A STUDY OF THE ORAL AND INTRAVENOUS GLUCOSE TOLERANCE TESTS AND THE INSULIN-GLUCOSE TOLERANCE TEST IN ANXIETY STATES

A THESIS

PRESENTED TO

THE FACULTY OF GRADUATE STUDIES

UNIVERSITY OF MANITOBA

IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE

MASTER OF SCIENCE

BY

LESLIE JEROME CERA

APRIL 1951

ACKNOWLEDGMENTS

It is a privilege to acknowledge the valuable assistance rendered by Dr. F.D. White, Dr. G.E. Delory, Dr. J. Matas and the nursing staff of Deer Lodge Hospital. Statistical advice was generously given by Dr. I.M. Thompson.

This work was carried out under the tenure of a National Research Council Fellowship.

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THEORETICAL & HISTORICAL CONSIDERATIONS

1. General Introduction

The opinion has been repeatedly expressed that, at a given time, there is some correlation between the glucose level of the blood and the emotional state of the subject under examination. The earliest attempt at a reasonable explanation of the variation in blood sugar in certain emotional states was the "flight or fight" theory of Cannon (1920). The essence of this explanation was that certain acute emotions (anger, fear, rage) were productive of stimulation of the sympathetic nervous system leading to increased adrenaline liberation with ultimate hyperglycaemia.

Following the monumental work of Cannon a voluminous literature arose which purported to show variations from normal in blood sugar in mental disease ranging from the psychoneuroses to the psychoses.

In recent years however, with advances in biochemical knowledge and techniques and with the increasing advances of

psychiatry, there has come a renewed interest in physiological and chemical aberrations in mental disease. In psychiatry as in other branches of medicine the knowledge of the laboratory scientist is being increasingly invoked as an ancillary agent in the study of the diagnosis and treatment, as well as the elucidation of the nature, of specific disease processes.

2. Glucose Metabolism In The Normal Human

a) Introduction

A detailed discussion of carbohydrate metabolism in the normal organism is not germane to the purposes of this study. Accordingly only the features necessary for interpretation of abnormalities will be considered, and these briefly.

b) Absorption of glucose

Absorption of glucose occurs almost entirely in the small intestine. No glucose is removed in the stomach from moderately concentrated solutions although some gastric absorption has been reported from solutions containing forty percent or more of the monosaccharide. Relatively small quantities are removed from the colon. The rate of absorption decreases progressively as glucose passes distally in the small gut. Within wide limits of concentration the absorption of glucose proceeds at a constant rate. It is generally conceded

that monosaccharide absorption is of two types: a specific mechanism which probably involves phosphorylation; a non-specific mechanism of diffusion governed by osmotic forces.

Van Liere and others (1946) have established that ingested glucose inhibits gastric motility, hypertonic solutions particularly accomplishing this inhibition. It has also been stated that sugar placed in the upper small intestine in adequate quantities inhibits gastric motility by stimulating the production of enterogastrone. Evensen (1942) has considered the relation of gastric motility to the absorption of sugar and had arrived at the following conclusions:

1. Since it has been demonstrated that four hours after ingestion of a large quantity of glucose, sugar may still be found in the stomach but not in the duodenum or small intestine, glucose which enters the duodenum must be absorbed almost instantly.
2. Under normal conditions the stomach empties slowly and rhythmically. Supposedly, only a minor part of the intestinal surface participates in the process of glucose absorption. If the emptying proceed too rapidly large quantities of glucose come into contact with a larger intestinal surface than usual so that increased absorption occurs. Presumably, by a similar process of reasoning, delayed emptying would result in decreased absorption over a given period of time.

c) Intermediate metabolism

Having been absorbed, glucose is carried in the portal stream to the liver where it is stored as glycogen or used in the course of the metabolic activity of this organ or sent into the general circulation to reach the tissues. In the tissues it may be stored as glycogen, utilized for energy, or transformed to fat.

Hepatic glycogenolysis with liberation of glucose into the blood stream is stimulated largely, according to Soskin (1941), by diminution in circulating blood sugar and is inhibited by a rise in concentration of this substance in the blood. Disturbance of this homeostatic function is a characteristic aberration in diabetes mellitus.

Carbohydrate metabolism is profoundly influenced by the internal secretions of the pancreas, anterior pituitary gland, adrenal and thyroid glands. Those endocrine and neurogenic factors affecting metabolism which concern this study will be considered in the appropriate places.

d) Excretion

Normally glucose is filtered by the glomeruli of the kidney and largely re-absorbed into the blood stream through the tubular epithelium.

The question of whether there is a "renal threshold"

for glucose is a vexed one. Although perhaps unsound physiologically it is firmly entrenched in the literature and from a practical standpoint it would seem satisfactory to assume that tubular reabsorption of glucose becomes incomplete and glycosuria occurs when the blood sugar concentration rises to an excessively high level. It is, however, recognized that the threshold is exceedingly variable.

Goldring and collaborators (1940) have demonstrated that in normal human subjects the maximum rate of glucose reabsorption in the kidney is 250 to 450 mg. per minute. It is now generally held that the normal kidney is capable of reabsorbing this amount of glucose per minute and that levels higher than these are productive of glycosuria.

3. The Endocrine Control Of Blood Sugar

Since the description in the second century A.D. of the disease now known as diabetes mellitus by Aretaeus the Cappadocian, there has been almost constant interest in the elucidation of the mechanisms controlling the level of blood sugar. It is impossible to trace precisely the steps in scientific accretion which dispelled the fog of ignorance and placed our knowledge (albeit still imperfect) on its present plane. Undoubtedly the most significant early contribution to the unravelling of the knot was the description of the islet

cells of the pancreas by Langerhans in 1869. Twenty years later Von Mering and Minkowski extirpated the pancreas of the dog producing a disease state resembling human diabetes mellitus. In 1922 Banting and Best demonstrated the "antidiabetic" hormone. Bruch (1950) has reviewed the work which led to the discovery of insulin.

In 1946 Soskin stated that there was not a single known hormone which did not exert some influence on carbohydrate metabolism. Even today this statement cannot be strongly contested but it is now generally agreed that the most important members of the governing glandular system are: the pancreas, the anterior pituitary gland, the adrenal cortex and medulla, the thyroid gland.

The functions of <u>insulin</u> in the normal body economy have recently been reviewed (Bruch, 1950). These functions are stated to be: promotion of the oxidation of glucose by the tissues, promotion of glycogen deposition in muscle and liver, inhibition of gluconeogenesis in the liver, promotion of the deposition of fat from dietary glucose, prevention of ketosis. Insulin has been shown to act at more than one metabolic site. In addition to participating in the hexokinase reaction it has been shown that it participates in reactions of the tricarboxylic cycle (Beaser, 1950). It has been suggested that an alpha cell hormone with a hyperglycaemic - glycogenolytic action is also produced in the pancreas, but, as pointed out

by Pincus (1950), there is as yet no direct proof that this factor is functional under physiological conditions.

The physiological effects of the Anterior Pituitary Gland on carbohydrate metabolism are as yet imperfectly understood. It would appear that its normal actions are: to retard the utilization of muscle glycogen, to reduce the capacity of the organism to oxidize carbohydrate and, to accelerate the formation of liver glycogen from protein. The pituitary hormones act, no doubt, through the adrenal cortex. The fact that they may exert some "diabetogenic" effect in the absence of the adrenal in dogs and toads indicates that they have a more direct action on carbohydrate metabolism not mediated through the adrenal cortex.

The adrenal cortical hormones exerting an effect upon carbohydrate metabolism are those possessing an oxygen atom attached to the eleven-carbon atom. The principal effects of these hormones appear to be stimulation of gluconeogenesis from protein and inhibition of tissue oxidation of glucose. The adrenal cortex would also appear to indirectly influence carbohydrate metabolism through its function of maintaining electrolytic homeostasis. The action of the adrenal medullary hormone, adrenaline or epinephrine, would seem to be to stimulate glycogenolysis in the liver in conditions of emergency. It has been claimed by Vogt (1944) that epinephrine directly stimulates the adrenal cortex. On the other hand Long (1947)

has stated that stimulation of the autonomic nervous system with release of adrenaline is a major factor in the release of ACTH from the anterior pituitary.

The hormone of the thyroid gland appears to accelerate the intestinal absorption of both glucose and galactose and to promote good utilization of absorbed sugar, apparently at an accelerated rate. It may also increase hepatic gluconeogenesis (Soskin, 1946).

4. The Homeostatic Theory

The chief proponent of this theory has been Soskin (1941, 1946, 1950). Briefly, he holds that under normal conditions as the blood sugar increases above the resting level glycogenolysis and gluconeogenesis in the liver decrease and tissue activity in the form of oxidation and storage increase. Conversely decrease in blood sugar is automatically followed by augmentation of the former processes and decreased tissue activity. This then is a theory of auto-regulation of blood sugar.

5. Neurogenic Mechanisms

The highest levels of cerebral control of blood sugar have not been adequately localized although there is considerable evidence that the hypothalamus probably acting through the

adenohypophysis is intimately concerned with the regulation of carbohydrate metabolism. Macht and Bard (1942) found that satisfactory carbohydrate metabolism could be maintained in the cat even when the pituitary-hypothalamus was converted into an island without any neural connections.

Portis (1950) has recently reviewed the autonomic nerve supply of the pancreas and has attributed to it a major roll in the mechanism of production of symptoms in a psychoneurotic group with fatigue as the main feature. There is evidence enough that the right vagus nerve supplies the pancreas but its function as a major agent in controlling blood sugar is in doubt. Houssay (1937) has shown that the pancreas can produce its internal secretion after all its extrinsic nerve supply has been removed.

6. Glucose Metabolism In Abnormal Mental States

The first suggestion that emotional aberration was perhaps associated with some abnormality of glucose metabolism was the observation of glycosuria attending such states. (Cannon et. al. 1912). This work however did not go uncontested for in 1922 Stewart reported that in his experimental animals excitement was not productive of a rise in blood sugar and in the same year MacLeod failed to find glycosuria in humans subjected to the stress of examinations.

Along with the aforementioned studies of the effects of emotion on laboratory animals and normal humans there came a number of blood sugar studies in humans suffering from mental disorders. Kooy (1920) reported a marked trend toward hyperglycaemia in people with mental disease and especially in melancholia. Uyematsu and Soda (1921) reported the average fasting blood sugar in thirty-two catatonic schizophrenics to be noticeably above their normal controls. On the other hand Raphael and Parsons (1921) reported that the fasting blood sugar in patients with mental disease was normal. This work was supported by the observations of Bowman in 1923 and of Bowman and Kasanin in 1929. The latter observers went on furthur to state that there existed no correlation between the mood of the patient and the height of the blood sugar.

The state of the blood sugar in neurasthenia was the subject of two separate investigations in 1922. In this year Langston reported an excessive hyperglycaemic reaction in neurasthenia and this report was supported by the work of Olmsted and Gay. However in 1929 L. Szondi and H. Lax published a well controlled report which seemed to refute the previously mentioned results. These observers demonstrated that in a neurotic state characterized by fatigue, prostration, apathy, anxiety, vertigo, and vasomotor lability, there was absence of the expected rise in blood sugar after glucose ingestion (flat glucose tolerance curve). They showed that

their neurasthenics were not ordinarily hypoglycaemic by demonstrating that the mean blood sugar levels in twenty-six normals and thirty-one patients were identical. After the ingestion of fifty grams of glucose the average rise in blood sugar was 69% in normals and 31% in neurasthenics.

The influence of the emotions on glucose tolerance was the subject of an investigation carried out by Diethelm in 1936. High early peaks in the blood sugar content, especially in cases of acute anxiety and fear, were reported. These findings were interpreted as representing unusually rapid absorption of glucose. It was further stated that in less acute states of tension the blood sugar failed to return to normal in the usual time. The latter result, Diethelm suggested, might be due to an overactive sympathetic-adrenal apparatus. One year later Martin and associates (1937) described a group of patients suffering from a syndrome which they called "symptomatic functional hypoglycaemia". But they clearly stated at this time that they did not believe hypoglycaemia to be the cause of the vague symptomatology manifested by the psychoneurotic individual. In the same year Dorst, as quoted by Stevens (1945), found that persons with neurocirculatory asthenia had a flat oral glucose tolerance curve which returned to normal after a course of insulin therapy. Further investigation revealed a flat curve in neurasthenic persons in general. Finally, Dorst expressed the opinion that the low blood sugar

levels produced the symptoms in this psychoneurotic syndrome.

Schizophrenia, manic depressive psychoses, senile psychoses, alcoholism and other addictive states were the diseases present in a group of patients studied with the oral glucose tolerance test in 1940 by Robinson and Shelton. The curves obtained were characterized by a decreased tolerance for ingested sugar (inordinate rise with delayed return to the fasting level). They felt that the abnormal curves resulted from malnutrition and they emphasized the importance of biochemical and physiological, in addition to psychological studies in these people in view of the fact that there was no universally accepted etiology in these states. Moreover it was known that the healthy brain utilizes only glucose as its fuel and that it is probably incapable of storing a glycogen reserve (Kerr, 1936). As a consequence, a continuous supply of sugar to the brain seemed obligatory for healthy metabolism. These matters considered, Robinson and Shelton concluded: "It is certainly not illogical to assume ... that disturbed carbohydrate metabolism of the organism as a whole can and will disturb the functioning ability of the brain to some degree."

Support for the contentions of these observers came from Katzenelbogen and Haws (1944) who demonstrated a relatively low sugar content of arterial (femoral) and venous (internal jugular) blood for certain schizophrenic patients; they suggested a lower intracranial carbohydrate metabolism in their

subjects than in normal people.

Rennie and Howard (1942) described a syndrome consisting of tension-depression and hypoglycaemia. They stated that the hypoglycaemia seemed secondary to the psychiatric disorder since it disappeared with the treatment of the latter condition.

One of the most energetic investigators of the relationship between glucose metabolism and emotions has been Portis. He has published a number of papers on this subject and has presented some observations which, if substantiated, will exert a profound influence on the management of a large group of psychoneurotic people (1943, 1944, 1950, 1951). Briefly, Portis holds that in certain emotional situations characterized by loss of zest and consequent failure to maintain normal vegetative tonus, there is a preponderance of vago-insular tonus over sympatho-adrenal tonus with consequent manifestations of hyperinsulinism. So extensive have been Portis' studies and so important are they with regard to the present work, they will be dealt with in greater detail at a later stage(SectionVI).

In 1945 Stevens studied forty patients with symptoms and signs typical of neurocirculatory asthenia and in whom organic disease had been excluded as far as possible. Six hour glucose tolerance tests were done on all patients after administration of one hundred grams of glucose. In this study sixty-five percent of the patients had flat sugar tolerance curves. He did not agree with Dorst that the low blood sugar values

were responsible for the asthenia for three reasons: not all neurasthenics had low values; none of the symptoms was improved by sugar feeding; one patient had an accompanying diabetes mellitus with hyperglycaemia. A symptom-complex simulating peptic ulcer, neurocirculatory asthenia and psychoneurosis has been described recently by Peskin (1948). This observer considers increased sugar tolerance to be a factor in the production of the complex and holds that patients in this group display symptoms of mild hypoglycaemia at relatively high blood sugar levels.

7. Statement Of The Problem

It is apparent from a survey of the historical background of the study of sugar variations in various emotional states that a clear pattern of change has not been demonstrated. The results reported are conflicting and confusing. A review of the subject in 1946 by Peters and Van Slyke stated: "There is no clear evidence that there are any disorders of carbohydrate metabolism characteristic of special types of mental disease." If, however, the evidence is not clear it is at least provocative.

The most plausible explanation of the impressive variation in reported results may be sought in the light of a politic statement by Peters: "When honest men disagree, the causes

of their differences are likely to be found in the conditions of their experiments, rather than in the accuracy of their data." On close examination of the evidence presented to date a number of sources of error or difference of opinion are apparent. These include:

1. Lack of clear separation of patients into homogeneous groups.
2. Inadequate or poorly controlled studies.
3. A tendency to apply results of animal experimentation to human beings in a haphazard fashion.
4. A failure to exclude factors which are known to profoundly influence the nature of the glucose tolerance curve. In particular failure to mention the diet and state of nutrition of the patients examined or to assure that medication (barbiturates, epinephrine et. al.) has not been administered may be mentioned. Another factor to be considered is the presence of concomitant disease states which might separately influence the state of glycaemia and activity of the patients considered.
5. In many cases no mention of renal excretion of glucose is made.
6. Different techniques and procedures have been used in the collection and analysis of blood specimens.

It was the purpose of this investigation to study various aspects of the metabolism of carbohydrates in a homogeneous group of carefully selected patients who were known to be

suffering from a psychoneurosis which may be designated as an anxiety state, in an attempt to aid in the resolution of conflicting opinions which have appeared in the literature. Throughout this study an attempt has been made to eschew the aforementioned errors and inconsistencies which have vitiated many of the reports of the past. With the accretion of knowledge over the years it is now possible to avoid many of the sources of difficulty which unknowingly plagued many previous investigators.

8. Scope Of The Thesis

The problem was undertaken in an attempt to contribute information about the following matters in particular, in the chosen patients:

1. The state of the fasting blood sugar.
2. The effect of ingested glucose on glycaemia and elimination of sugar in the urine.
3. The response to glucose administered parenterally on the level of blood sugar.
4. The effect of insulin on the level of blood glucose and the reaction to insulin-induced hypoglycaemia, if such occurred.

Since the patients available for the study were not primarily hospitalized for research purposes, the time alloted for investigation and the extent of investigation in sometimes re-

luctant subjects was limited. Tests were chosen which might be expected to provide much information about the absorption, metabolism, and excretion of administered glucose. Another factor in the choice of tests to be used was the hope that the information obtained would be of both academic and practical interest. Simplicity and practicability of performance were therefore further considerations.

SECTION II

CLINICAL CONSIDERATIONS

1. Introduction
2. The Psychoneuroses
3. Classification Of The Neuroses

1. Introduction

The present investigation concerned itself with the study of certain aspects of carbohydrate metabolism in a group of patients who suffered primarily from disturbances in the emotional sphere. These patients constituted a group usually classified by psychiatrists as psychoneurotic. They exhibited excessive emotional responses to the stresses of life and these faulty responses were accompanied by various physiological reactions. Although there was individual variability in the psychogenic stimuli to these reactions, the fundamental psychodynamic pattern in the development of these states was one of increasing inability to meet life situations.

2. The Psychoneuroses

The psychoneuroses are undoubtedly the commonest of all reaction types and constitute, by rough estimate, forty to fifty per cent of patients seeking medical advice. As a consequence they are a challenging problem to modern day medicine.

It is generally believed that in the neuroses the etiological mechanism is psychological and the resulting physiological abnormalities arise as a consequence. There is, at present, no cogent evidence that this belief is erroneous. However, there appears to be an increasing tendency to study physiological and biochemical factors in an attempt to increase our understanding of the neuroses. The situation has been well summarized by Nicole (1946) who has stated: "If we admit the importance of glandular activity and of autonomic tensions in the production of emotional disturbances ... we cannot afford to neglect any method that might possibly help us to arrive at a diagnosis ... we may some day find that chemistry and psychology are not as far apart as we might be inclined to suppose."

The bulk of evidence previously cited favors the opinion that there is some disturbance of carbohydrate metabolism in certain mental diseases. Unfortunately the precise nature of this disturbance is nebulous. Moreover evidence is conflicting on the question of whether this aberration is a cause or effect although it must be admitted that the latter appears more likely.

3. Classification Of The Neuroses

The problems of classification of the psychoneuroses continue to plague psychiatrists. Strecker and Ebaugh refer to attempts to date as "unsuccessful", "muddled", "artificial". This being the case any attempt to investigate patients by placing them into a definite group (e.g. anxiety neurosis,

neurasthenia, compulsion neurosis, conversion hysteria et.al.) is similarly likely to be castigated by these unwelcome adjectives. To eschew this lamentable fate patients were selected who were undoubtedly psychoneurotic and who, in the main, exhibited symptoms directly attributable to anxiety. In order to facilitate further description the disease in these people may be referred to as an anxiety state, but this does not connote that they are being rigorously separated in a group from other psychoneurotic reaction types. There is no doubt that if each of these patients were interrogated by many psychiatrists there would be considerable diagnostic difference of opinion as to classification.

For the purposes of this study the definition of anxiety given by Claghorn and Graham (1949) was eminently suited: "Anxiety is generally recognized as a condition of tense preparedness for action or a state of tension aroused by a sense of impending danger." These authors further point out that anxiety only becomes an abnormal reaction when precipitated by ordinarily insufficient agencies or when it is present in the absence of contemporary stimuli.

Most of the patients studied suffered from symptoms other than the usual nervousness and irritability attributable to anxiety. Fatigue was a very prominent symptom in the group and the usual pattern was that of great fatigue in the morning on awakening which improved somewhat as the day

progressed. Insomnia, dizziness, tension, light-headedness and depression were other manifestations of the anxious state. Finally, it should be stated that the psychopathologic process in these anxious patients was a chronic one. Every effort was made, during the testing, to avoid precipitating an acute anxiety reaction due to concern over needling of the veins and drawing of blood. Reassurance was freely given and diversion attempted in each patient during the tests.

SECTION III

TECHNIQUES

1. The Estimation Of Blood Glucose
2. The Oral Glucose Tolerance Test
3. The Intravenous Glucose Tolerance Test
4. The Insulin-Glucose Tolerance Test

1. The Estimation Of Blood Glucose

a) Introduction

An accurate method for the estimation of blood glucose was fundamental to this study. The conditions present further demanded that blood be transported from the site of collection to the site of analysis, this in turn necessitating an interval of from one to two hours between the drawing of samples and the estimation of glucose in these samples. Accordingly, important considerations in the experiment were collection of a sample suitable for transport and, inhibition of loss of glucose during the period of delay.

b) Historical

Innumerable methods for the determination of blood sugar have been published since Chaveau reported quantitative studies in 1856 and the last word on the subject has no doubt, still to be spoken. The early history of the subject has been reviewed by Peters and Van Slyke (1946)

c) Procedures

Most methods depend on the observation that at the temperature of boiling water glucose will reduce alkaline solutions of copper sulphate or potassium ferricyanide. The

amount of reduction, which will of course be, theoretically, a measure of blood sugar concentration, may then be determined titrimetrically or colorimetrically. Since colorimetric methods are more convenient, more readily applied to the use of small quantities, and, properly carried out, quite as accurate as the titration procedures, only the former need concern us here.

Modern colorimetric methods may be considered to have sprung from those of Folin and Wu which utilized an alkaline copper sulphate solution and measured the amount of reduction by determining the quantity of color developed by the subsequent addition of phosphomolybdic acid, which was reduced to a blue color by cuprous oxide. From time to time however, methods have been proposed which utilize ferricyanide, the amount of reduction being determined by measuring the amount of Prussian blue formed on the addition of ferric chloride to the ferrocyanide produced.

Later research has concerned itself with attempts to make the methods more specific for glucose and with increasing the sensitivity. Adaptation to smaller amounts of blood has also been a goal.

Earlier methods such as those of Folin-Wu also measured certain fermentable reducing substances, chiefly glutathione, present in the red cells and consequently gave results for blood sugar which were too high. This error could be eliminated by using as the protein precipitant a substance which also re-

moved the non- glucose reducing substances. Alternatively since glutathione is confined to the red cells, its effect on the subsequent procedure could be eliminated by carrying out the procedure in such a way that the cells were not hemolyzed.

However, although such methods are widely used, the method of Folin-Wu still continues to be popular in some places. It is not difficult to understand the continuing popularity of this test in view of the facts that the reagents are easily obtainable, the test is relatively simple to perform and, most important of all, wide experience with it has established certain standards of normality and recognition of aberration from these standards. To avoid the confusion that might otherwise arise, those methods which also measure non-glucose reducing substances are said to yield enhanced blood sugar values, while those in which these substances are removed are said to give true values. Experience has shown that the tests designed to give true values are as easily performed as the older tests. Moreover they are rapid and accurate.

The method used in this study was that of King and Garner (1947) which uses an alkaline copper sulphate solution in which the protein is precipitated by copper sulphate and sodium tungstate which also removes non-sugar reducing substances. The protein-free filtrate is heated with an alkaline copper sulphate solution and the cuprous sulphate formed is treated with an excess of arsenomolybdic acid to yield a

blue color. The latter acid is superior to phosphomolybdic acid used by Folin and Wu in that the blue color produced is more stable. The intensity of this blue color, which is a measure of the blood glucose concentration, is determined colorimetrically.

d) The Non-glucose reducing substances in the blood

As previously stated there exist in the blood certain reducing substances (chiefly glutathione) which are non-fermentable and which, unless removed, enhance the blood sugar values. When methods are used in which these non-glucose reducing substances are not removed a source of error is introduced which has been said to vary from ten to thirty mg. per 100 ml. blood. Mosenthal (1946) has reported that twenty-two percent of 196 blood sugar determinations in his laboratory showed levels of non-glucose reducing substances exceeding thirty mg. per 100 ml. blood. He has further reported results as high as seventy-eight mg. per 100 ml. for the substances. The criticism may be made that the method by which Mosenthal arrived at his results for non-glucose reducing substances (Folin-Wu values minus results obtained for true blood sugar estimations) was not an exact one since the two tests are not strictly comparable due to differences in the protein precipitants and reducing reagents used. The most acceptable method of estimating non-glucose reducing substances is to

estimate reducing substances before and after fermentation with yeast, the difference representing the non-glucose reducing substances. In spite of this criticism, however, there seems no doubt that the error inherent in Folin-Wu analyses is prohibitive beyond cavil.

e) Method finally adopted

The method used in this study was that of King and Garner (1947), designed to give true blood sugar values.

i. Reagents

1. Standard Glucose Solution - prepare a stock solution by dissolving 0.100 gm. pure anhydrous glucose in saturated benzoic acid solution. Make the volume up to exactly 100 ml. with more of the benzoic acid solution. Place 5 ml. of this stock solution in a 200 ml. volumetric flask and make up to the mark with saturated benzoic acid solution. This dilute standard solution contains 0.025 mg. of glucose per ml.

2. Harding's Copper Sulphate Reagent

Solution A - this is made up by adding 13 gm. $CuSO_4.5H_2O$ to a litre of water.

Solution B - to 700 ml. of water add 50 gm. pure sodium bicarbonate and, stirring, 40 gm. anhydrous sodium carbonate. When all is dissolved add a solution of 36.8 gm. potassium oxalate in 120 ml. of water which has been warmed to 60 degrees Centigrade. Finally, dissolve 24 gm. potassium sodium tartrate in water and add this also. Make the solution up to one litre with water. The solutions are stored separately and mixed in equal volumes to carry out the estimation.

3. Nelson's Arsenomolybdate Reagent

Dissolve 25 gm. ammonium molybdate in 450 ml. distilled water and add to this 21 ml. of concentrated sulphuric acid followed by 3 gm. sodium arsenate dissolved in 25 ml. water. Mix and allow to stand at 37 degrees Centigrade for 24 to 48 hours. This reagent should be stored in a glass - stoppered brown bottle.

4. Isotonic Sodium Sulphate Solution

(1.5 gm. anhydrous sodium sulphate per 100 ml. water)

5. Sodium Tungstate Solution (10 gm. per 100 ml.)

6. Copper Sulphate Solution (7 gm. per 100 ml.)

ii. Method and Calculations

Pipette 0.1ml. of venous blood into 3.5 ml. of isotonic sodium sulphate solution in a centrifuge tube. Then add 0.2 ml. of the copper sulphate solution and 0.2 ml. of the sodium tungstate solution. Mix by rotating the tube and then centrifuge. Into the first of three test tubes pipette 1.0 ml. of the supernatant fluid (equivalent to 0.025 ml. blood), into the second pipette 1. ml. of the standard glucose solution, and into the third 1. ml. of water (the "blank"). Into all three tubes pipette 1. ml. of Harding's Copper Reagent (a mixture of equal parts of solutions A and B). Place the test tubes in a boiling water bath for exactly ten

minutes. Then remove the tubes, cool in cold water, and add to each 1 ml. of the arsenomolybdate reagent. After a few seconds dilute to an appropriate volume (10 ml. for expected blood glucose values up to 200 mgs. percent; greater dilution for higher values). Read all the tubes in a photoelectric colorimeter. For this study the Coleman Junior Spectrophotometer was used at a wave length of 640 mu.

The quantity of dilute standard glucose solution taken contained 0.025 mg. of glucose. The supernatant liquid taken corresponded to 0.025 ml. of blood. The calculation was therefore as follows;

$$\text{Blood glucose (mg. per 100 ml.)} = \frac{\text{Density of Test-Density of Blank}}{\text{Density of Standard-Density of Blank}} \times 0.025 \times \frac{100}{0.025}$$

$$\text{or Blood glucose (mg. per 100 ml.)} = \frac{\text{Density of Test-Density of Blank}}{\text{Density of Standard-Density of Blank}} \times 100$$

2. The Oral Glucose Tolerance Test

a) Definition of glucose tolerance

The term glucose tolerance is used in the literature with different meanings. In this study it is considered to mean the definition of the degree and duration of the elevation of blood glucose caused by the administration of a given dose of

glucose.

b) Historical

The earliest studies of the changes in blood sugar following glucose ingestion were those of Liebman and Stern in 1906 and Baudouin in 1908. With the introduction of the micromethod of analysis by Bang more thorough studies came into being, including those of Bang and Jacobsen. In 1923 Gray published an important paper attempting to delimit the normal fasting blood sugar and the rise provoked by glucose ingestion. The literature on the subject up to 1932 was thoroughly reviewed by Herrman (1932) who also attempted to delineate standards of normality. In 1933 Cavette and Seljeskog published a report on the results of glucose ingestion in healthy young normal adults. One of the most important earlier studies published was that of Watson (1938). It was important because it had the merit of being relatively carefully controlled and because it dealt with a comparatively homogeneous group of young adults, all of whom were in good health. The early history of the investigation of glucose tolerance has been reviewed by Peters and Van Slyke (1946), while Mosenthal (1950) has recently suggested criteria for interpretation of normal glucose tolerance.

c) Technique

Venous blood was drawn from the chosen subject

after an overnight fast of twelve hours. This constituted the fasting blood specimen. Immediately following the venipuncture fifty gm. glucose in 250 ml. water flavored with lemon juice was drunk. Specimens were collected at thirty, sixty, one hundred and twenty, and one hundred and eighty minutes following the ingestion of the sugar solution. When possible two additional samples were collected at four and five hours. With each blood sample, a specimen of urine was obtained, when possible, to analyze for glucose. All blood samples were collected in fluoride and oxalate and kept refrigerated until analyzed.

d) Characteristics of the normal venous blood sugar curve

There is a considerable variation in the levels of blood sugar after glucose ingestion as reported by different observers. The differences may, at least in part, be attributed to differences in technique of collection and in methods of analysis of samples. Another, perhaps more important factor, is the failure to recognize the profound effects on the tolerance curve occasioned by the state of nutrition of the subject, the preceding dietary, medication and the presence of organic diseases. Today, the standards of normal response have been reasonably well established, although it must be admitted that stringent adherence to these standards is sometimes productive of error in diagnosis.

The responses considered normal by various observers

for fasting blood sugar and glucose tolerance, along with the time required to reach the maximal glycaemia and the duration of hyperglycaemia are indicated in Table I. The sugar values in these studies were enhanced and consequently, from the point of view of this study, the chief interest in this table is in the temporal aspects presented.

Cantarow and Trumper (1949) have recently succinctly summarized the normal features of the oral glucose tolerance test. They state the normal fasting blood sugar (Folin-Wu modified) to be 80 to 110 mg. per 100 ml. blood. Further experience has shown that these figures probably err on the side of slightly too-high values for true sugar estimation. The results now considered normal are 70 to 100 mgs.% for the Folin/Wu (modified) test (Normal Laboratory Values, 1950). Wooton, King et. al. (1951) have recently reported data on the normal frequency distribution of fasting blood sugar. They have found that 98% of their normal subjects have blood sugars between 55 and 109 mgs.%, 80% falling between 68 and 96 mgs.%. They state results outside the larger limits are definitely abnormal. After the administration of an arbitrary amount of glucose (50-200 gm.) per os, the blood sugar rises to a maximum of forty fo fifty mg. per 100 ml. above the fasting level within the first hour. Increasing the quantity of glucose ingested from 50 to 200 gm. has no significant effect upon the height the blood sugar attains although the

TABLE I

A COMPARISON OF THE RESULTS OF ORAL GLUCOSE TOLERANCE TESTS OBTAINED BY VARIOUS INVESTIGATORS

OBSERVER	AVERAGE FASTING BLOOD SUGAR	MAXIMAL BLOOD SUGAR LEVEL REACHED DURING TEST	TIME REQUIRED TO REACH MAXIMAL	DURATION HYPER-GLYCAEMIA	VARIATION IN PEAK VALUES OF BLOOD SUGAR
	mg. per 100 ml.	mg. per 100 ml.	minutes	minutes	mg. per 100 ml.
ENOCKSSON	98	152	27	65	130-194
HAGEDORN	84	147	33	64	119-176
SOISALO	89	153	33	64	129-178
EVENSEN	92.5	165.5	31	77	136-208

period of hyperglycaemia may be prolonged (Hansen, 1923). Finally, there is a return to a normal fasting level at the end of one and a half to two hours, a fall to a slightly sub-fasting concentration at about two hours, and a rise to the fasting level at three to four hours (Cantarow and Trumper, 1949). Mosenthal (1950) has summarized the criteria for true venous blood sugar: 100 mg. percent or less in the fasting specimen, an upper limit of 150 mg., and 100 mg. or less after two hours.

e) Factors influencing the normal curve

i. Diet

The question of the effect of diet on tolerance to glucose is of such practical importance and has occasioned so much discussion in the literature that it merits consideration at some length.

Himsworth (1934) reviewed the subject and presented a new theory purporting to explain the effect of diet on the assimilation of sugar. He noted that as early as 1877 Claude Bernard observed the decreased carbohydrate tolerance of a normal person deprived of food.

Prior to Himsworth's experiments on animals and humans, changes in tolerance had been attributed to variation in "tissue reaction" and variation in sensitivity of the insulin-secreting mechanism. Himsworth could not alter sugar tolerance

with long-continued administration of either sodium bicarbonate or ammonium chloride and hence could not subscribe to the "tissue reaction theory". Moreover his experiments showed that insulin exerted a greater effect on the blood sugar when it was preceded by ingestion of glucose and he believed that therefore glucose ingestion resulted in the production of a state of increased susceptibility to insulin or " ... each unit of insulin on injection has, actually or apparently, become more active." He could not subscribe to the second of the earlier theories.

In brief, Himsworth believed that the feeding of carbohydrate improved the sugar tolerance by making the animal more "sensitive" to the action of insulin and hypothesized that some activator of insulin was increased by carbohydrate feeding. By inference, starvation and fat feeding decreased the tolerance due to the absence of this activator.

Chambers (1938) has also expressed the belief that there is a change in sensitivity of the body to insulin with fasting. He further stated that no disturbance in the bodily mechanisms for handling of carbohydrate occured.

The subject of the effect of starvation on carbohydrate tolerance has recently been extensively reviewed, (Keys et.al. 1950). There would seem to be no doubt that complete starvation was attended by a hyperglycaemic response to ingested glucose. The results of semi-starvation were not so clear-cut.

The effect of a high protein diet on carbohydrate utilization has been placed intermediate to that of carbohydrate on one hand and fat on the other by several observers (Sweeney, 1927). Chambers (1938) thought that protein in abundance was capable of maintaining the carbohydrate-oxidizing ability of the body, although in a somewhat less than optimum state.

Heinbecker (1928) has studied the ability of the Eskimo, who lives on a relatively high protein, - high fat diet, to handle ingested glucose. He found normal tolerances during a control period and a hyperglycaemic response to ingested carbohydrate with a slow return to normal after eighty-two hours of fasting. In a comparable study Rabinowitch and Smith (1936) concluded that carbohydrate utilization was somewhat impaired in these people.

In the laboratory animal (rat), Haist (1944) had noted that the insulin content of the pancreas was reduced by fasting, lessening the caloric intake of a balanced diet or by fat feeding. He suggested that carbohydrate feeding was an important factor in maintaining a normal insulin concentration albeit other factors were surely involved.

The literature on the significance of antecedent diet on tolerance to glucose was reviewed in 1942 by Evensen. It was concluded that carbohydrate tolerance was reduced by fasting or by reduction of the quantity of carbohydrate in the diet even if caloric requirements were satisfied by fat.

Mosenthal (1950) has summarized the evidence as follows: "The diet preceding a glucose tolerance test in nondiabetics has a profound effect on the character of the resulting curve, though a closer scrutiny of the published observations points to a probably exaggeration of this influence."

ii. Obesity

The glucose tolerance curve in obese subjects may be essentially normal or may show evidence of increased tolerance, as illustrated by Lepore (1941), or decreased tolerance as demonstrated by Newburgh (1942). Unfortunately much of the evidence presented has been invalidated by failure to initiate a proper pre-testing dietary. In view of the conflicting results reported on the effect of obesity on glucose tolerance, such subjects have been excluded from this study.

iii. Age and Activity

It has been reported that in old age the height and duration of alimentary hyperglycaemia are increased (Spence, 1920). Deren (1937) has found in fifty tests on subjects over fifty-five years of age that the fasting blood sugar was normal and the peak of glycaemia was generally delayed (one to two hours). He also reported that the duration of the curve was prolonged. Deren observed that none of his patients showed a diuretic response after glucose ingestion.

Horvath and collaborators (1947) have reported that the carbohydrate metabolism of men in the age group sixty to

seventy was not significantly impaired although there was a tendency to slight diminution in tolerance.

Mosenthal (1950) has stated that sugar tolerance in his old age group (ten subjects from sixty-one to seventy-six years of age) did not differ from that in younger persons. He attributes previous findings of impaired tolerance in the aged to inactivity of the chosen subjects.

iv. Medication

Epinephrine, morphine and ether have been found to cause hyperglycaemia. The effect of atropine is not unequivocally established, some observers reporting increase and some decrease in blood sugar. Pilocarpine and physostigmine have been reported to induce hyperglycaemia and acetylcholine, hypoglycaemia. Other drugs said to induce hyperglycaemia are ergotamine tartrate, caffeine, quinine, emetine and dinitrophenol. The subject has been reviewed by Peters (1946).

The position of the barbiturates in this respect demands individual consideration for they are probably the most common drugs employed in the treatment of the subjects to be considered in this study. Amytal has been stated to be a hyperglycaemic agent. Rosenkrantz and Bruger (1941) have studied the effects of barbiturates on thirty-seven people, approximately half of whom were normal and half diabetic or "prediabetic". They found barbiturates had no specific effect on normal tolerance but tended to increase tolerance where

it had previously been diminished. The latter effect they ascribed to diminution in nervousness and anxiety and inhibition of hepatic glycogenolysis.

f) Experimental study of the reproducibility of the oral glucose tolerance test

i. Introduction

There have been conflicting opinions concerning the variation in the blood-sugar levels of individuals on repetition of the oral glucose tolerance test. One school of thought holds that so great is the "spontaneous" variability that the test has slight diagnostic value except in diabetes mellitus (Freeman et.al., 1942). Differences as great as 18.9 mg.% in the fasting specimen, 32.3 mg.% in the half-hour, 47.3 mg.% in the hour, 35.8 mg.% in the two hour and 29.6 mg.% in the three hour specimen have been reported (John, 1939). On the other hand Himsworth (1935), while investigating the dietetic factors influencing glucose tolerance in healthy men, found that if the diet be kept constant, the areas enclosed by separate tolerance curves were remarkably constant. The latter investigator noted at the time that existing methods for comparing repeated tests were unsatisfactory. Of all the studies made thus far on this particular subject none has been so strictly controlled nor so meticulous as that of Himsworth. Soisalo (1929) has reported results of repeated glucose

tolerance tests in seventeen healthy young subjects on a uniform diet. Repetition showed good agreement in nine cases and marked divergence in eight.

ii. Results of the present study

In this study six patients were subjected to repeated oral glucose tolerance tests after intervals of one week.

The calculation of the area bounded by a glucose tolerance curve is ideally accomplished by the use of a planimeter. Since this instrument was not immediately available, the area was calculated in the following manner. In the ordinary three hour glucose tolerance test let (a) represent the fasting blood sugar, (b) the half-hour value, (c) the one hour, and (d) and (e) the two and three hour values respectively.

Let (t) represent the time in half-hours. Finally let (o), (f), (g), (h), (i), represent the time markers, e.g. o = time of fasting, f = one-half hour, and so forth. The area of quadrangle oabf= $\frac{a+b}{2}(t)$; the area of quadrangle fbcg is $\frac{b+c}{2}(t)$; the area of quadrangle gcdh is $\frac{c+d}{2}(2t)$; the area of quadrangle hdei is $\frac{d+e}{2}(2t)$. The sum of the areas of the quadrangles represents the area bounded by the tolerance curve. The final calculation is easily simplified to a

$$\frac{a}{2} + b + \frac{3c}{2} + 2d + e.$$

The levels of blood sugar which obtained at given intervals in the subjects of this study have been indicated in Table II and a comparison of areas has been made in Table III.

TABLE II

MEAN VARIATION IN TWO PERFORMANCES OF THE ORAL GLUCOSE TOLERANCE TEST ON THE SAME INDIVIDUAL

NO. SUBJECTS	MEAN VARIATION IN GLUCOSE VALUES PER 100 ml. BLOOD				
	F.B.S	½ hour	1 hour	2 hour	3 hours
6	10.4	7	20.5	11	13

TABLE III

"SPONTANEOUS" VARIABILITY OF ORAL GLUCOSE TOLERANCE TEST

SUBJECT	DIAGNOSIS	AGE	AREA		DIFFERENCE	DIFFERENCE
			FIRST TEST	AFTER ONE WEEK		
			mg. - half hours		mg.-half hrs.	%
G.C.	normal	30	845	796	49	6.1
C. A.	normal	25	543	610	67	10.9
L.C.	normal	25	643	691	48	6.9
S.S.	paranoid state	32	609	605	4	0.65
M.H.	normal	24	587	575	12	2.0
S.Z.	normal	32	578	557	21	3.6

The claims of Freedman, Johns et. al. were not completely substantiated in that considerably less variation in results after repetition of the tests was found. The statement that the test is of slight diagnostic value except in diabetes mellitus is contested for Himsworth's area-comparison method has given results which do not vary greatly from test to test Table III). The present study seems to have confirmed the fact that the most significant differences on repetition occur in the one hour specimen, but the difference at this time was less than fifty percent of that reported by John (47.3 mgs.% : 20.5 mgs.%).

In summary then, it has been found in this study that the reproducibility of the oral glucose tolerance test is reasonably good, a relatively large difference occurring only in the one hour specimen. The latter difference is not considered to be justification enough for the restriction of this test to the diagnosis of diabetes mellitus.

3. The Intravenous Glucose Tolerance Test

a) Introduction

This is a test wherein the level of blood sugar is measured at intervals for an hour or two following an intravenous infusion of glucose solution. It has been used widely in an attempt to avoid the irregularities which are due to the absorptive processes.

b) Historical

This test has received sporadic attention for approximately the last quarter century. Ross(1938) has stated that the first study of the results of injection of sugar into veins was that of F.J.von Becker (1854) who produced glycosuria in rabbits by this means. The first clinical application of this test was in 1913 by Thannhauser and Pfitzer who followed the changes in blood sugar after injection of 7% glucose solution.

Rigler and Ulrich (1923) and Jorgensen and Plum (1923) were among the early investigators who followed the levels of glycaemia attending the administration of parenteral sugar and the latter claimed more regularly reproducible curves by this than by oral testing. Since the appearance of these studies many others have been reported including those of Davidson and Allen (1925), Thaysen (1929), Ross (1936), Fraser (1938). A complete review of the literature was published in 1940 by Tunbridge and Allibone.

The most recent study and one of the most extensive, has been that of Portis (1950).

c) Review of methods

There has been a great variability in the methods used by different investigators. In general, however, these methods have taken the form of continuous infusion or rapid

injection of a solution. Not only the rate of infusion but also the concentration of solution used have been exceedingly variable. Thus there are available results following administration of solutions of the following strengths: 7%, 7.5%, 10%, 20%, 25%, 30%, 40%, 50%, and 54%.

d) Evaluation of reported results

It is probable that in no other test so potentially useful as the intravenous glucose tolerance test has there been such lack of unanimity on procedure and interpretation of results. This, in large measure, has resulted as a consequence of differing techniques and improperly controlled test. It was soon apparent that nothing could be gained by strict adherence to the standards proposed in the past and accordingly the establishment of tolerance to intravenously injected glucose in normal people was one of the objectives of this study.

e) Factors influencing the test

Since these factors are essentially the same as those influencing the oral test, they will not be considered in detail. Suffice it to say that all precautions (diet, medication, et.al.) which were taken for the one, were taken for the other.

f) Method finally adopted

At first glance it would seem unnecessary to introduce still another method for this test when so many different techniques already exist. However, this step was finally agreed upon for the following reasons:

1. 5%, 10%, and 50% solutions of glucose are readily available in almost all hospitals at all times. The same cannot be said for 7.5%, 20%, 30%, and 54% solutions. Since one of our avowed aims was practicability of performance, the choice obviously was between the first three.
2. Although the 50% solution is probably the one most frequently used in performing the test at present, it has some disadvantages. These include the possible production of physico-chemical injury to the vascular endothelium. Christopher, in the fourth edition of his Textbook of Surgery recommends 50% glucose as a sclerosing agent for varicose veins. McPheeters and Anderson in their book on varicose veins state: "The sugar ... mixtures are very effective (for sclerosis) but ... quickly produce sloughs if injected perivascularly." In the few tests performed with this hypertonic solution in this study, complaints of arm cramps were not infrequently heard.
3. The injection of a 5% solution would be satisfactory but would take a greater length of time than a 10% solution and would have no obvious advantages over the latter.

Accordingly the choice was the 10% solution which was administered to the extent of 350 ml.. (35 gm.) over a period of twenty minutes by intravenous drip. Since the patients dealt with did not vary to a great extent in body weight, and since it has been recommended that even in cases with considerable variation a standard dose of glucose should be given as in the oral test (Tunbridge, 1940), 350 ml. were administered consistently.

Venous sampling was done during the fasting period immediately prior to the infusion, ten minutes after the infusion was begun, immediately the intravenous was terminated (twenty minutes), and at forty, seventy, one hundred and one hundred and thirty minutes from the start of the injection.

4. The Insulin-Glucose Tolerance Test

a) Introduction

The discovery of insulin by Banting and Best provided not only a powerful therapeutic weapon to the medical world, but also an agent of marked importance in the investigation of the metabolism of carbohydrate.

There have been many techniques described for the use of insulin in investigative and diagnostic procedures each of which has two objectives: to determine the effect of insulin administration to a given subject on the level of blood sugar

(insulin "sensitivity"); to study the response of the organism to the hypoglycaemia which usually follows its administration.

b) Review of methods

There have been at least three different methods advocated for the study of the varying responses of the body to parenteral insulin administration. They consist of:

a) the administration of insulin alone intravenously with serial blood sugar determinations - the insulin-tolerance test.

b) the simultaneous administration of glucose and insulin with serial blood sugar determinations - the glucose-insulin tolerance test.

c) the administration of insulin intravenously followed by glucose ingestion after thirty to sixty minutes or when the first symptoms of hypoglycaemia appear - the insulin-glucose tolerance test.

c) Evaluation of proposed tests

A. The Insulin-Tolerance Test

This test has been extensively used by Fraser and Smith in the diagnosis of Simmonds' Disease (1940). Engel and Scott (1950) have recently commented on this test and have criticized it as being "disappointing in those situations in which it should be most valuable i.e. conditions characterized by hypoglycaemic unresponsiveness." Moreover the intravenous injection of insulin in certain disease states (panhypopituitarism, adrenal insufficiency) is not without considerable

danger. The administration of a fraction of the dose recommended in order to avoid mishap is frequently productive of an equivocal response. Therefore it may be said that although the use of insulin alone is theoretically the foremost means for studying its action, certain practical considerations militate against its use in this manner.

B. The Glucose-Insulin Tolerance Test

This test was introduced by Himsworth (1939) with the object of discovering whether the hyperglycaemia of diabetes mellitus (hyperglycaemia unresponsiveness) was due to lack of insulin or insulin resistance, that is, whether in diabetes mellitus there was a relative deficiency or a normal supply of the hormone with resistance to its action. He tried to arrange for the insulin administered to balance the glucose ingested with, therefore, the subsequent production of a flat or nearly flat ensuing tolerance curve. Using Himsworth's method, cases of hypoinsulinism should also present a flat curve whereas in the presence of insulin resistance the test should approach the ordinary glucose tolerance test.

Himsworth's test was not considered suitable for the present study because it was early discovered that we were not dealing with a group of patients who manifested hyperglycaemia unresponsiveness. Also, the simultaneous administration of glucose and insulin by different routes inevitably introduces certain variables which tend to make the interpretation of results difficult.

C. The Insulin-Glucose Tolerance Test

This modification of the insulin tolerance test has been chosen for the present study because it overcomes the disadvantages of the former. It has been described by Engel and Scott (1950) and is based on Somogyi's observation that in normal people the administration of glucose thirty to sixty minutes after insulin resulted in greater hyperglycaemia than the administration of the same amount of glucose without insulin. Engel states that this result is presumably mediated by the hormones of the adrenal (medulla and cortex) and anterior pituitary, but this interpretation is largely speculative.

d) Method finally adopted

The patients were prepared in the same manner as for the preceding tests. In the morning after a twelve hour fast venous blood was withdrawn. Then 0.1 units Toronto insulin (Connaught Laboratories) per kg. body weight was injected intravenously. After thirty minutes or when the first symptoms of hypoglycaemia appeared (whichever was first) 50 gm. glucose were given by mouth. Blood samples were taken at thirty minutes (immediately prior to the administration of glucose), sixty minutes, ninety minutes, one hundred and twenty minutes, and one hundred and eighty minutes from the start of the test. Blood sugar levels were expressed as percent of the initial blood sugar which was considered 100%.

SECTION IV

EXPERIMENTAL CONSIDERATIONS

1. A comparison Of The King-Garner And Folin-Wu (True Sugar) Tests
2. The selection Of Psychoneurotic And Control Subjects
   a) The psychoneurotic group
   b) The control groups
3. Preparation For The Tests
4. The Blood Samples
5. The Tests Performed

1. A Comparison Of The King-Garner And Folin-Wu (True Sugar) Tests

This comparison was made for two reasons. The first was to confirm the accuracy of the more recently proposed procedure. The second was to eliminate the possibility of glycolysis occurring in the specimen which was stored in fluoride and analized after some two hours had elapsed. The Folin-Wu analyses were done after only a few minutes from the time of venous sampling. The results obtained with the two tests are seen in Table IV. From the examination of these results it was concluded that the Folin-Wu and King/Garner tests give quite comparable values and preservation with fluoride successfully inhibited glycolysis.

2. The Selection Of Psychoneurotic And Control Subjects

a) The psychoneurotic group

1. All subjects were male and in the age group twenty-two to forty-five. Only five however were over forty.
2. A history, physical examination, urinalysis, complete

TABLE IV

A COMPARISON OF THE RESULTS OF ANALYSES OF THE SAME BLOOD SAMPLE BY THE METHODS OF FOLIN-WU AND KING-GARNER

(results in mg. per 100 ml. blood)

SAMPLE NO.	FOLIN-WU (TRUE SUGAR) IMMEDIATE ANALYSIS	KING-GARNER DELAYED ANALYSIS
1.	81	80
2.	107	105
3.	80	80
4.	83	77
5.	83	79
6.	42	43
7.	103	104
8.	92	90

blood count, sedimentation rate, chest x-ray, and Wasserman test were performed routinely on each patient. If these examinations indicated further studies (B.M.R., Barium series, etc.) were necessary before a definitive diagnosis could be made, these studies were carried out.

3. All subjects were hospitalized in Deer Lodge Veteran's Hospital and were interviewed by the attending staff neuropsychiatrist.
4. The ultimate selection of patients depended on the demonstration that insofar as possible organic disease had been excluded and an opinion from the neuropsychiatric examiner that the cause of the presenting symptomatology was a psychoneurosis, with anxiety as the basic feature.
5. Patients either manifestly underweight or obese were rejected.
6. All patients were ambulant.

b) <u>The control groups</u>

For the oral glucose tolerance test two control groups were used. The first consisted of twelve subjects who were either apparently completely free of any disease (three subjects) or who were hospitalized for ailments which were not considered important with respect to this test. These ailments were: plantar warts (two subjects), varicose veins of the legs (four subjects), old gun-shot wounds of

the extremities (two subjects), inguinal hernia (one subject). All patients were ambulant and were taking a satisfactory diet. All were in a good state of nutrition. The second control group was made up entirely of psychotic patients. This group was chosen not to demonstrate abnormalities of carbohydrate metabolism in the psychoses primarily, but rather to provide a comparison between hospitalized psychoneurotic and psychotic individuals on identical diets.

The control group for the intravenous tolerance test consisted of four ostensibly normal medical students and internes and seven patients hospitalized with ailments inconsequential with regard to the test. All subjects were taking satisfactory diets, were ambulant and well nourished.

Ten hospitalized patients, again satisfactory with regard to the previous criteria, constituted the control group for the insulin-glucose test.

3. Preparation For The Tests

Having been selected as suitable for the investigation, the subjects were placed on the standard hospital diet for a minimum of four days preceding the tests. The ordinary hospital diet consisted of approximately 300 gm. carbohydrate, 130 gm. fat, and 80 gm. protein. While no careful surveillance of the patients was possible at each meal, both the patient's and nursing staff's assurance that the diet was being taken satisfactorily was sought and obtained before a test was performed.

No medication was administered from the time of entry into the hospital until the tests were completed.

4. The Blood Samples

The question of whether to use venous or capillary (arterial) blood was settled in favor of the former for the following reasons: transportation of the blood was obligatory and it was found more practical to obtain a sample of venous blood for this purpose; there was less objection from the sometimes recalcitrant patients to venipuncture than to stilette puncture; after glucose ingestion a large amount of glucose may be assimilated by the peripheral tissues and this assimilation is reflected in a lowering of the venous blood sugar but not in the arterial blood sugar.

The advantages of venous over arterial estimations have been reviewed recently by Mosenthal (1946, 1950).

All blood was withdrawn from a vein after light tourniquet application and was collected in fluoride and then refrigerated.

5. The Tests Performed

An oral glucose tolerance test was performed on each of the selected patients and on the normal and psychotic controls. Usually this was of three hours duration but, when circumstances permitted the blood glucose level was followed for five hours. Those people who had an oral test and who were

able to prolong their period of hospitalization or for whom this period would necessarily be lengthy, were subjected to an intravenous glucose tolerance and an insulin-glucose tolerance test.

It was apparent that the existing routine methods of performance and reported results of the intravenous glucose tolerance test were far from satisfactory. The standards for interpretation of this test are varied, often to a prohibitive extent. Accordingly it was found necessary to attempt to establish a normal set of standards to be used with the previously designated method for the performance of this test.

As previously mentioned, the insulin-glucose tolerance test used in this study was that proposed by Engel and Scott.

For those subjects on whom more than one of the tests was performed, repeated tolerance testing was done at a minimum of five days and usually seven days from the preceding test.

SECTION V

RESULTS

1. The Oral Glucose Tolerance Test
2. The Intravenous Glucose Tolerance Test
3. The Insulin-Glucose Tolerance Test

1. The Oral Glucose Tolerance Test

The individual results obtained in the performance of the oral glucose tolerance test have been recorded in tabular form. Table V shows the values obtained in twelve subjects who were ostensibly normal or who were suffering from maladies which would not be expected to affect the test (see page 51). Table VI records the values in thirteen psychotic subjects and Table VII the values in thirty-six psychoneurotic individuals. A comparison of the curve of the mean values of the psychoneurotic and normal groups is seen in Figure 1, and of the psychotic and normal groups in Figure 2.

Examination of Tables V, VI, VII, and Figures 1 and 2 reveals the following facts:

1. The mean fasting blood sugar in the normal group was 87 mgs. per 100 ml. blood, in the psychoneurotic group 84 mgs. and in the psychotic group 83 mgs. per 100 ml. blood. The largest value for fasting blood sugar in the normal group was 97 mgs.% and in the psychoneurotic group 103 mgs.%.

TABLE V

ORAL GLUCOSE TOLERANCE TEST IN NORMAL SUBJECTS

(blood sugar values expressed in mg. per 100 ml. blood)

SUBJECT	AGE	F.B.S.	½ HR.	1 HR.	2 HRS.	3 HRS.	4 HRS.	5 HRS.
T.W.	26	78	141	73	69	74		
A.T.	40	86	135	150	93	72	85	90
M.H.	33	95	155	110	95	97	79	90
W.D.	44	97	137	140	125	70	77	95
P.C.	29	85	140	87	67	83	70	87
D.M.	26	85	137	112	67	53	80	80
R.R.	30	83	145	160	75	75	83	85
S.D.	27	92	147	90	107	90	79	97
D.B.	31	80	110	145	80	80	83	94
H.H.	34	95	154	158	85	100	103	
L.M.	22	91	157	132	75	85	94	103
R.S.	25	79	120	125	80	87		

TABLE VI

ORAL GLUCOSE TOLERANCE TEST IN

THIRTEEN PSYCHOTIC SUBJECTS
(sugar values expressed in mg. per 100 ml. blood)

PATIENT	AGE	F.B.S.	½hr.	1hr.	2hr.	3hr.	4hr.	5hr.	DIAGNOSIS
J.B.	40	67	155	120	102	85			catatonic schizophrenia
C.C.	30	65	127	160	120	70			catatonic schizophrenia
L.M.	36	79	140	130	97	65	67	95	catatonic schizophrenia
L.C.	32	87	152	95	85	79	90		catatonic schizophrenia
L.S.	40	87	120	140	85	58	85	85	schizophrenia
V.S.	36	97	137	140	113	62			schizophrenia
N.Z.	25	79	152	147	50	67	58	70	schizophrenia
W.M.	60	65	112	137	72	68			"mental deterioration"
C.I.	19	92	140	195	120	70	79	90	manic-depressive (manic phase)
C.C	24	90	140	100	103	95	96		psychopathic personality
S.S.	39	88	160	120	80	65			paranoid psychosis
C.S.	39	80	150	105	85	88			paranoid psychosis
V.W.	37	102	145	175	160	95			depressed state

TABLE VII

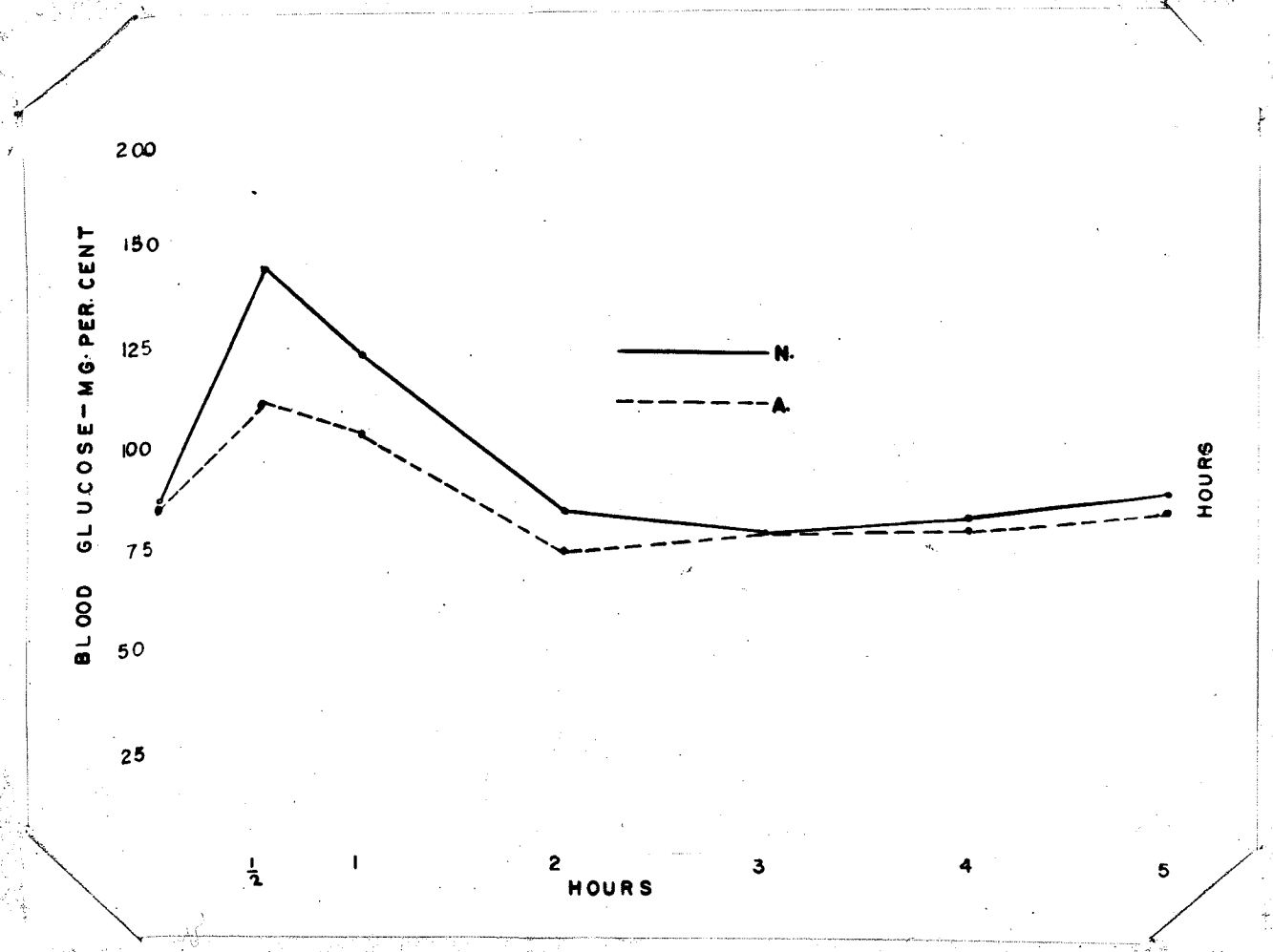
THE ORAL GLUCOSE TOLERANCE TEST IN

PSYCHONEUROTIC PATIENTS

(Blood sugar values in mg. per 100 ml.)

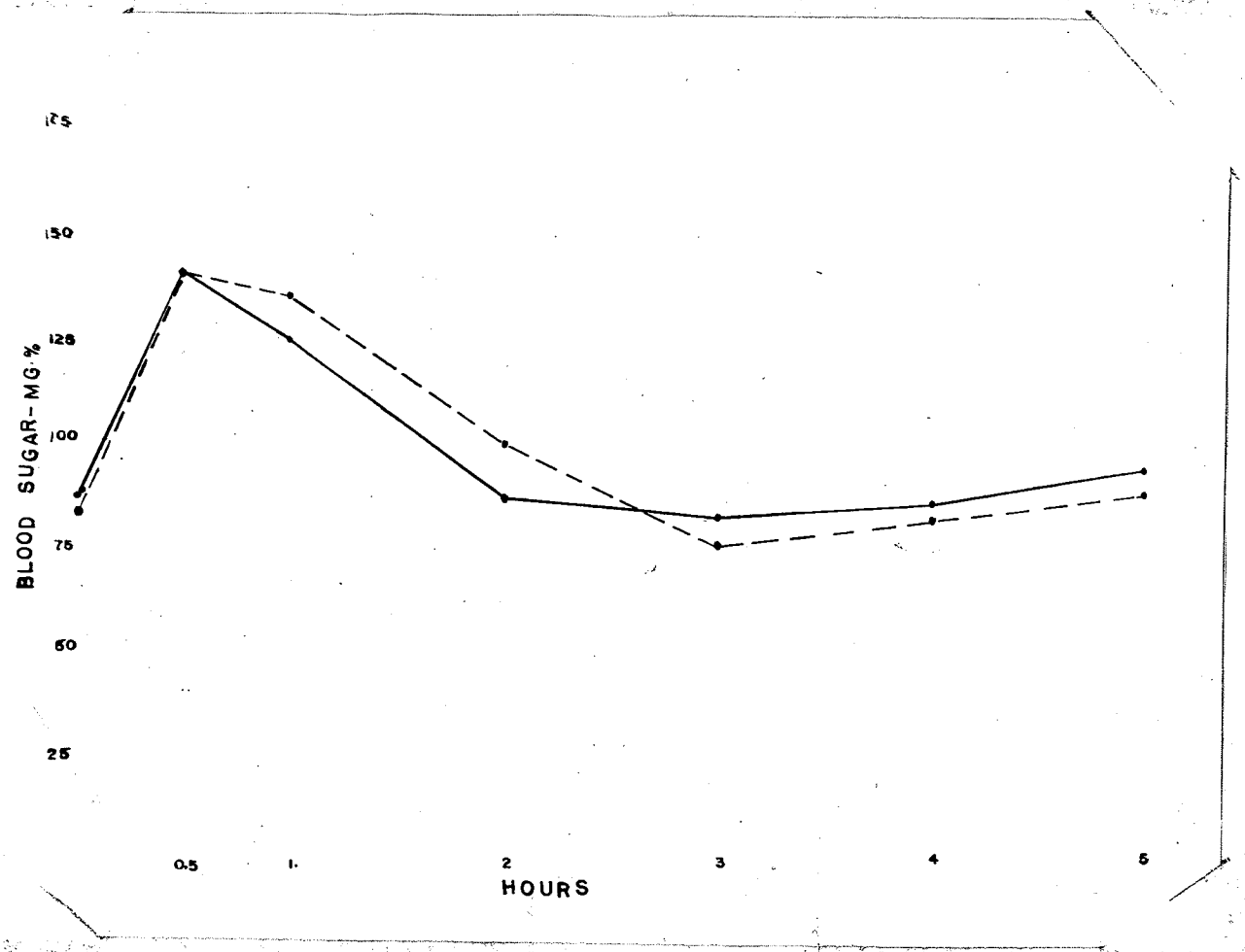
PATIENT	AGE	F.B.S.	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.
A.S.	28	72	102	70	60	83		
S.N.	29	90	120	105	90	105		
F.N.	33	77	95	115	70	62		
H.K.	29	80	83	88	65	80		
C.C.	45	80	85	72	80	83		
E.W.	33	91	130	125	95	72		
L.C.	30	92	112	70	83	85		
F.Y.	25	72	125	113	88	70		
M.M.	27	102	122	135	115	83		
L.A.	36	83	120	120	45	70		
R.H.	37	75	125	130	61	70		
B.L.	39	80	97	75	92	90		
S.W.	32	83	97	105	80	77		
V.K.	37	75	127	79	79	65		
W.W.	30	92	120	87	58	70		
V.B.	29	90	127	100	80	92		
A.B.	25	95	115	130	90	97		
T.K.	32	67	102	113	62	65		
Y.R.	35	70	80	92	87	92		
J.B.	35	92	124	134	106	74		
L.M.	41	72	80	112	60	90	92	87
I.D.	26	85	115	100	58	83	79	87
J.L.	25	92	137	95	97	95	87	95
T.T.	28	75	110	97	67	77	75	88
W.B.	27	95	105	95	85	80	80	85
J.J.	36	85	137	135	72	80	95	107
I.M.	41	72	80	112	60	90	92	87
Y.M.	41	85	137	112	67	53	80	80
V.J.	41	88	110	120	58	70	67	67
R.R.	22	83	95	107	58	75	79	87
A.A.	29	72	115	90	60	80	70	83
L.D.	25	83	115	97	72	83	70	80
R.B.	34	90	115	107	80	67	75	85
S.S.	28	92	140	95	80	100	107	110
F.J.	26	87	110	120	58	70	67	67
P.C.	25	103	107	80	85	92	90	100

FIGURE 1



The oral glucose tolerance curve in normal (N) and psychoneurotic (A) subjects. The mean values obtaining at the stated intervals of time are represented by heavy dots.

FIGURE 2



The oral glucose tolerance curve in normal (undivided line) and psychotic subjects. The mean values obtaining at the stated intervals of time are represented by heavy dots.

The lowest values recorded were 78 mgs.% in the former group and 67 mgs.% in the latter.

2. Following the ingestion of 50 grams of glucose the average rise in the blood sugar of the normal controls was 53 mgs.%, of the psychotic group 58 mgs.%, and of the psychoneurotic group 28 mgs.%. Nine (25%) of the thirty-six psychoneurotic subject demonstrated a rise in blood sugar greater than 40 mgs.%, the greatest rise being 53 mgs.% (one subject) and the average rise in these nine patients being 49 mgs.%. None of the subjects of either control group was found to exhibit any elevation in blood sugar less than 40mgs.% after ingestion of 50 gms. of sugar.
3. The maximal height of the blood sugar was found in the half-hour specimen in twenty-two of thirty-six psychoneurotic patients (61%). The remainder reached their zeniths at one hour. In the normal control group 50% achieved their peak at one-half hour and in the psychotic group the peak was reached in one-half hour in 52%. The remainder of both groups reached their peak in one hour.
4. Having reached their greatest heights, the tolerance curves (representing mean values) of the psychoneurotic and normal groups began to descend approximately parallel to one another (Figure 1). The mean value of the former group at two hours was 75 mgs.% (a fall of 37 mgs.% from the acme) and of the latter 85 mgs.% (a fall of 55 mgs.%).
5. At three hours the mean values of the two groups were identical (80 mgs.%).
6. At four hours the mean value for the patients was 81 mgs.%

and for the normal subjects 83 mgs.%.

7. At five hours the mean value for the patients was 87 mgs.% and for the normal group 91 mgs.%.
8. Only one of the psychoneurotic subjects (LA, Table VII) demonstrated considerable hypoglycaemia and he in only one specimen (two hour).
9. Five of the patients (14%) had a flat oral tolerance test i.e. exhibited no appreciable rise in blood sugar following glucose ingestion. These patients were: H.K., B.L., Y.R., C.C., P.C. - (Table VII).

2. The Intravenous Glucose Tolerance Test

The distribution of values obtained in the performance of this test on eighteen psychoneurotic subjects and eleven controls has been presented in Table VIII. The curves, representing the mean values obtaining at stated intervals are seen in Figure 3. Examination of Table VIII and Figure 3 shows the following:

1. The values of the fasting blood sugar in the two groups were within normal range. The mean blood sugar value in the anxiety group was 75 mgs.% and in the control group 75 mgs.%.
2. At ten minutes (half way through the infusion when 17.5 gms. of sugar had been injected) the mean blood sugar level of the control group was 178 mgs.% and of the patients 152 mgs.%. Thus infusion of an equal amount of sugar in the two groups was productive of a rise greater by 26 mgs.%

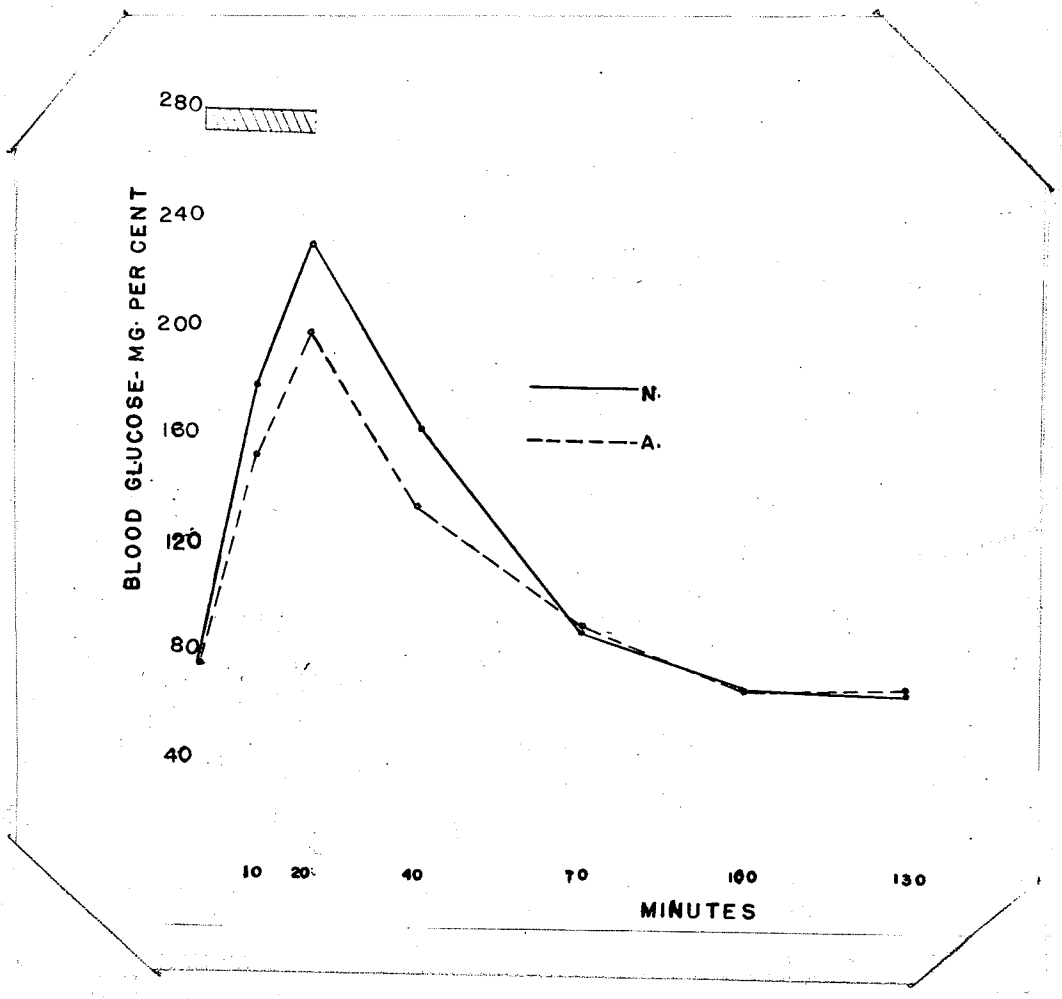
TABLE VIII

DISTRIBUTION OF VALUES OF INTRAVENOUS GLUCOSE TESTS

(Sugar values expressed as mg. per 100 ml. blood)

SUBJ.	AGE	F.B.S.	10min.	20min.	40min.	70min.	100min.	130min.
F.G.	29	64	181	273	124	112	50	50
E.S.	25	80		263	183	102	63	59
A.T.	22	75	181	238	142	54	71	77
J.S.	20	72	154	231	202	123	89	82
V.C.	27	77	191	216	114	48	51	58
R.R.	24	67	130	220	167	79	65	69
V.H.	30	68	213	220	150	70	63	63
A.C.	30	91		214	186	103	91	78
P.P.	29	77	192	211	146	63	72	64
R.S.	30	68	185	220	194	82	65	61
L.M.	25	82		241	183	131	62	44
V.K.	37	78	121	216	138	73	67	64
B.L.	39	75	139	162	102	78	79	74
D.D.	32	74	137	213	130	87	58	76
T.D.	26	78	176	215	145	73	63	58
J.L.	25	80	154	213	184	149	96	60
L.F.	25	79	153	191	143	99	63	73
J.J.	36	81		201	140	82	68	71
I.M.	41	79	147	195	120	60	46	50
Y.M.	41	70		226	158	84	64	113
D.M.	25	67		167	115	72	67	44
F.J.	26	88		152	125	81	85	75
R.R.	22	85	114	208	151	58	62	76
A.A.	29	65		184	98	75	47	73
L.D.	25	75	154	208	112	68	82	83
C.R.	29	66		195	119	118	59	68
R.B.	34	72		193	165	138	61	60
S.S.	28	77		231	166	156	64	48
L.W.	33	87	184	217	109	71	59	60

FIGURE 3



The intravenous glucose tolerance curve in normal (N) and psychoneurotic (A) subjects. The mean values obtaining at the stated intervals of time are represented by heavy dots. The cross-hatched area at the top of the figure represents the duration of the intravenous infusion.

in the control group at the time of the first sampling. As the infusion was continued to completion, although the disparity was seen to increase, quantitatively there was a much less significant increase in the level of the controls over the psychoneurotics (6 mgs.% from ten to twenty minutes). The mean levels at twenty minutes were 231 mgs.% in the normal controls and 199 mgs.% in the psychoneurotics.

3. Having reached their zeniths at twenty minutes the curves began to drop and by forty minutes the control group level had fallen 68 mgs.% to reach the lower level of 163 mgs.% and the level of the patients had fallen 65 mgs.% to reach the level of 134 mgs.%.

4. During the ensuing thirty minutes concurrence of the two curves was achieved which continued to the end of the test.

3. The Insulin-Glucose Tolerance Test

The distribution of values obtained after performance of this test on ten control subjects has been presented in Table IX. For purposes of analysis and comparison with other similar studies the values so obtained have also been recorded as a percentage of the fasting blood sugar (Table X).[1] Table XI shows the values in sixteen psychoneurotic subjects, again expressed as a percentage of the fasting blood sugar. In Figure 4 are seen the curves which chart the mean values

1. The reader's attention is particularly drawn to Table X. Table IX has been inserted to demonstrate the means by which the values in Table X are calculated.

TABLE IX

RESULTS OF INSULIN-GLUCOSE TOLERANCE TESTS IN NORMAL GROUP

(Sugar values expressed in mg. per 100 cm1. blood)

SUBJECT	AGE	F.B.S.	½ HR. OR Hypoglycaemia	1 hr.	1½hr.	2 hr.	3 hr.
A.T.	40	105	37	115	137	105	83
M.H.	33	95	34	83	100	103	100
W.D.	44	77	52	90	148	145	122
P.C.	29	85	35	62	87	70	85
D.R.	26	90	20	107	152	115	83
R.M.	30	85	23	79	83	140	110
R.D.	27	92	83	130	79	67	87
W.F.	29	86	65	85	144	130	100
W.D.	3 7	85	40	76	87	75	86
L.J.	27	80	38	114	127	118	74

TABLE X

RESULTS OF INSULIN-GLUCOSE TOLERANCE TESTS IN NORMAL GROUP

(Blood sugar levels recorded as per cent of fasting blood sugar)

SUBJECT	AGE	F.B.S.	½ hr. or HYPOGLYCAEMIA	1 hr.	1½hr.	2 hr.	3 hr.
A.T.	40	100	35	109	130	100	79
M.H.	33	100	35	87	105	108	105
W.D.	44	100	67	116	191	188	158
P.C.	29	100	41	73	102	82	100
O.R.	26	100	22	118	168	127	92
R.M.	30	100	27	93	97	164	129
R.D.	27	100	90	141	86	73	94
W.F.	29	100	75	98	167	151	116
W.D.	37	100	47	89	102	88	101
L.J.	27	100	47	142	158	147	92
Total			461	1066	1306	1228	1066
Mean		100	46	107	130	123	107

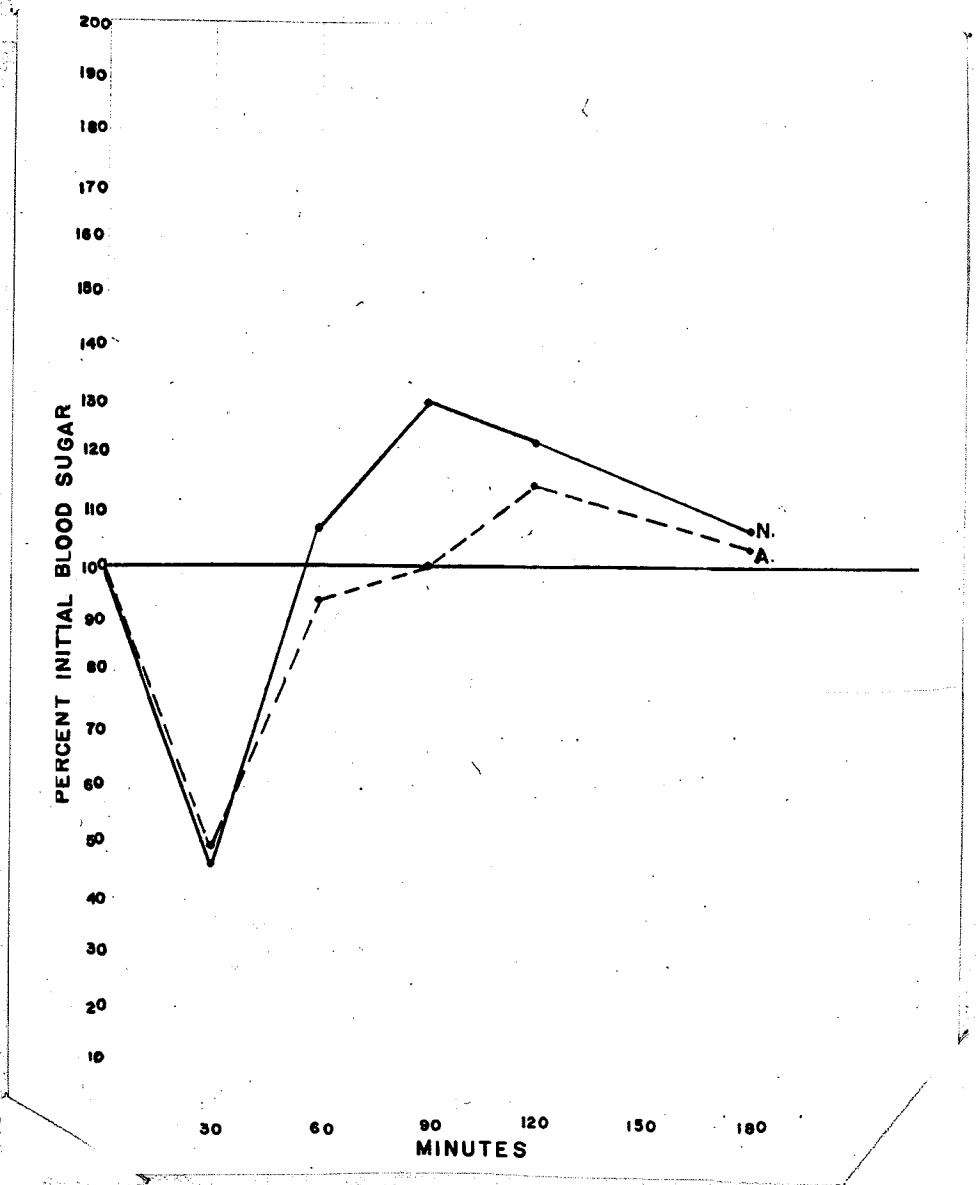
TABLE XI

INSULIN-GLUCOSE TOLERANCE TEST IN PSYCHO NEUROTIC GROUP

(Sugar values expressed as percent of fasting blood sugar)

PATIENT	AGE	F.B.S.	½ hr. or HYPOGLYCAEMIA	1 hr.	1½hr.	2 hr.	3 hrs
V.K.	37	100	76	146	108	111	92
B.L.	39	100	20	49	84	95	135
W.W.	30	100	62	90	138	76	65
I.D.	26	100	60	88	94	98	109
J.L.	25	100	91	137		186	102
L.M.	41	100	91	127	151	157	
Y.M.	41	100	27	93	97	164	129
O.M.	25	100	38	92	114	131	144
V.J.	36	100	39	95	95	92	100
R.R.	22	100	36	70	107	113	138
L.D.	25	100	29	63	96	108	86
E.W.	33	100	47	137	102	80	59
R.B.	34	100	53	71	104	121	156
S.S.	28	100	41	76	108	122	122
F.J.	26	100	39	95	95	92	100
P.C.	25	100	42	73	105	96	
MEAN		100	49	94	100	115	103

FIGURE 4



The insulin-glucose tolerance curve in normal (N) and psychoneurotic (A) subjects. All sugar values are expressed as a percent of the fasting blood sugar. The mean values obtaining at the stated intervals of time are represented by heavy dots.

of the psychoneurotic and normal groups at stated intervals of time.

Examination of Tables IX, X, XI, and Figure 4, revealed the following facts:

1. The mean value of the blood sugars of the control group one-half hour after insulin was administered (or when significant hypoglycaemic symptoms appeared) was 46 mgs.%, in the neurotic group 49 mgs. %.
2. At one hour (one-half hour after administration of glucose per os) the value in the normal group was 107 mgs.% and in the neurotic subjects 94 mgs.%.
3. At one and one-half hours from the start of the test the mean value in the former group was 130 mgs.% and in the latter 100 mgs.%.
4. At two and three hours the mean values expressed in mgs. per 100 mls. were respectively 123;115 and 107:103.
5. Within the psychoneurotic group (Table XIII) two patients (J.L., L.M.) were quite refractory to insulin and three (V.K., W.W., I.E.) did not respond with the expected fall in glycaemia. Three were apparently hypersensitive (B.L., Y.M., L.D.). In the normal group (Table XII) one patient (R.D.) was quite refractory to insulin and two were quite sensitive (D.R., R.M.). Two (W.D., W.F.) did not respond with the expected depression of blood sugar.

SECTION VI

DISCUSSION AND CONCLUSIONS

1. Introduction
2. Statistical Methods
3. Analysis And Discussion Of Results
4. Suggested Explanation Of The Results Of This Study
5. Comparison Of Results With Other Studies
6. Conclusions

1. Introduction

Having recorded the results of the three tests used in the study of a group of patients suffering from chronic anxiety and its attending manifestations, it was then deemed desirable to subject the results obtained to statistical analysis in order to ascertain whether or not there existed significant differences with respect to the metabolism of glucose in these subjects as compared with normal people.

2. Statistical Methods

The objectives of this study demanded that an efficient method for comparison of the data collected from the control and normal groups be utilized. Theoretically many methods of comparison could have been used. For example the rate of ascent and decline of the tolerance curves could have been estimated. Another alternative would have been to estimate and compare the areas bounded by tolerance curves. However, it was decided that the simplest method for the present study was a comparison of the mean values obtaining at different time intervals by means of the t-test.

The t-test is a test for the significance of the difference between two means, the probability of the observed difference (or a greater) occurring by chance being estimated from a t-table. This test is particularly adapted to the analysis of small samples. P (obtained from the t-table) is the probability (expressed either as a decimal e.g. P = 0.10, or as a percentage e.g. P = 10%) of a difference between the two means as great as (or greater than) the observed difference occurring by chance. The limits of significance (P = 0.05 or P = 0.01) which are purely conventional have been proved satisfactory by long experience. The test was originally proposed by "Student" in 1908 and has been modified since then to its present form by Fisher (1946).

3. Analysis And Discussion Of Results

A comparison of the data obtained on psychoneurotic patients and normal controls in the performance of the oral glucose tolerance test, the intravenous glucose tolerance test, and the insulin-glucose tolerance test has been presented in Tables XII, XIII and XIV respectively. The results of statistical comparison of the mean values of blood sugar at the given time intervals, have been indicated in the columns headed P_t in the latter Tables. It may be recapitulated that when P is less than 0.05 there exists a significant difference between the two means and when P is less than 0.01 there is a highly significant difference.

TABLE XII

A COMPARISON OF ORAL GLUCOSE TOLERANCE TEST IN PSYCHONEUROTIC PATIENTS AND NORMAL CONTROLS

PSYCHONEUROTIC GROUP					P_t	CONTROL GROUP				
MINUTES AFTER ADMINISTRATION	NO. OF SUBJECTS	GLUCOSE IN mg. per 100 cc. BLOOD				MINUTES AFTER ADMINISTRATION	No. of SUBJECTS	GLUCOSE IN mg. per 100 cc. BLOOD		
		MAX.	MIN.	AVERAGE				MAX.	MIN.	AVERAGE
0	36	103	67	84	$P > 0.05$	0	12	97	78	87
30	36	140	80	112	$P < 0.01$	30	12	157	110	140
60	36	135	70	104	$P \leq 0.05$ $P > 0.01$	60	12	160	73	123
120	36	115	58	75	$P > 0.05$	120	12	125	67	85
180	36	105	53	80	$P > 0.05$	180	12	100	53	80
240	16	107	67	81	$P > 0.5$	240	10	103	70	83
300	16	110	67	87	$P \geq 0.30$ $P < 0.40$	300	9	103	80	91

TABLE XIII

ANALYSIS OF DATA OF PATIENTS AND CONTROL GROUP IN INTRAVENOUS GLUCOSE TOLERANCE TEST

PSYCHONEUROTIC GROUP					P_t	CONTROL GROUP				
MINUTES AFTER INJECTION	NO. OF SUB-JECTS	GLUCOSE IN mg. per 100 cc. BLOOD				MINUTES AFTER INJECTION	NO. OF SUB-JECTS	GLUCOSE IN mg. per 100 cc. BLOOD		
		MAX.	MIN.	AVERAGE				MAX.	MIN.	AVERAGE
0	18	88	65	75	$P > 0.05$	0	11	91	64	75
10	12	176	114	152	$P < 0.05$ $P > 0.02$	10	8	191	130	178
20	18	231	152	199	$P < 0.01$	20	11	273	211	231
40	18	184	98	134	$P < 0.01$	40	11	202	114	163
70	18	156	60	90	$P > 0.05$	70	11	131	48	88
100	18	96	46	66	$P > 0.50$	100	11	91	50	67
130	18	113	44	68	$P > 0.50$	130	11	89	44	65

TABLE XIV

A COMPARISON OF INSULIN-GLUCOSE TOLERANCE IN PSYCHONEUROTIC SUBJECTS AND CONTROLS

PSYCHONEUROTIC GROUP					P_t	CONTROL GROUP				
MINUTES AFTER INJECTION	NO. OF SUB-JECTS	GLUCOSE AS PER CENT OF F.B.S. BLOOD				MINUTES AFTER INJECT-ION	NO. OF SUB-JECTS	GLUCOSE AS PERCENT OF F.B.S.		
		MAX.	MIN.	AVERAGE				MAX.	MIN.	AVERAGE
30(-)	16	91	20	49	$P > 0.5$	30(-)	10	75	22	46
60(-)	16	146	49	94	$P > 0.2$ $P < 0.3$	60(-)	10	142	73	107
90(-)	15	151	84	100	$P > 0.01$ $P < 0.02$	90(-)	10	191	86	130
120(-)	16	186	76	115	$P > 0.5$	120(-)	10	188	73	123
180(-)	14	156	59	103	$P > 0.5$	180(-)	10	158	79	107

The results of some seventy separate estimations of fasting blood sugar on thirty-six psychoneurotic subjects indicate that there is no significant difference between the mean values of these patients and the normal control groups in the post-absorptive state. Moreover the range of values of the neurotic group is within normal limits.

Following the introduction of glucose into the body either through the gastrointestinal tract or by vein, an interesting phenomenon is apparent in the great majority of the psychoneurotic group namely that whichever avenue of introduction is chosen, there is evidence of increased tolerance for this sugar i.e. the ensuing curve is lower than in the control group. Thaysen (1935) has suggested that unless the blood sugar rises more than 40 mgs.% after ingestion of sugar the ensuing curve is preternaturally low. Only nine of the thirty-six neurotic patients tested had a rise in blood sugar greater than 40 mgs.% (Table VII).

The t-test applied to the mean values obtaining at given intervals in the psychoneurotic and normal groups reveals that in the oral test there is a highly significant difference at one half hour (the neurotic subjects' values being the lower), a significant difference at one hour (again lower values for the neurotic subjects), and no significant difference at either two, three, four, or five hours (Table XII). It is also interesting to note that the

mean value of the psychoneurotic group at one half hour (112 mg. per 100 ml.) is considerably lower than the lowest average result of a quite large, well-controlled study of normal young adults found in the literature (Watson, 1938). Finally, it is of considerable importance that in the oral test there is no suggestion of hypoglycaemia at four or at five hours in either the control group or in the psychoneurotic subjects (Table XII).

In the intravenous tests the situation is similar (Table XIII). The fasting blood sugar values are normal in both groups but at ten minutes from the start of the infusion (17.5 gm. sugar injected) there is a significantly lower mean value in the psychoneurotic group, and at twenty minutes (infusion ended) a highly significant lower mean value in this group. Forty minutes from the start of the test the mechanisms for regulation of blood sugar are seen to have accomplished an almost identical lowering effect in the two groups but the mean level of the psychoneurotic group is still significantly lower than that of the normal group. During the next thirty minutes concurrence of the two curves has been achieved (Figure 3) and no statistically significant difference in the mean levels is present at seventy, one hundred or one hundred and thirty minutes.

In brief then, the psychoneurotic group exhibit an increased tolerance for injected sugar. Effacement of the variation is accomplished approximately within an hour.

In the case of the insulin-glucose tolerance test it is apparent that the neurotic group respond with approximately the same depression of blood sugar as the normal group after

insulin administration (Table XIV and Figure 4). Thus insulin "sensitivity" is the same in both groups. The two groups respond with quite comparable elevations of blood sugar one-half hour after glucose ingestion. However, one hour after drinking the glucose, the mean level of the psychoneurotic group is seen to be significantly lower than that of the control group (100 : 130 mgs.%). At two and three hours no significant difference exists. Finally, it may be noted that within the neurotic group the peak of the blood sugar tends to occur later (two hour specimen) than in the control group but even here the mean level is below that of the controls.

The foregoing facts may then be summarized as follows: the patients chosen for this study i.e. those people who were suffering from a psychoneurosis characterized mainly by chronic anxiety manifested an increased tolerance for orally and parenterally administered glucose and also an unsatisfactory response to insulin-induced hypoglycaemia. A discussion of the known causes of these phenomena is now necessary.

The first concept demanding consideration is that of poor or slow absorption of glucose from the digestive tract. Certain facts mediate strongly against the operation of this factor in the production of the observed phenomena. These facts are:

1. Glucose is one of the most readily absorbed substances. Evidence to this end has previously been cited (Evensen, 1942). Thaysen (1935) has quoted a case of gastro-colic

fistula and another with resection of four metres of small gut, each with normal tolerance.

2. Seventy-seven percent of the patients tested showed a return to the fasting level or even lower in two hours.
3. The anxious subjects demonstrated an increased tolerance for intravenously administered glucose. Here, of course, the variable factor of absorption had been eliminated.

The next possible explanation of the low curves, excessive renal excretion, may be summarily dismissed for, during the oral testing, no glycosuria was found on examination of any specimen of urine. Moreover all patients were routinely tested for glycosuria on admission and at two week intervals during their period of hospitalization. The subjects of the intravenous and insulin-glucose tests were chosen from the group who had been submitted to oral testing.

A group of endocrine disorders has been found to be associated with increased glucose tolerance. These disorders include: hyperinsulinism, adrenal cortical insufficiency, anterior pituitary hypofunction, and hypothyroidism (LePore, 1941). The evidence at hand does not permit the ascription of increased tolerance to primary endocrine dysfunction in the neurotic subjects of this study. Against <u>hyperinsulinism</u> are the following facts:

1. The absence of fasting hypoglycaemia; a proportion of the patients having had as many as four independent estimations of postabsorptive glycaemia.
2. The diagnosis of hyperinsulinism by means of the glu-

cose tolerance test rests on the demonstration of persistent postalimentary hypoglycaemia (Peters and Van Slyke, 1946) and it has already been shown that in none of the sixteen patients subjected to the five hour oral test was there any significant evidence of hypoglycaemia.

3. The insulin-glucose test in the subjects of this study dispelled the possibility that these people were excessively "sensitive" to insulin. Moreover, in proved cases of hyperinsulinism, the insulin test is said to almost invariably indicate increased "sensitivity" to insulin (Cantarow and Trumper, 1949). The subjects of this study were normally "sensitive" to insulin. This is held to be a further link in the concatenation of evidence against the operation of hyperinsulinism in these subjects.

Against adrenal or pituitary insufficiency are the following:

1. Normal fasting blood sugars.
2. Absence of increased "sensitivity" to insulin.
3. Absence of an exaggerated hypoglycaemic response during either the oral or intravenous glucose tolerance test.
4. Absence of clinical stigmata of these diseases.

There was no clinical evidence of hypothyroidism in any of the neurotic group. Although B.M.R. estimations were done

on many (for the primary purpose of eliminating hyperthyroidism), in no case did this test point to the existence of myxoedema. Moreover the flat type of glucose tolerance curve sometimes observed in myxoedema is usually held to be due to poor absorption of glucose and the evidence against this concept in these patients has already been stated.

Many other causes for increased tolerance to glucose have been propounded including malnutrition, vitamin B deficiency, and idiopathic steatorrhea. The method of selection of patients and their abundant diet makes discussion of these states unnecessary. Finally, the possibility that excessive carbohydrate feeding produced the observed increased tolerance is untenable because the control groups were fed identical diets and manifested normal glucose tolerance.

4. Suggested Explanation Of The Results Of This Study

The following hypothesis would seem to adequately explain the facts that although the postabsorptive blood sugar levels are normal in the anxiety neurotics, administration of a given amount of glucose by mouth or by vein is followed by evidence of increased tolerance for approximately sixty minutes and then a normal response for the remainder of the test, these phenomena obtaining in spite of the probable absence of disturbances of absorption of glucose, increased renal excretion, or major endocrine dysfunction.

It is generally held that the fall of the glucose tolerance curve is due largely to three factors: removal of sugar by the liver with glycogen formation; removal of sugar by the extra-hepatic tissues for oxidation and for glycogen and fat formation; diminished hepatic glycogenolysis and gluconeogenesis. The last proposition has been advanced by Soskin (1941) and at present is considered to be of fundamental importance in the homeostatic mechanism for the control of the level of blood sugar. Application of these tenets to the present study made obligatory the conclusion that increased tolerance for glucose in the psychoneurotic subjects was the result of either:
a) decreased glycogenolysis and gluconeogenesis in the liver.
b) increased tissue utilization (oxidation and /or storage).
c) a combination of both.

It has been established that in anxiety states the work of certain tissues is increased. Hickam and collaborators (1948) have shown that anxiety increases the pulse rate, the blood pressure, and the cardiac output. The presence of irritability, tension, tachycardia and tremulousness in these patients almost certainly calls for increased energy production to sustain the constantly hyperfunctioning heart, musculature, and perhaps other tissues although evidence for the latter is scanty.

It is postulated that in the psychoneurotic patients ordinarily the increased requirements of certain tissues are met by acceleration of the hepatic glycogenolytic process with

maintenance of normal levels of blood sugar at the price of a temporary but relatively large decrease in the glycogen stores of the liver. With the introduction of exogenous glucose, the hungry liver clutches the glucose until it is sated and stores it as glycogen in preparation for future demands. This results in a diminished supply of sugar immediately available to the blood stream and consequently lower blood sugar curves no matter what the avenue of introduction of the sugar. When a satisfactory equilibrium is established the glucose tolerance curve assumes its normal configuration. The normal liver, since it is not subjected to so great a functional strain does not abstract glucose so avidly. The basic tenet of this theory is, then, that there exists a more dynamic ebb and flow of hepatic glycogen stores in the psychoneurotic than in the normal subject due to the exhortations of certain extra-hepatic tissues in the former patients. The explanation of the failure to respond with hyperglycaemia after glucose ingestion in the insulin-glucose tolerance test is again found in the depleted hepatic glycogen stores.

Some support for this theory has been found in the work of Hinkle et.al. (1950) on the relation of stressful life situations to the concentration of ketone bodies in the blood of diabetic and normal individuals. These investigators found that stressful stimuli in the normal and in the diabetic individual were capable of producing a rise in the ketone body content of venous

blood both in the periphery and from the hepatic vein. They further showed that when emotional security was achieved the level subsided. They postulated that a stimulation of the pituitary-adrenal mechanism was responsible.

Mirsky (1937) believes that the essential stimulus to ketone formation by the liver is a decrease in liver glycogen and this belief is generally regarded as at least a partial explanation of ketosis. It follows that if the theory of greatly fluctuating glycogen stores be accepted, the explanation of the moderate rise in ketone bodies in Hinkle's patients is facilitated. Conversely the demonstration of a milk ketonemia in these patients is indirect evidence in favor of the theory of greatly fluctuating stores. It would also seem significant that one of Hinkle's patients in whom anxiety was a marked feature had a rise in blood sugar of only thirty-four mgm.% after ingestion of 100 gm. of glucose and two who were patently anxious (A.W., A.X.) has quite low blood sugar values at one, two and three hours, accompanying the rise in blood ketones.

5. Comparison Of Results With Other Studies

Although the question of glucose tolerance in mental disease has been the subject of many previous investigations, none of these investigations has concerned itself with this problem in patients whose fundamental complaints were stated to be anxiety. Within the psychoneurotic group of patients,

neurasthenic subjects seem to have constituted the most investigated class, but it has often been stated that anxiety was a prominent feature in these neurasthenics. The confusion which exists in the classification of the neuroses has already been elaborated and at present appears to be the chief stumbling-block to the accurate comparison of different studies. Although the symptomatic pattern of the various neuroses may be markedly different some justification for their comparison may be found in their common etiological patterns.

Examination of the levels of the fasting blood sugars of some seventy anxious subjects in this study seems to show beyond doubt that these subjects, like the neurasthenic patients of Szondi and Lax (1929) and many others are not ordinarily hypoglycaemic. Like the neurasthenic subjects of Dorst (1937), Steven (1945), and Portis (1950) they exhibit increased tolerance for a load of glucose. It is the latter study however, which merits closest attention at present for the following reasons: it is the largest study of its type recorded; it is a recent study which utilizes modern laboratory methods; it has many implications which, if substantiated, will profoundly influence the management of a large group of neurotic patients. Portis has stated that he believes life situations and emotions may produce a symptom complex resulting in fatigue. He believes the mechanism in the production of this state to be hyperinsulinism. He has stated: "Since the pancreas is an end

organ and subject to vagal stimulation I considered it logical to assume that the hypoglycaemia of psychoneurotic patients is due to long-continued stimulation of the right vagus nerve." His evidence for the belief that hyperinsulinism is the mechanism concerned may be summarized as follows:

1. He found that the average intravenous glucose tolerance curve of 157 patients with fatigue as an outstanding and unexplained symptom was "flat" in comparison with the average curve of 772 controls.
2. He found that of the "fatigue" patients, fifteen showed a tendency to return to normal response when the glucose tolerance test was performed after administration of 1/50 gr. atropine.
3. He found that therapeutic benefit resulted when the patients were treated with atropine in addition to a diet low in free sugar over a fairly long period.

The most patent criticism of the work of Portis lies in the performance of his study. He stated that 929 patients with psychosomatic illnesses formed the group studied, of which 157 had a "flat" curve. It seems clear that his control subjects were a heterogeneous group suffering heterogeneous complaints. Whether fatigue was present in the control group was not made clear. Moreover dietary preparation for the tests, medication, body weight et.al. were not mentioned. Nor was it stated whether the subjects or the controls or neither or both were

hospitalized. Furthermore, the validity of the conclusions reached by Portis is questionable. Hyperinsulinism has been rejected as the etiological agent in the group examined in this study and for similar reasons it cannot be accepted as the mechanism in the production of symptoms in Portis' patients. His arguments are based on the belief that because vagal fibres supply the pancreas, emotional impulses may stimulate these fibres with consequent liberation of increased amounts of insulin. The evidence for the nervous control of the pancreatic internal secretion in the normal body economy rests on shaking foundations. While it is true that vagal fibres supply the pancreas, their function in controlling blood sugar levels is not obvious. The one fact that seems inescapable is that an animal with a denervated pancreas can deal with sugar in an apparently normal fashion (Houssay, 1937). Even the work of LaBarre which Portis quotes in support of the vagal stimulation theory in the dog, has been contested (Jensen, 1938). Portis' suggestion that atropine selectively paralyzes the right vagus nerve thus diminishing response to emotional stimuli by decreasing insulin production is difficult to accept. It would seem more reasonable to propose that the effect of atropine is to upset the normal autonomic synergism in favor of the sympathetic system with consequent elevation of blood sugar due, at least in part, to increased glycogenolysis. However, since the effect of atropine on blood

sugar is still equivocal in the normal organism, it is difficult to be dogmetic about its effect in the neurotic subject. Close examination of his recorded data shows that if the half-hour blood sugar of his atropinized subjects be compared with the average half-hour value of the non-atropinized "fatigue" patients a rise of some twenty-two mgs.% is demonstrated in less than ten percent of the patients whereas the levels of the control group exceed the patients' levels by some fifty mgs.%. Although it is said that two fasting blood sugar specimens were taken (one before and one after atropine injection) it is not made clear which was recorded. Finally, Portis' patients manifested no evidence of fasting hypoglycaemia or persistent hypoglycaemia at any time during the tolerance tests, further evidence against the doctrine of hyperinsulinism.

6. Conclusions

The results of this study indicate that the majority of patients afflicted with a chronic anxiety state metabolize glucose in a manner which differs significantly from that of normal people. There is evidence that the subjects of this study have increased tolerance for glucose administered either orally or parenterally and that although these anxious subjects are normally sensitive to the hypoglycaemic action of insulin they fail to respond with the expected degree of hyperglycaemia after administration of glucose during the period of

insulin-induced hypoglycaemia. The results of this study agree fairly well with the results of other similar studies performed in the past, largely on neurasthenic patients, but the mechanisms invoked in explanation of the demonstrated abnormalities are different.

In keeping with recent advances in the knowledge of the mechanisms regulating blood sugar, an explanation of the observed abnormalities has been sought in the concepts of increased tissue utilization of glucose and compensatory acceleration of hepatic glycogenolysis. While therapeusis has played no partt in this investigation, it must be clear that although the demonstrated increased tolerance is considered to be an effect of the primarily psychological disturbance and not an etiological factor, rational therapy demands dietetic as well as psychotherapeutic considerations.

SECTION VII

SUMMARY

1. A study of tolerance to orally and intravenously administered glucose was performed on a group of patients who were suffering from a psychoneurosis, the principal feature of which was anxiety. The same patients were tested with the intent of discovering their glycaemic reaction to intravenously administered insulin and their response to insulin-induced hypoglycaemia, if such occurred. All studies were controlled.
2. A simple but practical method for performing the intravenous glucose tolerance test has been described and the results of the test in a group of normal people outlined.
3. The patients dealt with in this study manifested an increased tolerance to glucose administered either per os or parenterally.
4. The patients further manifested a normal response to the hypoglycaemic action of insulin but did not respond with a satisfactory elevation of blood sugar when glucose was administered orally during the period of hypoglycaemia.
5. A theory has been expounded to explain the observed abnormalities. The basic tenets of this theory are that there is increased utilization of glucose by certain tissues and compensatory increased hepatic glycogenolysis.
6. The recent work of Portis and associates which dealt with carbohydrate metabolism in emotional states characterized principally by fatigue (neurasthenia) has been extensively reviewed in view of the very important implications of this work and Portis' interpretations and conclusions have been criticized.

It has been shown that patients with anxiety as the main feature of their psychoneurosis manifest increased tolerance to glucose as do patients who suffer in the main from fatigue. It is suggested that the mechanisms in the two groups and indeed possibly in all neurotic reaction types are similar.

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