1955

Insulin : chemical, physiological and clinical considerations

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Submitted in Partial Fulfillment for the Degree of
Doctor of Medicine

College of Medicine, University of Nebraska

April 1, 1955

Omaha, Nebraska

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INTRODUCTION

Since the first papers on the isolation of insulin were published in 1922, many millions of words have been printed on the subject. In going over the literature, it is no simple matter to pick from the numerous bibliographies and papers which discuss the complete story of insulin, the various insulin modifications, and a study of the comparative actions and advantages and disadvantages of the many preparations commercially available. To say the least, to the doctor who does not have to deal with diabetes regularly in his practice, and especially to the medical student, whose mind is already so filled with such an array of connected and disconnected facts, the intelligent use of insulin becomes a rather complicated problem. As Gerritzen (1) states, "To choose the right insulin for a certain diabetic patient is not a simple matter. It requires a thorough study of the particular manifestations of the case, and a knowledge of the characteristics of the various insulins existing, to adapt the insulin therapy to the individual need. It would be of great value, therefore, if the physician could be guided in the choice of the most suitable insulin, or if the patient is already using insulin, in making a change that might be of benefit in the particular case."

This paper, therefore, represents an attempt to tell the story of insulin, and to discuss the most important of the insulin preparations available, so that treatment of diabetes by proper selection of the indicated insulin preparation will be simplified.

HISTORICAL ASPECTS

Diabetes mellitus, as a disease in man, has been known and recognized for many centuries. Much has been written about diabetes and various different methods have been used to control its clinical manifestations over the years. However, it was not until the dis-covery by Banting and Best of the active principal of the Islets of Langerhans insulin, that it became possible for persons with severe diabetes to lead acceptably normal and productive lives.

The Islets of Langerhans were first described by Langerhans in the year 1869. This man was a histologist and he made no attempt to ascribe any particular function to them. Von Mering and Minkowski in 1889 made the fundamental discovery that total removal of the pan-creas in dogs produced a disease which resembled ordinary human dia-betes mellitus, producing in their laboratory animals, glycosuria, hyperglycemia, ketonemia, and loss of body weight. La Guesse, a Frenchman, was the first man to refer to the pancreatic islets as Langerhans islets. This reference is found in papers published by La Guesse as early as 1893. The name insulin for the as yet hypo-thetical secretion of the islets appeared as early as 1909 (de Meyer). (2).

It was Dr. Banting, in the year 1921, who first conceived in his mind a workable plan for the isolation of islet tissue and hence its active principal, insulin. Dr. Banting was a not too successful practicing physician of a small Canadian town, who supplemented his

meager income from medicine, by giving lectures at a near college. It was during the preparation of some notes on a lecture he was to give on the pancreas that his now famous idea came to him. It was his idea that if the pancreatic ducts which emptied digestive enzymes into the duodenum were ligated, it would cause that part of the pan-creas that was responsible for the production of the digestive juices to atrophy, thus leaving only the Islets of Langerhans, which he hoped would still be in a normal productive state. He presented his idea to the head of the physiology department of the Univ. of Toronto, Pro-fessor MacLeod, and after a time, it was agreed that Dr. Banting might use the facilities of the university for his work during one summer when regular classes were not being held. It was also decided that C. H. Best, then only a student with an organic chemistry background, would be his assistant. Best is said to have asked Banting how much he would be paid and Banting is said to have answered, "Your salary will be twice mine and my salary is nothing." Thus was started the work on the isolation of the active principle of the Islets of Lan-gerhans, which culminated in the discovery of insulin, the perpetual fame of these two great men, and finally the actuality of normal, healthy and productive lives for the millions of persons suffering from diabetes the world over.

The first insulin injected into living animals, and which con-trolled blood sugar, was prepared from the atrophied pancreatic tissue of dogs. The pancreatic tissue was removed from dogs which had, some weeks previously, been subjected to the surgical procedure in which

the duct draining into the duodenum has been ligated. This material was placed in a mortar with some sand and thoroughly ground up and then an extract of the ground-up material was made and subsequently injected into other diabetic dogs. Banting and Best knew that an important milestone in medicine had been reached when they demonstrated the blood sugar lowering effect of this extract. It was soon found that a better yield of insulin could be gotten in a much less tedious manner by extracting insulin from fresh beef pancreas with alcohol, some of the fat being removed from the preparation with a fat solvent. The first human subjects to receive insulin were, first, Banting and then Best. The first diabetic patient to receive insulin was Leonard Thompson, a diabetic boy in a medical ward of the Toronto General Hospital. (3)

With the discovery of insulin, as has been so aptly stated by Campbell, (4) "Diabetes has been changed from a metabolic disease, incapacitating and progressing slowly or more rapidly to an inevitable end, to a metabolic defect. And this metabolic defect is controllable by suitable diet and skillful application of crutches called insulin; thus enabling the patient and his family to live long, happy, and useful lives. The physician who cannot use this weapon for the strengthening of his patients moral; who cannot make his patient see that science has discovered a way to train him to win the game is, indeed, to be pitied: he and his patient also, for hope well founded is the most precious thing that we can offer the suffering."

CHEMISTRY OF INSULIN

The exact structure of the insulin molecule is still to be worked out; however, great strides have been made in the past several decades toward the understanding of the structure of this complex protein molecule. The insulin molecule is built almost entirely of amino acids and i richer in the acid leucine, glutamic acid and cystine than most other protein molecules known. Chubnall concludes from his work that insulin is a system of 18 peptide chains of unlike composition. He has isolated the following amino acids and their approximate % concentration accounting for 95% of the insulin molecule.

arginnie	3.05%	amide ammonia	1.65%	proline	10.%
histidine	10.70%	leusine	13.4%	phenylalamine	1%
lysine	1.26%	glutamic acid	17.5%	serine	3.57%
cystine	12.5%	alamine	4.7%	threonine	2.66%
tyrosine	12.5%				

The maximum molecular weight of insulin when in a .4 - 9% solution at a PH of 7 - 7.5 is 48,000, but when more dilute solutions of the hormone are used at PH values below 4 or above 7.5, the insulin molecule dissociates into subunits having molecular weights of about 12,000. When insulin is oxidized with performic acid, the molecule is split into its seperate polypeptide chains. Of these, two fractions have been isolated; the first being an acidic fraction, containing no basic amino acids and, the second being a basic fraction. It is felt that these two physiologically inactive components are normally linked together by the - S - S bridges of cystine and perhaps by other as

yet unknown bonds. It s also thought that the 12,000 mol. weight fractions are composed of two identical acid chains and two identical basic chains. Jensen ( ) states that the insulin molecule contains 3.2% sulfur. When a dilute solution of insulin containing small amounts of salt is heated, a flocculant precipitate is formed. Waugh (7) has shown that insulin can be modified to yield fibrils. In fibril formation two reactions are involved. First the formation of active centers, and second the elongation of these into fibrils. The rate of fibril formation increases with increasing hydrogen ion concentration, salt and protein concentration, and temperature. The resulting fibrils have little or no anti-diabetic activity, but can be converted into active insulin by changing the reaction to the alkaline side. He also found that by seeding an insulin solution with fibrils a complete conversion of the active insulin into inactive fibrils was brought about. Insulin s hydrolized by pepsin, trypsin, kinase and papain and the physiological activity is permanently lost. The potency of insulin seems to be related to the intact structure of the molecule. During the hydrolysis of insulin with any one of the above enzymes, the physiological activity disappears much more rapidly than does the digestion of the protein. This fact would suggest, according to Scott and Fisher (5) that those linkages which are first attacked by the various enzymes are of special significance for the physiological action. With this in mind, attempts were made to follow the tyrosine content of insulin during hydrolysis by pepsin. In these experiments, this amino acid was one of the first to be split off, whereas the

cystine content of the protein remained practically unchanged during the early stages of digestion. It could not be established, however, that the loss of activity was directly related to the tyrosine fraction. Other experiments indicate that certain hydroxy, amino, or imino groups are concerned with the physiological activity of insulin.

Crystalline insulin can be prepared by the addition of metal salts of zinc, nickel, cobalt and cadmium. These crystals are slightly doubly refractive and have a refractive index of 1.58. Microscopically these crystals present the appearance of rhombohedra, approximating to cubes which are standing on one corner. It has also been shown that insulin will form crystalline products with each of the bases, iso-amylamine, n-amylamine, and piperidine. In general, these crystals have a needle-like appearance.

It is generally assumed that both endogenous and exogenous insulins are rapidly destroyed in the organism, but knowledge of the site and mechanism of this destruction is incomplete. Karelity and associates in 1930 showed that blood cells in vitro showed a greater inactivating effect on insulin than did plasma alone. This effect was multiplied two to three times if the blood cells were laked. It was concluded from this that inactivating factors in the blood were mainly intracellular.

PHYSIOLOGY OF INSULIN

As is true of the chemistry, much remains to be learned concerning the physiological role insulin plays in human metabolism. That insulin exerts a profound effect on blood sugar levels was demonstrated by Banting and Best in their early work. These workers could only guess, however, the exact mechanism of this action. Insulin is produced in the Beto cells found in the Islets of Langerhans and the Beta granules found there-in are the morphologic expression of the insulin content. The function of the alpha cells, also present in the islets, remains unknown.

Insulin promotes the oxidation of glucose by the tissues, thereby causing a corresponding reduction of the blood sugar. Insulin is necessary in deposition of glycogen in the liver and muscle. Insulin plays a part in the regulation of sugar formation from protein and fat in the liver. Insulin is also utilized in the promotion of the deposition of fat from glucose in the diet and prevention of hetasis.

Insulin is a hormone and as such does not initiate any new metabolic process in the body, but, rather, influences the rate of speed of existing processes by accelerating or inhibiting certain enzymatic reactions. Best ( ) states that one molecule of insulin accounts for the comp ete utilization of some 15,000,000 molecules of glucose per hour. In the metabolism of glucose the phosphorylation of glucose by ATP to glucose 6 phosphate takes place. This phosphory-lation is catylized by hexckinase and is a necessary reaction for

further biochemical oxidation of glucose. The transformation of glucose into clycogen is also dependent upon initial phosphorylation. It was observed in 945 that the hexokinase reaction can be inhibited by an extract of the anterior lobe of the pituitary gland and that this inhibition is abolished by insulin. It was later observed that certain hormones from the adrenal gland exerted the same inhibitory effect on the hexokinase reaction. It was also noted that certain pituitary and adrenal hormones tended by their action to increase carbohydrate formation from protein by the liver, and to diminish the peripheral utilization of glucose, while the pancreatic hormone, insulin, had an opposing action at both sites. Both of these effects of insulin are explained in terms of an increase activity of the enzyme hexokinase. The methods were devised for measuring hexo-kinase activity in extracts of muscle and other animal tissue. The addition of insulin to such preparations had no effect on the rate of the hexokinase reaction. It was found, however, that the rate of the hexokinase reaction could be depressed by the addition of certain protein fractions obtained from the pituitary gland and that inhibition of the reaction could be much more marked if the pituitary extract was supplemented with adrenal cortical extract. By itself, the adrenal cortical extract exerted no inhibition. The inhibitory effect of this combination of pituitary and adrenal extracts was shown to be completely abolished when insulin was added to the extracts. It was also shown that insulin which had been altered so as to lose its blood sugar lowering activity by the addition of a mild alkali, also lost

its ability to exert an effect on the hexokinase system in tissue extracts combined with the adrenal and pituitary hormones.

When hexokinase activity was measured in muscle extracts from diabetic animals, adrenal cortex extract alone was found to produce inhibition as great as that obtained by the addition of both pituitary and adrenal cortical hormone in normal tissue extracts. From this observation it is thought that there must be present in diabetic extracts an inhibitory factor of pituitary origin, in sufficient amounts to give rise to marked inhibition in insulin free extracts when adrenal cortical hormone is added.

This work has led Colowick (9) to the conclusion that insulin is not essential for glucose utilization, but serves rather to oppose the inhibitory actior of anterior pituitary and adrenal cortical hor-mones on this process. No insulin effect on glucose utilization could be obtained in these experiments unless these inhibitory factors were present. But the fact remains that insulin can also exert certain effects in the absence of pituitary and adrenal factors, as demonstrated by its profound hypoglycenic effect on adrenalectomyed and by hypophysectionized animals.

Of interest is the work reported by Houssay (10), who states that very different amounts of pancreatic tissue, with or without innervation or in grafts, secrete insulin at the exact rate needed to maintain the normal blood sugar. The blood sugar level can be kept normal by a pancreas reduced to one/seventh its original mass and also in a dog which has three pancreases grafted into its cir-

culation. On the other hand a reduced pancreas secretes sufficient insulin to maintain normal blood sugar, but it is unable to correct diabetic hyperglycemia.

THE VARIOUS COMMERCIALLY AVAILABLE INSULINS

As stated by Margolin (11) commercially available insulin preparations can be classified according to duration of action into three main categories, namely; 1. Rapid Acting Insulin, 2. Intermediate Acting Insulin, and 3. Prolonged Acting Insulin. The important insulin preparations to be considered in these categories include the following:

1. Rapid Acting
   (a) Regular Amorphous Insulin
   (b) Crystalline Insulin
   (c) Semi Lente Insulin (not available commercially in the U.S.)

2. Intermediate Acting
   (a) Globin Insulin
   (b) NPH Insulin
   (c) Lente Insulin

3. Prolonged Acting
   (a) Protamine Zinc Insulin
   (b) Ultra Lente Insulin (not available commercially in the U.S.)

To the student of diabetes and insulin therapy the above outline may seem to be rather incomplete, and it can be argued with probable justification that other preparations should be included for consider-ation. However, since the discovery of protamine insulin by Hagedorn, and its introduction into clinical usage in 1936, there have been a constant stream of modifications of insulin having the common attributes of prolonged action by virtue of differences in soluability in tissue fluids at the approximate PH of the body. Combinations of

insulin with protein precipitants (protamines, histones, globin, kyrin) or chemicals (hexamine, iso-cyanate, poly vinyl pyrroladone) have been used. Physical preparations vary from a clear to cloudy suspensions. By varying the quantity of agent used in proportion to the insulin, and the zinc content, or both, either in bulk, or ex-temporaneously by mixing, the preparation of a wide variety of com-binations having time activities ranging between the short profound action of regular insulin and the long slow effect of PZI is made possible. However, all preparations display the fundamental character-istic hypoglycemic reaction of insulin and they differ from one another only in their rate of onset of action and duration of effect. Thus far, duration of action has never been obtained except at the expense of rapidity of onset. Clinically the major problem has been to deter-mine which particular time activity best meets the average daily needs of the greatest number of diabetic patients.

AMORPHCUS INSULIN AND CRYSTALLINE INSULIN

As has been stated, the first insulin to be isolated was extracted from the pancreas of laboratory dogs by Banting and Best. This first insulin was essentially the same type of insulin as what is now known as unmodified or amorphous insulin. Banting and Best used somewhat crude methods for extraction of their preparations and as a result their amorphous insulin was not in as pure a state as present day commercially prepared amorphous insulin.

Most insulin is prepared from fresh beef or pork pancreas and is extracted from these organs with a 1/6 normal solution of acid alcohol. This acidic solvent has the effect of putting the insulin molecule in solution and thus washing it out of the pancreas along with some other similar protein materials. The alcohol is then dis-tilled off leaving an acidic solution of amorphous insulin. Commercial preparations contain .1% phenol as a preservative. PH of the preparation is 2.5 to 3.5. The biological activity of the insulin is assay on rabbits and mice. It is supplied in 10 cc vials in 40, 80 and 100 uni s per cc.

With the isolation of sizeable amounts of amorphous insulin, work was soon begun in an attempt to prepare a pure crystalline pro-duct that could be seperated from the impurities extracted along with insulin. Crystalline insulin was first prepared by Abel (12) in 1926. His method included the use of pyridine and brucine in order to pro-mote crystal formation, but it was tedious and time consuming with

poor yields and not applicable for large scale production. However, this was the first instance of a hormone having the properties of a protein being prepared in a crystalline form. In 1928 Harrington and Scott succeeded in forming a crystalline insulin employing saponin to promote crystal formation. Their method also proved unsatisfactory because different samples of reagents used to promote crystallization behaved quite differently in their power to promote crystal formation and also yields by their method accounted for only 5 - 15% of the activity of the crude insulin powder. However, their crystals had the same microscopic appearance and chemical analysis as those isolated by Abel. The results also showed that different batches of insulin crystals prepared by different methods had the same physiological activity, namely, 24 international units per mg.

During 1929-30 many experiments were conducted in attempts to improve the existing methods of crystallizing insulin. Further work indicated the presence of some substance, apparently unknown to all persons working on the problem that implemented crystal formation. It seemed probable that an inorganic substance played a definite role and since it was known that the pancreas contained appreciable amounts of cobalt, zinc, and nickel, it was not long before it was shown that the addition of zinc to an insulin preparation resulted in almost complate crystallization of the insulin. The amount of zinc necessary to affect crystallization was about 1 mg. of zinc to 1000 international units of insulin. The crystals obtained in this manner were found to have the same microscopic appearance and physiologic activity as the crystals that had been isolated by the earlier methods. Further

work demonstrated that a similar effect could be obtained using either of the metals cobalt, nickel or cadmium.

Thus a crystalline insulin in a highly purified state and free of foreign protein had been isolated by a method that was applicable to large scale production and which gave almost 100% recovery of the active product from the crude. Zinc Insulin crystals are slightly doubly refractive and have a refractive index of 1.58. Scott and Fisher (13) feel that zinc insulin is a metallic salt. Since there is no foreign protein presen in zinc insulin, it is preferable in allergic patients.

Commercially prepared zinc insulin is a purified aqueous solution of crystalline insulin. It contains .1% phenol as a preservative and has a PH of 2.5 to 3.5. It is supplied in 10 cc vials of 40 and 80 units per cc.

Crystalline insulin and amorphous insulin are similar in action. Peck, F. B. (14) states that there is no significant difference in the action of highly purified amorphous insulin and crystalline insulin and it is possible to interchange them at will in the treatment of diabetes whenever a rapidly acting preparation is desirable. Other workers, such as Ricketts and Wilder (15) and Marble and Vartiainen (16) report the same conclusions.

Crystalline and amorphous insulins exhibit a rapid onset of blood lowering effect which, on the average, is noticeable within an hour. Peak effect is noted in 3 to 4 hours and is ended by 8 to 12 hours. Duration of action can be varied somewhat depending on the

site of injection, size of dose, concentration, blood sugar level, and unknown factors affecting the sensitivity of the patient. Gerritzen (1) found in the study of the action of regular insulin on normal healthy students that this insulin caused the blood sugar to reach a low point after one hour and return to the starting point in 8 hours.

Because of their prompt and intense but rapidly waning action these preparations are now most commonly used to supplement modified insulins, such as PZI, in severe diabetes requiring large dosage. Haunz (35) resorts to the use of multiple doses of regular insulin exclusively, in his attempt to control the "brittle" diabetic. They are also widely used to treat diabetic emergencies, such as, pre-operative and post-operative management in surgery, acidosis and coma, or infections and injuries. They are also useful during the initial regulation of insulin dosage. They are used for shock therapy in schizophrenia where doses as high as 150 to 200 units are sometimes employed. In malnourished and depressed states they are employed in dosages ranging from 10 to 40 units to stimulate the appetite.

The usual mode of administration is subcutaneously 15 to 20 minutes before meals. They are the only preparations injected intra-venously in emergencies.

PROTAMINE ZINC INSULIN

Protamine zinc insulin was the first of the long acting insulins to be developed which has stood the test of time in the treatment of diabetes. Jamieson (17) reports that in 1951 PZI accounted for more than 65% of the total insulin preparations used in Canada. Over the last quarter of a century there has been a gradual evolution of methods of insulin therapy in the management of diabetes. The original short acting preparations required multiple daily injections in many cases in order that blood sugar levels could be controlled during the daylight hours when food was being taken. It was also found that in severe cases the blood sugar could not be satisfactorily controlled in the fasting state, when the patient was asleep, with the short acting preparations.

This problem of the control of nocturnal blood sugar occupied the minds of many, and much work was done in order to develop an insulin with delayed absorption which would be effective during the night. Various procedures were tried in order to slow down absorption. Insulin as a suspension or an emulsion in oil was tried. It was injected together with a vasoconstrictor substance. Sparing by soluable insulin compounds were developed. In order to get less soluability the insulin was combined with kyrin, histones, and
finally globins and protamines.

Much of the work with protamines was done in the laboratory of Hagedorn in Denmark. Protamines are protein molecules extracted from

the ripe sperm of fish. Protamines can be subdivided into monoprotamines, deprotamines or triprotamines according to their con-tent of the basic constituents of lysin, arginine, and histadine. When protamine is added to insulin the two molecules form a loosely bound compound molecule which precipitates out of solution at a neutral PH. The amount of protamine that combines with insulin is 1/10 the weight of the insulin according to Scott and Fisher.(5)

The first insulin preparations containing protamine were used clinically before the effect of adding zinc to further prolong the action of protamin insulin was known. The literature contains many reports concerning the use of protamine insulin without zinc. Many of the clinicians mixed their own protamine insulin preparations. Root (18) and his group reported that this early preparation had a prolonged effect, however, its effect was noted for less than twenty-four hours. They also complained about the inconvenience of having to mix the protamine and insulin.

Much speculation has arisen as to the exact chemical mechanism for the release of insulin from protamine after injection into the depot. Sindoni (19) reports that the soluability of protamine insulin after administration depends upon the amount of serum present in the depot. Protamine combines with tissue fluid protein. The tissue protein has the effect of displacing the insulin from the protamine and releasing it. He suggested that variations in the amount of protein in tissue fluid or variations in the amount of protamine present in the insulin would cause variations in the soluability

and rate of absorption of insulin from the depot. In other words, a high concentration of tissue fluid protein or a small portion of protamine would favor release of insulin, while a low concentration of tissue fluid protein or an excess of protamine would result in a slow release of insu in. Evidence was presented by other workers that the splitting o protamine insulin is an enzymatic process and that the actual enzyme is probably of the cathepsin type.

Not long after the in roduction of protamine insulin Scott, D.A. and Fisher, A. M. (15) reported that zinc was an essential constituent of crystalline insulin and was also present in large amounts in the pancreas. This led workers to investigate the effect of the addition of zinc to protamine insulin, for it was known that many metals form complex compounds with basic substances such as protamine. It was found that the physical stability of protamine insulin was much improved by the addition of any of several metals, and that some of these resulted in additional prolonged hypoglycemia. However, the only metals that had desirable effects without causing loss of potency were the metals zinc nickel, cobalt and cadmium.

Thus, protamine zinc insulin, as we know it today, was developed and rapidly became the foundation of treatment. It con-tains 1.25 mg. of protamine and .2 g. of zinc per 100 units of in-sulin. Commercial preparations contain .25% phenol as a preservative. It is buffered at a pH of 7.1 to 7.4 and forms a milky white suspen- sion on shaking. Theoretically, .67 mg. of protamine is sufficient for complete precipi ation of the insulin present, but in actual

practice complete precipitation does not occur. A small amount of insulin remained in soluti n. In order to precipitate ll of the small amount of insulin remaining and to place the soluability of the combination wel beyond the point where variations might result in significant alteration of the rate of activity, a substantial excess is added to the extent of 1.25 mg. The excess also has the effect of combining with tissue proteins about the depot of injection, thereby delaying absorption still further and increasing prolonged action of the produ t.

Protamine zinc insulin shows little effect on the blood sugar in the first 6 - 8 hours. Its effect then becomes more intense and a maximum effect is noted in 16 - 18 hours, with still some action present at 24 hours. Its effect has been reported to persist for as long as 30 - 36 hours. Gerritzen (1) reports in his study of the action of PZI on healthy students that it had a duration of action of 18 hours with the low point reached in 5 - 8 hours.

PZI is most commonly used to control moderately severe and severe diabetics and is excellent as a single injection where the dose is 40 units or less. In higher doses the preparation will cause hypoglycemic reactions at night. It is injected only sub-cutaneously, never intravenously or intramuscularly. Before use, it is recommended that the vial be rolled gently between the hands or shaken gently to resuspend the contents. Dosage is determined by the needs of the individual patient. It is recommended that the initial dose should be about 2/3 of the daily amount of unmodified insulin needed to keep the patient sugar free. Fasting blood sugars

are the best guide for requirements. The dose that gives a normal to somewhat subnormal fasting blood sugar is the highest dose that can be given without danger of causing severe insulin shock during the sleeping hours. The patients' diet and eating habits have to be adjusted to fit the pharmacology of the preparation. Some workers recommend that the total carbohydrate be divided so that 1/5 is taken at breakfast, 2/5 at lunch and 2/5 at supper. Diet problems vary from patient to patient and it may be necessary to resort to between meal feedings and a bedtime snack. PZI insulin is supplied in 10 cc vials of 40 unit and 80 unit strengths. The required dose is usually taken by the patient 1 to $1\frac{1}{2}$ hours before breakfast.

Margolin (11) feels that the chief importance of PZI is that nocturnal control of the blood sugar level makes it possible to eliminate night shots. He also states that the cumulative overlap effect, which is attained in 2 - 3 days after starting on this in-sulin results in a onstant supply of insulin. As a disadvantage to the use of PZI he states that allergic reactions are most often seen with this preparation. Bindoni (19) reports that patients using PZI develop a dread of hypoglycemic reactions at night. They awake with a tingling sensation around the mouth and on the fingers,
or in a mental stupor to be succeeded by profound insulin coma. These patients may develop signs of mental impairment, as lapse of memory, forgetfulness, blurred vision, loss of appetite, frequent headache, morning nausea and vomiting, weakness in the legs or marked fatigue.

He states that patients may carry a low blood sugar for some time without apparent signs or symptoms, and he points out that if the blood sugar supply is cut off for a few hours by insulin, irreversible nerve cell changes may occur. It has been shown in dogs that prolonged hypoglycemia can cause blindness, deafness, disturbed emotion, incoordination, spasticity and paralysis.

In spite of PZI with its control of nocturnal blood sugar there remained a group of severe cases which still required multiple doses of regular insulin along with PZI before both daytime and night time control could be established successfully due to the slow on-set of action of the PZI preparation. With their eyes always on the goal of preparing an ideal single daily shot preparation, the next step of the early workers was to find a combination of a short and long acting insulin that would give an early initial response coupled with the long action of PZI. Regular insulin was mixed with PZI in the same syringe and after much clinical trial it was decided that the 2:1 mixture (2 regular: 1 PZI) gave the most satisfactory control for most diabetics as was reported by Colwell. (20) The dosage of the mixture was estimated in accordance with the amount of urinary sugar present in the day specimens and the morning fasting blood sugar. The rapid acting portion being based on the daily urine sugar and the slow acting based on the fasting blood.

Such mixtures were far from ideal due mostly to the technical difficulty of their preparation. Both insulins had to be of the same unitage, either 40 or 80 units, and it was preferred that the same

manufacturer had prepared the insulins. The regular insulin was first drawn into the syringe, followed by the PZI. When the two are mixed a sizeable portion of the regular is precipitated by the excess prota-mine present in the PZI and becomes converted into the long acting insulin. This was undesirable due to the extreme care necessary in preparing such a mixture and also due to the fact that insulin activity varied fro batch to batch making it difficult to accurately predict just how a particular mixture would effect blood sugar levels.

After much work was done trying to perfect this type of mixture, there then followed a deliberate attempt by various workers to produce a preparation which was both stable and uniform, and which could duplicate the action of the 2:1 mixture in a practical manner.

NPH INSULIN

Neutral Protamine Hagedorn Insulin or NPH, as it is called, is an insulin preparation giving a prolonged hypoglycemic effect, which is similar to PZI in that both preparations contain protamine, zinc and insulin, but NPH by virtue of its own particular chemical structure is an insulin classified as intermediate in action.

In the preparation of NPH insulin an acidic solution of zinc insulin crystals and protamine in the proper amounts are placed in vials. This is then followed by an alkaline buffer solution, such that the acidity is raised to 7.2 and results in the precipitation of protamine zinc insulin crystals. One of the essential steps in the preparation is the use of purified protamine in a quantity such that it is sufficient to precipitate all the anti-diabetic activity of the preparation. NPH forms a white suspension upon shaking and is a buffered aqueous suspension of crystals. A small amount of nacc is included in the product as means of insuring the preparation of a uniform crystalline product. A small amount of glycerine is also present and metacresol is used as a preservative. All of the protamine present in the preparation is combined with insulin, differing in this respect from PZI which has an excess of protamine. The amount of protamine used is .5 mg. per 100 units of insulin.
The protamine is derived from the sperm and testes of fish of the family Salmonidae.

The action of NPH insulin is intermediate between regular insulin and protamine zinc insulin. Gerritzen (1) in the study of the action of various insulins in now diabetic healthy individuals, finds that NPH has a dura ion of action of 11 hours with a low point at 3 hours. Howev r, in diabetic therapy its effect is generally claimed to still be noted as long as 28 - 30 hours with a peak effect noted at 7 - 11 hours. Its action is considerably more rapid than PZI and is claimed to be usually prompt enough to control the after breakfast rise in lood sugar which is not possible with Protamine Zinc Insulin. Its length of action being what it is, it is, therefore, usually able to carry most patients through the period of nocturnal fasting, having le s of an overlapping effect than PZI.

One of the main advantages in the use of NPH is that there is no excess of protamine in the preparation. This enables the clinician to prepare mixtures of NPH and regular insulin in the same syringe, both insulins retaining their own respective actions, and the result-ing solution of the two insulins gives the same reaction as if sepa-rate doses of the two insulins were given at different sites at the same time. In this respect, NPH appears to be a satisfactory answer to the problem of giving more than one shot a day to a patient. In a study of the action of NPH on 41 patients Boganz, et. al. (21) were able to get better control with NPH than they had previously been able to achieve with only PZI. They found that control with NPH was similar to that gotten when a 2:1 mixture of Protamine Zinc Insulin and regular was used. Sharkey and King (22) in a series of

28 cases mixed NPH and regular insulins and were able to improve overall control and to reduce severity of the diabetes in these patients. Edwards, Vance and Mulholland (23) found that the carry over effect of NPH or 2:1 mixtures was a desirable factor in treating severe and complicated cases. They found that NPH showed remarkable uniformity of action during the 24 hour period. Greenhouse (24) states that reactions to NPH insulin tended to be less insidious than those resulting from PZI and were therefore easier to recognize and treat. However, he reported a lack of uniformity as to the time of the day that the insulin reactions occurred. In his series he noted that with NPH insulin 34.5% of the reactions occurred in the morning, 37.7% occurred in the afternoon, and 27.8 occurred in the evening. This phenomenon is rather surprizing since it is known that Protamine Zinc Insulin is prone to cause reactions in the early morning hours and globin insulin has its peak effect in the late afternoon. It is suggested that hypoglycemic reaction with NPH apparently follow individual variations in the patients response to insulin modified, of course, by diet and exercise.

NPH insulin is administered subcutaneously. It should never be injected intradermally, intramuscularly or intravenously. Since it is in the form of a precipitate and not in solution it must be mixed to insure a uniform suspension before being used. Vigorous shaking with frothing is not recommended, but rather, it is recommended that it be mixed by rotation or inversion. It should be stored in a refrigerator, but care must be taken so that it does not freeze. If

precipitated clumps are noted or if the crystals are seen to adhere to the sides of the vial, the insulin should be discarded.

If a mixture of NPH and regular insulins is desired, it is recommended that the unmodified insulin be drawn into the syringe first, followed then by the NPH. These mixtures are less variable in action than mixtures of Protamine Zinc Insulin and regular in-sulin.

Dosage of NPH is individualized to the needs of the particular patient as with other insulins. In new uncomplicated cases, it is recommended that the initial dose of NPH be 2/3 the amount of regular insulin needed to keep the patient sugar free, and if this is not known, ten units should be chosen for the first dose. The dosage is then increased daily or every few days by 3-5 units until adjust-ment is obtained as evidenced by urine and blood sugar levels. If a patient is on Protamine Zinc Insulin, it is recommended that 20% less NPH be used.

JLOaIN INSULIN

Globin insulin, as the name implied, is an insulin preparation to which purified g.obin, derived from beef hemoglobin, has been added. It was found in the laboratory by Reiner (25) that when insulin was mixed with a solution of globin, the resulting solution remained clear at PH of 4 or less and precipitated at Ph's of 5 to 8. He also found that when .2 - .3 mg. of zinc was added to a preparation containing 3.8 mg. of globin per 100 units of insulin, that the resulting hypoglycemic effect in diabetic subjects lasted twice as long as the effect of regular insulin.

The exact chemical nature of the insulin globin union is not known, and presumably when globin insulin is injected into a diabetic some precipitation of the solution occurs at the site of injection as the PH of the solution begins to approach that of the body. It seems possible that at the lower PH values there is very little reaction between the insulin and globin molecules. The resulting solution being only a homogeneous mixture of the two proteins. This could explain the relative rapidity of action of globin insulin, some of the insulin escaping into the circulation before the PH within the depot became high enough to cause precipitation of the insulin globin complex.

According to Sindoni (19) insulin is released from globin by simple solution and dissociation. He found that the soluability varied with PH, being least at PH 6 and that at body PH of 7.2,

there was considerable soluability, which he thought accounted for the rapid release of insulin. However, Reiner feels that the rate of absorption of the insulin is determined, not only by soluability, but other factors as well. He found that varying PH did not greatly effect duration of hypoglycemia, but that decreasing the amount of globin in the preparation shortened the action of this insulin and also that the amount of zinc present affected duration and intensity of action. At any rate, a prolonged acting insulin in the form of a clear solution was produced, which has the advantage that it does not have to be shaken in order to get a uniform suspension as do some of the turbid precipitated preparations.

Commercial globin insulin contains 3.7 mg. of purified beef globin and .31 mg. of zinc per 100 units of insulin. It is supplied in vials at both 80 units per c.c. or 40 units per c.c. The U80 preparation is a clear amber in color and the U40 preparation is water clear. It contains 1.5% glycerine to make the solution iso-tonic and to minimize local irritation and discomfort. It also con-tains .25% phenol and this, along with the PH, are said to contribute to the maintenance of sterility. It is very stable. It has been observed that no noticeable change in potency occurs even after several years storage in the refrigerator. However, it should not be stored at room temperature, as some loss of potency does occur when stored in this manner.

The action of globin insulin is intermediate between regular insulin and protamine zinc insulin. Its action begins two hours after

injection, with hei ht of activity being noted in 10 - 12 hours. There is some disagreement as to the exact length of duration of action; some authors feel that it is completely out of the system in 16 hours and others state that some activity can still be noted at 24 hours. However, it is pointed out, that with increased dosage, both intensity and duration of action will increase, but that with the average dose, there is no overlap with daily injection, so cumulative effect is not a factor. Bauman (27) saw the need for an insulin that exerted its maximum effect during the day when food was being assimilated; and by virtue of its relatively rapid action, globin insulin tends to give better control of past prandial glycosuria and because of its prolonged action also controls nocturnal hyperglycemia.

It is recommended that globin insulin be injected into the deep subcutaneous tissues rather than intramuscularly or into the skin. Sterile technique should be adhered to and the site of the injection should be varied. Care also must be taken not to inject this preparation into the vein, because it precipitates at the PH of the blood. It should not be used to treat diabetic coma.

Globin insulin is said to be the most efficient preparation unit for unit compared with other insulin preparations requiring only 3/4 the amount needed when other insulin is used to obtain the same control. As with the other insulins, dosage is based upon the amount necessary to provide an approximately normal blood sugar or sugar free urine before breakfast on the day following injection.

In starting a new patient on globin insulin, it is recommended that the patient be placed on a diet for several days, then blood sugar and urinary sugar studies be made. One worker recommends that 2/3 of a unit of globin insulin should be used for each gram of sugar excreted in the urine in 24 hours. If, after starting this schedule, it is found that the morning urine specimen contains too much sugar, it would mean that the patient was either getting too little insulin or that his diet was too high in calories, or that he was getting too many calories in his evening meal, or a combination of all three of these factors.

Bauman (28) in his series found that there was a hypoglycemic tendency which was usually seen in the late afternoon. This, he felt, could be adequately controlled by diet, large meals being taken at noon and evening, and an afternoon snack allowed if indicated. He recommended injection one hour before breakfast in order that the effect of the insulin would be felt by the time the morning meal was being assimilated. Some diabetics were found to react rather slowly to globin insulin, the height of reaction coming late in the evening. In these cases a bedtime snack was recommended. In following the effect of globin, it is recommended that a fasting blood sugar, and also a blood sugar, when the effect of the insulin is greatest, be taken. These samples show if hypoglycemic reactions are imminent and also indicate if the patient can tolerate higher doses, if it appears that the action is not prolonged enough.

It is cited a a point in favor of the use of globin, that there is greater freedom from the danger of allergic reactions. Globin in the amoun' used in the preparation is only weakly anti-genic and Bauman states that he has seen no evidence of allergic manifestations with its use. In patients who have a proven allergic tendency toward protamine, the use of globin insulin appears to offer distinct advantages.

THE NEW INSULINS; SEMI LENTE, LENTE AND ULTRA LENTE

Although significant progress has been made in the past twenty years with regards to modifying the action of insulin, and preparing various types of insulins with different ranges of action, it has not been felt by the insulin chemists or clinicians that any insulin preparations yet available were the complete answer as far as diabetic therapy was concerned. Thus the work of preparing new and different types of insulins has been carried out in laboratories in different parts of the world in the hope that finally a one shot preparation could be perfected which would answer the problem of making it possible for the diabetic organism to approach more closely the finely regulated metabolism of the normal organism.

The laboratory of Hallas-Møller of Denmark has been the scene of some of the most intense and worthwhile work on the problem of insulin preparations. These workers for the past fifteen years have been studying insulin chemistry and preparing and testing various insulin preparations and in 1951 they worked out the process for the production of Lente insulin, which appears at the present time to be a rather substantial contribution to the problem of insulin therapy.

In the past few years Hallas-Møller and his group had concentrated on trying to elusidate the fundamental role which zinc plays in connection with insulin and the interaction between insulin and zinc. It was first discovered that phosphate ions used in the protamine zinc insulin preparations are able to influence the physical chemical relation between insulin and zinc. Over the years it had

been the habit of various workers to use the phosphate ion as a buffer in insulin preparations. In his series of experiments Hallas-Møller showed that in order to prepare long acting insulin which was insoluable at the PH of the blood it was necessary to add protamine to the preparation when phosphate buffer was employed in the preparation. However, when acetate buffer was used instead of phosphate, zinc insu in could be made to precipitate out in the preparation which was insoluable at the PH of the blood without the usual necessity of adding protamine to the preparation also. The amount of zinc used to attain this new zinc insulin was 2 mg/1000 units of insulin and the insulin attains just as high or a higher degree of insoluability as insulin in combination with protamine. The zinc insulin contains chemically combined zinc. It is felt that when phosphate buffer is used the affinity of zinc for phosphate is greater than for insulin, this having the effect of not allowing the zinc to become chemically bound to the insulin, thus necessitating the added step of supplying protamine to the preparation before precipitation at blood PH takes place. However, when phosphate interference is eliminated by using the acetate buffer, zinc is allowed to combine chemically with insulin and a precipitate is formed at blood PH without the necessity of adding protamine to the preparation also. The physical state of this insulin preparation varied depending on the PH of the solution from an amorphous material to crystals of different size. Biological experiment with dogs revealed that the new preparation demon trated prolonged activity. These insulin crystals

when suspended in a solution of zinc salt are preserved intact and are completely undissolved in the PH range from 5 - 8 provided that substances that may interact with the zinc are not present.

It had not been possible before this time to prepare insulin crystals with more than .8% zinc, however, this new insulin has been shown to contain more than 2% zinc. Apparently the structure of the insulin crystals permits su stances to enter into the crystals by diffusion and react chemically with the insulin within the crystal lattice. The insulin crystals with an increase zinc content are, contrary to the ordinary crystals, insoluable in water at the neutral point and retain their excessive zinc content, but by suspending them in a neutral phosphate buffer they dissolve as the zinc is liberated and precpitated as zinc phosphate. By increasing the zinc content of the cry. tals not only the chemical but also the biological properties are altered. The action of ordinary insulin crystals suspended in water is not significantly different from that of ordinarily dissol ed insulin, but with crystals of increased zinc content a striking prolongation of insulin action is found. Of interest also is the finding that by varying the physical state of this new insulin and its zinc concentration, it is possible to make insulin preparations ranging in activity from ordinary insulin to one exceeding the prolonged action of the protamine - zinc insulins. Insulin prepared in this manner will appear as an amorphous modification at the moment of precipitation, but at the PH interval from 5 to 6 it is transformed into a crystalline modification after the

passage of a certain time. This means that the amorphous state is stable only outside this ph interval, whereas the crystalline form, once it is established, will be stable over the entire precipitation zone. This chemical phenomenon forms the basis for the introduction of three new insulins into the armancentarium of the physician treating diabetes.

Semilente insulin is a suspension of crystalline insulin in the amorphous state and has an action range of 12 to 14 hours. It is prepared by the rapid adjustment of the PH of a solution of insulin and zinc upward to he neutral point.

Ultralente is a suspension of crystalline insulin crystals of a size of 10 - 20 microns and has an action range beyond 30 hours. It is found when the zinc insulin solution is carefully adjusted to a PH range of 4.8 to 5.7.

Lente, which has the widest range of application with an action of about 24 hours, is a suspension of crystalline insulin, partly in the amorphous (30%) and partly in the crystalline (70%) state.

The zinc content of all three preparations is 2 mg./1000 units of insulin or the same as PZI. The insulin employed is crystallized at least three time. in order to insure purity and tolerance. It is felt that tolerance to the lente preparations should prove to be excellent, due to the fact that, apart from the acetate buffer and sodium chloride added to obtain isotonicity, the preparations contain no foreign substances.

The first clinical trials with the new insulins were carried out at the Hvidore Hospital in Copenhagen, Denmark. In previous studies M. Jersild of that hospital had shown that there may be great differences in the way that different patients react to the same insulin preparation. In studies with NPH insulin it was shown that some patients react quickly to the preparation, the effect of which is to make the night insulin supply inadequate. This was called an A reaction and can be graphically represented thus, ◡ . Other patients reacted slowly to the NPH, the supply of insulin being inadequate during the day. This was called a C reaction and was graphically represented thus ◠ Other patients obtained good twenty-four hour control with the preparation. This was called reaction B and was graphed as a — . This same picture was observed when patients were given clinical trials with semilente and ultralente preparations. When Semilente insulin was used alone a predominence of ◡ A reactions was seen and when Ultralente was used a predominence of C ◠ reactions was noted. It was these findings that prompted these workers to prepare the mixture of Semilente and Ultralente which is called lente and which to date has proved to be the most useful from a clinical standpoint.

Peck and his group (30) found the action of Lente insulin to be similar to that of NPH insulin. This group transferred their patients from NPH to Lente as a unit for unit basis and report that the transition occurred smoothly and with no untoward effects. He reports one patient who complained of pain at the site of

injection with the Lente insulin. However, Oakley (31) reports that a change to Lente insulin completely relieved persistent painful reactions in a patient who had previously been using PZI. In his series of cases he obtained good control with the Lente preparations and feels that, if with further study, these preparations prove themselves, they should be put on the market at which time PZI and globin should be simultaneously withdrawn. Murray and Wilson (32) in their series concluded that Lente insulin has an action similar to a mixture of soluable insulin and PZI and thus avoids the difficulty of having to prepare the extemporaneous mixture. They also reported that upon changing to Lente insulin the problem of local allergy was eliminated in every case which had previously presented this problem.

The question of allergy is, I believe, the main talking point in favor of the new preparations. Many patients present the problem of being allergic to the added foreign protein of the protamine and globin preparations In some cases severe local reactions occur 3 - 9 months after the injections have started. In the great majority of cases protamine and globin preparations have been used without positive evidence of damage, however, long term toxicity is notoriously difficult to establish. Amt (33) sites cases as representing toxicity to protamine evidenced by unexplained edema, and signs of renal insufficiency, the symptoms of which are said to disappear on withdrawal of protamine insulin. The fact that some cases show a tendency toward thrombotic phenomenon coupled with the knowledge that protamine is an antagonist to heparain

would seem reason encugh to question the use of protamine. There is also some evidence which would indicate that protamine may play a part in the genesis of atherosclerosis. Even without concrete proof of the detriment effect of protamine and globin, the elimination of a daily injection of foreign protein on theoretical grounds alone would seem very desirable. The Lente preparations are the only long acting preparations now available which seem to be the answer to this problem.

Thus a new group of insulins are introduced to the clinician which have the disadvantage of adding to the number of usable preparations which has the effect of adding more confusion to a confusing situation. However, if these preparations prove satisfactory, it should become apparent that several of the older preparations could be discarded al together. The discards could include Globin Insulin, NPH Insulin and Protamine Zinc Insulin, and Lente with its comparitively simple chemistry and understandable mode of action would be a welcome replacement. Nabarro and Stowers,(34) however, do not feel that diabetes, at present controlled on a single daily injection of PZI, globin or NPH, will benefit in any way from transfer to the new insulin unless there is an allergy problem. They feel that indications for transfer to Lente would include: 1. poor control with other insulin; 2. patients at present needing morning and evening injections; 3. patients requiring PZI and soluable insulin in the morning by seperate injection and; 4. patients showing allergy to other preparations.

SUMMARY

The intelligen use of insulin in treating diabetes is no simple matter because of the large variety of commercially avail-able insulin preparations. Therefore, an attempt is made to cover the most important of these preparations, along with historical, chemical and physiological considerations, so that proper selection of the indicated insulin preparation needed for a particular patient will be simplified.

The history of the development of insulin therapy, including the early work of Banting and Best on up to the present developments by the Danish workers on Lente insulin is discussed.

Concerning the chemistry of the insulin molecule, it is pointed out that complete elucidation of the various elements and complex linkages of the various amino acids included in the molecule remains to be completely worked out. However, much progress has been made along these lines. A list of 13 common amino acids is given which is said to account for 95% of the insulin molecule.

As is true of insulin chemistry, much remains to be learned concerning the physiology of insulin in normal persons as well as in diabetic subjects. Much has been learned however, and work by such men as Best, Colowick, and Houssay is discussed. These men along with others have done much to bring light to a dark and confusing problem.

The various commercially available insulins are discussed. The insulins included in this paper are not a complete listing of all insulins available in the world today, but only include those insulins which are widely used in the United States along with two preparations which are not commercially available in this country, namely, Semilente and Ultralente insulins. The insulins have been classified according to rate of action into 1. rapid acting insulin 2. intermediate acting insulin and 3. prolonged acting insulin. Included in the rapid acting insulins, amorphous insulin, crystalline zinc insulin and semilente are discussed. Of the intermediate preparations NPH, globin insulin and semilente are discussed. Protamine zinc insulin and ultralente are included as prolonged acting preparations. Points discussed concerning the various insulins include method of preparation of insulin, length of action, advantages and disadvantages in their use, indications for use, and rules for administration.

The problem of allergy to insulin and to foreign protein added to certain of the preparations is briefly discussed and the desirability of using an insulin that is free of added foreign protein is pointed out. The new insulin, Lente, which contains no added foreign protein, is discussed and offered as the insulin of choice where allergic manifestations to foreign protein are a problem.

CONCLUSIONS

1. All of the insulin preparations available display the same fundamental characteristic hypoglycemic effect of insulin and differ only in rate of onset of action and duration of effect.

2. With all of the preparations increase dosage causes an increase in both intensity and duration of action.

3. Available preparations are classified according to rapidity of onset and duration of action. Rapid acting preparations include amorphous Insulin, Crystalline Zinc Insulin, and Semilente Insulin. Intermediate acting preparations include NPH Insulin, Globin Insulin, and Lente Insulin. Prolonged acting insulins include PZI and Ultralente Insulin.

4. Crystalline Zinc nsulin and Globin Insulins are clear solutions, all of the other preparations being suspensions which must be resuspended by shaking before being drawn into a syringe.

5. The long acting preparations have no use in the treatment of diabetic emergencies. The short acting preparations have the most overall utility and with the exception of Semilente are the only preparations injected intravenously.

6. Good diabetic therapy presupposes not only the intelligent use of insulin, but also a proper dietary regulation along with regulation of activities. All of these factors must be individu-

lized to fit the needs of each patient.

7. The new Lente preparations appear to be a useful addition to the armementarium of the physician. The simplicity of their preparation tends to help clarify much of the speculation as to the exact chemical nature and mode of action of prolonged acting preparations.

8. The elimination of foreign protein in a prolonged acting preparation is very welcome, especially where allergy is a problem, and even where there is no overt allergic manifestations, elimination of daily injection of a foreign protein can be heartily approved of on theoretical grounds alone.

9. With the introduction of the Lente preparations it may be possible in the future to eliminate the other prolonged acting insulins from usage, thus minimizing the confusing number of preparations from which to choose.

10. Further research into the chemistry and physiology of insulin is still needed. Daily injection is a traumatic procedure both from the physical and psychological standpoint. The preparation of an insulin, which would only be released from its depot when the blood sugar was high and which would remain in the depot when insulin was not needed, is the ultimate target. With such a preparation daily injection would be eliminated and possibly large depots could be established

which would only have to be replenished at monthly intervals or even longer. This type of insulin would also ideally eliminate the necessity of dietary and activity control and put the diabetic on equal footing with his non-diabetic brother.. All of this is doubtless for the distant future. In the mean-time the development of an insulin which could be taken orally would be a welcome addition to the list of insulins.

ACKNOWLEDGEMENT

I wish to thank Dr. Morris Margolin for the assistance rendered to me by him in the preparation of this paper. It was with his help, friendly advice and the donation of hours of his valuable time that this paper was brought to completion.

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