A CLINICAL STUDY OF THE USE OF HYPERTONIC SOLUTIONS AND NEW METHODS FOR THE ANALYSIS OF SUGARS AND SUGAR ALCOHOLS IN BIOLOGICAL MATERIALS

by

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INTRODUCTION

It is the purpose of this thesis to outline the available information regarding the clinical application of hypertonic solutions. An important observation concerning the clinical action of hypertonic solutions is brought out. This is the chief contribution to the future research worker in this field.

A review of the use of hypertonic solutions is presented under two divisions, the first concerning the dog and the second concerning the human. This review presents a basis upon which to evaluate the clinical data obtained.

The bibliography is of such a diffuse nature that a future investigator will be able to obtain information on the subject of diuresis in almost every field. This thesis has presented a foundation from which future research may begin in the field of diuresis. Studies were carried out concerning the action of the human kidney, the differential anatomy, the renal function, the normal and pathological diuresis and factors which control diuresis. The author has questioned and criticized many investigators and wishes to leave himself open for criticism. Therefore this thesis is left as a stimulus for continued investigations concerning the use of hypertonic solutions.

Part II, which is less important from the clinical viewpoint, concerns the potentiometric applications for the determination of sugars and sugar alcohols. It presents a preliminary study from which renewed research can be carried out on ceric sulphate and ferricyanide reagents. The use of ceric sulphate as an oxidising agent may present an entirely new physico-chemical field to the biochemist in the study of sugars and their polymers.

PART I

A REVIEW OF THE KIDNEY

The kidney is an organ in man, composed of approximately 1.5 million functioning units called nephrons. Each nephron is composed of a plasma filterer called a glomerulus, and numerous reabsorptive surface areas called tubules. Wearn and Richards, (1), in 1924, followed by the works of Walker, Hudson, Findley and Richards, (2), Montgomery and Pierce, (3), and White and Schmitt, (4), studied the differential activity of the nephron. It was found that plasma completely passes through the glomerulus into the proximal convoluted tubule. Here water and glucose are reabsorbed. The tubule is then converted into the descending and the ascending loop of Henle which is characteristic for mammals. Some water is thought to be absorbed in this part but experimental evidence is lacking. The tubule then enters its last phase of activity in the distal convoluted portion. Here water, urea, creatine, sodium, potassium, chloride, phosphates, calcium and carbonates are reabsorbed to a threshold level. Here too, in the epithelium, or in the peritubular venous spaces, ammonia, creatinine and hippuric acid are formed and excreted. The complex renal tubular function regulates the urinary pH, the body buffer systems, the ionic balances, all the threshold substances, and the water balance.

Since the time of the work of Ludwig, (5), in 1844, and Cushney, (6), in 1917, the investigation of the kidney was based upon that of function and factors which regulate nephritic activity. Smith, (7), in 1937,

reviewed and correlated most of the investigations to this date. It is of great interest to note that the physiology and renal function of the cat, rabbit, dog, and man are entirely different. Bloom, (8), has pointed out that a transposition of even minute pathological conditions is seldom possible. As a result of this free transposition from animal and human experimental work there has resulted a profound clinical confusion. These statements will be confirmed and some of these differences will be pointed out later in this paper.

At the present time it is known in the case of the human that the kidney does not show pathological renal tests until less than 700,000 nephrons are functioning. The normal blood supply to the kidneys is about 1,000 cc per minute. The blood passes directly from the renal artery to a short afferent arteriole. This breaks up in the glomerulus into a network of approximately twenty non-anastomotic capillaries. Here sixty percent of the blood volume, composed of plasma, is passed into the renal tubule. The efferent glomerular arteriole is then much smaller, but other characteristics are present which are of great anatomical significance. The walls of this vessel are composed of numerous smooth muscle bundles, well supplied with sympathetic and parasympathetic nerve fibers. The neurological or hormonal regulation of the efferent arteriole may be a direct factor of glomerular intermittency of function. Spanner,(9), in a serial section study of kidney tissue states there is a possibility of a normal arterio-venous anastomosis. This would shunt blood

directly from the afferent arteriole to the peritubular plexuses. The functioning aglomerular kidney of hypertension may be of this type. The peritubular venous plexus which completely surrounds all of the tubular epithelium receives its blood from the efferent glomerular arteriole.

Renal Functional Tests

Present renal function tests are based upon plasma clearances. These are dependent upon the accuracy of analysis of the substance used in the blood and the urine. Recent work shows that it is no longer necessary to have a maximum or a minimum clearance test as advanced by the historical work of Möller, McIntosh, and VanSlyke. (10). These authors advanced the following formula:

$$\text{Clearance} \qquad \frac{U(\text{conc. gms in urine}) \times \text{Vol.}(\text{vol. of urine/ min})}{P\ (\text{conc. gms in blood plasma})}$$

Clearances are now considered to be the minimal volume of blood required to furnish the quantity of substance excreted into the urine per minute. This formula may be used to determine the clearance tests for urea, inulin, phenol red, iodine complexes, and creatinine.

Present glomerular renal tests all center about the use of compounds easily analysed and not reabsorbed by the tubules. Theoretically glucose may be used if the patient has been phlorizinized, but this is impractical. Inulin, a polysaccharide of 32 fructose molecules, with a molecular weight of 5,000 has been found to be of great value. Inulin is unchanged

in the body and has been shown by Shannon and Smith, (11), Miller, Alving, and Rubin, (12), to be completely filtered by the glomerulus. Pfanstiehl Chemical Company has purified inulin so that it is now available for clinical administration with no toxicity. This substance has a complete glomerular clearance, independent of high or low plasma concentrations in both normal and pathological conditions. The tubular excretion or reabsorption is negligible. A review of recent literature shows that a ratio of inulin to the urea clearance test will be of great value since Alving, Rubin and Miller, (13) have developed a rapid analytical procedure for inulin analysis.

Tubular Renal Tests

Among the foreign substances excreted by the tubules are exogenous creatinine, phenol red, and numerous organic iodine preparations. Many of these iodine drug preparations have been shown by Shannon, (14), Chasis, Ranges, Goldring and Smith, (15), Smith and Ranges, (16), and Cheeley and Cheeley, (17), to be almost entirely excreted as a result of tubular function. Clinical tubular renal function tests are based upon the studies by Smith, Goldring, and Chasis, (18), Cheeley, Connell, Cheeley, Kats and Glisson, (19), using phenolsulphonpthalein, diodrast, hippuran, and creatinine. The best substance found was diodrast which did not combine with the blood protein and was non-toxic. Theoretically diodrast reached a complete tubular clearance with no storage in the renal tubules.

Interpretation of Renal Function

Clinically most renal function tests are run using either urea, or phenol red. The fallacy of these tests has been pointed out by a preliminary report by Ranges, Chasis, Goldring and Smith, (20). They hope to show a true kidney test by a combination of the glucose threshold test and the diodrast tubular clearance. This will then take into consideration the normal physiological intermittency of glomerular activity which was first noted by Richards and Schmidt, (21). The use of glucose at its tubular threshold level will show the number of glomeruli that are functioning at any one time. While the use of a diodrast clearance may show the possibility of about one-half of the nephrons being aglomerular but having a normal blood supply by the arterio-venous shunt of Spanner, (9).

The interpretations of the present routine kidney function tests are inadequate. This is due to the failure of the clinician to realize the physiological and the pathological conditions present at the time the test is being run. The patient must not be in acidosis or alkalosis and must have essentially a normal blood chemistry in respect to ions and serum proteins. The urea clearance test, which is one of the most commonly used tests, is no longer considered of real value when used alone for specific glomerular function. Urea is easily reabsorbed by the tubular cells and rapidly diffuses throughout all bodily tissues. Barker, (12), Smith, (7), Dem, (23), Wohl, Brust and Freed, (24),(25), have shown that wide variations in urea excretion may occur in hyper-

tension, congestive heart failure, hyperthyroidism, following intestinal hemorrhage, in the syndrome of non-renal azotemia, in shock, and following drug therapy of adrenalin, caffeine, and digitalis. It is due to these wide discrepancies in the results using urea alone that the use of inulin plus urea and the determination of the ratios of these substances excreted is rapidly replacing the urea test.

THE EXCRETION OF WATER

In order fully to understand the excretion of water and the response of the kidney to hypertonic solutions, a review of factors which control diuresis and water balance must be considered. This is divided into the pathological syndromes of Diabetes Insipidus and Addison's Disease. These are followed by a generalized discussion of normal processes and the clinical uses of substances which apply to diuresis.

Diuresis and Diabetes Insipidus

Diabetes Insipidus is a clinical syndrome characterized by a tremendous thirst (polydipsia), and a urinary output of 10 to 30 liters per day (polyuria). This has been produced in animals by lesions of the floor of the third ventricle, and the infundibular stalk of the pituitary gland. Dandy, (26), has recently reported a case in a human produced by a division of the hypophyseal stalk without an injury to the portal circulation to the hypothalmus.

In a review of the experimental work on the diuresis due to the pituitary gland, Smith again points out the wide diversity of results. Most of the observations on renal function were made with animals in the state of anesthesia. In no other physiological field has the use of morphine, atropine, phenobarbitals and ether caused a greater confusion. Many investigators have concluded that anesthetised animals are so highly abnormal that results obtained on them have little bearing upon the normal. All diuretic observations must be made upon unanesthetised animals without pain, psychic or physiological disturbances. With this point of view as a criterion of analysis, only clinical and carefully controlled animal work is reviewed.

Veil, (27), (28), (29), first tried to classify diabetes insipidus according to the type of the blood chloride curve and water elimination. Later Ambard, (30), studied the chloride excretion in diabetes insipidus. This was followed by the extensive work of Findley and White, (31), (32), (33), who studied the action of pitressin on the normal and the diabetic cases following water and salt ingestion. They concluded that diabetes insipidus is due to a deficiency of the anti-diuretic principle excreted by the pars nervosa of the hypophysis. The principal hormone acts on the renal tubules enabling the reabsorption of water from hypertonic solutions in the lumen. If a normal person is given sodium chloride intravenously the plasma protein is slightly diluted, diuresis is transient, and only 20 percent of the sodium chloride is eliminated in the first 24 hours

and another 40 percent in the next 24 hours. The serum chloride curve rapidly falls to normal in three hours, showing the diffusibility of the ions into the tissues. In a case of diabetes insipidus exactly the same results occur as in the normal despite the tremendous urinary output. Pitressin therapy has had no effect upon the serum chloride curves or the urinary chloride output in the normal or the pathological condition. But pitressin is a definite anti-diuretic factor in both for a period of four to six hours. Pitressin has no effect upon a normal person if the urine is concentrated, or if the individual is under salt or mercurial diuresis. Hirsch and Kaats, (34), have administered salyrgan to a diabetes insipidus case with a resultant decrease in the urinary output. After pitressin was given to the patient salyrgan produced no diuresis nor did it decrease the urinary output.

What effect this hormone has upon the normal is still in an experimental state. It may alter plasma concentration which is believed by many to be the stimulus to a sudden diuresis. Or the true stimulus to diuresis may be that of a reflex neurological activity. Fischer, Ingram, Hare, and Hanson, (35), have studied the fiber tracts of the hypothalamic nuclei communicating by the tractus supra-optico-hypophyseus by way of the infundibular stalk to the pituitary pars intermedia and pars posterior. With these numerous hypothalamic interconnections diuresis may be associated with the vasomotor centers which pass down the cord by way of the reticulospinal formation according to the work of Allen, (36). Recent observations of Materius, (37), and Salk and Weinstein, (38), help to advance

this hypothesis by their demonstration of a vaso-constrictal renal nerve pathway. Further evidence at present is lacking for a diuretic hormone from the pituitary gland.

We may then state that the syndrome of Diabetes Insipidus is due to a hormone which regulates the reabsorption of water in the tubules. The pituitary may not contain a diuretic hormone. Diuresis may be due to a reflex neurological action. The vasomotor reticulospinal system may be the chief factor in the control of sudden constriction of the efferent arterioles causing an intermittent glomerular activity and a greater blood flow to the tubules.

Diuresis -- and Addison's Disease

The interrelationship of the adrenal cortex to the pituitary gland is probably one of great significance. Diuresis and anti-diuresis are closely related. Seldom does one think of the adrenal gland as a potent regulator of kidney function for our lack of knowledge of this function has recently been studied.

The syndrome of Addison's was first described by Thomas Addison in his classical paper on the constitutional and the local effects of diseases of the suprarenal capsule in 1855. Since this time the clinical studies and the hormonal interrelationships of the cortex and the medulla have been studied with great strides. Addison's Crisis was controlled by administering an extract of the adrenal cortex by Rowntree and Green, (39), Swingle and Pfiffner, (40), and later Kendall, (41).

The direct relationship of the adrenal gland to the kidney is still not understood. The work of Loeb, (42), Harrop et al, (43), (44), (45), and Allers et al, (46), (47), showed in the dog that primarily the chemical abnormalities are in the balance of the sodium and the chloride ions. In the dog the sodium and the chloride excretion are increased in the urine, more sodium is lost than chloride, and the potassium ion takes the place of the sodium ion in the blood plasma. Other constituents of the blood indicate the effect of hemoconcentration with an increase in the urea content. Recent clinical investigations by Thorn, Howard and Emerson, (48), show that in the human there is seldom a change in the blood non protein nitrogen unless there is an extreme dehydration and crisis. Careful studies of the urinary sodium and chloride outputs have shown that the ratio of the sodium to the chloride is 1 to 1. This would indicate a species difference between the human and the dog.

Swingle and Pfiffner, (49), and Harrop, Pfiffner, Weinstein and Swingle, (50), first indicated that the hormone of the adrenal cortex has some function concerning urinary excretion. The only respect in which the behavior of adrenalectomized cats receiving the hormone differs from that of the normal cats seems to be in the frequency of urination. Diminutions of the urinary excretions occure progressively if the hormone be withdrawn. Urea, chloride, and inorganic phosphate excretion is suppressed, and finally when the animal is in a critical state, creatin, creatinine, and injected phenolsulphonthalein are retained. Injection of the cortical hormone is followed by diuresis and excretion of the retained substances.

Recent experimental studies with the use of desoxy-corticosterone acetate by Reichstein and Buw, (51), Thorn and Eisenberg, (52), and Thorn, Howard and Emerson, (48), tend to prove the relationship of a hormone of the adrenal gland to the tubular mechanism of the reabsorption of potassium and the failure to readsorb sodium and chloride ions. The important clinical symptom in Addison's disease is a dehydration of the individual, due primarily to an increased loss of fluids and the imbalance of electrolytes. When treatment is first instigated there is a weight gain. During the first two days of treatment however there may be a diuresis despite the retention of sodium chloride. Diuresis is thought to be due to the increased potassium output. If in the course of treatment the administration of the drug is stopped, diuresis will again recur with an increased sodium chloride excretion.

It can then be stated that the adrenal cortex may produce a hormone which acts upon the renal tubules, regulating the balance of sodium, potassium and chloride ions. Diuresis has been noted prior to the use of desoxy-corticosterone acetate, but this drug has produced clinical proof. Diuresis is associated with an excessive loss of either sodium or potassium and chlorides.

Diuresis and Blood Pressure

It was previously held that another factor in the control of water excretion is the regulation of glomerular filtration. According to this

theory an increase or a decrease of filtration rate is dependent upon the number of functioning glomeruli and the pressure exerted by the blood stream. This misconception was due to the work of Richards and Plant, (53), on the perfused kidney. An increase of renal arterial pressure increased the urinary output, but it is to be remembered that again we are using a kidney which is not in a normal state of metabolic processes. Any tissue deprived of oxygen, glucose and serum proteins has such an altered permeability that fluids and electrolytes may be freely diffusible. Chasis, (15), and Smith, (54), have studied this problem in clinical cases which were given spinal anesthesia. The effects of the fall of blood pressure in such studies did not affect the clearance tests in any way. Increased blood pressure did not show an increase in the urine flow. From the data based on the inulin and the diodrast clearance tests it was shown that anesthesia had no effect upon the renal blood flow. These observations substantiate the view that urinary output is entirely controlled by tubular activity.

Diuresis and Drugs

The field of diuretics and the application of drug therapy is one that has been studied by the clinical man. In a review of this subject there is a confusion of terminology between the physiologist and the clinician. By definition diuresis is an increased excretion of urine. Diuretics are those substances which produce diuresis. As stated in the

papers dedicated to Christian, (55), the ideal drug and diuretic substance is one which will produce an increased urinary output over a 24 hour period. The physiologist in his reports holds to the true definition of the term, but the clinician does not. The physiologist generally studies a diuretic over a period of hours and usually on partially or wholly anesthetized animals, while the clinician carries out his studies on unanesthetized patients over a period of days. It is obvious that great precautions should be used in the interpretation of animal work.

The massive amount of clinical literature has been reviewed by Kennedy, (56). The primary modes of action are a change in the plasma composition, an increased rate of glomerular filtration, and a decreased rate of water reabsorption in the renal tubules.

The changes in the plasma concentration and composition during diuresis and body dehydration still are in the research phase. Harris and Gibson, (57), have applied recent studies on blood volume during diuresis. Their results show the possibility of a sudden shift of the water binding powers of the proteins. Reed, (58), has shown that this may be due to an electrolytic disbalance between the plasma and the tissues. Such electrolytic disbalance may be caused by salts such as ammonium and potassium chloride according to Keith and Bingen, (59).

Ammonium salts may act as diuretics due to their acidifying action and slightly as the result of the production of urea. Potassium is easily eliminated from the normal body and may act as a diuretic. The chief

action of potassium chloride, sodium sulphate, urea and many other compounds is due to the fact that all act to increase the osmotic pressure in the tubules. The rate of the water excretion is dependent upon the concentration of the salt in the tubule. This osmotic effect and diuresis due to osmosis has a maximum height of water retention above which no more water can be retained. Thus the peritubular plexuses will reabsorb water no matter what the tubular osmotic pressure may be if the body is in a state of dehydration.

Xanthine Diuretics

The general group of drugs which are thought to cause an increased rate of glomerular filtration are the xanthine derivatives. Smith points out the confusion in the literature associated with this group of drugs. Caffeine may cause a marked diuresis in the rabbit, no action in the cat, a very slight action in the dog, and a moderate action in man. According to this author poorly controlled experiments have not proven that an increased blood flow was essential for diuresis. Recent work on the determination of the blood flow by the filtration rates does not support the theory that these drugs cause an increase of the glomerular filtration rate. On the other hand, the chief action of such drugs may be due to a stimulation of the central nervous system, and a hindrance of the anti-diuretic hormone.

Mercurial Diuresis

A decrease in the rate of water reabsorption in the tubules may be brought about by the use of mercurial drugs. The best mercurial drug now available according to Marvin, (60), is salyrgan. The work of Bartram, (61), and Blumgart, Gilligan, Levy, Brown and Volk, (62), shows that salyrgan has an almost specific action in the distal convoluted tubules. It is known that its action can be limited to one kidney. It causes no increase in the renal blood flow, and no change in the urea clearance test. Diuresis with salyrgan is usually accompanied by a tremendous urinary chloride output, so that ammonium chloride is an advantageous drug to administer simultaneously. A clinical review of the use of salyrgan by Tarr and Jacobson, (63), has shown that only one case in 8,000 may have an idiosyncrasy or produce renal damage. In the administration of this drug at this institution usually one or two cc of a 10 percent solution are given intravenously. A mixture of blood with salyrgan at the time of the injection prevents sclerosis or pain in the vein injected. Mixtures of theophylline and salyrgan have been advocated by DeGraff and Batterman, (64), but clinically Uhlaman, (65), has not shown any diuretic advantage. Ethridge, Myers and Fulton, (66), have shown that acid producing salts must be administered before salyrgan is an effective diuretic.

A REVIEW OF HYPERTONIC SOLUTIONS

With this general review of some of the factors which may go to govern diuresis in mind, the subject of hypertonic solutions and their physiological and clinical applications may be taken up. The following clinical uses of hypertonic solutions based upon physiological observations are: as diuretics, for dehydration, to decrease intracranial pressure, for asthma, for alcoholism, in barbital poisoning, in confusional states, in delirium tremens, in pulmonary edema, in shock, in Stokes Adams Syndrome, and to almost every patient in coma when other treatment has failed. The hypertonic solutions to be considered are sodium chloride, glucose, sucrose and sorbitol.

A Review of Sodium Chloride and Glucose

Weed and McKibben in 1919, (67), (68), first studied in normal cats the results of the intravenous injection of hypertonic saline solutions. This was followed by the confirmations of Cushing and Foley,(69), Foley and Putnam,(70), who indicated that in the normal animal hypertonic solutions of saline caused a shrinkage of the brain and the parenchymatous tissues. This observation was quickly applied to numerous intracranial pathological conditions by Dowman, (71), Keegan, (72), Bedell, (73), Haden, (74), Bennett, (75), and other investigators. The clinical results did not confirm the animal investigations. Finally Hoff, (76), Fay, (77), (78), Milles and Hurwitz, (79), and Browder, (80), showed

that hypertonic sodium chloride in the normal and the pathological condition is only transitory in activity. The increased blood osmotic pressure causes a release of fluid from the cell only over a short period of time. Following this a diffusion takes place into the tissues and cells, this in turn causes an increase in intercellular fluid and an edema greater than before. Since these first studies it has been definitely shown that the administration of sodium chloride intravenously may cause edema.

Dextrose was first studied because a 50 percent solution could be administered intravenously with little or no toxicity. Due to the transitory diuresis produced, dextrose was thought to cause a tissue anhydremia, and therefore possibly to be of value in decreasing intracranial pressure. Hoff, (76), first disputed the use of hypertonic dextrose in 1930. He stated that the central nervous system when injured did not react the same physiologically as the normal. The choroidal plexus became more permeable to glucose which was the cause of the secondary spinal fluid pressure increase when glucose was used. This was followed by the work of Jackson, Kutsumai, Leader and Joseph, (81), who pointed out some of the failures of clinical investigations. Dextrose had been injected at random according to these workers without any knowledge of the blood sugar curves or diffusion into the cerebrospinal fluid. Studies were then carried out on 20 pathological cases giving 100 cc or 200 cc of 50 percent or 25 percent glucose intravenously. The blood pressure, temperature, pulse, respiration and the cerebrospinal fluid pressure were carefully followed.

It was found that hypertonic solutions caused an immediate rise in the cerebrospinal fluid pressure in about one-half of the cases, while it was noted a slight reduction occurred in the other half. A secondary rise in all of the cases occurred at the end of 15 to 30 minutes following the injection. The secondary spinal fluid rise was due to a diffusion of dextrose into the brain cells and the tissues with a resulting edema. This same observation was again repeated by Wassermann, (82), (83), in 1934. He showed a primary spinal fluid pressure rise at the time of the injection, a secondary fall, and then the secondary rise. This work was the prime factor for the abandonment of hypertonic glucose and saline in intracranial injuries.

Sucrose, Actions in the Dog

Sucrose, a disaccharide which is composed of glucose and fructose, was first applied clinically as a diuretic in 1928. At this time it was found by Keith and Wholen, (84), that if large amounts of sucrose were administered intravenously to an anesthetized dog there was a marked loss of water, urea, chlorides and sodium. With a dosage five times that clinically applicable a hyperpyrexia resulted. Walker and Keith, (85), noticed following intravenous injections a marked diuresis, dehydration and a tendency for bowel movements. If following sucrose injections, acacia was given to an anesthetized animal, a marked diuresis occurred even though the animal was dehydrated. Such an observation is of importance in that diuresis may be produced by a substance which is not found in the glomerular filtrate.

Bullock, Gregersen, and Kinney, (36), using anesthetized dogs set out to prove that hypertonic solutions of sucrose could be used to reduce the cerebrospinal fluid pressure without a secondary rise. The average dosage was 6 gms per kilogram, which is twice the average clinical dose. These observations showed that following a sucrose injection in anesthetized dogs there was a fall of the spinal fluid pressure for a period of five to eight hours. The lowering of the pressure was dependent upon the amount of the sucrose given, yet no secondary rise was observed even after a 12 hour period. Injections of sucrose produced an active diuresis equal to four times the intake of fluids. Diuresis was active over a three hour period. Hypertonic solutions were then considered to be effective in a prolonged reduction of cerebrospinal fluid pressure in the supposedly normal anesthetized dog. Gregersen and Wright, (37), showed that this action may be due to the fact that sucrose does not diffuse into the cerebrospinal fluid while glucose does.

The work of Bullock et al is not confirmed by the work of Schwartz and Elman, (38), who compared sorbitol and sucrose in the anesthetized dog. Sucrose injections produced a fall in the cerebrospinal fluid pressure which rapidly returned above the basal in 1½ hours. A secondary rise was noted with sucrose and does not confirm Bullock. Diuresis was dependent in these observations upon the sucrose concentration in the blood as was the urinary output.

In the normal individual he made a comparison of the diuretic action of ammonium chloride, urea, sucrose, organic mercury, and theophylline. The most marked diuretic results were obtained with organic mercury, urea, and the ammonium salts. Minimal diuresis was observed for digitalis and theophylline, but no definite data was given concerning sucrose.

Keith, Wakefield, and Power, (92), next studied the excretion and the utilization of sucrose when injected intravenously in man. In this study three normal patients were used and three patients with impaired kidney function. Sucrose caused some subjective symptoms in the patients during the injection, while during the excretion there was a loss of small quantities of protein in the normal urines. The clinical dosage of sucrose used was the same as the present standard, 0.6 to 1.6 Gms. per kilogram. A mild diuresis was reported, but the actual data is not given and it is not known whether this is a transient diuresis or a clinical diuresis of 24 hours duration. In the normals 97 to 98 percent of the sucrose was recovered in the urine during the first 24 hour period. The blood plasma curves showed that the maximum excretion was within the first 12 hours. Among the cases with renal insufficiency there was found a delay of excretion in the urine and a delay in the blood sucrose levels. In these cases it required 72 to 96 hours to excrete 88 to 98 percent of the sucrose.

Keith, Power, and Peterson, (93), (94), observed that sucrose given to dogs diffuses into the tissue spaces and that only 70 to 80 percent is ever recoverable. The repetition of the recoverability of sucrose at a

constant level in man leads to the possibility of a simple renal function test. The renal clearance tests for sucrose, xylose, urea, and inorganic sulphates were compared. Sucrose has a plasma renal clearance test of 100 cc., xylose 89.6 cc, urea 72.2 cc, and sulphates 85.5 cc per minute. With this data we may compare the renal functional tests. Inulin is completely filterable through the glomerulus at a rate of 120 cc per minute, compared to sucrose at a rate of 100 cc per minute. This is based upon the formula of Möller, McIntosh, and Van Slyke,(10). With this in mind it can be said that sucrose is not completely cleared from the blood plasma, and that 10 to 20 percent is readsorbed by the renal tubules.

Massermann, (95) considered sucrose might be used to advantage as a hypertonic solution and undertook a clinical study of its action on 35 normal subjects. He recorded the cerebrospinal fluid pressure, the urinary excretion, the blood chemistry, the bleeding and clotting time, the blood cytology, blood pressure, the pulse rate, and the dextrose and the sucrose content of the spinal fluid. The only changes of great significance were in the spinal fluid and the urine. It was first found that massive doses of sucrose, 300 to 500 cc of 50 percent, had to be given before any effects were noticeable. Thus when 100, 200, or 300 cc of sucrose were administered the diuretic effects were slight. If 500 cc of 50 percent sucrose were given, in the first four hours there was a urinary output of 2,300 cc. Microscopic examination did not reveal blood or protein. The elimination of sucrose during the first four hours ran as high as 80 percent. A markedly decreased spinal fluid pressure was ob-

served when 500 cc were given, but this was only of transitory nature lasting only three and one-half hours. Fluid pressures were not recorded in Wassermann's experiments after the first four hours.

Davis, (96), Glass, (97), Murphy, Hershberg, and Kats, (98), immediately suggested the application of sucrose in pathological cases. Hahn, Ramsey, and Kohlstaedt, (99), reported a series of three cases. None of the criteria of clinical study as outlined by Jackson et al and Wassermann were followed, nor were routine spinal puncture pressures, or urinary sucrose determinations made. The clinical symptoms may have been masked due to the administration of other drugs and lumbar taps withdrawing spinal fluid. It is to be noted in case (1), that even though sucrose was given every three hours a daily urinary output of only 600 cc was recorded despite fluid hyperdermoclysis. The author states that immediate diuresis took place in every case following sucrose administration.

Recent investigations of Keith and Power, (100), show that 60 per cent of all sucrose is excreted in the first two hour period. It is of interest to note in a review of their article that the diuretic effect of sucrose over a 24 hour period is slight. No mention of this is made nor has any significance been made in the literature of this observation.

Personal communications with the Cook County Hospital services confirm the inapplicability for the useof sucrose in intracranial injuries. Fantus, (101), has recently published the mortality rates of the neurological and emergency services. All previous methods including the use of hypertonic solutions showed a mortality rate of 38 percent. With the

present routine method of treatment for shock with glucose and saline, later followed by direct lumbar tapping and decompression, the mortality rate has been decreased to 15 percent.

Renal Pathology following Sucrose, Glucose or Sorbitol

Helmholz in 1935, (102), reported a clinical case which had received sucrose intravenously. At autopsy there was found a hydropic degeneration of the renal tubules. He then confirmed this effect of sucrose by a poorly controlled experiment on rabbits. Such a valuable observation was quickly lost in the literature. Cutler, (103), revived this work by doing routine renal sections on all cases at the Mayo Clinic in which sucrose had been given. Of all the cases investigated 97 percent exhibited hydropic degeneration of the renal tubules, and 70 percent of all the cases which had received sucrose had tubular degeneration. This was followed by observations on four patients to whom 200 cc of 50 percent sucrose was administered. At death all four showed degeneration. Other cases failed to show any tubular damage when 100 cc of 50 percent sucrose was administered.

Helmholz and Bollman, (104), continued to study this pathological renal damage in a group of rabbit experiments. Lindberg, Wald, and Barker, (105), compared sucrose, glucose and sorbitol. Dogs were used because the rabbit's kidney is so easily susceptible to renal damage. Renal biopsies were taken before and after the administration of 50 percent sucrose, glucose and sorbitol. Sucrose administered over a short period showed a

transient morphological hydropic tubular degeneration. Over a prolonged period there was a permanent evidence of glomerular change and a failure of tubular restitution. The animal experiments using 50 percent glucose and sorbitol failed to show any kidney damage. The clinical research carried out by this author is not conclusive, for the phenolsulphonthalein test will not depict slight tubular functional changes.

Sorbitol, Its Action in the Dog

Sorbitol, a hexahydric alcohol, was first produced in Germany as a waste product of wood pulp. Clinically it was first applied by Heidpreim, (106), and Thannhauser and Meyer, (107), as a substitute for glucose in the treatment of diabetes mellitus. Sorbitol and its application to diabetes has been reviewed and studied by Manville, (108).

Sorbitol was first introduced as a clinical diuretic by West and Burget, (109), in 1936. Sorbitol was found to be non toxic and rapidly excreted. It possesses 1.88 times the osmotic pressure of an equal percentage of sucrose. The first experimental work was carried out on anesthetised dogs, from which the urine volumes could be measured over a 15 minute period following the intravenous administration of 50 percent sorbitol. Comparisons were made of sorbitol and sucrose; sorbitol produced greater diuresis than sucrose when used in equal concentrations.

Schwartz and Elman, (88), compared the effects of sorbitol and sucrose on the cerebrospinal fluid pressure and the urinary output. Dogs were used which had been placed under the influence of barbital anesthesia. Clinical

equivalents of 50 percent sorbitol and sucrose were administered simultaneously to nine pairs of dogs. Following the intravenous injection there was a fall of the cerebrospinal fluid pressure with both substances. Sorbitol caused a greater depression and reached the normal in 2.75 hours as compared to 1.25 hours for sucrose. Sorbitol also caused less secondary rise of cerebrospinal fluid pressure. The urinary peak was greater with sorbitol than with sucrose, but both returned within two hours to the basal level.

Todd, Myers and West, (110), studied the metabolism following the intravenous administration of mannitol and sorbitol in dogs. In the dog 40 to 50 percent of the injected sorbitol could be recovered in the urine within the first 24 hours. The other 50 to 60 percent apparently was metabolized. Such a view was substantiated by blood glucose curves. The blood sorbitol clearance showed that the basal level was reached within the first two hours. The urinary collections were done on normal female dogs by the use of urethral catheters. This is the only article found in which the authors have observed the anesthetic factor. A review of the experimental data on these dogs showed that a diuresis was produced over a 24 hour period.

Rosmer and Bellows, (111), have reported sorbitol studies in the aqueous humor and the cerebrospinal fluid of dogs. Dogs under anesthesia showed that sorbitol diffuses into the aqueous humor and the cerebrospinal fluid. Sorbitol is rapidly eliminated within a three hour period, and there is a temporary rise in the blood glucose level. A critical analysis of this article tends to show an extreme degree of discrepancy of tabulated

data. These may be attributed to the inherent errors of the periodate methods for sorbitol, a procedure not as accurate as the method of Todd, Vreeland, Myers and West, (112).

Sorbitol, Its Clinical Application

Sorbitol was applied to one clinical case of anuria, and was reported in the literature by Strohm, (113), as an effective diuretic. The only other application has been reported by Bellows, Puntenney, and Cowen, (114). Sorbitol was administered intravenously over a period of 48 hours to decrease intraocular pressure. Cases of glaucoma were completely relieved from pain by repeated dosages of 100 cc of 50 percent solution.

Summary - Sucrose in Dog

All of the animals used have been under anesthesia of some type. Massive dosages of sucrose have produced a dehydration and a greater 24 hour output than normal. 70 to 80 percent of sucrose is the maximum amount recoverable in the urine of the experimental animal. There is no confirmatory proof in the literature regarding the prolonged action of sucrose on the cerebrospinal fluid pressure. Bullock et al claimed that sucrose decreased the cerebrospinal fluid pressure for a 12 hour period. Schwartz et al showed a secondary rise in 1.25 hours. Increased intracranial pressure may decrease the urinary output. Pathologically sucrose has been shown to cause a tubular hydropic degeneration.

Summary - Sucrose in Man

There is no proof in the literature that sucrose is a clinical

diuretic. When massive dosages are given sucrose may produce dehydration. An intermittent sucrose diuretic has been observed dependent upon the blood concentration. There is no clinical evidence and proof that sucrose is of definite benefit in pathological cases of increased intracranial pressure. Massive dosages of sucrose decreased the intracranial pressure for only 3.5 hours. 88 to 99 percent of sucrose is recovered in the urine of a normal individual during the first 24 hours. In cases of renal damage 88 to 99 percent is recoverable in 72 to 96 hours. Sucrose has been shown by Cutler in his series to produce tubular degeneration in 97 percent of the cases when the administered dose is over 1.0 gm per kilogram.

Summary - Sorbitol in Dog

Sorbitol has been shown to produce greater diuresis, and to produce a greater 24 hour output than normal. Sorbitol decreases intracranial pressure but there is a secondary rise and a diffusibility into the aqueous humor and the cerebrospinal fluid. 40 to 50 percent of sorbitol is recovered from the urine in the first 24 hours and about 50 percent metabolized.

Summary- Sorbitol in Man

Sorbitol was reported to have relieved a case of surgical anuria. Sorbitol definitely decreased intraocular pressure over a period of 48 hours in cases of glaucoma.

A CLINICAL EXPERIMENTAL STUDY

It has been observed that no reports are available concerning the diuretic action of sorbitol, glucose and sucrose over a 24 hour period. Because of the sparsity of adequate data on the diuretic value of these materials, an extended program of research was begun on hospitalized patients at Multnomah County Hospital in cooperation with Dr. Charles Kennedy.

Patients exhibiting a variety of conditions were used. Patients were studied having edema from the following causes: chronic passive congestion of heart failure, reversal of the albumin-globulin ratios, with nephrotic and nephritic syndromes, with liver damage and abdominal ascites, and others due to mediastinal obstruction. A large series of patients were used who had an essentially normal blood chemistry picture. Several cases were studied in coma and in the terminal stages of eclampsia. A case of undetermined edema was thought to be due to Mediastinal Hodgkin's Disease. This patient had an entirely normal blood chemistry picture and offered an excellent subject for a long continued study of diuresis. Phenolsulphonphthalein and urea clearance tests were run on many of these patients. A normal test was considered as indicative of a normal kidney; however, in some cases the necropsy did not prove this to be true.

During the experimental periods on each patient the fluid intake and output were recorded prior to the injections. During the injection period hypertonic solutions were allowed to run in by the use of an intravenous setup at the rate of 8 to 10 cc per minute. Each patient was care-

fully observed during the course of the injections for unwarranted symptoms of headache, nausea, paresthesias, pain, increase in pulse rate, flushing of the face, and any anxiety or other discomfort that could be elicited.

Blood Sorbitol Studies

The potentiometric determination of sorbitol by the ferricyanide reagent is reviewed in Part II of this paper. It has been shown to be applicable to blood and urine determinations with as great an accuracy as the previous iodometric method of Todd, (112). The saving in time is an advantage, for such determinations are time consuming.

It should be understood that the determination of sorbitol in blood or in the urine is rather indirect. That is, total reduction to ferricyanide does not represent only glucose plus sorbitol but also reduction due to many non sugar reducing molecules. For this reason the basal reduction in blood or urine must be determined, and increases in these figures can then be calculated as sorbitol or other reducing substances employed in the experiment. In the determination of sorbitol in the blood and the urine samples mercury filtrates are prepared according to the method of West, Scharles and Peterson, (115). The glucose is estimated by the Shaffer-Hartmann method, (116), and sorbitol by the ferricyanide procedure outlined in Part II of this thesis. The sorbitol content of the blood or urine is calculated from the difference in the above determinations.

Studies were carried out on six patients. 100 or 200 cc of 50 percent sorbitol were administered intravenously after taking a basal blood sample. Blood samples were then collected every one-half hour or hour over a three and one-half hour period. Table I and II, and Chart I and II represent the sorbitol and the glucose concentrations of the blood samples.

The most interesting curves are those of case (4) and case (5), which represent liver damage and positive galactose tolerance tests. In neither of these cases did the blood sorbitol curve return to the basal levels at the end of a three hour period. The other four patients in this group had returned to a normal basal level at the end of a two hour period. The blood glucose curves as represented in Chart I show that glucose is also maintained higher in cases of liver damage than in the others. The failure of the patient to maintain himself in a fasting state often upset the blood glucose curves.

The fact that the blood glucose curve did rise in fasting patients following sorbitol administration, substantiates the observations of Todd in the dog. This observation would indicate that sorbitol was changed probably in the liver to glucose or stored as glycogen. This is substantiated by the observation that about 85 percent of injected sorbitol is metabolized or retained in the human body. The increase of blood glucose did not reach the renal threshold and the slow conversion of sorbitol to glucose would probably allow the use of this substance intravenously in the diabetic. This observation may be of tremendous value in cases of shock, in which sorbitol in 10 percent solutions could be administ-

ered with isotonic saline, producing a constant source of glucose.

In general the blood sorbitol curves also correspond to the work of Todd in the dog. The sorbitol peak was reached in one-half hour with a drop to the basal level at the end of two hours after its injection. The amounts injected did not seem to influence the rate of disappearance.

In comparing cases (1) to (4) with cases (5) and (6), it is to be noted that the two groups showed great differences in blood chemistry. Cases (1), (2) and (3) had disturbances in the albumin globulin ratios, while case (4) had severe abdominal ascites and edema of the extremities. The blood serum proteins were normal in cases (5) and (6). In cases (1) to (4) the reduction of the blood to ferricyanide was very high compared to cases (5) and (6). This indicated the presence of some material which would not reduce the Shaffer-Hartman but did cause reduction in the more alkaline solution employed with the ferricyanide reagent. (See column one in Table I and II. Miller and Van Slyke, (117), have observed a similar finding with this same type of reagent in a less alkaline media. These authors attributed this to the presence of excess urea in the blood. This cannot be the cause of the present findings in these patients and in other cases with altered albumin-globulin ratios, for normal blood urea concentrations were obtained.

TABLE I

BLOOD DETERMINATIONS

FOLLOWING THE ADMINISTRATION OF 100 cc OF 50 PERCENT SORBITOL INTRAVENOUSLY

Milligrams percent total reduction with the ferricyanide method

Case	Basal	30	Minutes following injection 60	90	120	150	180
1. Myocardial Failure-W.W.	120	188	148	128	112	100	
2. Myocardial Failure-E.R.	160	288		160		140	
3. Tuberculosis E.McN.	120	146	130		100		130
4. Cirrhosis Liver-V.G.	100	240	180		152		152

Milligrams percent blood sugar by the Shaffer-Hartman method.

1. Myocardial Failure-W.W.	51.98	61.02	69.6	66.5	67.8	65.1	
2. Myocardial Failure-E.R.	105	109.4		79.1		65.5	
3. Tuberculosis E.McN.	63	80	80		72		83.6
4. Cirrhosis Liver-V. G.	65	81.4	99.4		88.14	88.2	88.3

Calculated Sorbitol from the milligrams percent difference between the ferricyanide and the Shaffer-Hartman methods.

1. Myocardial Failure-W.W.	6842	126.9	78.4	61.5	44.2	34.9	
2. Myocardial Failure-E.R.	55	178.62		80.9		74.5	
3. Tuberculosis E.McN.	57	66	50	54			46.4
4. Cirrhosis Liver-V.G.	35	158.6	80.6		63.8		63.8

TABLE II

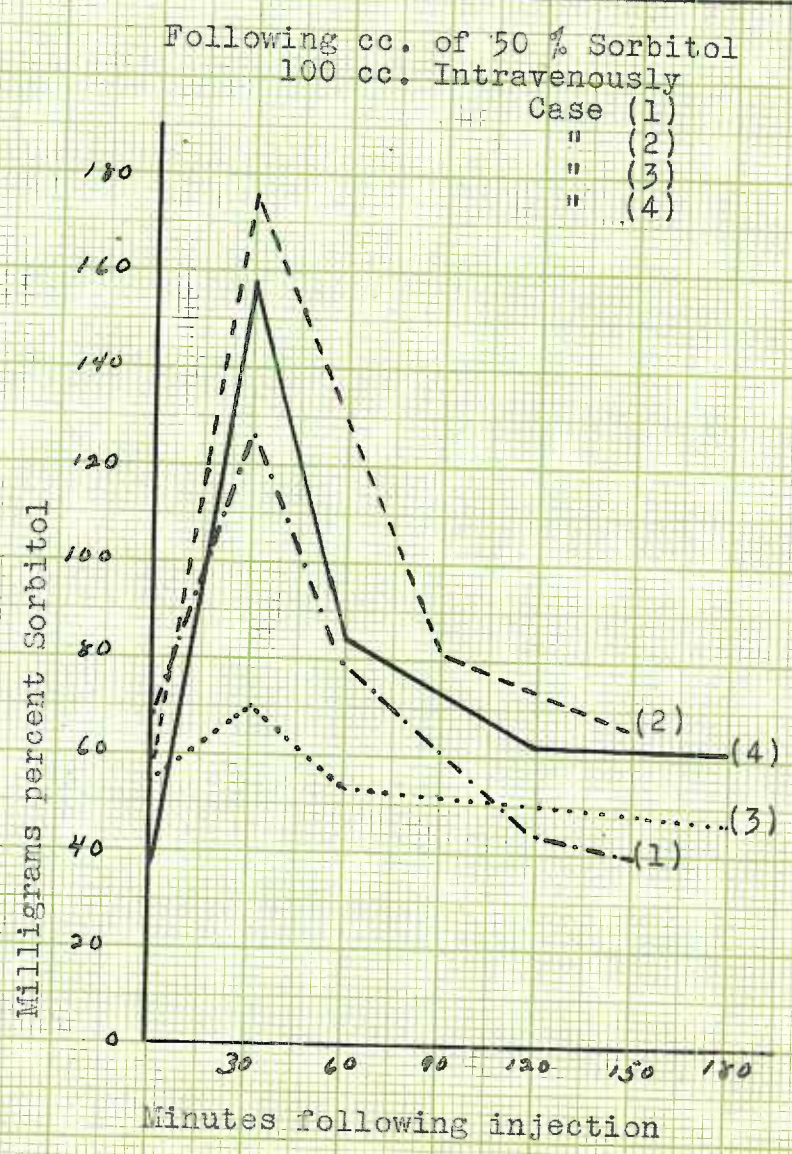
BLOOD DETERMINATIONS

FOLLOWING THE ADMINISTRATION OF 200 cc OF 50 PERCENT SORBITOL

INTRAVENOUSLY

Case	Basal	30	90	150	210
Milligrams percent total reduction with the ferricyanide method.		**Minutes following the injection**			
5. Hodgkin's N.O.* --A-	192	282	200	192	164
N. O. --B-	88	280	180	120	120
N. O. --C-	146.7	288	208	160	130
6. Banti's F.A.	92	300	184	176	144
Milligrams percent blood sugar by the Shaffer-Hartman method.					
5. Hodgkin's -A-	178.54	167.3	154.6	154.6	128.8
-B-	74.58	92.66	97.58	103.96	106.22
-C-	133.34	165.04	115.3	115.2	115.2
6. Banti's F.A.	81.4	92.66	99.44	92.66	74.58
Calculated Sorbitol from the milligrams percent difference between the ferricyanide and the Shaffer-Hartman methods.					
5. Hodgkin's -A-	13.46	114.76	45.42	37.4	35.2
-B-	13.42	187.34	82.42	16.04	13.78
-C-	13.36	122.96	92.74	45.8	14.8
6. Banti's F. A.	10.64	207.34	84.56	83.34	69.42

CHART I
CALCULATED BLOOD SORBITOL CURVES



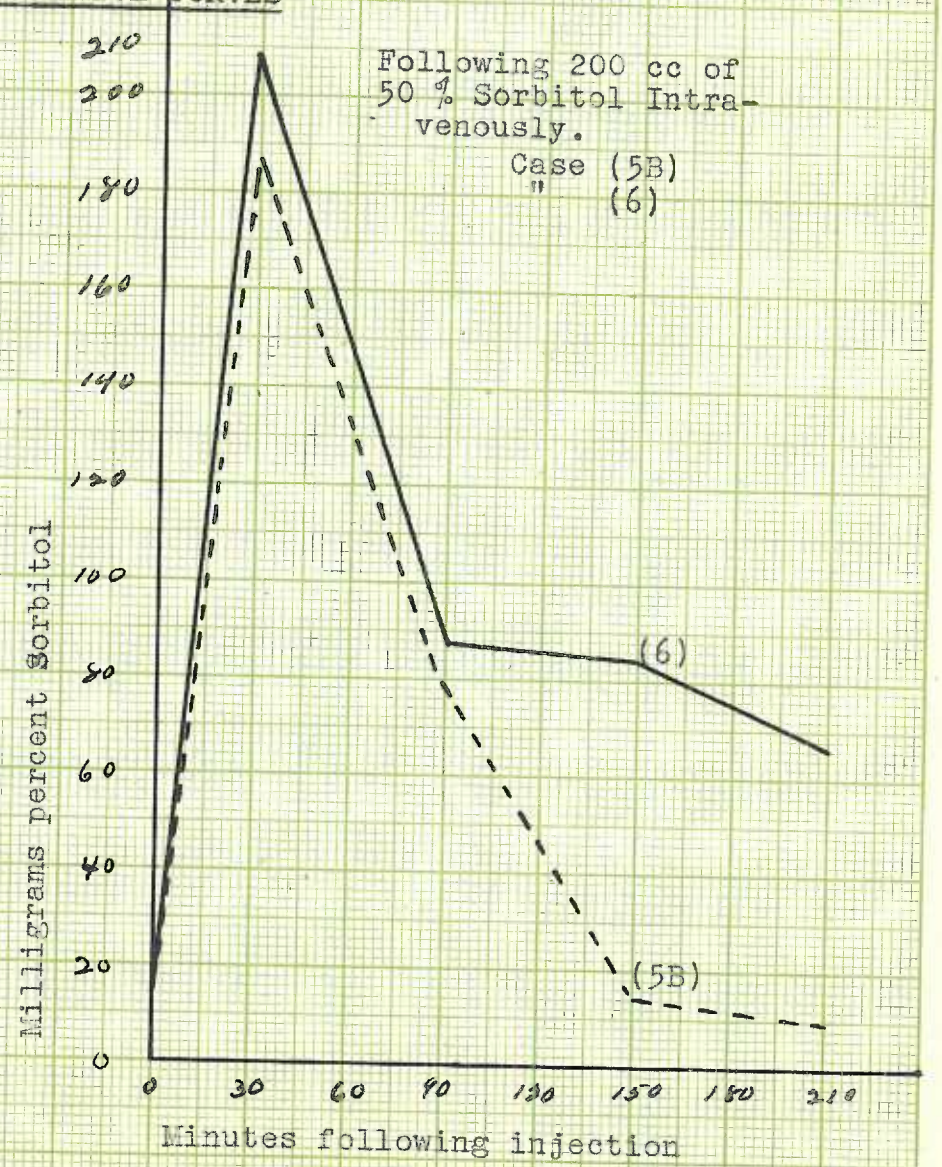
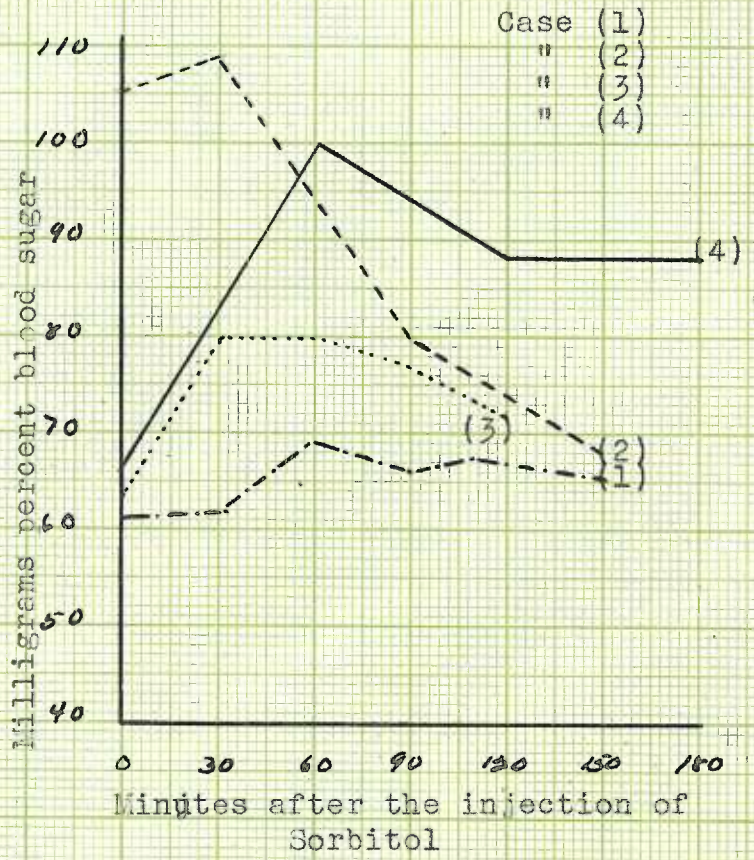


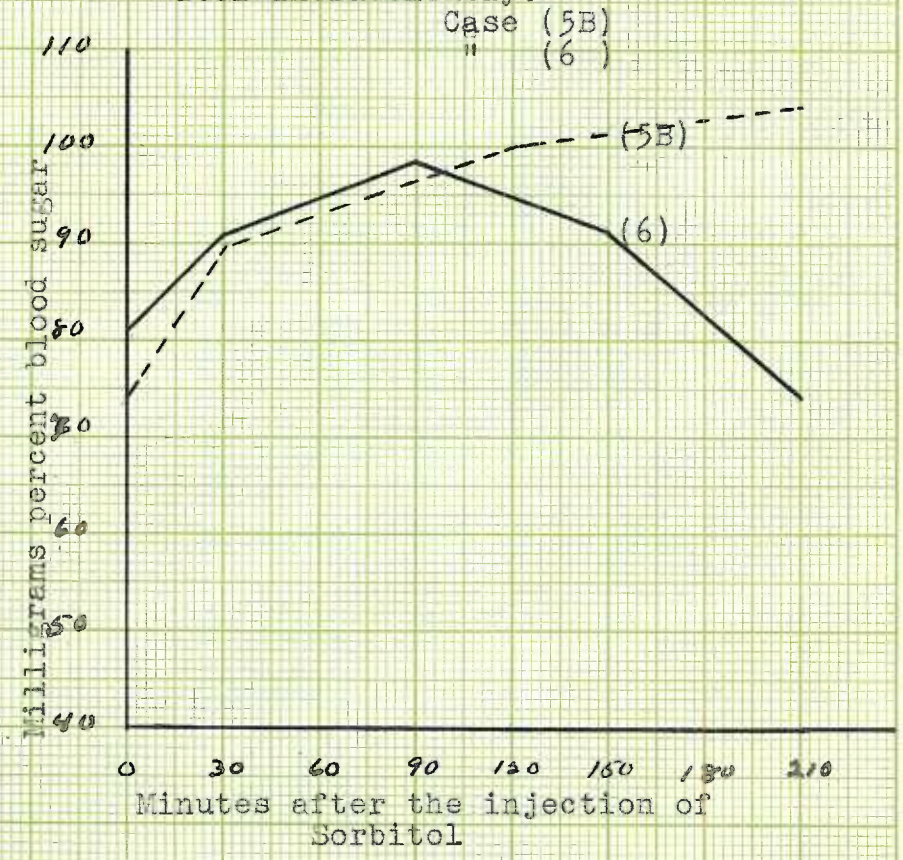
CHART II

BLOOD SUGAR CURVES FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 50 PERCENT SORBITOL

Following 100 cc of 50 % Sorbitol Intravenously



Following 200cc of 50 % Sorbitol intravenously.



Urinary Excretion of Sorbitol

Prior to each sorbitol study, the patients' urines were collected for two or three days to determine the basal urinary volume. A total reduction was run by the ferricyanide method as well as the Shaffer-Hartman to determine reducible substances. The average amount of total reducible substance in a 24 hour specimen of urine which was not removed by the mercury precipitation was found to be one to two grams, only 0.15 grams of reducing material calculated as glucose was present.

100 to 200 cc of 50 percent sorbitol was administered intravenously and the urinary volumes collected every hour in four cases, following the injection. Table III presents the data as found on two cases with liver damage and two cases without liver damage. In the two cases with liver damage 25 to 27 percent of the injected sorbitol is excreted within the first hour. In 24 hours, 37 to 47 percent of the total sorbitol is recoverable. This would indicate that the lack of liver conversion allowed a greater amount of unchanged sorbitol to be excreted by the kidneys.

In the two normal cases in Table III, 40 to 50 percent of all the recoverable sorbitol was excreted within the first hour. In 24 hours only 12 to 17 percent of the total injected sorbitol is recovered in the urine. No sorbitol was excreted after the first 24 hours, for the ferricyanide failed to show an increase above the basal values of reducible substances. Therefore in the normal patients about 25 percent of all sorbitol injected

TABLE III

URINE DETERMINATIONS

FOLLOWING THE ADMINISTRATION OF 100 or 200 cc OF 50 PERCENT SORBITOL INTRAVENOUSLY

** 200 cc Sorbitol

Urinary volume samples-cc.

Case	24 hrs. Basal	Minutes following the injection 30	60	90	120	150	180	210	270	300
1. Myocardial Failure-W.W.	1,500	140	140		48		40			48
2. Tuberculosis Ed.McN.	1,300	465		35		40				
4. Cirrhosis Liver-V.G.	1,400	125		70		20		40	40	
6. Banti's ass F.A.**	1,670	380	202		102	62				

Grams of reducible substance in the urinary volumes as determined by the ferricyanide reagent.

1. Myocardial Failure-W. W.	1.2	1.23	1.22		.36		.31			.31
2. Tuberculosis Ed.McN.	1.5	5.12		.47		.34				
4. Cirrhosis Liver-V. G.	1.4	3.8		2.66		.73		1.1	1.05	
6. Banti's F. A.**	2.09	6.46	6.1		3.	1.1				

Milligrams of reducible substance in urinary volumes as found by the Shaffer-Hartman reagent.

1. "-W.W	45	7.9	5.4		4.1		6.0			10.3
2. "-EdMcN.	240	27.		34		33				
4. "-V.G.	33.2	65.3		37.4		7.7				
6. "-F.A.**	61.7	23.8	38.7		28.8	15.7				

Total calculated urinary sorbitol recoveries 24 hours

	Ferricyanide Reduction	Basal Ferricyanide	Basal Shaffer-Hartman	Sorbitol Grams	Sorbitol Percent
1.* -W.W	7.52	1.2	0.0486	6.26	12.5
2."-Ed.McN.	8.13	1.5	0.3059	6.36	12.11
4.- V.G	18.8	1.3	0.117	17.32	34.63
5.- F.A.**	50.03	2.09	0.1629	47.84	47.84

is metabolized in the body. A total 24 hour output of about 15 percent of administered sorbitol was confirmed in twenty other cases.

Shaffer-Hartman determinations were run simultaneously with an essentially normal basal output. The amounts of the sorbitol injected did not seem to influence the total excretion of the sorbitol or cause a glycosuria. The above observations on the excretion of sorbitol did not correspond with the work of Todd. This author showed that 40 to 50 percent of all the injected sorbitol was recoverable in a 24 hour period from the dog.

Clinical Diuresis with Sorbitol, Glucose and Sucrose

Theoretically a physical osmotic agent is one which is capable of bringing about body dehydration. As has been shown previously in this review there is a failure to distinguish between physiological and clinical data. There is a misinterpretation of terminology between diuresis and clinical diuresis. Animal experimental work is seldom judged separately, and the conditions carefully studied. White, (118), states that hypertonic solutions have proven of no value in clinical dehydration. In the studies reported here similar results have been observed.

According to Todd, (119), when sorbitol is administered to non-anesthetized dogs in a clinical equivalent dose there is produced a

clinical diuresis which lasts over several days. By the term clinical diuresis is meant a greater 24 hour urinary output than normally under controlled conditions of fluid intake.

It was therefore thought desirable to study the clinical diuretic results in both the normal and edematous patients. The regulation of the fluid intake and the collection of each specimen of urine was made possible through the cooperation of the Nursing Staff and Dr. Charles Kennedy of Multnomah County Hospital. Several days prior to each injection of hypertonic solution, 24 hour samples of urine were collected and the volume accurately charted. The day following the injection this same procedure was carried out.

100 or 200 cc of 50 percent sorbitol solution were administered to each of 25 hospitalized patients. Patients presented diseases related to the cardiovascular system, to the kidneys, the liver and to the neurological system. Table IV summarizes the diuretic results obtained on ten of these patients, along with the percent sorbitol excretion and the percent rise in the blood sugar. In only one case out of 25 patients was there observed following the injection of sorbitol alone a definite 24 hour urinary increase above the pre-experimental average. In fact 99 percent of the cases studied with the administration of hypertonic sorbitol alone showed a definite decrease below the normal 24 hour urinary output that was previously recorded. Severe water retention was the most marked in those cases which had an altered ratio of serum proteins. These experiments did not indicate that sorbitol is a clinical diuretic or that it is able to produce clinical

dehydration. However, during the first four hours (see Table IV) after sorbitol, a diuresis resulted from the hypertonicity in the blood and the urine. This diuresis would be of great benefit if it occurred over a longer period of time. To produce an extended diuresis would require multiple injections which would be prohibitive at the present cost. Following the first four hour period there is anuria and retention of body fluids.

Such an observation with sorbitol warranted further investigation with hypertonic glucose and sucrose. A preliminary study on two patients is given in Table V. Both glucose and sucrose produced the same results as those obtained with the use of sorbitol. Glucose caused a greater retention of fluid than either sorbitol or sucrose over a 24 hour period. These observations are contradictory to the present clinicians viewpoint, and further clinical and experimental work must be carefully carried out.

The Combined Action of Diuretics

Evans and Gibson, (120), showed that during diuresis with hypertonic solutions there was increase of the blood volume. Cardoso,(121), demonstrated that salyrgan, the best diuretic yet available, produced a constant diminution of the blood volume. Salyrgan in combination with a hypertonic solution may be of great value. The increased osmotic pressure in the blood stream caused by hypertonic solutions draws fluid from the

TABLE IV

URINARY VOLUMES, SORBITOL EXCRETION AND BLOOD SUGAR RISE

FOLLOWING THE ADMINISTRATION OF 50 PERCENT SORBITOL INTRAVENOUSLY

Case and Diagnosis	cc. of 50% Sorbitol	Urinary Volumes: Ave. 24 hr. before sorbitol	Urinary Volumes: 24 hr. after sorbitol	Urinary Volumes: 4 hr. after sorbitol	% sorbitol excreted	% Blood sugar rise
1. Congestive myocardial failure	100	1,500	720	272	15	34
2. Pulmonary tuberculosis Hypoproteinemia	100	1,300	740	540	16	27
3. Arteriosclerotic heart disease	100	1,090	970	220	12	
4. Hodgkin's Disease	200	1,800	760	250	9	31
5. Hypertensive cardiovascular disease	100	1,800	1,000		11	
6. Arteriosclerotic heart disease	100	860	550	210	15	
7. Cirrhosis of liver	100	1,400	865	265	38	52
8. Banti's Disease	200	1,670	1,300	750	46	22
9. Infectious arthritis	200	2,300	3,450	610	13	
10. Multiple Sclerosis	200	2,000	2,050	670	27	

TABLE V

A COMPARISON OF THE CLINICAL DIURESIS OF HYPERTONIC GLUCOSE, SORBITOL AND SUCROSE

Case	Medication	Urinary Volumes: Basal 24 hr. urinary vol.	Following Medicat. 4 hrs.	Following Medicat. 24 hr. total
D: Hypertensive cardiovascular disease				
1-4-40	Aminophy. 10cc.	1050		
1-5-40	Aminophy. 10cc. *Glucose 100cc.	1050	360	660
1-9-40	Digitalis 10 gr. Aminophy. 10cc. *Sucrose 100 cc.	1100	400	880
1-12-40	*Sorbitol 100cc.	1110	380	980
W: Uterine Fibroids Normal Patient				
1-18-40	*Glucose 100cc.	1500	490	705
1-22-40	*Sorbitol 100cc.	1650	380	1245
1-27-40	*Sucrose 100cc.	1550	500	1300
2-2-40	Salyrgan 2 cc. *Sorbitol 100cc.	1125	360	2100

* 50 Percent Solutions

tissues, while salyrgan acts immediately upon the proximal convoluted tubules to produce a prolonged diuresis. It was felt that the presence of the hypertonic solution in the renal tubules in combination with salyrgan might produce a rapid diuresis with increased dehydration.

The administration of 1 or 2 cc of a 10 percent solution of salyrgan intravenously is a routine procedure in Multnomah County Hospital. Many of the patients respond to this drug over a period of time, and then suddenly lose their sensitivity. Case E, which is given in Table VI, represents a patient with arteriosclerotic heart disease with pitting edema of the extremities. This patient did not respond over a three week period to the administration of ammonium chloride, potassium chloride, aminophylline, or salyrgan. 200 cc of 60percent sorbitol was administered and one-half hour later 1 cc of salyrgan was given intravenously. The diuretic effects were immediate, and dyspnea and cyanosis were relieved. The data obtained on three cases as given in Table VI indicates that the combined action of salyrgan is more effective than when used alone. Salyrgan and sucrose also removed large amounts of fluid rapidly, and reduced edema with no distress to the patient. Several other cases not reported in this paper have proved that the combination of a hypertonic solution and a mercurial diuretic are beneficial for the rapid removal of edematous fluid.

This is the first observation as far as can be determined concerning the combined action of hypertonic solutions and salyrgan. Salyrgan has been shown to exert its clinical diuretic action probably on the proximal convoluted renal tubules. This is thought to be true because Moller, (122),

has shown there may be an increase in urinary chlorides associated with mercurial diuresis. Table VII represents the data concerning the daily 24 hour urinary chloride excretion. Each patient was on a limited chloride intake. A normal chloride output for such patients would be lower than that given in the standard textbooks. The normal chloride output was found to be 3 to 6 grams per 24 hours. Case 3, which is given in Table VII is the only patient in our series of 26 cases receiving sorbitol alone who responded with an increased 24 hour urinary output. This patient's clinical diuresis was associated with a 24 hour increase of urinary chloride. Other cases which received only hypertonic solutions had a normal urinary chloride output. In every case in which salyrgan was given there was noted an increased chloride excretion. A hypertonic solution and salyrgan produced a greater diuresis and also an increased chloride output. Salyrgan had no effect on the excretion or the retention of sorbitol. Salyrgan in combination with sorbitol did not cause the excretion of glucose in the urine. The loss of chlorides following the use of salyrgan may be great, but most of the patients studied had been on medications of ammonium chloride. Blood chlorides in these patients were within the normal limits. It is doubtful whether intravenous administration of potassium chloride or sodium chloride in combination with salyrgan and sorbitol would produce greater diuresis than salyrgan alone. Chart III presents a graphic picture of the urinary chloride output daily of case 6 in Table VII for 20 days.

TABLE VI

COMBINATION OF HYPERTONIC SOLUTIONS AND SALYRGAN

Case R: Arteriosclerotic heart disease	Diuretic	Urine Volumes		% sorb. or Sucrose exc. in 24 hrs.
		24 hr. after diuretic	4 hr. after diuretic	
Date: 8-27-39	2cc. Salyrgan	1200 cc		
8-31-39	1cc. Salyrgan 200cc. Sorbitol	3900	2600	23
9-27-39	1cc. Salyrgan 100cc. Sucrose	2200		91
9-30-39	1cc. Salyrgan 100cc. Sucrose	2900	1600	90
10-4-39	1cc. Salyrgan 100 cc. Sorbitol	3800	1350	18
10-11-39	100cc. Sucrose	600	220	49
10-15-39	1cc. Salyrgan	4600		
Case S: Hodgkin's Dis.				
9-6-39	1cc. Salyrgan 200cc. Sorbitol	2350	1150	10
9-7-39	2cc. Salyrgan	1800		
9-9-39	200cc. Sorbitol	760	250	9
9-13-39	2cc. Salyrgan 200cc. Sorbitol	4500	1830	12
9-22-39	2cc. Salyrgan 100cc. Sucrose	5800	3000	
Case P: Arteriosclerotic heart disease				
11-5-39		1150		
11-6-39	100cc. Glucose	890		
11-8-39	1cc. Salyrgan 100cc. Sorbitol	2000	1010	17
11-12-39	2cc Salyrgan 100cc. Sorbitol	3250	2335	16
11-14-39	2cc. Salyrgan	2250	510	

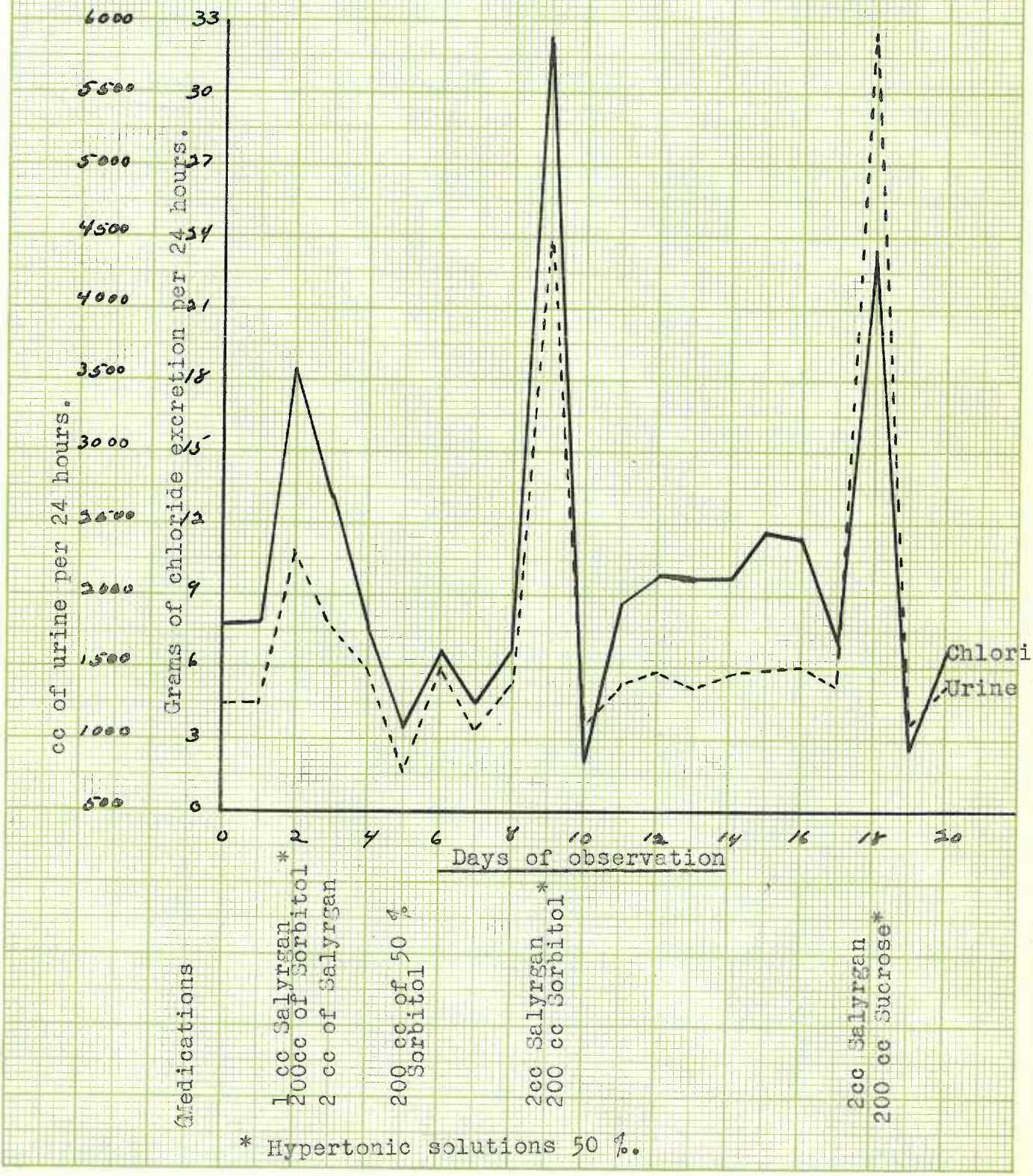
TABLE VII

RELATIONSHIP OF CHLORIDE EXCRETION TO DIURESIS

Case:	Diuretic	24 hr. Urine Vol.	Diuresis with Grams chloride excretion			
			Basal	3 hrs	21hrs.	Total
1. Myocardial failure	100 cc. Ser.	720	5.0	0.9	2.88	3.79
2. Multiple sclerosis	200cc. Ser.	2050	6.8	2.58	2.31	4.9
3. Poly-arthritis	200cc. Ser.	3450	8.0	2.4	11.97	14.37
4. Banti's disease	200cc. Ser.	1300	8.35	3.37	3.3	6.67
5. Myocardial failure	1cc. Salyr. 200cc. Ser.	3800	4.9	15.75	7.56	23.31
6. Hodgkin's disease	200cc. Ser.	760	8.4	1.1	2.24	3.34
7. Hodgkin's 9-6-39	1cc. Salyr. 200cc. Ser.	2350	8.2	9.3	9.6	18.8
8. Hodgkin's 9-7-39	2cc. Salyr.	1800				12.96
9. Hodgkin's 9-13-39	2cc. Salyr. 200cc. Ser.	4500	8.3	10.98	11.6	32.58
10. Hodgkin's 9-22-39	2cc. Salyr. 100cc. Ser.	5800	8.3			23.2

CHART III

THE RELATIONSHIP OF CHLORIDE EXCRETION TO DIURESIS



* Hypertonic solutions 50 %.

Aminophylline and Hypertonic Solutions

The diuretic uses of aminophylline are usually limited to the ambulatory patient in the first stages of edema. Seldom does aminophylline retain its ability to produce diuresis in the chronic edematous patient. 10 cc of aminophylline and 100 cc of 50 percent sorbitol were given intravenously to two cases of eclampsia in the terminal stages. In the first case a complete anuria occurred during the first three hours following the injection. The total sorbitol excretion was only 0.44 percent with a urinary output of 100 cc in 24 hours. The second case responded similarly, and both cases expired the next day. Sorbitol and aminophylline failed to produce clinical diuresis in cases of edema due to myocardial failure, and in other eclamptics.

Clinical Effects from Hypertonic Solutions

During the injections of hypertonic solutions of sorbitol there was noted in almost every case some of the following symptoms: epigastric distress, nausea, flushing of the face, chest pain, dyspnea, headache, and a tingling and numbness of the extremities.

However, case H (see table VI) had the most pronounced post intravenous symptomatology as yet observed. On 10-11-39 this patient was administered 100 cc of 50 percent sucrose over a 10 minute period. This resulted in a severe excruciating lower back pain requiring morphine for relief. The patient became apprehensive, dyspneic, cyanotic and the temperature rose to 101 for a thirty minute period. These symptoms persisted with a gradually lessening severity for 24 hours. The next 48 hours the

patient had a residual dull backache. Careful physical and laboratory tests indicated that this pain after the sucrose injection had a renal origen. Only 600 cc of urine and 50 percent of the administered sucrose was excreted in the urine during the first 24 hours. For the next three days following the injection the urinary output was very low. 1 cc of salyrgan given on the fourth day after the disappearance of the renal symptoms caused excretion of 4,800 cc of urine.

Analytical procedures to determine the amount of sucrose excreted in 24 hours with salyrgan are the same as those used by Keith, Power and Peterson, (94). The data in Table VI records findings similar to those observed by these authors in normal and pathological conditions. It was found in the cases studied that with combinations of salyrgan and sucrose, 90 to 98 percent of the sucrose injected is excreted within the first 24 hours.

A Discussion of the Clinical Work

Recent investigations by Gilligan, Altschule and Volk,(123), Altschule and Gilligan, (124), and Ellis and Faulkner, (125), helps to clarify the physiological action of hypertonic solutions in the human. It was first shown that following hypertonic solutions there was a rise in the venous pressure and an increase in the blood and the plasma volume to a maximum level of 20 percent above the normal. This volume of fluid which is drawn into the vascular system would tend to diffuse into the tissue spaces if not rapidly eliminated. Blood volume determinations

have proven this to be the case. On the basis of the available knowledge, a flow of fluid to the tissue spaces is enhanced by decreasing the colloidal osmotic pressure and increasing the venous pressure. A fall in the venous pressure which occurs in some cases is accompanied by a peripheral vaso-dilatation. These phenomena may be the cause and one of the potent factors in the failure of sorbitol, glucose and sucrose to act as a clinical diuretic. Further studies must be carried out following the injections of hypertonic solutions to determine this possibility of tissue diffusion. Peripheral dilatation was thought to be noted in our observations with symptoms of headache, epigastric distress, nausea and flushing and tingling of the face and forehead.

Whether hypertonic solutions will ever be of importance in clinical dehydration is doubtful. Browder and Meyers, (126), questioned the use of hypertonic solutions in head injuries. Bamberger, (127), states that with the use of hypertonic glucose and sucrose the supposed dehydration is not sufficient to obtain the desired results. This author has noted that unless some other diuretic drug is used to eliminate the excess fluid drawn into the blood by hypertonic solutions there may be a reversed process which results in a fluid accumulation in the tissues. In order to increase the urinary output in intracranial injuries this author has used one of the organic mercury compounds intramuscularly on the same basis that they are used for cardiac edema. In this thesis it is suggested that a combined mercurial drug and hypertonic solution be used in cases of edema of the brain and intracranial injuries. Drug dehydration of

intracranial injuries should never be relied upon, for the mortality rate is much lower when the routine spinal tap is carried out in all cases. Investigations are now being carried out, using the combined action of salyrgan and hypertonic solutions and are to be reported at a future date. It is to be pointed out that in studying any case of intracranial pressure relief by diuretics, it is difficult to judge the results without actually measuring the pressure.

Whether a hypertonic solution alone should ever be given a patient is still questionable. Hypertonic solutions will not decrease edema or produce a clinical diuresis with the present clinical dosage. Further clinical investigation must be carried out on the pathological conditions of edema, especially the blood volume determinations, the tissue fluid pressures, and the arterio-venous pressure differences. It is also questionable whether hypertonic solutions should be administered in cases of pulmonary edema, for the relief given to such a patient may be due to the progressive peripheral vaso-dilatation occurring during the course of and following the injection.

If sorbitol, glucose or sucrose are given to a patient followed in two or three days by salyrgan a tremendous diuresis results. The pathological effects of a hypertonic solution on the renal tubules has previously been pointed out in the case of sucrose to be an epithelial hydropic degeneration. The very nature of a hypertonic solution in great concentration in the renal tubules would lead to a difference in the osmotic pressures exerted across the tubular epithelial membranes and possibly

produce an epithelial swelling and sensitivity. This may account for the ease with which salyrgan may work following the administration of a hypertonic solution.

It is interesting that this is the first observation of the difference in the action of hypertonic solutions of sorbitol in the dog and in man. By comparing the urinary output it was found that sorbitol produces a clinical diuresis in the dog but not in man. Such a difference of activity is easily explainable if the anatomy of the kidney of man is compared to that of the dog. Man has a larger kidney per surface area. The nephrons in man are fewer; however each nephron has 50 percent more reabsorptive tubular area than that of the dog. This is easily proven by the results of the phenol red and the diodrast clearance tests. And lastly, the renal blood flow in man to the glomerulus and the tubules is twice as great as in the dog. Such anatomical differences undoubtedly account for the differences in the action of hypertonic solutions in these two species.

PART II

Introduction

The data on sugar and sorbitol of blood and urine in Part I of this thesis were determined by a potentiometric method described in the following pages. The ferricyanide reagent will be discussed in the following manner: the historical development, the theoretical physico-chemical basis, the method, the procedure, its application to blood and urine followed by a generalized discussion in which some of its analytical faults are pointed out.

The last part of Part II is completed by a short discussion of a method for the determination of sugars, sugar alcohol, and polysaccharides by the use of ceric sulphate in acid medium. This method is still in the experimental stage, but its range is wide and its possibilities are great.

POTENTIOMETRIC APPLICATION OF THE FERRICYANIDE REAGENT

Historical Development

Todd, Vreeland, Myers and West,(112), first suggested that a highly alkaline modification of the Hagedorn-Jensen,(128), ferricyanide reagent could be used for the determination of sugar alcohols. The alkaline solution of potassium ferricyanide is an oxidising reagent. Heating this reagent in the presence of a sugar alcohol or glucose will reduce the ferric iron to the ferrous state, thus form-

ing an oxidation-reduction system. The ferrocyanide that is formed can be completely removed by the addition of zinc sulphate, and the ferricyanide remaining can be determined iodometrically. Such a method has several variables and the duplication of results is difficult without giving due consideration to temperature effects.

Wood, (129), Shaffer and Williams,(130), and Hoy and West,(131), have applied potentiometric measurements to the ferricyanide system and have studied the analysis of sugars quantitatively. Since the above method of Todd, Vreeland, Myers and West, (112), is essentially this same type of oxidation-reduction system, a direct potentiometric application to the quantitative analysis of sorbitol in a highly alkaline media seemed feasible.

Theoretical Discussion

It is to be remembered that in the process of oxidation electrons are lost, and in reduction electrons are gained. Sugar alcohols and glucose are capable of giving up electrons and therefore are oxidized. The electrons which are lost are readily taken up by the ferricyanide ion which is then reduced to the ferrocyanide.

$$Fe(CN)_6^{\equiv} \quad e \rightleftarrows \quad Fe(CN)_6^{\equiv\equiv}$$

Nernst in 1889 first recognized that there may be a potential difference between a solution and a metal immersed in it (E). Thus in a series of known metals each will have a metal solution potential which is constant (E°). In order to reach

a standard potential from time to time other factors must be constant. These factors are represented in the following manner: (R), is the gas constant, (T), is the absolute temperature, (F), is the current in Faraday units, and (n), is the ionic charge during the reaction. A formula embodying the above factors and expressing the relation between the electrode potential and the ferro-ferricyanide concentration in a solution has been developed. It is as follows:

$$E = E^{0} - \frac{RT}{nF} \ln \frac{Fe(CN)_6^{\equiv}}{Fe(CN)_6^{\equiv}}$$

By substituting the appropriate numerical values in the above equation the desired calculations may be made. The above discussion is taken mainly from Getman and Daniels, (132), and from Kolthoff and Furman, (133).

By the use of the customary apparatus consisting of an inert electrode, such as platinum, which is placed in the solution and connected in circuit with a potentiometer, galvanometer and a reference electrode such as the calomel cell, the potentials of the ferro-ferricyanide system may be studied.

In order to determine the ratio of the ferric/ferrous ions, an inert electrode of platinum must dip into the alkaline ferri/ferrocyanide solution. When the platinum electrode dips into the solution it is surrounded by ferric and ferrous ions. Ferric ions on colliding with the platinum will tend to take electrons from the electrode to form ferrous ions and leave the electrode positively charged. Ferrous ions will tend to give electrons to the electrode, making the electrode negatively charged. It is this ratio of ferri/ferrous ions which will determine the electromotive force. The electrical circuit is completed by the use of a saturated calomel half cell which is connected to the ferri/ferrous solution by means of a 3 percent agar saturated potassium chloride bridge. The saturated calomel half cell when standardized against the standard hydrogen electrode will give a positive E. M. F. of 0.246 volts which must be added to each E. M. F. reading when calculating the formula as derived. Each milligram of sugar will produce a definite ratio of the ferri/ferrous ions which in turn is dependent upon the number of the electrons lost by each sugar during its oxidation.

Method

Reagents

1. 1.06 per cent of Potassium ferricyanide in freshly distilled water. Normality of 0.0064.
2. 5 percent Sodium sulphate in 3.33 Normal Sodium hydroxide.

Essential Apparatus

1. Leeds and Northrup Student's type of Potentiometer.

2. Enclosed lamp and Galvonometer with scale.

3. Standard Weston Cell.

4. A saturated calomel half cell, and saturated Potassium chloride in 3 percent agar bridges.

Procedure

Pyrex tubes (25 x 200 mm) are charged with 5 cc samples of a solution containing 0.1 to 1.0 mg of glucose or sugar alcohol. Then 5 cc of Reagent (1) are added to each tube followed by 5 cc of Reagent (2). The tubes are immediately covered with glass bulbs and after the contents are mixed they are placed in a boiling water bath. After heating for 30 minutes the tubes are removed and placed in cold water, where they are allowed to remain until they reach the temperature of 22° C. They must be kept at this temperature, which is also the temperature of the saturated calomel half cell and a specially constructed pyrex tube (25 x 50 mm) into which the contents of the oxidation tubes can be placed to make potentiometric readings. With each sugar alcohol known solutions must be used and a graph constructed, plotting the E. M. F. against the milligrams of reducible substance. Using this graph as a standard, unknown solutions may be determined since each E. M. F. reading corresponds to a definite amount of reducible substance.

Comparison with the Iodometric Method

There was not available in the beginning of our experimental study an accurate method for the standardisation of potassium ferricyanide. Curves had to be constructed for each sugar alcohol and for glucose with each sample of reagent made. This was the same procedure of standardisation as used by Todd, Vreeland, Myers and West, (112). The results by the potentiometric method were compared to those obtained by the iodometric titration method. Unknown solutions were run in this manner. It was found that the results by the potentiometric method were more reproducible than those obtained by the iodometric determination of the ferricyanide remaining after the oxidation. The potentiometric method for sorbitol determination was used because of its rapidity.

Recovery of Known Solutions

Recovery of known amounts of glucose and sorbitol from distilled water solutions ranged from 90 to 100 percent. However, when tap water was used without boiling the range of the recovery increased from 90 to 120 percent. Mercury sulphate and barium Carbonate precipitation did not interfere with these recoveries.

Urine And Blood Determinations

The method outlined below is essentially the same as that which was used by Todd, Vreeland, Myers and West, (112). Urine filtrates were prepared in the following Manner: 10 cc of urine are added to

to 75 cc of water in a 250 cc Erlenmeyer flask and 15 cc of mercuric sulphate reagent (2.5 percent mercuric sulphate in 2N. sulphuric acid) are added; the mixture is neutralized with barium carbonate (about 2.5 gms.) and filtered; 1 gm of zinc dust per 15 cc of filtrate is added to remove traces of mercury. After filtering again through a fine filter paper (Whatman No. 42) the filtrate is suitably diluted and the reduction is estimated by the procedure as directed.

Blood filtrates are made in a similar manner, 5 cc of blood are added to a flask containing 90 cc of water. When laking of the blood has taken place 5 cc of mercuric sulphate reagent are added and the mixture neutralized with barium carbonate. Zinc dust is added and the mixture is filtered. The final filtrate is diluted as necessary before analysis.

It is to be remembered that other substances besides glucose and sorbitol reduce the ferricyanide reagent. Basal determinations must be run before any comparative analysis can be made. The amount of glucose that is present in the sample can be run by the Shaffer-Hartman method. The more highly alkaline ferricyanide reagent is more sensitive to non-sugar reducing substances. Miller and Van Slyke,(117), have pointed out this fact by the direct titration of the amount of ferrocyanide with ceric sulphate. These authors have shown that reducible substances may be present in distilled and undistilled water, in sulphuric acid, and thus it may be assumed that reducing substances may be present

in the barium carbonate used as a precipitating agent and on unclean glassware. It is therefore necessary to check every reagent used for reducible substances before accurate determinations can be made.

Recovery from blood and from urine corresponded with the iodometric method and were found to be 90 to 100 percent. Errors in the recoverability were occasionally noticeable when less than 0.5 mgs. of reducible material were present. This was thought by West, (134), to be due to hydrogen peroxide formed by the reaction of the zinc dust in the filtrate. Titanium sulphate, which is one of the most sensitive tests for peroxide, failed to demonstrate its presence in zinc filtrates. However, dissociation of hydrogen peroxide is rapid in acid media and oxygen saturation of the filtrate could occur. Further investigation proved that this difference arose from another source.

Table VIII presents the E. M. F. data obtained with the alkaline ferricyanide reagent against known milligrams of reducible substances. Glucose, sorbitol, mannitol, dulcitol, inisitol, and pentaerythritol were all studied. Chart IV represents the curves obtained by graphing millivolts against the milligrams of the substances studied. Chart V represents the curves graphing the cc of titration difference against the milligrams of sugars by the iodometric procedure. For each sugar alcohol the corresponding curves are very similar. With the potentiometric method the curves for sorbitol could be

repeated under the standard conditions as outlined with the same reagents at any time during a six week period.

The millivolt difference may be used as an indication of the ferri/ferrocyanide ratio. Using the data in Table VIII a curve may be constructed graphing the millivolt change against the milligrams of glucose and sorbitol. This curve is represented in Chart VI and demonstrates that with this ferricyanide reagent there is not a uniform millivolt change per unit weight of sorbitol and glucose. Therefore further studies upon the nature of the reagent were carried out.

Discussion of the Reagent

The previous work shows by the nature of the millivolt change that the reagent has probably a much greater proportion of ferri than ferrocyanide after oxidation. For such an oxidation-reduction system to be accurate there must be a large millivolt and uniform change in each determination. This is not the case with the present reagent as Chart VI shows. It was found by experimental study that to reduce completely all of the ferricyanide to ferrocyanide there must be 2.8 milligrams of glucose present per 5 cc. This reagent is three times the desired strength and the potentiometric curve is different from that which is obtained when less ferricyanide is used. The lack of time has made it impossible to continue this study.

TABLE VIII

SUGAR DETERMINATIONS

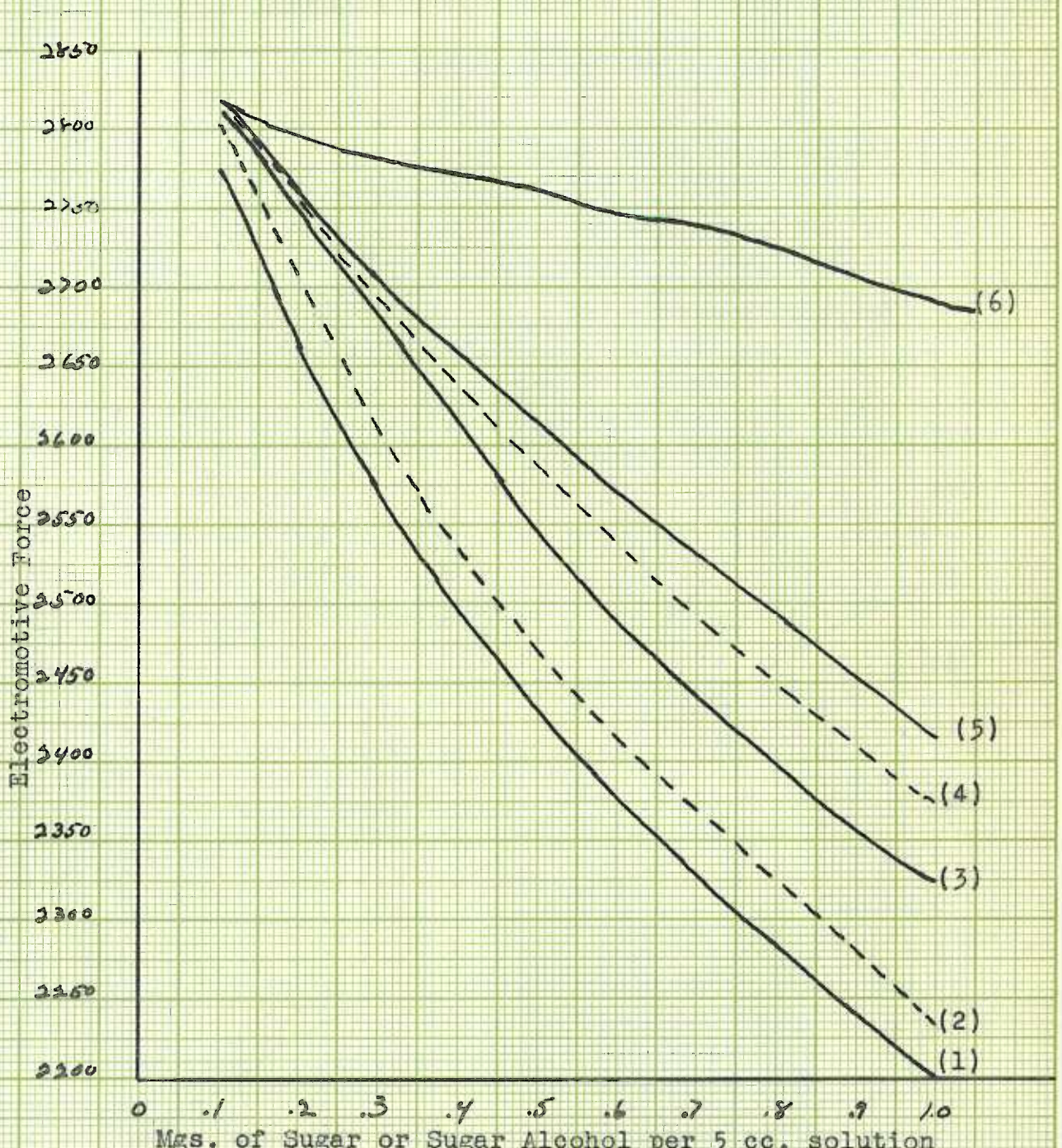
ELECTROMOTIVE FORCE OF ALKALINE FERRICYANIDE REAGENT

Mgs.	Sugars					
	Glucose	Sorbitol	Mannitol	Dulcitol	Inositol	Penta-Erythritol
1.0	.2288	.2245	.2242	.2293	.2425	.2709
0.9	.2251	.2236	.2255	.2427	.2462	.2745
0.8	.2292	.2283	.2408	.2456	.2500	.2780
0.7	.2338	.2332	.2448	.2492	.2536	.2746
0.6	.2382	.2423	.2492	.2524	.2575	.2755
0.5	.2429	.2481	.2532	.2563	.2615	.2770
0.4	.2495	.2532	.2602	.2629	.2662	.2775
0.3	.2545	.2615	.2679	.2695	.2706	.2785
0.2	.2655	.2709	.2743	.2760	.2753	.2792
0.1	.2775	.2802	.2805	.2803	.2810	.2815

Determination Constants
1. Temperature—22 C.
2. Heating Time-30 minutes.
3. Standardization of Potentiometer with Weston Cell.

CHART IV

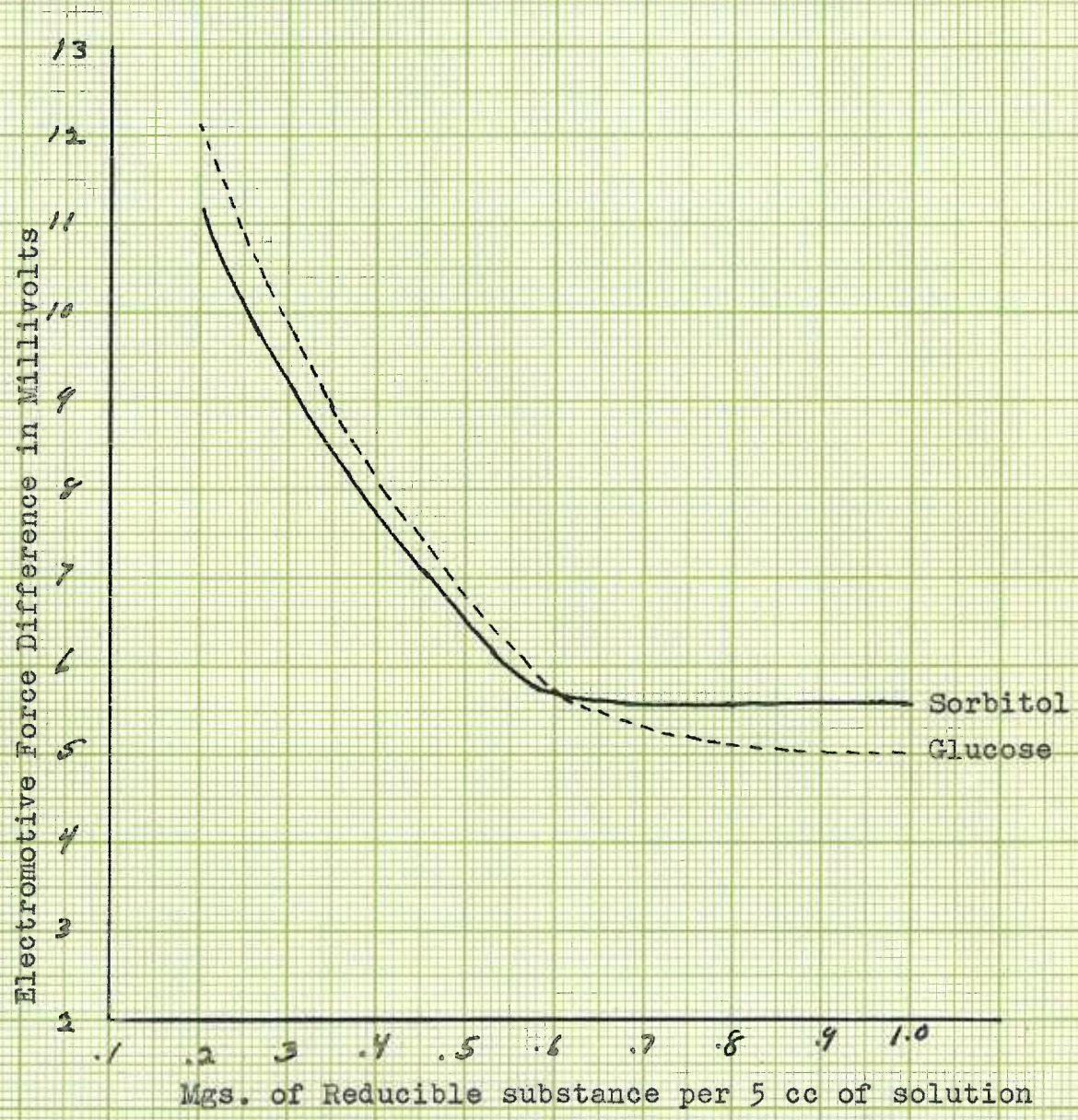
POTENTIOMETRIC RELATION OF CONCENTRATION TO OXIDATION



(1) Glucose
(2) Sorbitol
(3) Mannitol
(4) Dulcitol
(5) Inisitol
(6) Penta-erythritol

CHART VI

RELATIONSHIP OF THE MILLIVOLT CHANGE TO THE MILLIGRAMS OF REDUCIBLE SUBSTANCE



Preparation and Standardization of Ferricyanide

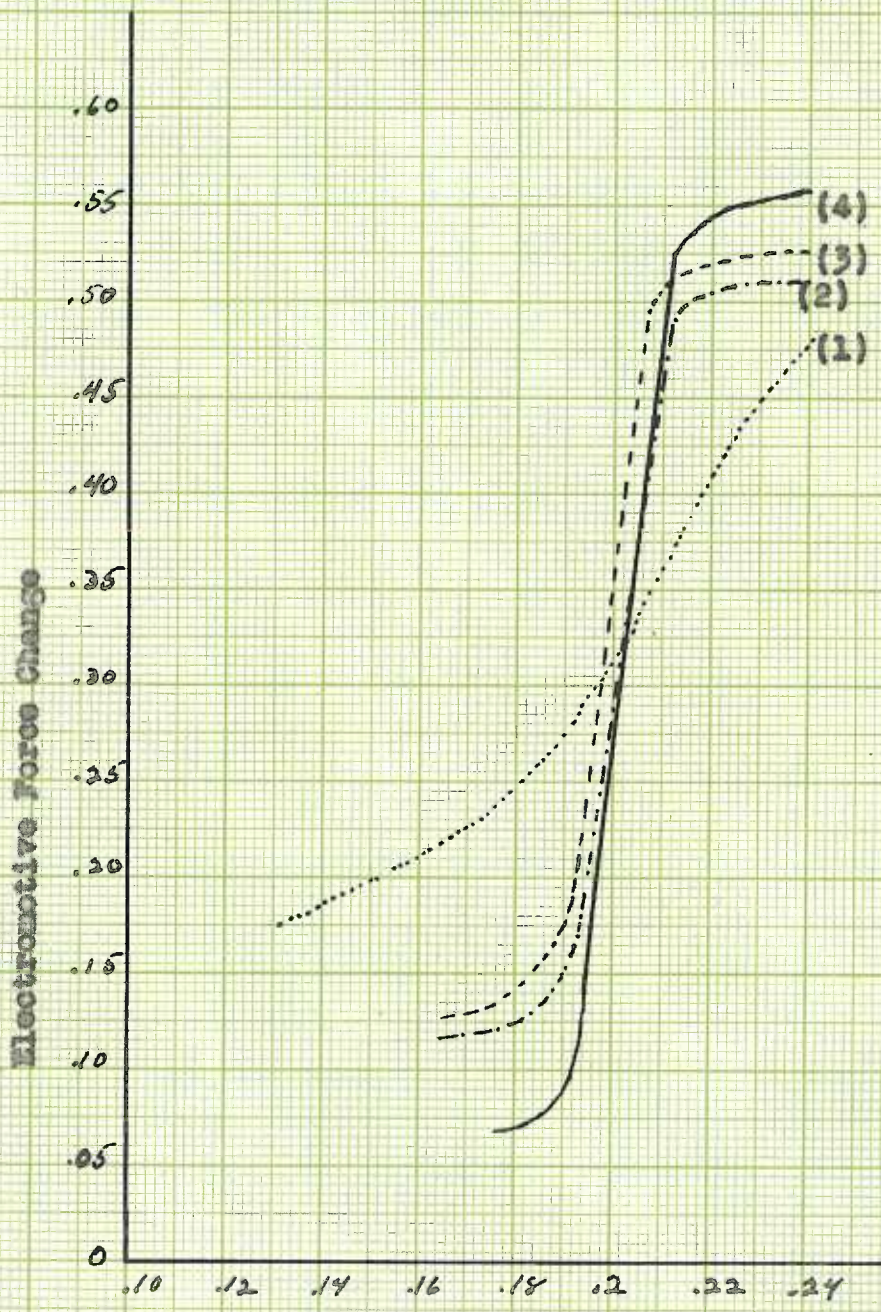
This previous work illustrated the fact that there would be little need of repeating the curves with each new sample of reagent if an effective method for the standardization of potassium ferricyanide was available. By developing a method one would be able to titrate directly the amount of ferricyanide left after the oxidation which is necessary for the standardization of the potentiometric method. Frequent checks on the stock reagent of ferricyanide must be done each week, for a definite change in the reagent is noted after a six week period.

It was first thought that solutions of cerous sulphate or titanous sulphate might be used to standardize potassium ferricyanide, but these procedures were found to be impractical. A method was finally applied in this laboratory for the standardization of ferricyanide by the potentiometric titration with stannous chloride. It was found that a reagent quality of stannous chloride dihydrate (Mallinckrodt Chem. Co.) gave satisfactory results. This material must be finely ground and kept in an atmosphere of dry air. Solutions were made up to 0.02 normal by placing 0.01 gm. mol. wt. in 50 cc of concentrated hydrochloric acid and making up to one liter volume by the addition of freshly distilled water previously boiled to expel oxygen.

It was soon found that the potassium ferricyanide must be finely ground and placed in a dessicator three days prior to weighing. When stannous chloride was potentiometrically titrated against ferricyanide in the presence of air, there were widely varying results. The substitution of a closed vessel with an atmosphere of nitrogen removed this difficulty. Since acids react with ferricyanide to liberate hydrocyanic acid slowly, different concentrations of acid had to be studied to determine the errors which might result. This data is presented in Chart VII, which shows the E. M. F. change of a solution of stannous chloride plotted against the cc of potassium ferricyanide. Potassium ferricyanide was made to 0.05 N. by weight for the potentiometric titration against stannous chloride. The reliability of the results can be determined by taking the average equivalent of the potassium ferricyanide from the curves (2), (3) and (4) in the Chart. These curves demonstrate an equivalent value of 2.01 cc to 5 cc of 0.02 N. stannous chloride and a value of 0.0498 for the normality of the potassium ferricyanide. Stannous chloride can in turn be checked against ceric sulphate, ceric sulphate can be standardised against Mohr's Salt. Stannous chloride was found to be an effective reagent for the standardization of potassium ferricyanide in an atmosphere of nitrogen.

CHART VII

POTENTIOMETRIC TITRATION OF POTASSIUM FERRICYANIDE WITH STANNOUS CHLORIDE



Curves

(1) -2 N Hydrochloric acid.
(2) -3 N " "
(3) -4 N " "
(4) -6 N " "

CERIC SULPHATE AND ITS APPLICATION TO THE DETERMINATION OF SUGARS

Development

Ceric sulphate was first used as an oxidising agent by Lange in 1861, (135). It was found to be applicable to numerous organic and inorganic compounds by Willard and Young, (136), and Furman, (137). Its wide use has been limited due to the expense of purification and the lack of a suitable method. Recently the Smith Chemical Company has placed ceric sulphate on the market in both a pure and economical form. As a chemical oxidising agent it has numerous advantages which may make it preferable to any other known oxidising reagent. It is stable over a wide range of sulphuric acid concentrations and for short periods of time in high concentrations of hydrochloric acid. Ceric sulphate solutions can be standardized against sodium oxalate, ferrous sulphate, ferrous ammonium sulphate or stannous chloride. In many of the above reactions a large number of indicators can be employed such as diphenylamine, diphenylbenzidene, methyl red, methylene blue, eric glaucine, eric green and methyl violet. Electrometric titrations are applicable to oxidation-reduction methods using ceric salts.

Lejeune, (138), (139), (140) first showed that ceric salts in an alkaline medium may be used to oxidize glucose, galactose and

fructose. Potentiometric readings showed that a method might be devised for the determination of sugars. Ghosh and Rakshit,(141) proved that ceric hydroxide at room temperature was able to oxidise glucose and levulose. Krantz and Carr, (142), first reported an iodometric method with the direct use of ceric sulphate as an oxidising agent in 2 N sulphuric acid for the determination of isonamide. Many indirect methods are now available,but no direct data was found in the literature concerning the use of ceric sulphate as an oxidising agent for sugars.

Potentiometric titrations by Furman and Evans, (143) have shown a close relationship between the potential of the ceric (Ce^{++++} - cerrous (Ce^{+++}) system to that of the ferric-ferrous system. The potentiometric application of the ferric-ferrous system has been previously pointed out in this thesis. The advantage of applying an oxidising agent in an acid media such as ceric sulphate in sulphuric acid is that it would combine hydrolysis of more complex sugars with oxidation. Ceric sulphate is stable in sulphuric acid and is not sensitive to light or air, but it is highly sensitive to reducing substances to form the cerous salt. With this in mind, the potentiometric application to the ceric-cerous system seemed feasible and a preliminary experimental study was made.

Experimental Application

Ceric sulphate in an acid medium was used to determine glucose, sorbitol, mannitol, dulcitol, isomannide, sucrose, mannose, fructose, galactose, inulin and glycogen. It was apparent that ceric sulphate is such a strong oxidizing agent that it reacts with a great variety of sugars and sugar-like compounds.

Ceric sulphate was found to be unstable to heat in the presence of 0.25 N sulphuric acid. A whitish precipitate which was formed was thought to be a mixture of ceric and cerous hydrates and cerous hydroxides and carbonates. Chart VIII represents the solubility of the cerous sulphate salts in their relationship to heat and sulphuric acid concentration. These curves demonstrate that the acid concentration of the ceric sulphate solution must be above 0.7 N. It was found that at this acidity no precipitate formed and all of the cerous-ceric salts were retained in solution. Thus it was easy to determine with the potentiometer or by direct titration of the ceric salt with ferrous ammonium sulphate the required concentration of ceric sulphate for any sugar concentration.

A reagent was finally developed containing 0.018 N ceric sulphate in 1.5 N sulphuric acid. 5 cc of this reagent was used with 5 cc of a sugar solution containing not more than 1.2 milligrams of glucose. Direct titrations of the ceric salt left or of the cerous salt formed after oxidation was easily accomplished with the use of

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A reagent was finally developed containing 0.018 N ceric sulphate in 1.5 N sulphuric acid. 5 cc of this reagent was used with 5 cc of a sugar solution containing not more than 1.8 milligrams of glucose. Direct titrations of the ceric salt left or of the cerous salt formed after oxidation was easily accomplished with the use of

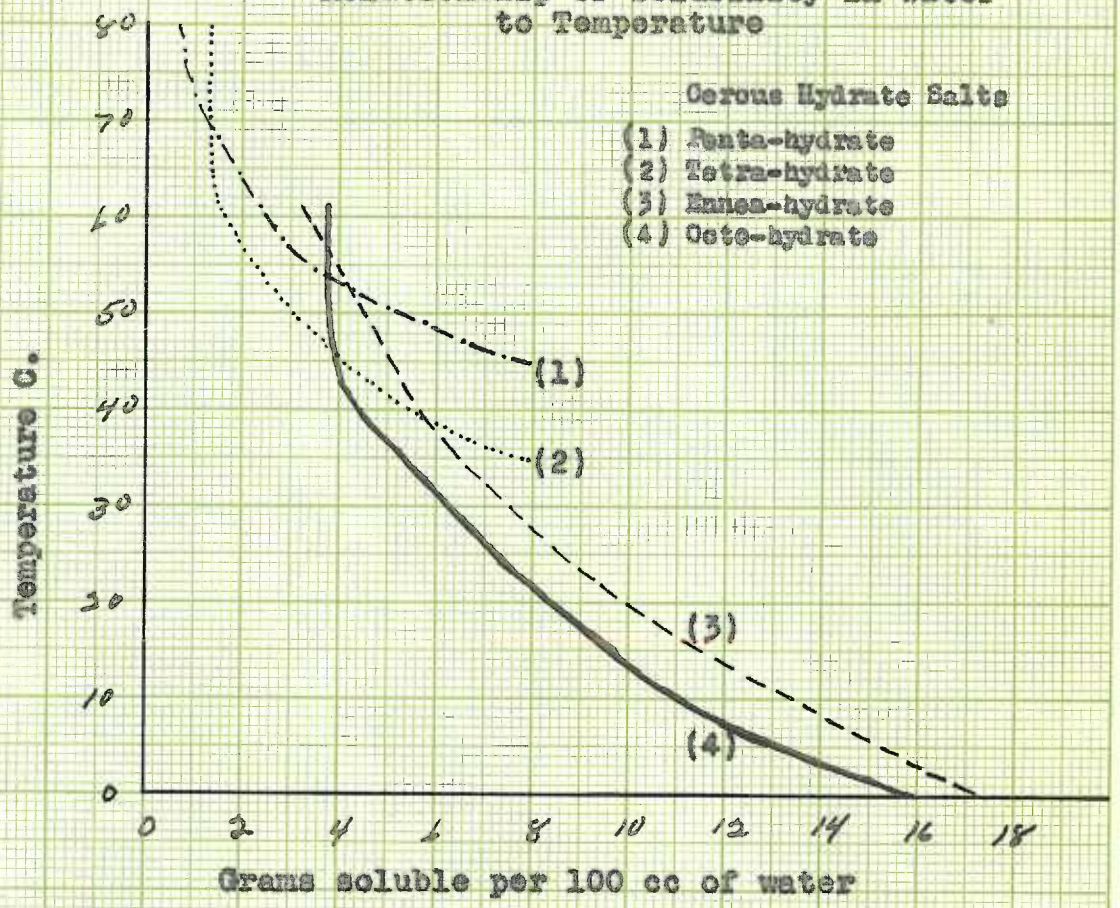
0.005 N ferrous ammonium sulphate or 0.005 N ferric sulphate. It was found that with glucose after heating for 15 minutes no further oxidation took place. This method offers a direct procedure for the study of the electronic change in the oxidation of glucose, sorbitol, mannitol, inisitol and the hydrolysis and oxidation of sucrose, inulin and glycogen. This same reagent was found to be applicable to blood and urine filtrates. Thus sucrose in blood and urine was determined directly by this technique. The results were comparable to those obtained by the well known hydrolytic methods.

An iodometric method was tried similar to that which was reported as applicable for blood and urine determinations of isomannide. The ceric sulphate was made up in 2 N sulphuric acid with an excess of the ceric salt. This procedure was found to be inapplicable. The end point was difficult to obtain and a direct combination of the ceric sulphate with sodium thiosulphate is known to take place. It is questionable whether the results obtained by Krantz and Carr, (142) are acceptable until their method and data are published concerning the direct use of ceric sulphate as an oxidising agent.

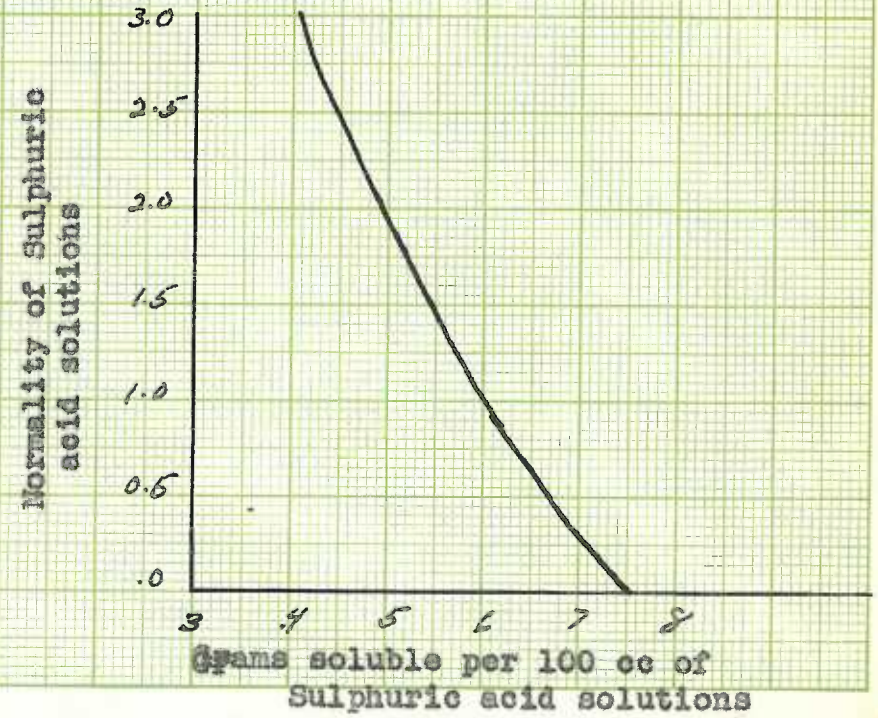
CHART VIII

SOLUBILITY OF CEROUS SULPHATE

Relationship of Solubility in water to Temperature



Relationship of solubility to Sulphuric acid concentration



CONCLUSION

Part I.

There is presented a review concerning the human kidney and its functions. The present renal function tests are still in a state of confusion. The use of inulin and diodrast ratios is becoming a standard renal test for the glomerular and the tubular function. The renal blood flow is an important factor concerning the intermittency of glomerular function. Further investigation may prove whether or not there is an arterio-venous shunt from the afferent arteriole to the peritubular spaces. There is also presented a review of many of the factors involved in diuresis. Diabetes insipidus is a syndrome in which the excretion of water is due to the lack of a hormone called pitressin. Pitressin is produced by cells of the pituitary gland and acts as an antidiuretic in both normal and pathological conditions. Pitressin may act on the vasomotor center or it may act directly to regulate the reabsorption of water in the renal tubules.

Addison's disease is definitely associated with diuresis. The cortex of the adrenal gland produces a hormone which acts on the body tissues and the renal tubules to regulate the balance of the sodium or potassium and chloride ions. Diuresis in Addison's disease occurs when there is an excessive loss of sodium or potassium and chlorides.

fell rapidly and reached the pre-experimental basal level in 2 hours. In cases of liver damage the blood sorbitol did not fall as rapidly and had not reached the basal level at the end of a 3 hour period. Blood glucose curves showed an increase following the injection of sorbitol in all the cases studied. 85 percent of all the sorbitol was retained or metabolized in the human body except in the cases of liver damage. There was also noted in the blood of individuals with a disturbed albumin-globulin ratio some substance which did not reduce the Shaffer-Hartman reagent but did cause reduction in the more highly alkaline ferricyanide reagent. This material was not urea.

In 24 cases there was an average urinary excretion of only 15 percent of the administered dosage. The amounts administered did not alter the excretion of sorbitol. In cases of liver damage it was noted that 37 to 47 percent of the injected sorbitol was recoverable in the first 24 hours. The following 24 hours did not reveal an increase in the ferricyanide reduction above the pre-experimental basal level.

100 to 200 cc of 50 percent sorbitol were administered to each of 25 patients. Out of this group only one showed a greater 24 hour urinary output than previously. In the other 24 cases

there was a diminution in the urinary output. Diuresis which did occur during the first 4 hours was dependent on blood and urine sorbitol concentration. Following this diuresis there occurred a relative anuria for the following 21 hours with a retention of fluid in 96 percent of the cases. Similar observations were noted with the use of hypertonic glucose and sucrose. The administration of clinical dosages of hypertonic solutions to the patients studied with diseases related to the cardio-vascular system, to the kidneys, to the liver and to the neurological system failed to produce a clinical dehydration.

The use of 1 or 2 cc of 10 percent solution of salyrgan in combination with sorbitol or sucrose intravenously produced a rapid excretion of urine followed by a continued urinary output which resulted in a clinical diuresis and tissue dehydration. This combination was effective in the reduction of edema due to a hypoproteinemia or a reversal of the albumin-globulin ratio. Its application should be effective over a prolonged period in the reduction of increased cerebro-spinal pressure due to edema of the brain.

In every case studied in which clinical diuresis was produced by salyrgan alone, or in one case with sorbitol alone, and in cases with the combined action of salyrgan and sorbitol or salyrgan and sucrose, there was an increase in the urinary chloride output. Therefore there seems to be a direct relationship between the chloride excretion and clinical diuresis.

Aminophylline in combination with hypertonic solutions did not produce a clinical diuresis in any of the cases studied.

Following the intravenous administration of 50 percent sorbitol at the rate of 8 to 10 cc per minute there was noted in almost every case epigastric distress, nausea, flushing, tingling of the face, chest pain, dyspnea and headache. Sorbitol produced a complete anuria for 8 hours following its administration in a case of eclampsia. The administration of 50 percent sucrose produced a similar picture and in one case an anuria for 4 hours following its administration.

PART II

Ferricyanide Reagent

Time was the limiting factor in the completion of the potentiometric method for the determination of sugar alcohols. With a potentiometric study of the ratio of the ferric to the ferrous ions it will be possible to speculate upon the electronic shift in the oxidation of any sugar.

The potentiometric method corresponded with the iodometric method and was applied to the study of 25 clinical cases. In these cases sorbitol determinations were repeated with consistent results in the urine and blood at different intervals. It was felt that the analytical procedure was adequate for this type of clinical study.

Further investigation should be carried out with this reagent using the principles as are pointed out in this paper.

Ceric Sulphate

Ceric sulphate offers a new method for the determination and the physico-chemical study of sugar oxidation. Ceric sulphate was found applicable to blood and urine filtrates. Sucrose in blood and urine was determined directly by this technique. The results were comparable to those obtained by the hydrolytic methods.

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