - minute time point of OGTT. Analysis of plasma glucose was done using a standard microtechnique called glucose oxidase/peroxidase method (Trinder, 1969) described in 2.2.5.1 below.

N.B. The rats were in a restraint device throughout the period of blood collection (Plate 2).

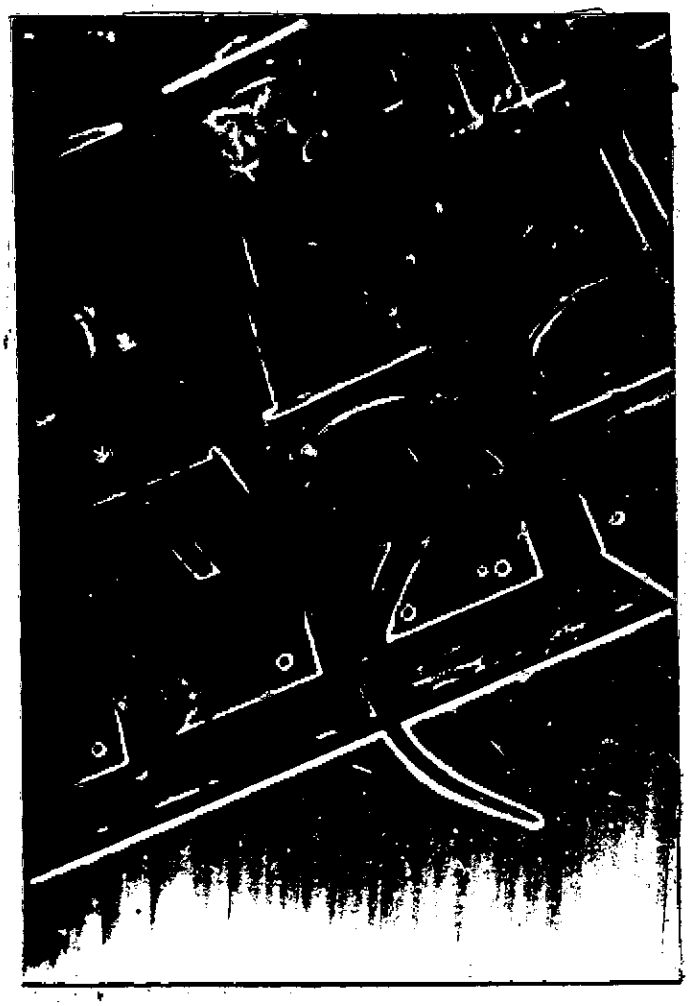

Plate 2. The Ratogram(manufactured locally): An animal restraint device used in blood collection $\times \frac{1}{3}$

2.2.5.1 Glucose Oxidase/Peroxidase Method For Quantitative Determination of Plasma or Serum Glucose

A glucose oxidase /peroxidase (GOD/POD) reagent kit essentially consists of the enzymes glucose oxidase and peroxidase and chromogen (4- aminophenazone/phenol). The glucose in plasma (or serum), in the presence of glucose oxidase reacts with oxygen to form gluconic acid and hydrogen peroxide. The peroxide oxidizes the chromogen in the presence of peroxidase to form a pink color which is measurable by spectrophotometry. The absorbance of the sample (Asample) and of the standard (100mg% glucose) was then read against the blank using the following equation.

$$\text{Plasma Glucose Concentration} = \frac{\text{A sample}}{\text{A standard}} \times \frac{100}{1} \text{mg/100ml.}$$

The detailed method of peraparation of GOD/POD reagent is presented in Appendix 3.

2.2.6 Study on the Influence of Genetic Factors on Glucose Tolerance in Two Isolated Colonies of Sprague -Dawley (SPD) Strain of Rats For Three Generations

The two isolated colonies are maintained at the Biological Garden, University of Lagos, Akoka and the Laboratory Animal Center, College of Medicine, University of Lagos, Idi-Araba. The Akoka colony was derived from a small stock secured from the Idi-Araba colony in August,1982.

2.2.6.1 Selection, Caging and Treatment of Animals

Animal samples consisting of 2 males and 8 females were randomly selected from each colony. The animals from each colony were then sub-grouped into 2 in the Experimental Room of the laboratory Animal Centre, Idi-Araba. Each sub-group was made up of 1 male and 4 females per cage for harem mating to take place. The two cages containing the two sub-groups of animals from each colony were labelled as "Nondiabetic" and "Diabetic" respectively. These animals were taken to be the parental generation. The presence of sperm which was observed on the vaginal smear and several mucus plugs on the cage floor was indicative of intromissions and successful mating. Pregnant females were later separated out and caged individually. After paturition and weaning of young ones at the age of about 5 days, parents from the nondiabetic category were subjected to glucose tolerance test (GTT) after fasting for 24 hours. Those from the diabetic category were pre-treated with alloxan to induce diabetes before carrying out GTT on D5 as described previously (See Section 2.2.5.).

2.2.6.2 Treatment of First and Second Generation Offspring

To see the contributory effects of genetic and environmental factors to the differences in the pattern of glucose tolerance observed in the parental generation between the animals from the two different colonies, similar experiments as in the parents were conducted on the first and second generation offspring.

2.2.7. Data Analysis

Glucose Tolerance Index (GTI) for each rat computed by adding the fasting - , one - , two -, and three- hour plasma glucose concentrations (PGCs), was used to assess glucose tolerance according to the methods of previous workers (Beach et al, 1956; Reaven, 1983; Onokpite, 1987). Overall results were expressed as Mean ± S.D or Mean (S.D) unless otherwise stated. Statistical significance for comparison of results was determined with the student t-test. Regression and other analyses were carried out when pertinent. P values less than 0.05 were considered significant.

3.0 RESULTS

3.1 Glucose Tolerance in Normal and Alloxan-Treated Rats: Establishment of Nondiabetic and Diabetic Categories.

The plasma glucose concentrations (PGC) of alloxan - treated rats were strikingly higher than the PGC of the controls at every time - point of oral glucose tolerance test (Fig.2). The mean fasting plasma glucose concentration (FPGC) of alloxan-treated animals was 240±55.2 mg%. This value was significantly higher ($P<0.001$) than 118±12.6 which was the mean FPGC of those animals not injected with alloxan (Table 1). The highest plasma glucose level attained by alloxan-treated rats during glucose tolerance test was 620±61.7 mg%. This peak plasma glucose concentration (PPGC) was attained at 120-minute time-point of glucose tolerance test (GTT) as compared with the significantly lower PPGC ($P<0.001$) of animals not given alloxan which was 217±3.7mg% and which occurred at 90-minute time-point of GTT. After 180 minutes, the plasma glucose concentration (PGC) of untreated rats has been brought down to 139±27.0mg%. This PGC value was significantly lower than the PPGC of the group ($P<0.05$) and was very significantly lower than the PPGC of alloxan-treated rats which was 572±61.4mg% ($P<0.001$). The 180-minute PGC was however, not significantly different from the PPGC in the alloxan-treated group (Table 1).

When glucose tolerance was assessed by glucose tolerance index (GTI), it was revealed that glucose tolerance in

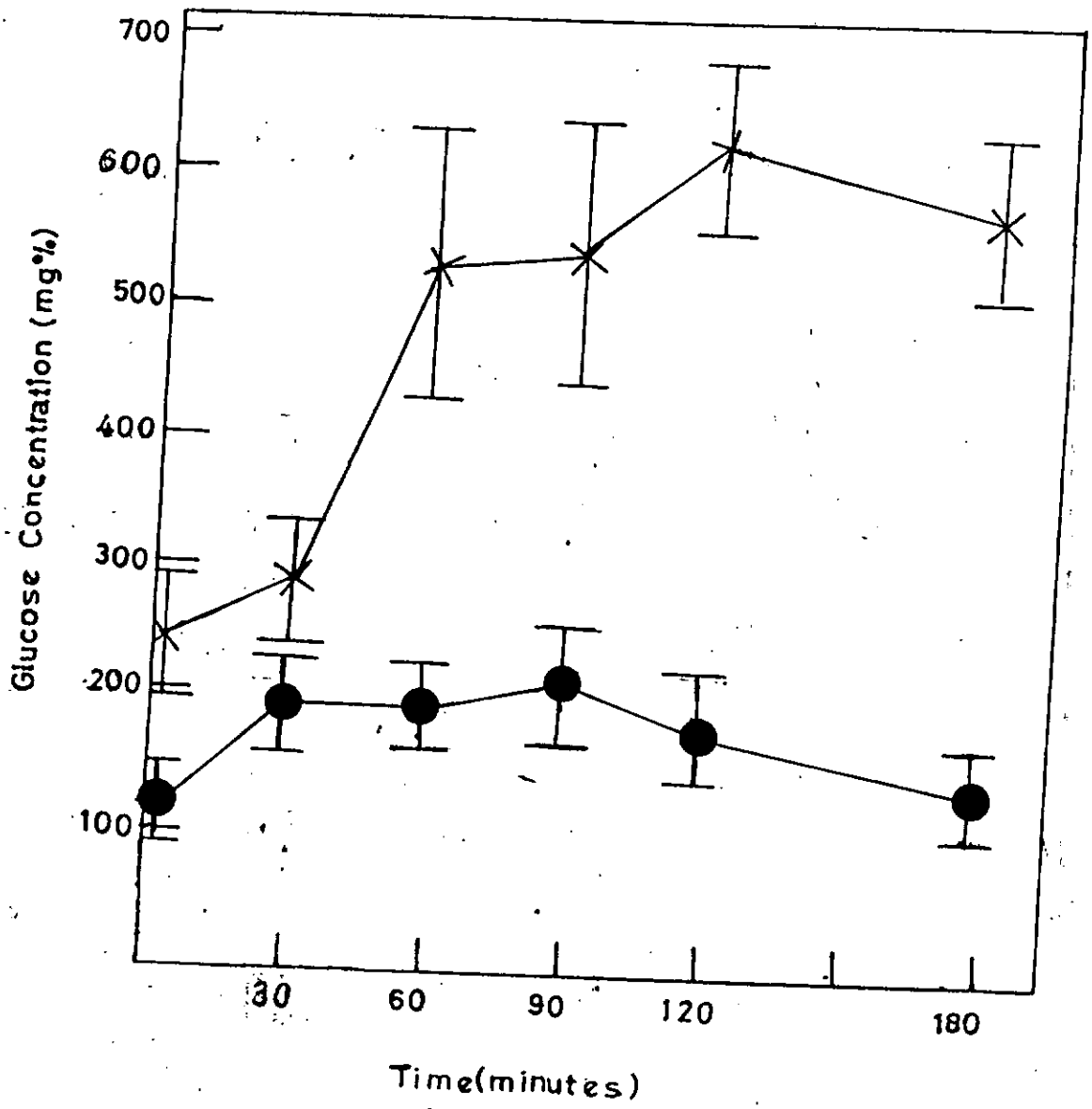

Fig. 2. Glucose tolerance pattern in nondiabetic (●) and alloxan-diabetic (X) rats. Vertical bars represent ± S.D.

Group	Number of Rats	Plasma Glucose Concentration(mg%)						GTI
		0 min	30min	60min	90min	120min	180min	
Nondiabetic (Normal)	21	118 (12.6)	195 (22.9)	198 (28.0)	217 (35.7)	181 (32.4)	139 (27.0)	636 (80.8)
Alloxan-Diabetic	19	240* (55.2)	293* (45.8)	532* (104.3)	538* (99.7)	620* (61.7)	572* (61.4)	1965* (226.2)

Table 1. Glucose tolerance in normal and alloxan - treated (alloxan - diabetic rats.

N.B. Asterisk (*) indicates significant difference ($P<0.001$). Results are presented as means (S.D.).

GTI = Glucose Tolerance Index.

alloxan-treated rats was grossly abnormal as compared with those not treated with the substance. The GTI of 1965± 226.2 of alloxan-treated rats was significantly higher than 636±80.8, which was the GTI of animals not given alloxan ($P<0.001$). Infact it could be noted that the GTI of alloxan-treated rats was three times greater than that of animals not treated with the substance. A close examination of Fig.3 and the raw data in Appendices 4A,B,5A and B will show that the highest GTI in the animals not treated with alloxan is quite lower than the lowest GTI in alloxan-treated animals. This fact further underscores the severe glucose intolerance present in the alloxan-treated animals.

The foregoing results of glucose tolerance in the alloxan-treated and untreated rats strongly suggests that the establishment of the two broad animal categories, viz: "nondiabetic (normal) control" and "alloxan-diabetic (diabetic) control" have been successfully accomplished. This suggestion was corroborated by the fact that the fasting plasma glucose concentrations (FPGC) and two-hour PGC of animals in the nondiabetic and diabetic categories satisfy the WHO (1985) recommendations for human nondiabetic and diabetic states. According to WHO (1985), an FPGC of 140 mg% and above, with a two-hour PGC of 200mg% and above is sufficient for the diagnosis of diabetes mellitus. In the present study, the FPGC of alloxan-induced diabetic rats which ranged between 173-348mg% (Appendices 5A&B) and the two-hour PGC which was 503-706 mg% met the WHO standard for diabetic state. In the same vein, the FPGC of animals not given alloxan which ranged

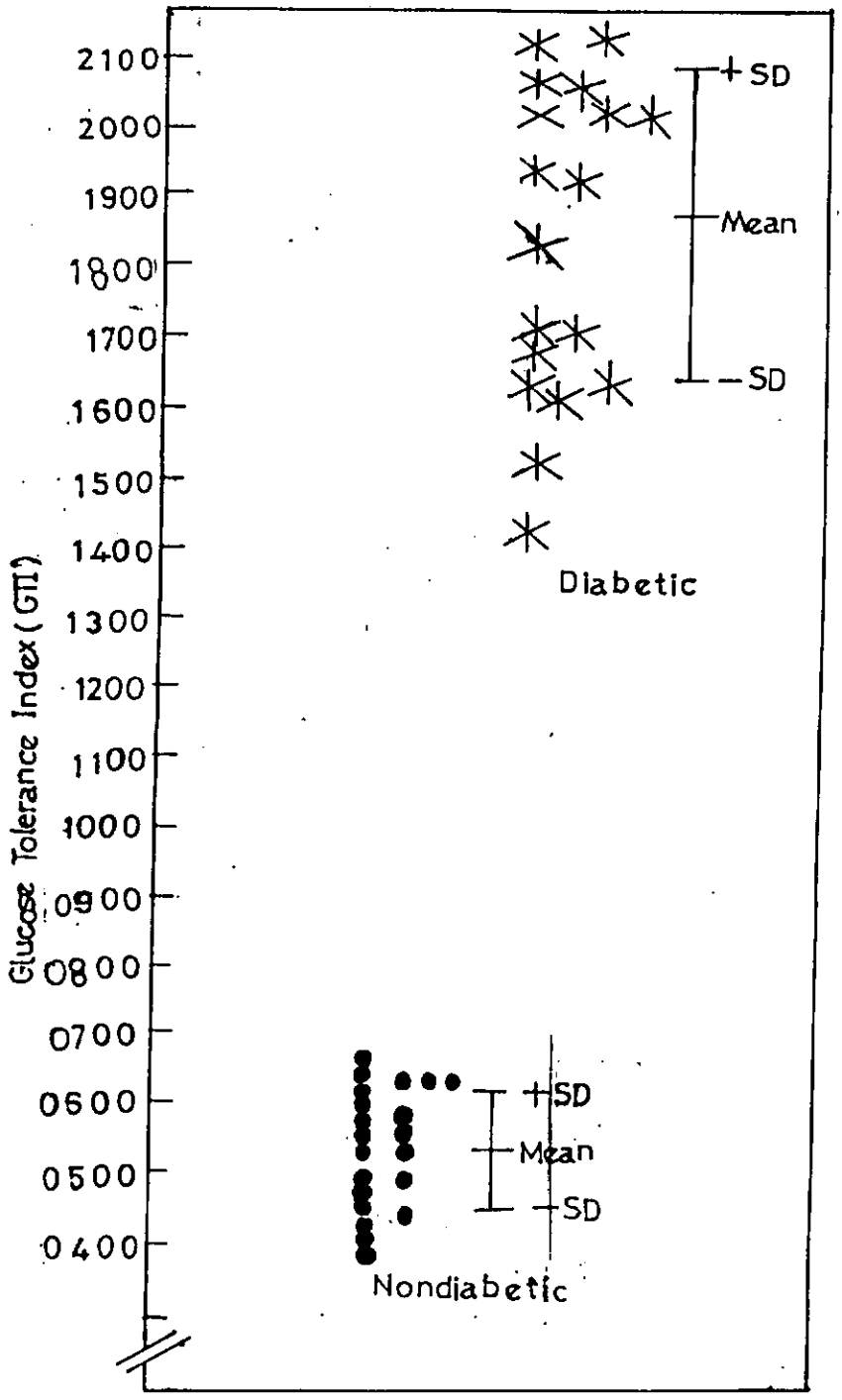

Fig. 3. Glucose tolerance indices in nondiabetic (●) and alloxan-diabetic (✳) rats. The means are significantly different ($P < 0.001$).

100-138mg% while the two-hour PGC of 128-239mg% satisfied the WHO criteria for nondiabetic states.

3.2 Influence of Sex and Body Weight on Glucose Tolerance in Nondiabetic and Diabetic Control Animals

3.2.1 Influence of Sex

As assessed by glucose tolerance index, no significant difference was found in the pattern of tolerance between males and females in both the nondiabetic and diabetic categories (Table 2). The mean GTI of 613±74.6 in nondiabetic male rats was not significantly different from that of their female counterparts which was 654±83.9 ($P > 0.05$). Also in the diabetic category, the mean GTI of male and female rats which were 1961±252.3 and 1970±208.4 respectively were not significantly different ($P > 0.05$).

3.2.2 Influence of Body Weight

The animals used in this study were non-obese with body weights within the normal range of 150-200g. As revealed by Tables 3a&b, nondiabetic animals with lower body weights (150-170g) had a mean GTI of 612±66.8. This value was not significantly different ($P > 0.05$) from that of animals with higher body weights (180-200g) with a mean GTI of 655±85.6. Also in the diabetic rats, the mean GTI between these animals grouped according to body weights (lower and higher) were not significantly different (2068±229 vs 1903±243.7; $P > 0.05$). Regression analyses further indicated

Group	Male		Female	
	GTI Mean (SD)	No of Rats	GTI Mean (SD)	No of Rats
Nondiabetic (Normal)	613(74.6)a	9	654(83.9)a	12
Alloxan-Diabetic	196(252.3)b	10	1970(208.4)b	9

Table 2. Influence of sex on glucose tolerance as assessed by glucose tolerance index (GTI).

N.B. GTI values with the same letters are not significantly different ($P > 0.05$) from each other. Results are presented as mean (SD).

150 - 170g	180 - 200g
540	553
595	633
674	546
507	746
637	710
653	565
676	590
	739
	738
	730
Mean GTI 612 ± 66.8	655 ± 85.6

Table 3a. Influence of body weight on glucose tolerance in nondiabetic rats as assessed by glucose tolerance index (GTI).

N.B. The mean, GTI in the two body weight class of ('150 - 170g' and '180 - 200g') are not significantly different ($P > 0.05$)

150 - 170g	180 - 200g
2324	2187
2156	1547
1808	1810
2284	1950
2038	2069
1796	2114
	1646
Mean GTI 2068 ± 229	1903 ± 243.7

Table 3b. Influence of body weight on glucose tolerance in alloxan-diabetic rats as assessed by GTI.

N.B. The mean GTI in the two body weight class ranges (150-170'g and '180-200g') are not significantly different ($P > 0.05$),

that no consistent or significant association could be established between body weight and GTI. A perusal of Fig.4 will reveal that the data-points in the scatter diagram describing the association of GTI with body weight do not conform to any specific pattern in both the non-diabetic and alloxan-diabetic categories. Moreover, the correlation coefficient (r) of the association was 0.8 and -0.3 in nondiabetic and diabetic rats respectively. Students t test indicated that these values were not significantly different from 0.

3.3 Influence of Genetic Factors on Glucose Tolerance in Two Isolated Colonies of Sprague-Dawley (SPD) Strain of Rats Monitored For Three Generations.

The two isolated colonies were maintained in the Biological Garden, Unilag, Akoka and the Laboratory Animal Centre, College of Medicine, University of Lagos, Idi-Araba. The Akoka colony was derived from a small stock secured from the Idi-Araba colony in August 1982. The rats were bred (mass breeding) in the animal house of the Biological Garden, Akoka since 1982 without accessions from elsewhere. Glucose tolerance pattern was studied in nondiabetic and alloxan-induced diabetic rats from these two colonies for three generations (Parental, F_1 and F_2).

3.3.1 Glucose Tolerance in Nondiabetic and Alloxan-Diabetic Parent Rats

No significant difference in the pattern of glucose

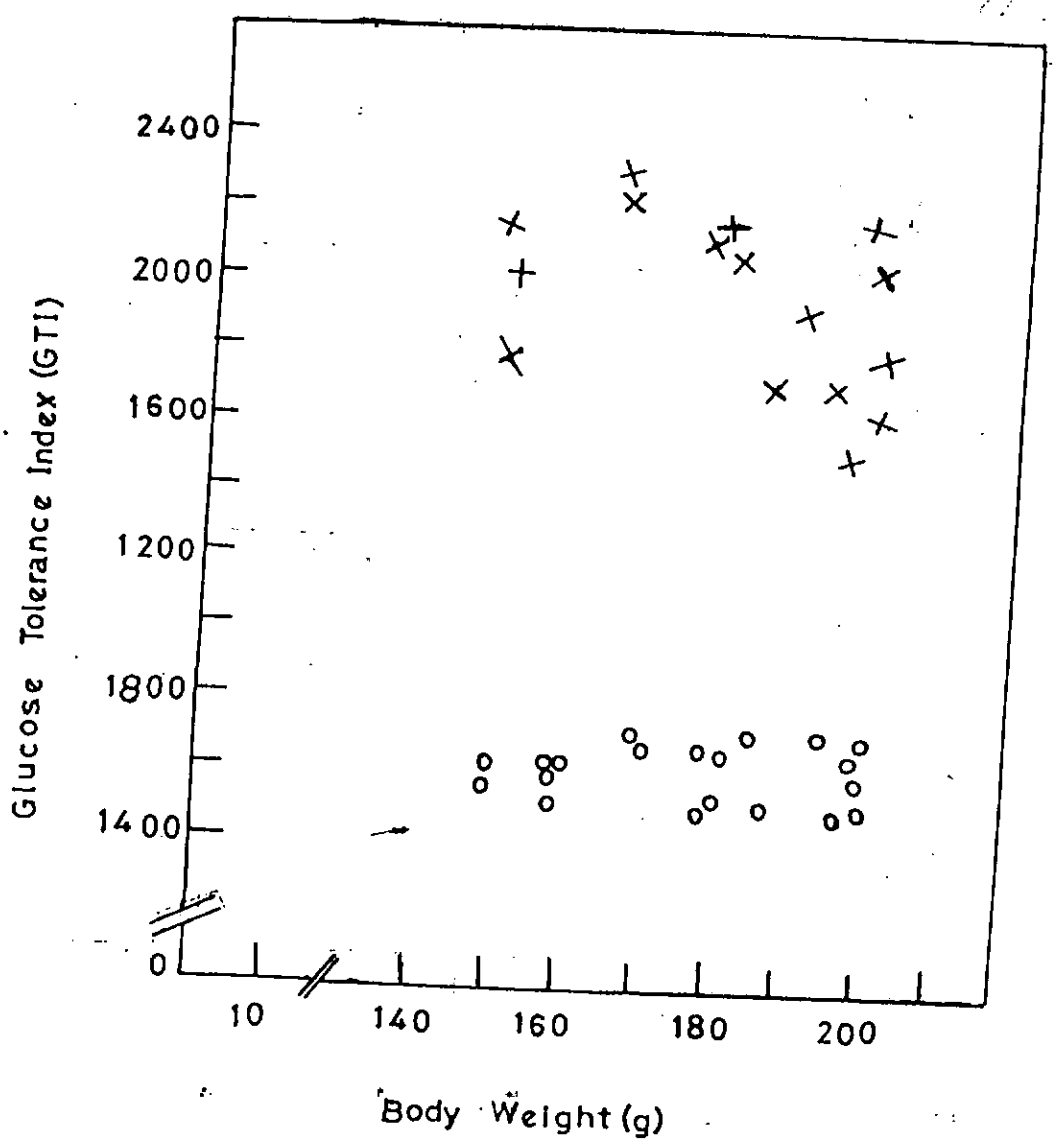

Fig.4. Relationship between body weight and glucose tolerance in nondiabetic (o) and alloxan-diabetic (X) rats as assessed by glucose tolerance index (GTI). Note: The data points representing GTI values do not conform to any specific pattern indicating lack of relationship between body weight and glucose tolerance in both categories.

tolerance was found between the parent animals from Akoka and Idi-Araba colonies in the nondiabetic category (Table 4, Figs 5a&b).

In the alloxan category, however, striking difference in glucose tolerance was observed between Akoka and Idi-Araba animals. Those from Akoka had a lower mean fasting plasma glucose concentration (FPGC) of 134±20.2mg% when compared with that of animals from Idi-Araba with FPGC of 245±28.9mg%. This difference was found to be statistically significant ($P<0.01$). The mean glucose tolerance index (GTI) of Akoka rats was also correspondingly lower) $P<0.01$) than that of Idi-Araba rats (1601±39.1 vs 1940±74.6).

3.3.2 Glucose Tolerance in Nondiabetic and Alloxan-Diabetic F_1 and F_2 Generation Offspring

The extent to which the pattern of glucose tolerance observed in the parents was genetic and/or environmental needs to be evaluated. This is particularly so in the case of alloxan-diabetic category where important differences in glucose tolerance was observed between animals from the two colonies. Thus, glucose tolerance test was carried out in the F_1 and F_2 generation offspring. Cognizance was taken of the fact that, unlike the parents, the offspring were bred and maintained in the same rigidly controlled environment (Experimental Room, Laboratory Animal Centre, CMUL, Idi-Araba).

In the nondiabetic group, no significant difference in glucose tolerance was found between animals from the two

Generation	Group	Idi-Araba Colony			Akoka Colony		
		FPGC(mg%)	GTI	No of Rats	FPGC(mg%)	GTI	No of Rats
Parental (P)	Nondiabetic	117 a (10.9)	467 b (57.2)	4	122 a (17.5)	486 b (53.8)	5
	Diabetic	245c (28.9)	1940d (74.6)	4	134 e (20.2)	1601 f (39.1)	4
First Generation Offspring (F_1)	Nondiabetic	120 a (9.9)	450 b (39.5)	10	113 a (16.6)	440 b (49.7)	10
	Diabetic	241 c (27.9)	1931 d (59.5)	8	131 a (16.1)	1621 e (79.3)	11
Second Generation Offspring (F_2)	Nondiabetic	121 a (14.7)	459 b (51.9)	7	116 a (13.5)	450 b (47.4)	10
	Diabetic	248 c (31.3)	1952 d (109.2)	8	134 a (9.7)	1631 e (93.0)	9

Table 4. Fasting plasma glucose concentration (FPGC) and glucose tolerance indices (GTI) in nondiabetic and alloxan-diabetic rats from Idi - Araba and Akoka Colonies.

N.B. GTI values with the same alphabets are not significantly different ($P>0.05$) from one another. However, those with different alphabets are significantly different ($P<0.05$). Results are presented as mean (SD).

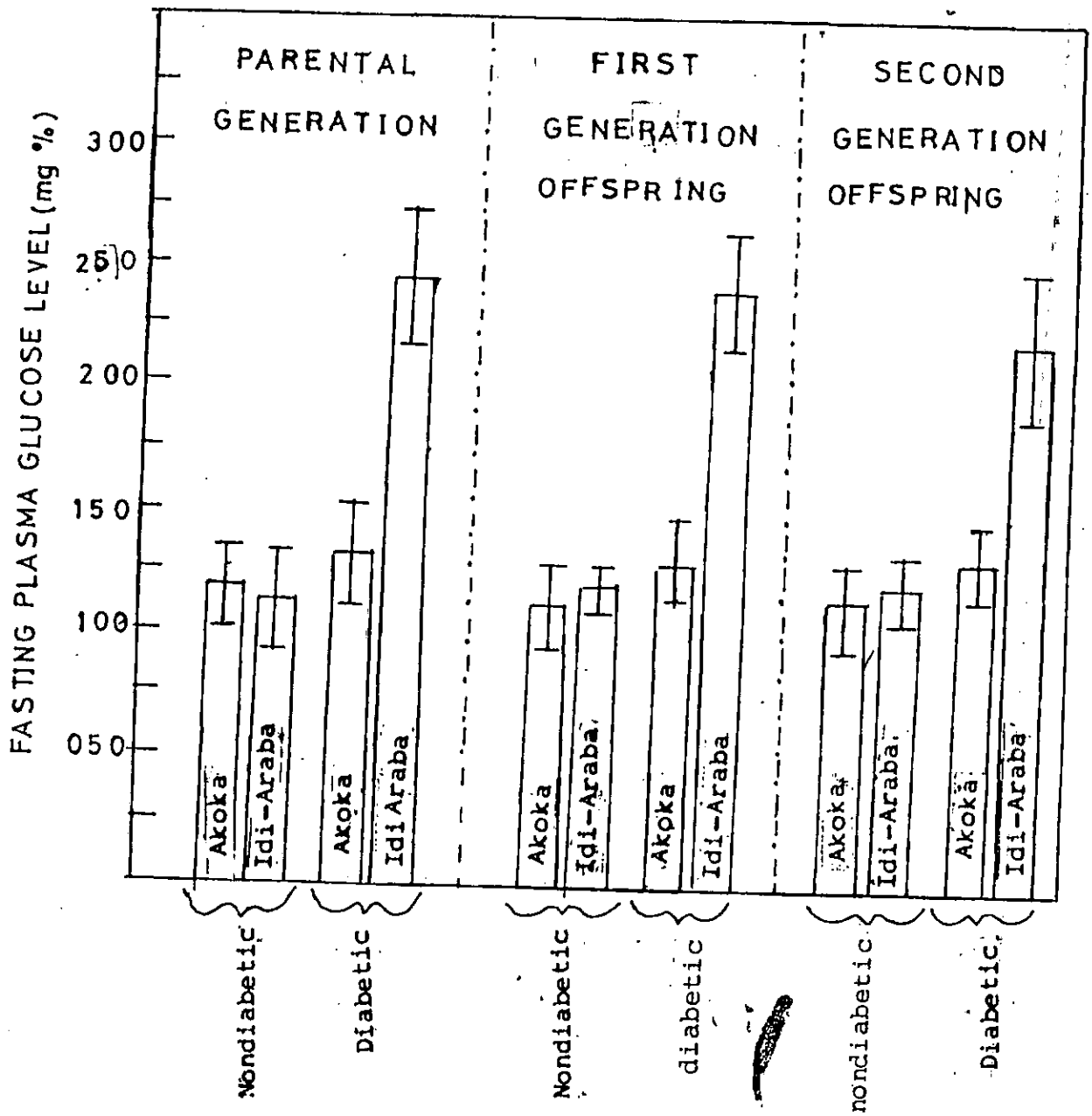

Fig.5a. Fasting plasma glucose concentrations (FPGC) in rats maintained at Idi-Araba and Akoka. Note: While there was no significant difference in FPGC at 0.05 level between Idi-Araba and Akoka rats in the nondiabetic category, significant difference (P 0.05) was observed in the diabetic category.

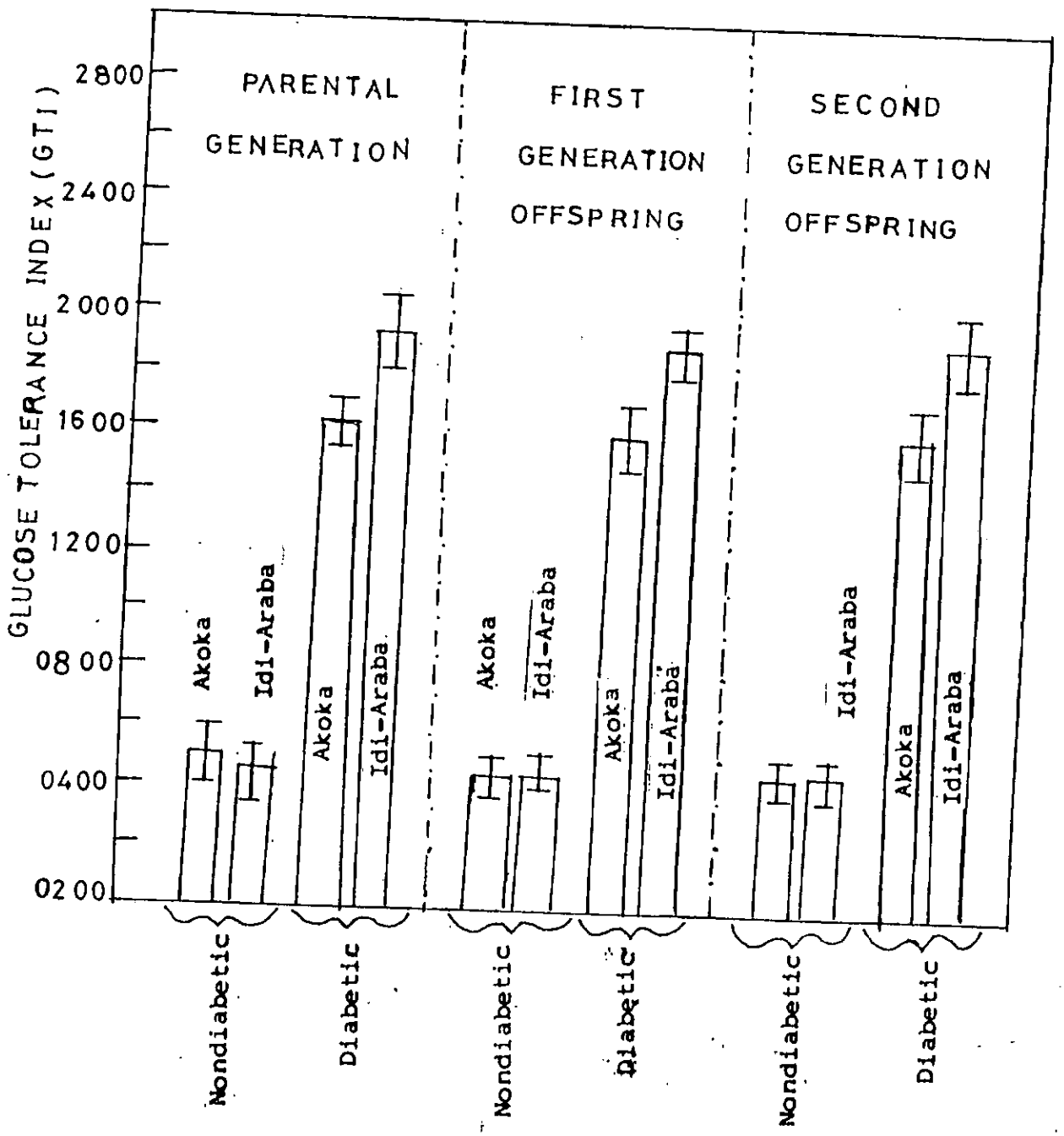

Fig.5b. Glucose tolerance indices (GTI) of rats maintained at Idi-Araba and Akoka. Note: While there was no significant difference in GTI at 0.05 level between Idi-Araba and Akoka rats in the non-diabetic category, significant difference was observed in the diabetic category (P 0.05).

colonies in the F_1 and F_2 offspring. The fasting plasma glucose concentrations and glucose tolerance indices of the offspring (F_1 and F_2) were not significantly different from those of the parents (Table 4; Figs 5a&b). It therefore appeared that, in the present study, glucose tolerance in the nondiabetic state is not of genetic or environmental importance.

In the diabetic animals, important differences were observed between the offspring from the two colonies. The pattern of result was similar for the F_1 and F_2 offspring and the parent animals (Table 4; Figs 5a&b). The FPGC of Akoka F_1 diabetic offspring was 131±16.1mg% while the GTI was 1621±79.3. These values were significantly lower than those of Idi-Araba F_1 diabetic offspring with FPGC of 241±27mg% and GTI of 1931±59.5 ($P<0.05$). In the F_2 generation, Akoka rats had FPGC of 134±97 and GTI of 1631±93.0 compared with the significantly higher FPGC and GTI of Idi-Araba rats which were 248±31.3mg% and 1952±109.2 respectively ($P<0.05$). It should be noted that this pattern of results is similar to that of the alloxan-diabetic parents (Figs 5a&b). It therefore appeared that glucose tolerance in alloxan-diabetic rats is largely genetic. The explanation may lie in genetic differences in sensitivity to alloxan in the rats maintained at these two different colonies.

3.4 The Influence of Dietary Substances on Glucose Tolerance.

3.4.1 The Influence of Three Varieties of C.annuum (Chilli Peppers)

3.4.1.1 C. annuum Var. fasciculatum (Cluster Peppers)

In the nondiabetic animals treated with this pepper variety, the peak plasma glucose concentration (PPGC) was 166±22.5mg%. When this value was compared with the PPGC of the nondiabetic control rats which was 181±32.4mg%, the difference was significant ($P<0.05$). The mean glucose tolerance index (GTI) of the pepper - treated non-diabetic animals was also significantly lower than that of the control (566±74.7 vs 636±80.8; $P<0.05$); see Table 5 and Fig.6a. Also, the mean glucose tolerance curve of the non-diabetic control rats was found to lie below that of the untreated animals (Fig.6a).

The pattern of results in the diabetic category was somehow similar to that of the nondiabetic. This is because pepper significantly lowered plasma glucose concentions in this category also. This plasma glucose reducing effect of cluster peppers was found to persist for three hours during glucose tolerance testing (Fig.7a). The mean PPGC of rats treated with this pepper variety was 410±42.1mg%. This value was significantly lower than the PPGC of the diabetic control animals which was 620±61.7mg% ($P<0.01$). The mean GTI in the pepper - treated group was 1202 ±69.9 as compared with the significantly higher GTI of 1965±226.2 in the diabetic control rats. This effect is desirable in diabetes because the primary problem of a diabetic is the control of postprandial (after feeding) hyperglycaemia.

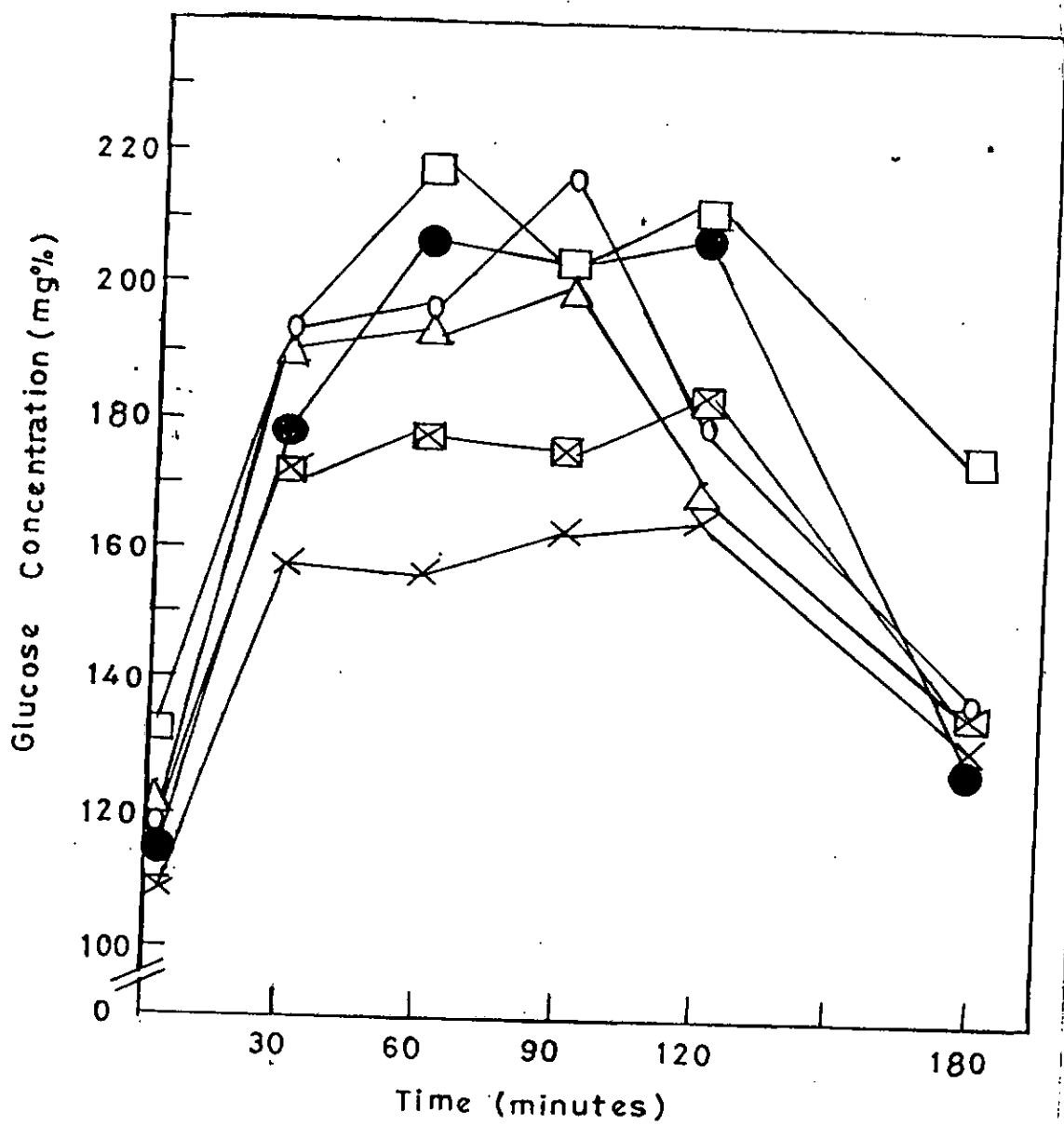

Fig. 6a.. The effect of common salt and three varieties of chilli peppers on glucose tolerance in nondiabetic rats. N.B.: (o) control; (X) Capsicum annuum var. fasciculatum; (●) C annuum var. abbreviatum; (Δ) c. annuum var grossum, (⊡) Common salt; (⊠) Common salt combined with C. annuum var. fasciculatum.

3.4.1.2. C annuum Var. abbreviatum (Wrinkled Peppers)

The mean glucose tolerance index (GTI) of the non-diabetic treated rats was 649±73.4. This value was not significantly different from the mean GTI of the nondiabetic control animals which was 636±80.8 ($P>0.05$). No significant difference was also observed between the treated and untreated rats when the plasma glucose concentrations at every time-point of glucose tolerance test (GTT) was compared (Table 5).

In the diabetic animals, this variety of pepper was observed to affect glucose tolerance significantly. The mean GTI of 1553±170.2 in the treated rats was significantly lower ($P<0.05$) than that of the control which was 1965±226.2 (Table 6; Fig.7b). The plasma glucose reducing effect of C. annuum var abbreviatum was, however, observed to be lower than that of C. annuum var fasciculatum when the plasma glucose concentrations and glucose tolerance indices were compared (Table 6; Figs 6a,b,7a&b). Thus the glucose tolerance curve of animals treated with C. annuum var abbreviatum was found to lie above the curve of those treated with C. annuum var. fasciculatum (Figs.6a,7a). Wrinkled peppers may therefore not be efficacious in the treatment of diabetes.

3.4.1.3. C. annuum var grossum (Sweet Peppers).

Point - to - point comparisons of plasma glucose levels did not show that this pepper variety had any significant effect on glucose tolerance in the nondiabetic animals. The mean glucose tolerance index GTI of the nondiabetic rats

Treatment	No of Rats	Plasma Glucose Concentration (mg%)						GTI
		0.min	30 mins	60 mins	90mins	120mins	180mins	
Glucose only (control)	21	118 (12.6)	195 (22.9)	198 (28.0)	217 (35.7)	181 (32.4)	139 (27.0)	636 (80.8)
Glucose and CAF	12	108 (18.6)	159 (24.1)	157 (37.9)	163 (22.4)	166 (22.5)	131 (30.9)	566* (74.7)
Glucose and CAA	9	116 (31.0)	179 (31.0)	207 (31.0)	217 (27.6)	199 (49.0)	127 (16.4	649 (73.4)
Glucose and CAG	9	122 (8.7)	190 (41.3)	193 (50.0)	199 (42.1)	169 (23.1)	136 (12.7)	620 (85.0)
Glucose and Salt	8	132 ((6.8)	194 (26.2)	219 (6.6)	204 (11.5)	210 (13.6)	174 (21.0)	734* (33.9)
Glucose CAF and Salt	9	118 (25.3)	172 (32.0)	177 (14.5)	176 (25.4)	183 (17.2)	136 (31.0)	597 (48.2)

Table 5. Effect of C. annuum var. fasciculatum (CAF), C. annuum var. abbreviatum (CAA), C. annuum var. grossum (CAG), common salt (NaCl), and CAF combined with NaCl on glucose tolerance in nondiabetic rats

N.B. Results are presented as mean (S.D). Asterisk (*) indicates significant difference from the control ($P < 0.05$).

Treatment	No of Rats	Plasma Glucose Concentration(mg%)						GTI
		0 min,	30 min	60min	90min	120min	180min	
Glucose only (Control)	19	240 (55.2)	293 (45.8)	532 (104.3)	538 (99.7)	620 (61.7)	572 (61.4)	1965 (226.2)
Glucose and CAF	12	208 (11.0)	244 (29.9)	295 (33.7)	348 (63.8)	410 (42.1)	288 (34.9)	1202** (69.9)
Glucose and CAA	10	182 (23.4)	260 (73.6)	459 (40.2)	470 (137.0)	492 (91.0)	420 (38.6)	1553* (170.2)
Glucose and CAG	9	239 (74.9)	270 (62.9)	401 (73.1)	525 (63.0)	545 (8.7)	571 (76.0)	1757 (220.9)
Glucose and Salt	10	242 (61.5)	328 (36.9)	564 (125.0)	567 (120.5)	640 (51.5)	580 (54.6)	2026 (217.5)
Glucose CAF and Salt	9	210 (82.1)	250 (52.0)	335 80.6	450 92.9	493 (93.7)	387 (101.0)	1425 (304.2)

Table 6. Effect of C annuum var. fasciculatum (CAF), C. annuum var. abbreviatum (CAA), C. annuum var. grossum (CAG), common salt (NaCl), and CAF combined with NaCl on glucose tolerance in diabetic rats.

N.B. Results are presented as mean (SD). Asterisk (*) indicate significant difference from the control: * < 0.05; ** $P < 0.01$).

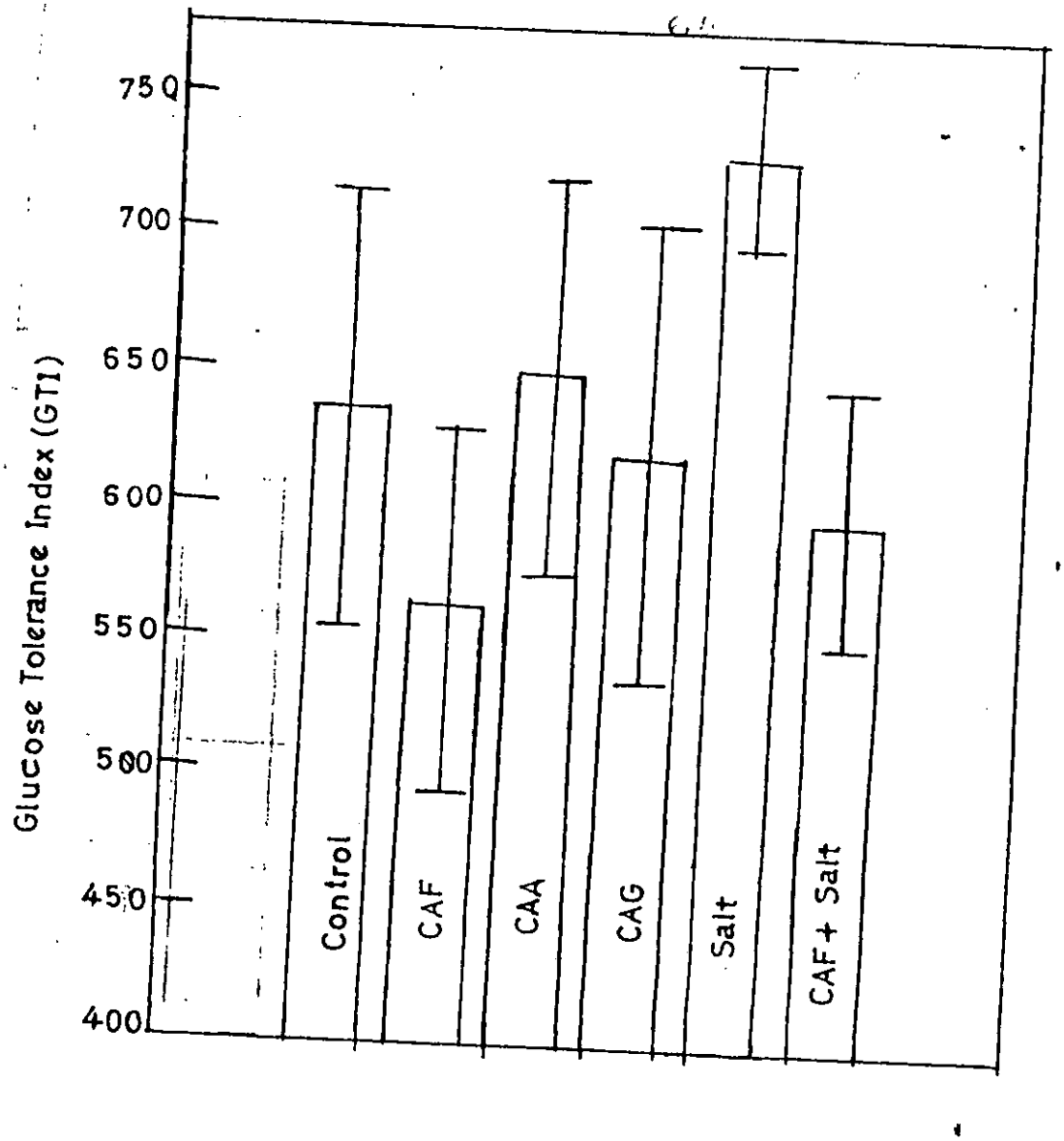

Fig.6b. Glucose tolerance index (GTI) in nondiavetic rats treated with dietary substances. N.B. CAF = Capsicum annuum var fasciculatum, CAA = C: annuum var abbreviatum; CAG = C. annuum var grossum. Vertical bars represent ± S.D.

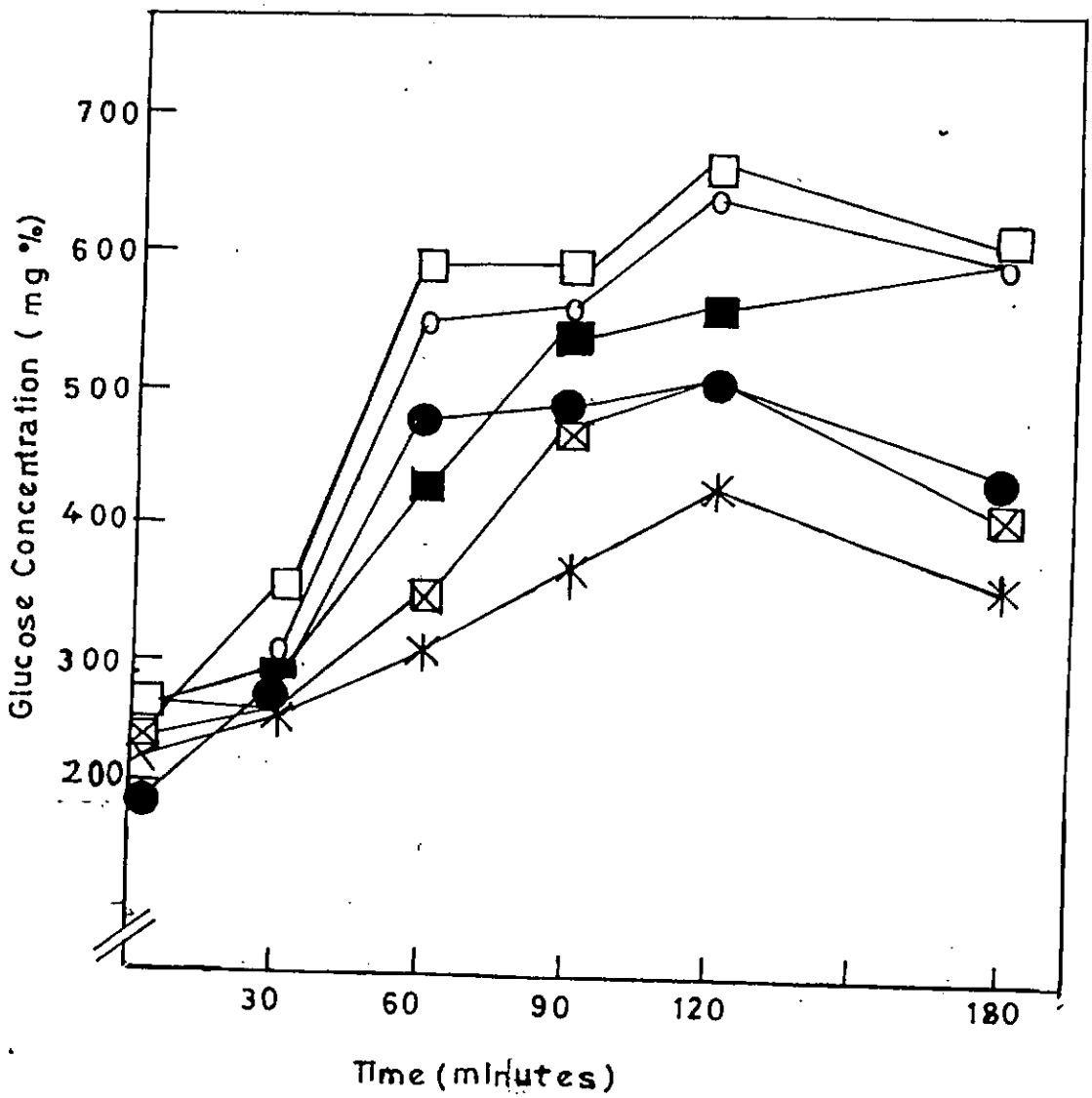

Fig.7a. The effect of common salt and three varieties of chillie peppers on glucose tolerance in alloxan-diabetic rats. N.B.: (o) control; (*) Capsicum annuum var fasciculatum; (●) C. annuum var abbreviatum; (■) C. annuum var grossum; (□) common salt; (⊠) common salt combined with C. annuum var. fasciculatum.

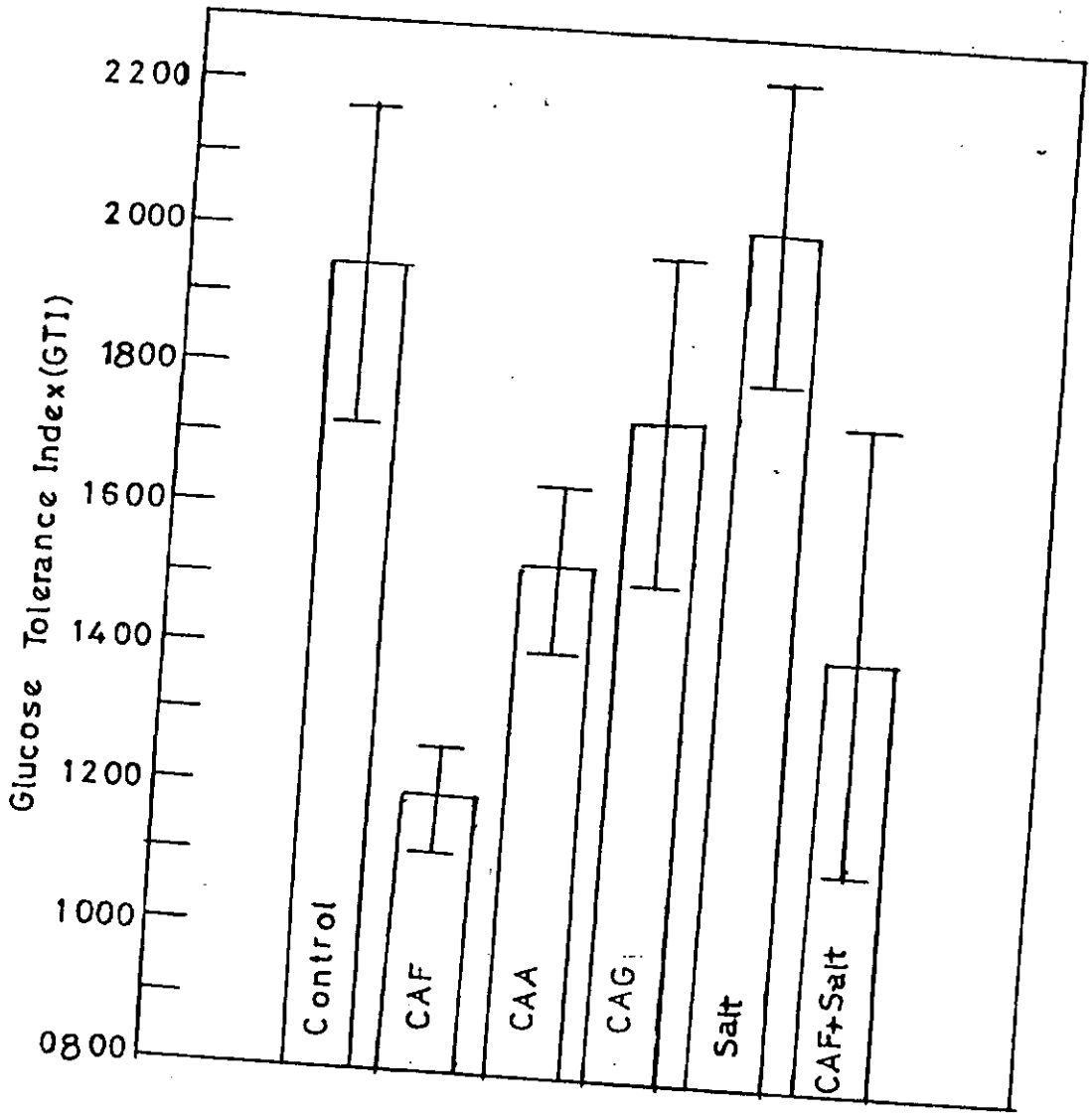

Fig.7b. Glucose tolerance index (GTI) in diabetic rats treated with dietary substances N.B. CAF = Capsicum annuum var. fasciculatum, CAA = C: annuum var abbreviatum; CAG =C. annuum var grossum. Vertical bars represent + S.D

treated with sweet peppers was 620±85.0. It was not significantly different from 636±80.8 which was the mean GTI for the nondiabetic control animals ($P>0.05$).

In the diabetic category also, no statistically significant difference could be established between the mean GTI of animals treated with sweet peppers which was 1757±220.9 and that of the diabetic control which was 1965±226.2 (Tables 5 & 6; Figs 6b & 7b).

Thus, among the three common varieties of pepper considered in this study, C. annuum var. fasciculatum (cluster pepper) had the most considerable plasma glucose reducing effect during glucose tolerance testing. This effect was consistent in the nondiabetic and diabetic states. C. annuum var abbreviatum (Wrinkled Peppers) reduces plasma glucose level during glucose tolerance testing only in the diabetic state while C. annuum var. grossum (sweet peppers) had no effect in both the nondiabetic and diabetic conditions. C. annuum var. fasciculatum therefore appeared to be the only likely candidate that may be efficacious in diabetic therapy.

3.4.2. Influence of Common Salt (NaCl) on Glucose Tolerance

Unlike C. annuum var. fasciculatum, salt increased glycaemic levels in nondiabetic and diabetic rats. The glucose tolerance index of 734±33.9 in nondiabetic salt - treated rats was significantly higher ($P<0.05$) than 636±80.8 which was the GTI of nondiabetic controls (Table 5). These consistent increases in plasma glucose concentrations during glucose tolerance testing indicated that salt increased

glycaemic response to glucose challenge in the animals (Fig.6a).

In the diabetic rats salt also increased glycaemic response to glucose challenge during GTT. The glucose tolerance index (GTI) of 2026±217.5 was significantly higher than that of the diabetic controls which was 1965±226.2 (Table 6; Fig.7b). These results indicate that salt may not have any desirable effect in diabetes.

3.4.3 Joint Effect of C annuum var. fasciculatum and Common Salt (NaCl) on Glucose Tolerance

At the time of completing this investigation, it was gathered that common salt and cluster peppers (C. annuum var. fasciculatum) are usually included as condiments in anti-diabetic preparations by many Nigerian Herbalists. The results of this study had, however, shown that these two dietary substances (out of the four considered) had consistent but opposite effects on glucose tolerance in both the nondiabetic and diabetic states. It was, therefore, of major interest to see the effects of a combined solution of common salt and cluster peppers on glucose tolerance.

In the nondiabetic category, the mean glucose tolerance index (GTI) of the animals given the combined solution was 597±48.2. This value was observed to be between that of salt - and cluster pepper - treated animals which were 734±33.9 and 566±74.7 respectively (Table 5; Fig.6b). The trend is similar with diabetic animals. The mean GTI of the

group administered with the combined solution was 1425±304.2. This value was between 2026±217.5 and 1202±69.9 the glucose tolerance indices of rats given salt and cluster peppers respectively (Table 6; Fig.7b).

The mean glucose tolerance curves of animals treated with the combined solution were found to lie between those of animals treated with only salt and only pepper solution in both the nondiabetic and diabetic rats (Figs 6a&7a).

4.0 DISCUSSION

The fact that alloxan diabetogenicity lies in its direct toxic action on the pancreatic beta cells is not an issue in debate (Beach et al, 1956; Howell, 1967; Lundquist and Rerup, 1967). It is well known that alloxan toxicity causes beta cell injury which results in the loss of insulin secretory function of the pancreas. Since insulin plays a very important role in glucose homeostasis because of its hypoglycaemic effects, an insufficiency or inefficiency of this hormone (insulin) causes hyperglycaemia - the most crucial diagnostic feature of diabetes mellitus (Baird and Strong, 1974; Cahill, 1979). The hyperglycaemia is due to two main mechanisms: a reduction in the rate of glucose removal from the blood by the peripheral tissues and an increase in the rate of release of glucose from the liver into the circulation. The latter mechanism consists of hepatic gluconeogenesis and lipolysis that follow as compensatory reactions to insulin lack under the influence of such hormones as growth and adrenocortical hormones (Baird and Strong, 1974).

The poor state of glucose metabolism in diabetes implies that the rate of intestinal absorption of glucose and other food substances will surpass body fuel utilization. This condition is most easily and clearly assessed by Glucose Tolerance Test (GTT) whereby the blood level of a glucose load administered orally is followed for a period of time (Baird and Strong, 1974; Cahill, 1979; WHO, 1979). Thus, the significantly higher plasma glucose concentrations (PGCs)

of the alloxan-treated rats as compared with those of untreated rats during GTT in this study showed that experimental diabetes has been successfully established.

Apart from glucose intolerance which was considered in this study as the main indicator of diabetes, other important symptoms that showed the successful establishment of diabetes in the alloxan-treated rats were also present. These include the presence of abnormally wet cages which might have resulted from polyuria (profuse urination). Moreover, it was frequently necessary to re-fill the water bottles of alloxan - treated rats suggesting the presence of polydipsia (excessive thirst). The mechanism responsible for these observations have been reported by Baird and Strong (1974). In the hyperglycaemic state, glucose concentration in the blood may exceed the renal reabsorption capacity for glucose, and glycosuria (excretion of glucose in urine) results. The level of blood glucose at which this happens in the majority of people is approximately 180mg/100ml. No information could be found on the renal reabsorption thresholds in experimental animals including rats. The presence of glucose in the glomerular filterate increases its osmolality. Thus, water reabsorption is prevented as the filterate passes down the renal tubular system. There is marked increase in the volume of urine (polyuria) and the loss of water and minerals causes excessive thirst (polydipsia).

In this study sex did not influence glucose tolerance significantly in the nondiabetic and alloxan-diabetic animals. Therefore, on the basis of oral glucose tolerance assessment,

sex difference in sensitivity to the diabetogenic effect of alloxan do not exist. This observation was consistent with that of Lukens (1948). Later, Beach et al (1951) noted more severe and higher incidence of glycosuria and ketonuria (ketones in urine) in alloxan-diabetic female rats when compared with males. They pointed out the similarity of this observation to that of humans where diabetes appears much more frequently among women than men. However, Beach et al (1951) could not show any sex difference in the postprandial (after meal) blood sugar concentrations. Thus, the parameter used to assess the influence of diabetes matters; while glycosuria and ketonuria may show greater susceptibity to alloxan toxicity in female than male rats, glucose tolerance or plasma glucose determinations may not.

Obesity is a common clinical disorder associated with insulin resistance. This fact has been repeatedly demonstrated in human and animal experimental studies (Rizza et al, 1981; Horton, 1983). Diabetes develops if there is a beta cell defect which hinders insulin secretory capacity to compensate for the insulin resistance caused by obesity (Horton, 1983). Since variation in body weight within normal range does not contribute to insulin resistance or glucose intolerance in man, it was not surprising that the body weights of the non-obese rats used in this study did not significantly influence glucose tolerance.

No difference in the pattern of tolerance was found between the parents and offspring from Idi-Araba and Akoka colonies in the nondiabetic category. In contrast, glucose

tolerance assessment showed that alloxan - diabetic animals from Idi-Araba had significantly reduced tolerance to glucose when compared with their Akoka counterparts ($P < 0.001$). This difference in glucose tolerance was largely due to the significantly higher fasting plasma glucose concentration (FPGC) in the alloxan-diabetic rats from Idi-Araba (Table 4; Fig.5a). This difference may be due to variation in sensitivity to alloxan toxicity between the two colonies. If this is the case, animals from Idi-Araba must be more sensitive than those from Akoka to alloxan action.

The difference in sensitivity to alloxan indicated above seemed largely genetic because first and second (F_1 and F_2) generation offspring showed remarkably the same pattern of tolerance as their respective parents. The evidence in support of the presence of genetic factors is strengthened by considering the fact that the offspring were bred and maintained in the same rigidly controlled environment thereby minimizing or even eliminating the effects of environmental factors.

These results complement the observation in human beings that genetic predisposition to environmental causative factor is crucial in the development of type 1 (IDD) and type 2 (NIDD) diabetes (Barnett et al, 1981). Similar explanation of genetic susceptibility has been postulated (Kambo et al, 1989) for the third recently described form of diabetes (WHO, 1985) known as Tropical or Malnutrition Related Diabetes (MRD). It is believed that cassava (Manihot esculenta)

consumption is an important environmental factor in MRD. The presence of cyanogenic glycosides in this plant is thought to cause exocrine pancreatic damage in persons taking low protein diets (McMillan and Geevarghese, 1979).

The present study, however, further suggests that the interaction between genetic and environmental factors in diabetes also operates at the population level. If it is possible to have two isolated colonies of rats that differ in their genetic predilection to alloxan diabetes, it seemed reasonable to expect diverse cultural and racial differences in incidence and severity of diabetes in human populations. Long ago Mills (1930) found that the incidence of diabetes is low in Ireland, but higher among the Irish in Boston. Spellberg and Leff (1945) similarly found low incidence (3 percent) in New Orleans and a high incidence (45 percent) in New England. More recently, Cahill (1979) reported the rarity of diabetes in Eskimos and its prevalence in certain American Indians such as the Pima in Arizona where 50 percent of the population may develop diabetes. The disease is not common among the Chinese, in whom it is mild and accompanied by supersensitiveness to insulin; however, high rates were observed in Asian Indians who have moved to South Africa.

Moody (1962) pointed out that random breeding populations have a tendency to maintain genetic equilibrium and this must be overcome if any change is to occur. Thus, any factor which tends to break up large populations into smaller ones are likely to cause change. If two parts of one population

are separated by some barrier, they therefore no longer share from a common gene pool. This means exchange of genes between the populations so isolated is prevented. Therefore, the occurrence of new mutations, genetic drift and the action of natural selection in one population will be different from the other.

It is quite difficult to explain the divergence in susceptibility to alloxan between the rats selected from the two isolated colonies on the basis of a single hereditary difference. Quantitative or polygenic inheritance seems more probable. Nevertheless, more studies are still needed to characterise the genetic component of alloxan - diabetes in rats and other animals more accurately.

Physiologic saline (0.9g% NaCl) increased plasma glucose response during oral glucose tolerance test (OGTT) in the present study. Several years ago Clifford (1936) showed that sodium chloride accelerated *in vitro* hydrolysis of pure raw starch by salivary and pancreatic amylases. Acceleration of starch digestion by stimulating amylase activity may then explain why moderate addition of salt increased plasma glucose and insulin responses to bread and lentils (Thorburn *et al*, 1986). In this study, glucose was used as the carbohydrate and similar results were obtained. Therefore, acceleration of small intestinal absorption of glucose may be an additional mechanism through which salt increases glycaemic response.

Oral rehydration fluid replaces the electrolytes and water lost during diarrhoea. The sodium in this

fluid is known to help the transport of glucose by the sodium/potassium-dependent adenosine triphosphate accross the small intestine (Thorburn et al, 1986). This mechanism may also fascilitate the transport of glucose during glucose tolerance testing thereby leading to increase in glycaemic response as observed in this study.

Salt, by virtue of this effect on glucose tolerance, may not be efficacious in the treatment of human diabetes mellitus. Infact salt may worsen an already established diabetes or even accelerates its progress towards mortality. It is also quite likely that chronic excess salt ingestion may precipitate diabetes in a subject who appears normal but is predisposed to the disease for genetic reasons. It may therefore be advised that the general population, particularly the diabetics, should restrict their salt intake.

Cluster peppers (C. annuum var. fasciculatum), unlike salt, reduced glycaemic response during oral glucose tolerance test (OGTT). This explains why the mean glucose tolerance curve and GTI of rats treated with a combined solution salt and cluster peppers could be located somewhere between those of animals given either salt or cluster pepper solution alone (Figs.6a,6b,7a,7b). It is likely that cluster peppers achieved its effect on oral glucose tolerance (OGT) by delaying gastric emptying into the duodenum. This implies that a low level of glucose will be passed to the small intestine for absorption in a particular period of time. The active and pungent principle of pepper, capsaicin, is known to be toxic to the

intestinal mucosa (Sirsatnik and Khanokar, 1960). Thus, cluster peppers may be irritating to the duodenum thereby automatically depressing the pyloric pump. This is achieved by an enterogastric reflex from the duodenum to the stomach which inhibits the degree of antral peristalsis in the stomach. The irritation can also cause intestinal release of enterogastrone, a hormone which passes through the blood to the stomach also to inhibit the pyloric pump activity by depressing antral peristalsis (Guyton, 1961).

Moreover, the toxicity of pepper on the intestinal mucosa (Sirsatnik and Khanolkar, 1960) may lead to destruction of some of the intestinal absorptive cells . The surface area available for glucose absorption from the intestine would therefore be reduced considerably. It is also propable that capsaicin interferes in some manner with the sodium pump mechanism which facilitates active uptake of glucose from the gut to the blood (Guyton, 1961). It may also be that cluster peppers increases pancreatic insulin release or/and enhances insulin - mediated glucose uptake by the periperal tissues.

The plasma glucose reducing effect of cluster peppers during glucose tolerance test suggests that this pepper variety may play a desirable role in the treatment of human diabetes mellitus. However, this desirable effect must be balanced against the possible toxic action which this variety of pepper may have on the intestinal mucosa. Intensive insulin and sulphonurea drug therapy increase the risk of hypoglycaemic encephalopathy (Young, 1985) and other deleterious

conditions (Baird and Strong, 1974; Cahill, 1979). The immediate benefits derived from such dietary regimes that are rich in cluster peppers cannot be overemphasized. This is because the pepper will reduce insulin and hypoglycaemic drug demand thereby reducing adverse reactions and complications associated with the use of these agents.

Wrinkled peppers (C. annuum var. abbreviatum) significantly reduced glycaemic response in the diabetic condition only. As compared with cluster peppers (C. annuum var. fasciculatum), wrinkled peppers had a lower plasma glucose concentration reducing effect during OGTT in both the non-diabetic and alloxan - diabetic rats. Sweet peppers (C. annuum var. grossum) did not affect glucose tolerance in both the nondiabetic and diabetic animals. It is significant to note that the magnitude of effects of these three varieties of chilli peppers varied directly according to the degree of their pungency. Thus, the pungent principle of peppers, capsaicin, may be the ingredient responsible for the effects observed in this study. It will of course be interesting to study the effect of pure capsaicin on glucose tolerance.

The exact mechanism(s) through which these dietary substances influenced glucose tolerance in this study is still unclear. It will, therefore, be elucidating to focus future research efforts on the effects of these dietary substances particularly common salt (NaCl and cluster peppers) on the following physiological processes:

(i) gastric emptying in rats and other experimental animals,

(ii) in vitro absorption of glucose using the everted gut sac (Crane, 1960),

(iii) insulin response (Jimenez et al, 1986), and

(iv) in vitro insulin - stimulated glucose uptake in muscle and liver tissues (Le-Marchand-Brustel, 1978).

SUMMARY

Diabetes was successfully induced in experimental rats by intravenous (jugular vein) administration of alloxan at 4.0mg/100g body weight as 0.8g% solution.

Based on oral glucose tolerance test (OGTT), rats from Idi-Araba were more sensitive to alloxan than those from Akoka. This difference in alloxan sensitivity was found to be largely genetic because this same pattern of glucose tolerance was observed in two consecutive generations of offspring bred and maintained in the same environment.

Out of the three common varieties of chilli peppers considered in this investigation, cluster peppers (C. annuum var. fasciculatum) appeared to be the only likely candidate for the treatment of human diabetes mellitus. This is based on the fact that it most significantly lowered glycaemic response in both the nondiabetic and diabetic animals when compared with other varieties of peppers viz: wrinkled peppers (C. annuum var. abbreviatum and sweet peppers (C. annuum var. grossum). Salt (NaCl) increased glycaemic response during glucose tolerance test in both the nondiabetic and diabetic rats.

Thus, cluster peppers may have desirable effects in diabetes while NaCl may worsen the disease. The different incidence and severity of diabetes in different populations may be due largely to genetic factors.

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ABSTRACT

The influence of genetic and some dietary factors on glucose tolerance in normal (nondiabetic) and diabetic rats (Rattus norvegicus) has been investigated. Diabetes was induced by intravenous (jugular vein) administration of alloxan at 4.0mg/100g body weight as 0.8g% solution. It was observed that the mean glucose tolerance index (GTI) of the alloxan - treated rats was 1965 ± 226.2. This was significantly higher than the mean GTI of the nondiabetic rats which was 636 ± 80.8 ($P < 0.01$). No significant association of glucose tolerance could be established with either sex or body weight.

Alloxan - treated animals of Idi-Araba colony were found to have significantly higher fasting plasma glucose concentration (FPGC) of 245±28.9mg% when compared with the mean FPGC of those from Akoka which was 134±20.2mg% ($P<0.05$). The mean GTI of Idi-Araba alloxan-diabetic rats was therefore, correspondingly higher than that of Akoka animals (1940±74.6 vs 1601±39.1; $P<0.05$). That this difference in glucose tolerance between animals from the two isolated colonies was largely genetic was indicated by the first (F_1) and second (F_2) generation offspring having a pattern of observation that was remarkably similar to that of the parents.

In the nondiabetic category, treatment with cluster peppers (C. annuum var. fasciculatum) resulted in significantly lower mean GTI of 566±74.7 as compared to 636±80.8 which was the mean GTI of the nondiabetic control animals ($P<0.05$). The mean GTI of alloxan-diabetic rats treated with cluster peppers was also significantly lower than that of the alloxan-diabetic

controls (1202±69.9 vs 1965±226.2; P<0.01). Common salt (NaCl) on the other hand had an opposite effect on glucose tolerance in that the mean GTI of salt - treated nondiabetic and diabetic rats were significantly higher than those of their respective controls. Expectedly, the mean glucose tolerance curves of rats (nondiabetic and diabetic) treated with a combined solution of common salt and cluster peppers were located somewhere between the curves of those treated with either salt or cluster pepper alone.

Wrinkled peppers (C. annuum var. abbreviatum) lowered GTI significantly in the diabetic category alone (P<0.05) while sweet peppers (C. annuum var grossum) had no significant effects on glucose tolerance in both the nondiabetic and diabetic rats. Thus, as compared with wrinkled and sweet peppers, cluster peppers appeared to have the strongest and most consistent plasma glucose reducing effect during glucose tolerance test in both the nondiabetic and diabetic states.

It could therefore be suggested that the different incidence and severity of diabetes in different human populations may be due largely to genetic factors. Moreover, if the results of the effects of the dietary substances are confirmed by other animal and human experimental studies, cluster peppers should be of value in the treatment of diabetes mellitus. On the other hand, salt consumption should be restricted in the general population, particularly in the diabetic

1.0 INTRODUCTION

According to WHO Study Group on Diabetes Mellitus (1985), diabetes is recognized by elevation of the blood glucose concentration (hyperglycaemia) resulting from insulin insufficiency or inefficiency. The classical symptoms of diabetes include severe thirst (polydipsia), profuse urination (polyuria) and weight loss (Baird and Strong,1974). Two important key factors have been identified in the development of the disease; these are the genetic constitution and the environment (Mills, 1930; Horton, 1983). It is generally believed that an individual inherits a susceptibility to develop diabetes and that one or more environmental factors can eventually precipitate the disease. In the absence of effective treatment, diabetes culminates in coma and death (Baird and Strong,1974; Cahill, 1979; WHO, 1985).

The clinical diagnosis of diabetes is often prompted by the classical symptoms (Baird and Strong, 1974). In this circumstance, according to WHO (1985), a single plasma glucose estimation in excess of 200mg% is sufficient to establish diabetes. However, a random plasma sugar estimation below 200mg% does not exclude the disease and in this case, standardization of the conditions under which the blood sugar estimation is done is necessary. As a result, the Oral Glucose Tolerance Test (OGTT) has been of fundamental importance in the diagnosis of diabetes (Junod et al, 1969; Baird and Strong, 1974; Cahill, 1979; WHO, 1979; WHO, 1985).

To perform oral glucose tolerance test (OGTT) in human subjects, WHO (1985) recommendations need to be followed. The test should be carried out in the morning after an overnight fast of 10-16 hours during which water may be drunk. After collection of the fasting blood sample, the subject should drink 75g of glucose dissolved in 250-300 ml of water over the course of 5 minutes. Blood samples must be collected 2 hours after the glucose loading; if appropriate, samples may also be taken every half an hour during this period. A fasting plasma glucose concentration (FPGC) greater than 140mg% and a 2-hour plasma glucose level of 200mg% and above indicates diabetes mellitus.

For the past few years, studies on glucose tolerance have attracted considerable interest for many reasons. For instance, glucose tolerance has genetic significance because of the association of a large number of genetic diseases with a high incidence of abnormal glucose tolerance. These genetic diseases, caused by chromosomal aberrations and inborn errors of metabolism include the Prader - Willi syndrome, sexual ateliotic dwarfism, Schmidt's syndrome, Friedriech's ataxia, optic atrophy, nerve deafness and Turner's syndrome (Cahill, 1979). Moreover, glucose tolerance is important as a test system for screening of newly discovered antidiabetic agents or modifying substances (Beach et al , 1956; Junod et al , 1969; Reaven, 1963).

There is evidence that the overall worldwide prevalence of diabetes is gradually increasing and this has continued to generate much concern (Gill, 1990). The gravity of this

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APPENDIX 1

Composition of Animal Feed from Pfizer and the Proportion of the Major Food Substances Present According to the Manufacturer.

Composition.

Maize
Groundnut
Wheat Middling
Fish Meal
Brewer's Fried Grain
Vitamin Premix
Mineral Premix
Bone Meal
Oyster Shell
Salt
Anti - Oxidant

Proportion of Major Food Substances.

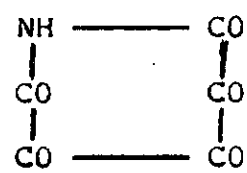
Protein	14%
Fat	3%
Fibre	8%

APPENDIX 2

Formular and structure of Alloxam (2,4,5,6 - Tetraoxypyrimidine or 5,6, Diouracil)

Formular: $C_4(NH)_2\ O_4$

Structure:



APPENDIX 3

Glucose Oxidase/Peroxidase (GOD/POD) REAGENTS.

Reagents.

Vial 1: Buffer/Enzymes/Chromogen

Bottle 1A: Phenol

Standard: glucose 100mg%

Preparation of Working Solution.

Solution (1): Add the contents of one vial 1 to one bottle 1A. Mix untill completely dissolved.

Procedure.

Pipette into test tubes

	Blank	Standard	Sample
Sample	-	-	0.02ml
Standard	-	0.02ml	-
Solution (1)	2.50ml	2.50ml	2.50ml

Mix and incubate at 37°c for 15 minutes or allow to stand at room temperature for at least 30 minutes.

Read the absorbance of the sample (A sample) and of the Standard (A standard) against the blank.

Wave length: 505nm (500 - 550nm)

Cuvette: 1cm light path

Temperature: 37°c or room temperature (not less than 20°c)

Reading: against blank

APPENDIX 4A

OGT in Nondiabetic (Normal) Control Male Rats.

Rat No	B.Wt.	PGC (mg%)						GTI
		0min	30min	60min	90min	120min	180min	
1	200	125	174	163	161	146	119	553
2	200	107	196	176	192	206	144	633
3	180	114	195	201	238	133	98	546
4	160	103	162	180	193	154	103	540
5	200	114	185	196	210	185	141	636
6	195	120	202	214	253	227	186	747
7	200	118	196	208	238	213	171	770
8	188	108	173	179	185	159	119	565
9	198	110	177	185	194	168	127	590
Mean	191	113	184	189	207	177	134	613
S.D.	13.6	6.9	13.6	16.7	30.1	32.7	29.5	74.6

APPENDIX 4B.

OGT in Nondiabetic (Normal) Control Female Rats.

Rat No	B.Wt.	PGC (mg%)						GTI
		0min	30min	60min	90min	120min	180min	
1	150	134	220	183	188	167	111	595
2	185	135	220	261	262	200	143	739
3	165	138	217	233	202	167	130	674
4	150	132	173	144	-	133	98	507
5	161	117	193	197	223	170	153	637
6	185	102	217	221	247	239	176	738
7	175	100	144	150	142	128	113	491
8	179	106	211	216	242	200	171	693
9	160	119	195	199	225	180	155	653
10	182	138	214	218	244	200	154	730
11	170	122	240	228	288	216	144	710
12	155	114	190	200	220	212	150	676
Mean	168	122	203	204	226	184	143	654
S.D.	13.1	14.7	25.7	33.5	39.1	33.2	25.7	83.9

APPENDIX 5A

OGT in Diabetic Control Male Rats.

Rat No	B.Wt.	PGC (mg%)						GTI
		0min	30min	60min	90min	120min	180min	
1	200	292	252	626	628	662	607	2187
2	197	195	205	320	348	558	472	1547
3	200	204	267	456	475	609	539	1810
4	193	231	299	524	538	620	575	1950
5	200	157	351	726	605	619	567	2069
6	180	290	-	638	-	665	521	2114
7	165	315	323	682	619	694	633	2324
8	150	292	299	616	569	658	590	2156
9	150	235	251	483	469	586	504	1808
10	200	219	227	416	419	550	461	1646
Mean	184.5	243	475	549	519	622	547	1961
S.D.	22.1	51.9	46.9	129.6	97.4	47.9	57.4	252.3

APPENDIX 5B

OGT in Diabetic Control Female Rats.

Rat No	B.Wt.	PGC (mg%)						GTI
		0min	30min	60min	90min	120min	180min	
1	194	179	-	412	-	538	621	1749
2	186	192	252	424	443	604	521	1741
3	150	348	330	482	478	590	618	2038
4	200	269	316	591	598	649	597	2108
5	180	197	309	574	719	706	638	2135
6	165	301	362	571	668	731	681	2284
7	178	269	332	534	614	677	640	2117
8	191	173	272	460	506	567	558	1758
9	150	205	342	571	452	503	517	1796
Mean	177	237	314	513	560	618	599	1970
S.D.	18.4	61.8	36.5	69.7	104.4	77.3	56.2	208.4

APPENDIX 6A

OGT in the Parental Generation of Nondiabetic Rats from Akoka.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30min	60min	90min	120min	180min	
1	M	150	136	174	102	148	121	94	453
2	F	190	144	122	89	117	108	102	425
3	F	150	110	116	175	187	130	115	530
4	F	160	102	142	142	148	123	100	467
5	F	170	124	152	194	133	124	111	553
Mean		164	122	150	140	147	121	104	486
S.D.		16.7	17.5	19.7	45.2	25.9	8.1	8.5	53.8

APPENDIX 6B

OGT in the Parental Generation of Nondiabetic Rats from Idi-Araba.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30min	60min	90min	120min	180min	
1	F	175	102	131	131	119	101	103	437
2	F	180	120	167	132	127	115	91	458
3	M	200	119	131	132	108	102	72	425
4	F	165	128	175	178	165	149	96	551
Mean		180	117	151	143	130	117	91	468
S.D.		14.7	10.9	23.3	23.2	24.8	22.4	13.2	57.2

APPENDIX 7A

OGT in Akoka Diabetic Patent Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30min	60min	90min	120min	180min	
1	F	200	147	246	297	525	574	563	1581
2	M	195	155	194	284	494	561	571	1571
3	F	150	121	233	370	564	583	584	1658
4	F	161	113	214	337	525	576	569	1595
Mean		177	134	222	322	527	574	572	1601
S.D.		24.7	20.2	22.7	39.1	28.7	9.2	8.8	39.1

APPENDIX 7B

OGT in Idi-Araba Diabetic Parent Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30mins	60mins	90mins	120mins	180mins	
1	F	150	217	267	513	526	554	583	1867
2	F	165	285	247	514	594	568	662	2029
3	M	170	234	267	514	514	500	643	1891
4	F	205	243	311	560	562	602	567	1972
Mean		173	245	273	525	549	556	614	1940
S.D.		23.3	28.9	27.0	23.2	36.3	42.4	45.9	74.6

APPENDIX 8A

OGT in First Generation Nondiabetic Offspring of Akoka Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30min	60min	90min	120min	180min	
1	F	165	82	170	98	112	93	74	347
2	M	150	119	123	119	140	120	107	465
3	M	165	135	129	140	130	107	102	484
4	F	187	114	151	146	122	118	94	472
5	M	175	119	149	131	132	109	98	457
6	F	185	131	161	142	141	115	106	494
7	F	200	101	125	113	123	100	86	400
8	F	160	125	155	137	135	112	102	476
9	F	190	107	131	119	126	103	90	419
10	M	187	95	119	107	120	97	82	381
Mean		176	113	141	125	128	107	94	440
S.D.		15.9	16.6	17.9	16.3	9.2	9.1	10.9	49.7

APPENDIX 8B

OGT in 1ST Generation Nondiabetic Offspring Of Idi-Araba Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30min	60min	90min	120min	180min	
1	F	155	103	149	114	130	106	78	401
2	F	150	136	166	121	131	112	92	461
3	F	175	118	150	152	132	127	115	512
4	M	170	117	149	111	115	110	83	421
5	M	180	123	157	130	130	117	96	466
6	F	190	131	163	140	136	123	104	498
7	F	165	113	148	115	121	107	84	419
8	M	166	127	160	135	133	120	100	482
9	M	178	117	151	120	124	111	88	436
10	M	190	110	145	110	118	104	80	404
Mean		172	120	154	125	127	114	92	450
S.D.		13.4	9.9	7.2	13.9	7.0	7.7	11.9	39.5

APPENDIX 9A

OGT in 1ST Generation Diabetic Offspring Of Akoka Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30min	60min	90min	120min	180min	
1	M	150	118	219	442	529	581	674	1815
2	M	175	110	220	365	559	578	569	1622
3	F	180	160	195	289	495	566	576	1591
4	F	165	142	241	280	530	569	5588	1549
5	M	200	143	243	290	522	571	560	1564
6	F	200	140	190	287	497	564	575	1566
7	M	160	118	230	367	564	590	581	1656
8	F	154	116	217	340	528	579	572	1607
9	M	165	136	220	320	525	576	570	1602
10	F	170	117	209	289	512	568	561	1555
11	F	190	145	229	373	544	582	603	1703
Mean		175	131	219	331	528	575	582	1621
S.D.		17.3	16.1	16.7	51.2	22.1	8.0	33.0	79.3

APPENDIX 9B

OGT in First Generation Diabetic Offspring Of Idi-Araba Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30min	60min	90min	120min	180min	
1	M	190	238	306	555	557	597	562	1952
2	F	200	230	272	510	533	505	648	1893
3	M	165	280	242	509	589	563	650	2082
4	F	170	208	272	518	539	559	579	1856
5	M	150	220	260	510	529	557	584	1871
6	M	200	282	244	511	593	565	659	2017
7	F	195	237	260	517	625	503	646	1903
8	F	180	240	308	557	559	597	559	1953
Mean		181	241	271	523	566	556	611	1931
S.D.		18.3	27.9	25.1	20.4	33.9	35.7	43.4	59.5

APPENDIX 10A

OGT in Second Generation Nondiabetic Offspring Of Idi-Araba Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30min	60min	90min	120min	180min	
1	M	150	131	163	140	137	124	101	496
2.	F	165	100	140	103	112	101	82	386
3	M	163	115	150	120	125	110	92	437
4	F	175	145	170	160	147	127	110	542
5	F	180	110	147	118	120	106	87	421
6	M	165	120	154	130	135	114	95	459
7	F	157	125	154	137	135	118	95	475
Mean		165	121	154	130	130	114	946	459
S.D.		10.1	14.7	9.9	18.4	11.8	9.4	9.1	51.9

APPENDIX 10B

OGT in Second Generation Nondiabetic Offspring Of Akoka Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30min	60min	90min	120min	180min	
1	F	165	117	146	130	133	109	100	456
2	F	170	105	131	115	118	103	90	413
3	M	175	125	156	140	143	113	105	483
4	M	174	94	121	105	108	97	85	381
5	M	169	139	176	155	158	125	117	536
6	F	160	109	136	120	123	105	91	425
7	M	180	103	126	110	113	101	87	401
8	F	175	113	141	125	128	107	94	439
9	M	160	121	151	135	138	111	101	468
10	M	170	129	166	145	148	115	107	496
Mean		170	116	145	128	131	109	98	450
S.D.		6.6	13.5	17.0	16.0	16.0	7.9	10.1	47.4

APPENDIX 11A

OGT in Second Generation Diabetic Offspring Of Idi-Araba Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30min	60min	90min	120min	180min	
1	M	165	205	247	499	531	517	575	1796
2	M	175	253	279	531	578	568	623	1975
3	F	175	298	320	577	650	540	690	2105
4	F	165	265	287	539	590	582	634	2020
5	F	200	241	271	523	566	556	611	1931
6	M	180	229	263	515	555	543	600	1887
7	F	180	217	255	507	542	529	587	1840
8	F	175	277	295	547	602	596	648	2068
Mean		177	248	277	530	576	554	621	1952
S.D.		10.9	31.3	24.0	24.9	38.0	26.8	36.7	109.2

APPENDIX 11B

OGT in Second Generation Alloxan-Diabetic Offspring Of Akoka Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30min	60min	90min	120min	180min	
1	F	175	132	220	335	524	573	582	1622
2.	F	173	139	227	365	641	585	600	1689
3	M	189	140	223	348	533	590	610	1688
4	M	177	115	186	269	498	560	556	1500
5	M	170	130	202	300	512	563	569	1562
6	F	190	142	230	382	649	594	611	1729
7	F	200	136	210	324	529	569	565	1594
8	F	165	125	194	283	505	557	560	1525
9	M	165	147	235	399	754	601	620	1767
Mean		178	134	192	333	571	577	586	1631
S.D.		12.2	9.7	65.9	44.4	88.7	16.1	24.6	93.0

APPENDIX 12

C. annuum Var. *fasciculatum* and OGT in nondiabetic Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30mins	60mins	90mins	120mins	180mins	
1	M	190	112	177	70	186	198	190	570
2	F	180	146	133	176	181	180	135	637
3	M	200	109	183	187	186	181	172	649
4	F	195	111	165	164	169	165	117	557
5	M	200	113	167	192	150	144	116	615
6	M	180	78	131	133	144	145	110	466
7	F	210	106	159	190	170	181	154	631
8	F	200	87	183	124	112	115	82	408
9	M	200	101	162	180	174	171	152	604
10	M	200	121	125	117	144	154	109	501
11	F	210	87	192	174	155	175	108	544
12	F	195	126	126	180	182	177	124	607
Mean		197	108	159	157	163	166	131	566
S.D.		9.61	18.6	24.1	37.9	22.4	22.5	30.9	74.7

APPENDIX 13

C. annuum Var fasciculatum and OGT in Diabetic Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30mins	60mins	90mins	120mins	180mins	
1	M	165	229	217	372	282	384	287	1172
2	M	195	203	-	269	390	444	293	1209
3	M	165	208	215	326	444	472	300	1306
4	F	155	228	219	273	282	383	289	1173
5	F	165	207	-	327	445	469	300	1303
6	M	170	205	291	268	392	443	293	1209
7	F	180	204	248	353	-	404	257	1123
8	F	150	213	290	273	293	365	330	1181
9	M	170	200	249	349	382	401	250	1200
10	M	170	213	-	279	293	376	339	1307
11	M	165	197	220	275	311	437	212	1121
12	F	190	194	247	274	315	343	310	1121
Mean		170	208	244	295	348	410	288	1202
S.D.		13.0	11.0	29.9	33.7	63.8	42.1	34.9	69.9

APPENDIX 14

C. annuum Var. *abbreviatum* and OGT in Normal Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30min	60min	90min	120min	180min	
1	M	160	96	180	207	210	276	134	713
2	M	150	139	165	161	195	151	149	600
3	F	140	70	150	271	180	145	121	607
4	F	160	112	135	210	-	195	133	650
5	F	175	161	-	185	224	226	100	672
6	M	155	84	190	187	-	145	109	525
7	F	170	100	-	217	239	172	118	607
8	M	165	132	209	197	-	226	136	691
9	F	180	148	221	227	254	253	145	773
Mean		162	116	179	207	217	199	127	649
S.D.		12.5	31.0	31.0	31.0	27.6	49.0	16.4	73.4

APPENDIX 15.

C. annuum var. abbreviatum and OGT in Diabetic Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30min	60min	90min	120min	180min	
1	M	160	152	260	499	470	481	394	1526
2	F	150	185	287	435	520	399	404	1423
3	M	150	207	314	483	569	478	421	1589
4	F	180	182	340	418	621	609	483	1686
5	M	175	150	367	402	669	363	366	1281
6	F	160	161	179	421	320	406	385	1 .73
7	F	175	172	-	440	420	449	404	1465
8	M	200	194	152	478	270	535	442	1649
9	M	195	205	233	497	-	578	423	1703
10	F	165	216	206	516	368	621	480	1833
Mean		171	182	260	459	470	492	420	1553
S.D.		17.3	23.4	73.6	40.2	137.0	91.0	38.6	170.2

APPENDIX 16

C. annuum var. *grossum* and OGT in nondiabetic Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30min	60min	90min	120min	180min	
1	F	145	131	190	189	200	183	145	648
2	F	150	131	174	156	185	165	129	581
3	F	160	121	161	193	169	171	138	623
4	M	160	109	146	284	150	200	152	744
5	M	180	117	129	143	139	151	115	526
6	M	170	112	206	137	215	133	122	504
7	F	165	117	221	165	229	142	131	555
8	F	175	127	34	221	246	183	143	674
9	M	200	132	251	249	260	192	150	723
Mean		167	122	190	193	199	169	136	620
S.D.		16.6	8.7	41.3	50.0	42.1	23.1	12.7	85.0

APPENDIX 17

C. annuum var. *grossum* and OGT in Diabetic Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30min	60min	90min	120min	180min	
1	F	150	195	270	314	525	553	468	1530
2	M	220	178	247	409	502	537	622	1746
3	F	210	340	224	484	479	540	621	1985
4	M	185	238	201	402	456	550	572	1762
5	M	160	138	178	295	433	533	463	1435
6	F	170	160	293	331	548	537	499	1533
7	M	150	202	316	369	-	541	535	1647
8	F	175	274	339	437	571	549	607	1867
9	F	180	310	-	467	593	554	643	1974
10	M	190	346	362	505	618	559	679	2089
Mean		179	239	270	401	525	545	571	1757
S.D.		23.4	74.9	62.9	73.1	63.0	8.7	76.0	220.9

APPENDIX 18

Common Salt and OGT in nondiabetic Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30mins	60mins	90mins	120mins	180mins	
1	M	165	133	181	215	205	214	165	722
2	F	175	130	222	218	196	191	187	726
3	M	150	133	196	233	225	225	192	783
4	F	200	137	224	225	213	217	205	784
5	M	210	143	188	213	196	206	138	700
6	M	200	120	175	217	200	205	158	700
7	F	195	127	150	217	188	192	176	712
8	F	200	132	219	215	206	227	172	746
Mean		187	132	194	219	204	210	174	734
S.D.		21.0	6.8	26.2	6.6	11.5	13.6	21.0	33.9

APPENDIX 19

Common Salt and OGT in Diabetic Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30mins	60mins	90mins	120mins	180mins	
1	F	160	356	360	522	508	610	628	2111
2	F	160	277	346	631	628	669	607	2184
3	F	170	225	339	614	749	726	648	2213
4	M	230	163	381	766	635	639	577	2145
5	M	200	298	-	673	-	685	531	2187
6	M	165	297	282	666	658	682	617	2262
7	F	205	186	-	452	-	558	631	1827
8	F	170	200	282	464	473	624	531	1819
9	M	199	205	335	360	378	578	482	1625
10	M	180	212	297	496	505	629	549	1886
Mean		184	242	328	564	567	640	580	2026
S.D.		23.5	61.5	36.9	125.0	120.5	51.5	54.8	217.5

APPENDIX 20

Joint Effect Of Common Salt and C. annuum var. fasciculatum on OGT in Diabetic Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30mins	60mins	90mins	120mins	180mins	
1	M	220	258	250	437	450	593	471	1759
2	M	150	167	231	233	407	392	260	1052
3	F	185	250	212	447	364	641	532	1870
4	F	200	171	193	219	321	342	213	945
5	M	210	208	174	336	493	493	317	1454
6	F	180	190	269	285	536	443	339	1257
7	F	185	233	288	386	-	546	439	160
8	M	160	210	307	336	579	491	392	1429
9	F	160	206	326	340	-	492	421	1459
Mean		183	210	250	335	450	493	387	1425
S.D.		23.8	32.1	52.0	80.6	92.9	93.7	101.0	304.2

APPENDIX 21

Joint Effect of Common Salt and C. annuum var. fasciculatum on OGT in Normal Rats.

Rat No	Sex	B.Wt.	PGC (mg%)						GTI
			0min	30min	60min	90min	120min	180min	
1	M	190	114	168	167	172	168	120	569
2	F	210	84	121	206	205	198	92	580
3	M	220	119	148	165	129	165	140	589
4	F	225	86	170	171	159	178	152	587
5	F	195	113	178	170	188	200	190	575
6	M	170	106	195	175	191	205	143	629
7	M	200	160	221	184	189	168	112	624
8	F	160	87	140	163	151	166	107	523
9	F	180	137	204	193	201	200	165	695
Mean		194	118	172	177	176	183	136	597
S.D.		22.0	25.3	32.0	14.5	25.4	17.2	31.0	48.2

APPENDIX 22

Statistical Analysis of Data.

The analysis of data obtained in this study was done using the following conventional statistical methods:-

(a) **Mean** $(\bar{X}) = \frac{1}{n}\sum x$

where n= number os observations

and $\sum x = X_1 + X_2 + ... + X_n$ or $\sum_{i=1}^{n} Xi$

(b) **Variance** $(S^2) = \frac{1}{n-1}\sum (x-\bar{x})^2$

Where the summation on the right-hand side is calculated by:

$$\sum (x - \bar{x})^2 = \sum x^2 - \frac{1}{n}\left\{\sum x\right\}^2$$

(c) **Standard Deviation** $(s) = \sqrt{\text{variance}}$

(d) **Variance - Ratio (F) Test:** This test is performed to know whether the variances of the two samples to be compared can be assumed equal.

$$F = \frac{S_1^2}{S_2^2}$$

where the samples are labelled so that S_1 is greater than S_2.

Then find from table of F - distribution (Apendix) the appropriate value of F for the chosen level of significance correnspondingto F_1 = $n_1 - 1$ degrees of freedom in the numerator and $F_2 = n_2 - 1$ exceeded in the data, the result is significant and the unknown variances should not be assumed equal when comparing the means of the samples by student t test.

(e) Student t test. For comparing the means of two small samples from normal populations with (unknown variance assumed equal),

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

where $s = \frac{\sum 1 \ (x - \bar{x}_1)^2 + \sum 2 \ (x - \bar{x}_2)}{n_1 + n_2 - 2}$

i where t, with $n_1 + n_2 - 2$ dgrees of freedom, can be read from Appendix 23 according to the probability required.

ii Unknown variance not assumed equal

$$d = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

Now treat as beign distributed like 'Student's' t with f degrees of freedom, the latter being given by:

$$f = \frac{1}{\frac{u^2}{n-1} + \frac{(1-u)^2}{n^2-1}}$$

where $u = \frac{s_1^2/n_1}{s_1^2/n_1 + s_2^2/n_2}$

(f) Regression Analysis

(i) Correlation coefficient (r) was calculated by the formular:

$$r = \frac{(x - \bar{x})(y - \bar{y})}{\sqrt{(x - \bar{x})^2 \quad (y - \bar{y})^2}}$$

(ii) Significance test for Correlation Coefficient was carried using:

$$t = \frac{r\sqrt{n-2}}{\sqrt{1 - r^2}}$$

The value of 't' can be reffered to in the usual Student t table (Appendix 23). The degrees of freedom (d.f.) in this case in n - 2.

The need to work out 't' using the equation in 'fii' above can be avoided by using the table in Appendix 24. The table gives the value of 'r' which must be exceeded for significance test at various levels. The degrees of freedom is n - 2 as usual.

APPENDIX 23

'STUDENTS' t-DISTRIBUTION (Bailey, 1981).

Degrees of freedom	*Value of P*					
	0.10	0.05	0.02	0.01	0.002	0.001
1	6.314	12.71	31.82	63.66	318.3	636.6
2	2.920	4.303	6.965	9.925	22.33	31.60
3	2.353	3.182	4.541	5.841	10.21	12.92
4	2.132	2.776	3.747	4.604	7.173	8.610
5	2.015	2.571	3.365	4.032	5.893	6.869
6	1.943	2.447	3.143	3.707	5.208	5.959
7	1.895	2.365	2.998	3.499	4.785	5.408
8	1.860	2.306	2.896	3.355	4.501	5.041
9	1.833	2.262	2.821	3.250	4.297	4.781
10	1.812	2.228	2.764	3.169	4.144	4.587
11	1.796	2.201	2.718	3.106	4.025	4.437
12	1.782	2.179	2.681	3.055	3.930	4.318
13	1.771	2.160	2.650	3.012	3.852	4.221
14	1.761	2.145	2.624	2.977	3.787	4.140
15	1.753	2.131	2.602	2.947	3.733	4.073
16	1.746	2.120	2.583	2.921	3.686	4.015
17	1.740	2.110	2.567	2.898	3.646	3.965
18	1.734	2.101	2.552	2.878	3.610	3.922
19	1.729	2.093	2.539	2.861	3.579	3.883
20	1.725	2.086	2.528	2.845	3.552	3.850
21	1.721	2.080	2.518	2.831	3.527	3.819
22	1.717	2.074	2.508	2.819	3.505	3.792
23	1.714	2.069	2.500	2.807	3.485	3.767
24	1.711	2.064	2.492	2.797	3.467	3.745
25	1.708	2.060	2.485	2.787	3.450	3.725
26	1.706	2.056	2.479	2.779	3.435	3.707
27	1.703	2.052	2.473	2.771	3.421	3.690
28	1.701	2.048	2.467	2.763	3.408	3.674
29	1.699	2.045	2.462	2.756	3.396	3.659
30	1.697	2.042	2.457	2.750	3.385	3.646

The table gives the percentage points most frequently required for significance tests and confidence limits based on 'Student's' *t*-distribution. Thus the probability of observing a value of *t*, with 10 degrees of freedom, greater in *absolute value* than 3.169 (i.e. < -3.169 or $> +3.1691$ is exactly 0.01 or 1 per cent.

APPENDIX 24

THE CORRELATION COEFFICIENT (Bailey, 1981)

Degrees of freedom	*Value of P*				
	0.10	0.05	0.02	0.01	0.001
1	0.9877	0.99692	0.99951	0.99988	0.9999988
2	0.9000	0.9500	0.9800	0.9900	0.9990
3	0.805	0.878	0.9343	0.9587	0.9911
4	0.729	0.811	0.882	0.9172	0.9741
5	0.669	0.754	0.833	0.875	0.9509
6	0.621	0.707	0.789	0.834	0.9249
7	0.582	0.666	0.750	0.798	0.898
8	0.549	0.632	0.715	0.765	0.872
9	0.521	0.602	0.685	0.735	0.847
10	0.497	0.576	0.658	0.708	0.823
11	0.476	0.553	0.634	0.684	0.801
12	0.457	0.532	0.612	0.661	0.780
13	0.441	0.514	0.592	0.641	0.760
14	0.426	0.497	0.574	0.623	0.742
15	0.412	0.482	0.558	0.606	0.725
16	0.400	0.468	0.543	0.590	0.708
17	0.389	0.456	0.529	0.575	0.693
18	0.378	0.444	0.516	0.561	0.679
19	0.369	0.433	0.503	0.549	0.665
20	0.360	0.423	0.492	0.537	0.652
25	0.323	0.381	0.445	0.487	0.597
30	0.296	0.349	0.409	0.449	0.554
35	0.275	0.325	0.381	0.418	0.519
40	0.257	0.304	0.358	0.393	0.490
45	0.243	0.288	0.338	0.372	0.465
50	0.231	0.273	0.322	0.354	0.443
60	0.211	0.250	0.295	0.325	0.408
70	0.195	0.232	0.274	0.302	0.380
80	0.183	0.217	0.257	0.283	0.357
90	0.173	0.205	0.242	0.267	0.338
100	0.164	0.195	0.230	0.254	0.321

The table gives percentage points for the distribution of the estimated correlation coefficient r when the true value ρ is zero. Thus when there are 10 degrees of freedom (i.e. in samples of 12) the probability of observing an r greater in *absolute value* than 0.576 (i.e. < -0.576 or $> +0.576$) is 0.05 or 5 per cent.

APPENDIX 25

5 PER CENT POINTS OF VARIANCE-RATIO (F) DISTRIBUTION (Bailey, 1981).

The table gives the 5 per cent points of the distribution of the variance-ratio, $F = s_1^2/s_2^2$, where the numerator and denominator have f_1 and f_2 degrees of freedom respectively. Thus if $f_1 = 2$ and $f_2 = 15$, the probability that the observed value of F is greater than 3.71 is exactly 0.05 or 5 per cent.

APPENDIX 26

1 PER CENT POINTS OF VARIANCE-RATIO (F) DISTRIBUTION (Bailey,1981).

f_2 \ f_1	1	2	3	4	5	6	7	8	9	10	12	15	20	30	∞
1	4052	4999	5403	5625	5764	5859	5928	5982	6022	6056	6106	6157	6209	6261	6366
2	98.50	99.00	99.17	99.25	99.30	99.33	99.36	99.37	99.39	99.40	99.42	99.43	99.45	99.47	99.50
3	34.12	30.82	29.46	28.71	28.24	27.91	27.67	27.49	27.35	27.23	27.05	26.87	26.69	26.50	26.13
4	21.20	18.00	16.69	15.98	15.52	15.21	14.98	14.80	14.66	14.55	14.37	14.20	14.02	13.84	13.46
5	16.26	13.27	12.06	11.39	10.97	10.67	10.46	10.29	10.16	10.05	9.89	9.72	9.55	9.38	9.02
6	13.75	10.92	9.78	9.15	8.75	8.47	8.26	8.10	7.98	7.87	7.72	7.56	7.40	7.23	6.88
7	12.25	9.55	8.45	7.85	7.46	7.19	6.99	6.84	6.72	6.62	6.47	6.31	6.16	5.99	5.65
8	11.26	8.65	7.59	7.01	6.63	6.37	6.18	6.03	5.91	5.81	5.67	5.52	5.36	5.20	4.86
9	10.56	8.02	6.99	6.42	6.06	5.80	5.61	5.47	5.35	5.26	5.11	4.96	4.81	4.65	4.31
10	10.04	7.56	6.55	5.99	5.64	5.39	5.20	5.06	4.94	4.85	4.71	4.56	4.41	4.25	3.91
11	9.65	7.21	6.22	5.67	5.32	5.07	4.89	4.74	4.63	4.54	4.40	4.25	4.10	3.94	3.60
12	9.33	6.93	5.95	5.41	5.06	4.82	4.64	4.50	4.39	4.30	4.16	4.01	3.86	3.70	3.36
13	9.07	6.70	5.74	5.21	4.86	4.62	4.44	4.30	4.19	4.10	3.96	3.82	3.66	3.51	3.17
14	8.86	6.51	5.56	5.04	4.69	4.46	4.28	4.14	4.03	3.94	3.80	3.66	3.51	3.35	3.00
15	8.68	6.36	5.42	4.89	4.56	4.32	4.14	4.00	3.89	3.80	3.67	3.52	3.37	3.21	2.87
16	8.53	6.23	5.29	4.77	4.44	4.20	4.03	3.89	3.78	3.69	3.55	3.41	3.26	3.10	2.75
17	8.40	6.11	5.18	4.67	4.34	4.10	3.93	3.79	3.68	3.59	3.46	3.31	3.16	3.00	2.65
18	8.29	6.01	5.09	4.58	4.25	4.01	3.84	3.71	3.60	3.51	3.37	3.23	3.08	2.92	2.57
19	8.18	5.93	5.01	4.50	4.17	3.94	3.77	3.63	3.52	3.43	3.30	3.15	3.00	2.84	2.49
20	8.10	5.85	4.94	4.43	4.10	3.87	3.70	3.56	3.46	3.37	3.23	3.09	2.94	2.78	2.42
21	8.02	5.78	4.87	4.37	4.04	3.81	3.64	3.51	3.40	3.31	3.17	3.03	2.88	2.72	2.36
22	7.95	5.72	4.82	4.31	3.99	3.76	3.59	3.45	3.35	3.26	3.12	2.98	2.83	2.67	2.31
23	7.88	5.66	4.76	4.26	3.94	3.71	3.54	3.41	3.30	3.21	3.07	2.93	2.78	2.62	2.26
24	7.82	5.61	4.72	4.22	3.90	3.67	3.50	3.36	3.26	3.17	3.03	2.89	2.74	2.58	2.21
25	7.77	5.57	4.68	4.18	3.85	3.63	3.46	3.32	3.22	3.13	2.99	2.85	2.70	2.54	2.17
26	7.72	5.53	4.64	4.14	3.82	3.59	3.42	3.29	3.18	3.09	2.96	2.81	2.66	2.50	2.13
27	7.68	5.49	4.60	4.11	3.78	3.56	3.39	3.26	3.15	3.06	2.93	2.78	2.63	2.47	2.10
28	7.64	5.45	4.57	4.07	3.75	3.53	3.36	3.23	3.12	3.03	2.90	2.75	2.60	2.44	2.06
29	7.60	5.42	4.54	4.04	3.73	3.50	3.33	3.20	3.09	3.00	2.87	2.73	2.57	2.41	2.03
30	7.56	5.39	4.51	4.02	3.70	3.47	3.30	3.17	3.07	2.98	2.84	2.70	2.55	2.39	2.01
40	7.31	5.18	4.31	3.83	3.51	3.29	3.12	2.99	2.89	2.80	2.66	2.52	2.37	2.20	1.80
60	7.08	4.98	4.13	3.65	3.34	3.12	2.95	2.82	2.72	2.63	2.50	2.35	2.20	2.03	1.60
120	6.85	4.79	3.95	3.48	3.17	2.96	2.79	2.66	2.56	2.47	2.34	2.19	2.03	1.86	1.38
∞	6.63	4.61	3.78	3.32	3.02	2.80	2.64	2.51	2.41	2.32	2.18	2.04	1.88	1.70	1.00

The table gives the 1 per cent points of the distribution of the variance-ratio $F = s_1^2/s_2^2$, where the numerator and denominator have f_1 and f_2 degrees of freedom respectively. Thus if $f_1 =$ and $f_2 = 15$ the probability that the observed value of F is greater than 4.14 is exactly 0.01 or 1 per cent.