INTRODUCTION
Diabetes Mellitus is a disease known since the time of Hippocrates. Four thousand years ago the Egyptians chronicled the symptoms of disease. Two thousand years later physicians (of the period of Christ) described it in more detail, and at the time of Aretaeus the Coppadocian (the second century A.D.), it had acquired at least part of the name under which it is known to-day.

Clinical description of the disease came first. Aretaeus wrote that "Diabetes is a wonderful affection, being a Melting down of the flesh and limbs into urine, the patients never stop making water but the flow is incessant, as if from the opening of aqueducts. The patient is short lived, for the melting is rapid and the death speedy. Moreover, life is disgusting and painful, thirst unquenchable, drinking excessive." In naming the disease "diabetes" (="to pass through".) the Greeks gave recognition to one of its most conspicuous symptoms: the rapid passing of water through the body. Later the Romans noticed that the urine of diabetics had a sweet taste and that bees swarmed around it, so they elaborated the name by adding "Mellitus" the latin word for "sweet as honey".

Diabetes Mellitus was known to the physicians of ancient India. Two books published over two thousand years ago (Charaka Samhita and Sushruta Ayurveda) give detailed descriptions of
the aetiology, diagnosis and treatment of the disease. Diabetes or Madhumeh was one of the 20 varieties of urinary disorder resulting from provocation of the body humours. This was confirmed by Thomas Willis an English physician in 1670, about 1200 years later.

**ETIOLOGY:**

There are number of factors which disturb the normal carbohydrate metabolism so it will be convenient if the etiology of diabetes is summarised in short.

**The Pancreas:** In 1632 Bruner (Bruner, 1662) successfully depancreatised animals. But it was only in 1885 that Mehring and Minkowski (Mehring and Minkowski, 1885) observed disturbed carbohydrate metabolism in depancreatised animals.

Paul Langerhans (Langerhans, 1869) was the first to demonstrate the "Islets of Langerhans", which were named after him, twenty years later by Laguesse (Laguesse, 1895). The idea that the beta cells or the probable site of anti-diabetic hormone came from Homans and Allens (Allen, 1922; Homans, 1915). Insulin is elaborated by the beta cells of the islets of Langerhans. The hyperglycaemic factor which is secreted along with insulin was isolated as glucagon by Murlin in 1923 (Murlin, 1923).
Glucagon is secreted by the alpha cells of Langerhans, and its role in the etiology of diabetes is not elucidated.

The name "insulin" was given to the hormone of the islets of Langerhans (de Meyer, 1909) 12 years before the proof of existence was obtained by Banting and Best in 1922 (Banting and Best, 1922) and 16 years before it was isolated and obtained in crystalline form (Abel et al, 1927). It was the most sensational and historical achievement in advance of diabetes that insulin was isolated in-pure state. It is discharged into the pancreatic-duodenal vein, carried in the blood into and through the liver and then distributed throughout the body. It is a highly complicated protein molecule, has a molecular weight about 6000 or the multiples of it. The biological activity of this is dependent upon the integrity of the disulphide bridges between cysteine residues in adjacent polypeptide chains. The structure has been investigated by Sanger (1945-55), the work is a great tribute to his foresight, persistence and experimental ability. The metabolism of the beta cells is also relevant to the problem of insulin synthesis. Many enzymes have been detected in the beta cells by Lacy in 1961 (Lacy, 1961). It may be that certain co-factors
produced as a result of the carbohydrate metabo-
olism of the beta cells themselves become
available for protein synthesis and so influence
synthesis of insulin. It may be that some of the
intermediary products of metabolism, such as
citrate, could act as chelating agents for zinc,
releasing free insulin from its zinc-protein
complex in which it exists in the pancreas (Maske,
1957). About 200 units of insulin or more can be
obtained from human pancreas. The stimulus for the
insulin secretion in the body is rising blood sugar
concentration (Foa, 1956). In the blood it inter-
acts with the Beta-globulins which picks up insulin
and puts it down at the site of action.

In-spite of the fact that 46 years have
been elapsed since the discovery of insulin and
the role of this agent still is not very clear.
This may be because of the factors which govern
blood glucose concentration are too numerous,
complex and independent. There are two main views
regarding the mechanism of action of insulin.
According to Levine and Goldstein (1950 and 1953)
insulin acts in enhancing the penetration of
glucose into the cell and in diabetics glucose
is not utilized because of its inability to enter
the cells, where it is phosphorylated and utilized.
Cori and co-workers (1939 and 1940) hypothesise that insulin counter reacts the pituitary inhibition of glucokinase.

The overall effects of insulin can be summarised as follows:

It enhances the normal breakdown of glucose to pyruvate which is ultimately oxidised into carbon dioxide and water. It appears to aid in the synthesis of amino acids and their incorporation into body protein and fat storage. In normal animals insulin tends to decrease liver glycogen and increases in diabetes. It also enhances the peripheral utilization of glucose which was demonstrated by using the arteriovenous difference in blood sugar as a criterion. The problem of precise action of insulin has been discussed and debated for many years but as yet no single mechanism has been put forward which will satisfy all the observed effects of insulin. (Fisher, 1960; Randle and Young, 1960; Park et al, 1959; Goldstein and Levine, 1960).

Current view favour a primary action on cell permeability rather than an effect on intracellular enzymes concerned directly with glucose utilisation, (Goldstein and Levine, 1960). They showed that insulin speeds the movement of many
sugars into the cell and this movement is not dependent upon the subsequent metabolic fate of the sugar. This is in contrast to the theory proposed by Cori (1945) who suggested that insulin acts primarily on enzyme hexokinase within the cell, accelerating the phosphorylation of glucose to glucose-6-phosphate.

Until recently, diabetes was assumed to be simply due to an absolute deficiency of insulin secretion by the pancreas. The diminished insulin content of the pancreas may merely reflect the exhausted state of the beta cells after vain attempts to neutralize excessive amounts of circulating antagonists. The various antagonists of insulin may be summarised as follows.

Glucagon: The name glucagon was given in 1923 to a factor found in a pancreatic extract which caused a rise in blood sugar (Kinball and Murlin, 1923). The hyperglycaemic action of glucagon is the result of mobilization of liver glycogen (Burger and Kramer, 1929), with subsequent outpouring of free glucose from the liver (Myers et al, 1952). The action of glucagon on phosphorylase stimulation is similar to that of adrenaline. Adrenaline, however, also activates the phosphorylase of skeletal muscle. The significance of glucagon in the general metabolism of the body is not entirely clear. The most
reasonable hypothesis concerns its counterplay with insulin in glucose homeostasis: hypoglycaemia caused by insulin is followed by an increase in hepatic glucose output, stimulated by glucagon. But there is nothing to suggest that glucagon plays a part in the aetiology of human diabetes or that it affects its clinical course. Glucagon has been found to have two other actions in the body:

(i) The motility both of the stomach and of the colon is drastically reduced within a few minutes of the injection of glucagon and gastric secretion is diminished (Sporn and Necheles, 1956). This effect persists while glucagon is infused, but ceases within a few minutes of termination of the infusion.

(ii) Glucagon also enhances the renal clearance of a number of electrolytes, but again, this effect is transient. It is thought to be due to an action on the renal tubules and not related to the hyperglycaemia (Elrick et al., 1958).

The Adrenal glands: Lukens and Long (1936) showed that extirpation of adrenal glands ameliorated pancreatic diabetes in animals. With these observations the importance of adrenal cortex in diabetes was firmly established. The increase of glycosuria induced by 11-oxosteroids is due to an increase in
gluconeogenesis. Epinephrine is responsible for transient hyperglycaemia as a result of increased glycogenolysis and also depression rate of peripheral utilization of glucose (Somoyi M. 1955). But there is no convincing evidence suggesting that adrenal medulla plays any part in the initiation of diabetes mellitus.

The Pituitary gland: In 1937, Young showed that permanent diabetes could be produced by administering extracts of anterior pituitary lobe continuously for four weeks. This was due to exhaustion of beta cells of pancreas.

The Thyroid gland: About 3.2% of diabetes was reported by Welder (Welder et al, 1940) in a series of patients with hyperparathyroidism. However, permanent diabetes cannot be established in animals by thyroid administration.

INSULIN RESISTANCE

It has been often observed that certain percentage of diabetics who had very favourably responded to insulin therapy in the beginning failed to respond after certain length of the treatment. Such individuals have been found to tolerate as much as thousand units of insulin
without any hypoglycaemic response. Investigations on such individuals have revealed the presence of certain insulin antagonists causing this resistance phase. Some such substances have been described below.

**Insulin Antibodies**: They may be regarded as distinct from the insulin antagonists, because they appear only when insulin has been administered to a patient. As a protein, insulin can be expected to have antigenic properties. Nevertheless, it came as a surprise to most clinicians to learn that antibodies can be demonstrated in the serum of most patients on insulin therapy, because clinical manifestations attributable to this type of antibody reaction appear to be rare. These serum antibodies are present in the globulin fraction of the serum protein. They bind insulin tightly, preventing its transfer from the blood stream to its site of action, and may account for the severe insulin resistance of some patients whose plasma could bind hundreds of units of beef insulin (Berson and Yalow, 1960). This type of insulin resistance responds to treatment with cortisone, the steroid presumably interfering with the association of insulin with the antibody. The management of such patients presents many difficulties. During the resistant phase they may require over 1000 units of insulin per day to control
the hyperglycaemia and ketosis; but, severe and persistent hypoglycaemia may occur when the resistance suddenly ceases and large amounts of insulin, previously bound to the anti-body, are set free in the plasma. There is little species specificity in these circulating antibodies, so that attempts at treatment with insulin from different animal sources meet with little success.

"Conventional" allergic reactions to insulin due to 'reagin' type antibodies may also occur. They take the form of local or, rarely, generalized, skin reactions, after insulin injections. This type of antibody can be demonstrated by patch testing on the skin. It is not certain, however, that these reactions are due to insulin at all, because highly purified, crystalline insulin preparations from the same animal species do not provoke such reactions in the same patients. It is, therefore, almost certain that some other protein in the commercial insulin preparation causes these reactions.

Experimentally, insulin antibody formation has been induced in guinea-pigs by sensitizing them to beef insulin (Moloney and Coval, 1955). The serum from such guinea-pigs is used in the immuno-assay of plasma insulin and for the experimental production of acute insulin deficiency in rats and other animals (Armin et al, 1960).
Insulin antagonists in plasma:

Although plasma antagonists interfere with the absolute measurement of insulin, it has been possible to turn this to good account in defining the different types of antagonists present in the plasma and in assessing their possible role in the development of diabetes.

In uncontrolled diabetic patients, there appears to be three hormone dependent antagonists.

Vallance-Owen has studied an antagonist associated with the albumin fraction of plasma. (Vallance - Owen et al, 1958). This factor may depend on the presence of pituitary gland and on adrenal cortical steroids. A high level of the antagonists occurs not only in uncontrolled diabetic patients, but also in the plasma of prediabetic subjects. The factor inhibits the action of insulin on muscle, but not in the adipose tissue.

In this connection it is of particular interest that the fat pad technique often indicates high levels of plasma insulin, even in young, untreated, ketonic diabetics, in whom the rat diaphragms assay shows apparently reduced insulin levels. These observations by Steinke et al (1961) have led to the suggestion that in some subjects there may be a genetically determined over production of insulin antagonists and that overt diabetes
develops when the beta cells of pancreas are no longer able to produce enough insulin to neutralize the circulating antagonists. (Vallance - Owen and Lilley, 1961).

Hormone dependent antagonists have also been found in the alpha-1 and alpha-2 globulin fractions (Field and Stetten, 1956; Taylor, 1961). They appear to be related to diabetic ketosis. The alpha-1 antagonist disappears when ketosis is brought under control, but the alpha-2 antagonist has occasionally been found in the serum of normal subjects. The alpha-1 fraction inhibits insulin stimulated glucose uptake of the rat diaphragm; the alpha-2 fraction is capable of reducing glucose uptake of rat diaphragm below control levels whether or not insulin is present in the incubating medium.

It is interesting to reflect on the possible meaning behind this bewildering variety of so called antagonists. It is known that, in common with other peptides, only part of complete molecule is necessary for biological activity. The remainder of the molecule is capable of interacting with other proteins which may act as the carriers for the essential part of the molecule. Perhaps different carrier proteins react at different rates, both in their formation and
dissolution of the complex and this may be of value in preventing too sudden a metabolic change following a release of insulin from the pancreas.

It may be that insulin can act on a tissue only if it has first been attached to it by a tissue-specific carrier— a concept analogous to the tissue specificity of iso-enzymes (Annotation, 1960). In this connection it is of great interest that immunological differences have been demonstrated in one and the same animal between its circulating plasma insulin and the insulin extracted from its own pancreas (Slater et al., 1961). These differences may reflect the association of insulin with different carrier proteins.

A further possibility is that insulin—antagonists complexes exist to protect insulin from degradation or to hold insulin in the plasma to keep it available as an emergency store, ready for immediate use.

Protein synthesis is under close genetic control. Small changes in protein structure, genetically determined, may be sufficient to alter the kinetics of insulin antagonists 'binding' and play an important part in the development of diabetes.
Several workers have classified diabetes mellitus in the following categories.

**Adult type** :- This occurs usually during the middle age i.e. between 40 - 60 years. In most cases it is due to obesity. It can be of mild or severe type depending on the amount of insulin secreted in the system. In mild cases glycosuria occurs while in severe cases ketosis etc., are main symptoms. In mild cases patients require about 20-40 units of insulin daily besides the obesity control.

**Juvenile type** :- The type that has its onset in childhood or adolescence is often severe and is frequently complicated by ketoacidosis. Sulphonylureas apparently act by discharging endogenous insulin, they would be expected to be clinically efficacious only in maturity onset diabetes and early juvenile diabetes, while there is some insulin in the pancreas.

**Insulin antagonist type** :- Plasma antagonists interfere with the absolute measurement of insulin and produce hyperglycaemia e.g. pituitary gland, adrenal cortical steroids etc.

**Maturity onset diabetes** :- The diabetes that develops later in life is mild and ketoacidosis is rare. Maturity onset diabetes occurs much
more frequently is obese people and weight reduction improves glucose tolerance.

Lipotrophic diabetes: It is rare but interesting disease. Patients with this syndrome have deficient fat depots. They become diabetic, probably because 30% - 40% of ingested glucose normally taken by adipose tissue remains in the blood stream, and the resultant hyperglycaemia eventually exhausts the beta cells.

Scope and comment on insulin therapy.

Despite the fact that insulin was the first ray of hope for the sufferers of this malady, it did not fulfil all the requirements of an ideal anti-diabetic agent which could be applied to all cases of diabetes mellitus which could be employed by all routes of administration and which could be used with perfect safety, irrespective of the age, sex, stage and course of the disease. The greatest drawback of insulin is its non-availability by the oral route, besides, a very careful watch is necessary on the blood sugar level of the patient during insulin therapy. All these considerations have inspired the workers in the field to discover a substitute for insulin which could be employed orally and which could fulfil all the requirements of an ideal antidiabetic agent. A large number
of substances have been tested for their ability to reduce blood sugar, although on rigorous screening and by clinical trials, many of these substances have been found to be of less value as therapeutic agents. A better understanding, in recent years, of some of the complex and manifold actions of insulin has stimulated further search of some of these compounds. When insulin itself is so effective, a search for a substitute for the hormone however may be questioned. But the discovery of a similarly acting drug, which could be administered orally would have more advantages.

A satisfactory substitute for insulin should have the following characteristics:–

a) Activity by oral route,
b) Low toxicity without any undesirable side reactions, and
c) Rapidity in producing hype-glycaemia.

Substances that effect the blood sugar may either raise or lower its level. A search for such substances reported in literature makes an interesting reading because of their quite unrelated and varied nature. These include plant extracts, crude drugs like gums and dyes, some hormones, nitrogenous substances, heavy metals like antimony, bismuth and synthetic organic compounds.
Alcohol has been reported to stimulate gastric and insulin secretion (Hunt, 1930) and thereby reduce blood sugar. Alkalies, phosphate, salts, sodium selenite, creatine, ergotamine, acetylcholine, bile acids, barbituric acid, yeast, extracts of spleen, vitamins (Mukherjee et al, 1957) and nicotinamide (Henry et al, 1947 and Banerjee et al, 1949) have been reported from time to time as blood sugar reducing agents. Small doses of colloidal sulphur injected intraperitoneally in animals (Bucciardi, 1928) has been claimed to produce hypoglycaemia. Clinical improvement in diabetics through the use of some preparations using, zinc, mica, iron, cobalt and copper has also been reported (Ajgankar, 1956 and Chowhan et al, 1957).

**SUBSTANCES OF PLANT ORIGIN IN DIABETES**

Extracts of plants have been used for a long time as traditional remedies for diabetics in many parts of the world. Extracts from roots, barks, leaves and fruits have been used to reduce sugar in the urine. Seeds and bark of Jaman (Eugenia Jambolina), Gurmar (Gymnema Sylvestris), Gular (Ficus glomerata), Kundru (Coccinia Indica), Bijasar (Pterocarpus Marsupium), bitter gourd or Karela (Memordica Charantia) have been used for
their hypoglycaemic activity (Chopra et al, 1926). Extracts of Pariwinkle, Mistletoe and the Nicker berry were studied by Hugh Jones (1955). Many other plant derivatives have been tested and some have been found to possess marked hypoglycaemic properties, e.g. the two alkaloids, of galegine (Muller, 1925) from the seeds of Galega Officinalis and lupamine (Clemanti and Torrisi, 1934) from the seeds of Lupinus albus reduce the blood sugar of normal individuals and diabetic patients, and other materials have been extracted from a wide variety of plant tissues ranging from cabbage and celery to yeast (Dubin and Corbitt, 1923).

Two polypeptides, hypoglycin A and B, have been isolated from the unripe 'ackee' the fruit of a plant, Blighia Sapida which probably produces vomiting sickness, seen in the poorer classes in Jamaica (Hassall et al, 1954). Both produce a marked hypoglycaemic action when given orally and although they are hepatotoxic, they are of considerable interest as insulin is a polypeptide which is not effective by mouth. The chemical structure of hypoglycin A has recently been confirmed (Ellington, 1959).
Recently a large number of indigenous plants were screened for their hypoglycaemic activity in laboratory animals by Aiman (1960 and 1961) like Ficus bengalensis bark, Ficus Glomerate bark, Linaria ramosissima leaves and twings, Vinca Rosea leaves and Eugenia Janbolana fruit and seeds which are found to be promising hypoglycaemic materials. Memordica Charantia and fresh Kerala juice were also claimed to be good hypoglycaemic agents without much untoward toxic effects (Sharma et al, 1960 and Vad, 1961).

**ORGANIC COMPOUNDS IN DIABETES**

The earliest synthetic compounds to be tried as oral anti-diabetic drugs were salicylates. As early as 1877, Muller reported that the new well known analgesic aspirin and salicylic acid for the treatment of diabetic patients. Recently there is a renewed interest in salicylates as hypoglycaemic agents. Reid and workers (Reid et al, 1957) have found that aspirin given in large doses (5 to 10 G. daily) produces beneficial results in diabetic patients. However, such large doses, at the same time produce undesired side effects such as deafness, vomiting etc., thereby eliminating its clinical usefulness. The mechanism of action of salicylates is not quite clear. Probably it acts at some stage
of carbohydrate metabolism by intra-cellular phosphorylation.

Occasionally compounds like indole-3-acetic acid (Mirsky et al, 1956) and mesoxylate of sodium and calcium (Shigera Ohashi et al, 1953 and 1955) and mesoxalic acid are reported to be good hypoglycaemic agents. According to the Shigera Ohashi (1953) the glucose tolerance curve during the treatment was lower than that of pre-treatment when mesoxalate was administered. Adrenaline induced hyperglycemia was also checked by one week continuous administration of mesoxalate. The decrease and disappearance of glycosuria by mesoxalate was explained by its enhancement of carbohydrate tolerance.

GUANIDINE DERIVATIVES IN DIABETES

The search for effective hypoglycaemic agents has an interesting and varied history. Claims that they were of value in the treatment of diabetes were made for innumerable compounds before insulin became available. Many were quite valueless; some caused severe damage to the liver or kidney. Other examples of effective hypoglycaemic agents of pre-insulin era are guanides, synthalin A and synthalin B. The accidental discovery by
Watanabe in 1918 of the hypoglycaemic activity of guanidine in rats has stimulated further studies in this series of compounds. Breslan (1926) working in Minkowskis clinic subjected the guanidine compounds to clinical trials. Although it was found to be effective, was also toxic to the liver. Frank and co-workers (Frank et al, 1926) studied the various derivatives of guanidine for their hypoglycaemic activity and also in diabetic patients clinically. They have found that 1-Guanido-4-amino butane which closely resembles arginine and had previously been isolated from Herring Sperum in 1913 by Kossel possessed marked hypoglycaemic activity and it was found that hypoglycaemic activity, up to a certain extent depends upon the length of the methelene chain. Thus butyl derivative was found to be less active than amyl derivative. On the basis of these results insulin was regarded even as a derivative of Guanidine (Glaser et al, 1928). Of the various derivatives of Guanidines which has been tried during this period mention may be made of two synthetic compounds.

\[
\text{Synthalin A: } \quad \text{H}_2\text{N} - \text{C} - \left(\text{CH}_2\right)_n - \text{NH} - \text{C} - \text{NH}_2
\]

\[
\text{Synthalin B: } \quad \text{H}_2\text{N} - \text{C} - \left(\text{CH}_2\right)_n - \text{NH} - \text{C} - \text{NH}_2
\]

\[n = 10 \quad \text{Synthalin A}
\]
\[n = 12 \quad \text{Synthalin B}
\]
Following the publications of Frank and Collaborators (1926 and 1928) synthalin was given an extensive clinical trial and held away for sometime as an oral anti-diabetic agent. Its clinical use was however, short lived. Subsequent reports regarding its toxic action from various laboratories, it led into disuse. Large doses may produce histological changes in the liver and kidney of animals (Bertram, 1927). Impairment of liver function was demonstrated in rabbits on account of their inability to deaminase glycine when given synthalin and dextrose (Lublin, 1926). Histological evidence of parenchymatous degeneration in liver was shown by Hornung (1928). Post-mortem examination in such animals showed liver congestion and fatty degeneration.

A good deal of work has also been done on physiological activity of the diguanides. Its effect on liver and muscle glycogen resembles insulin. But in many other respects such as oxidation of glucose it is different from insulin. Although various synthetic biguanides were claimed in 1929 to have beneficial hypoglycaemic effect in experimental animals (Slotta et al, 1929), they were not subjected to clinical trials probably due to an adverse report on synthalin. It was only
recently after a period of thirty years in 1957, the hypoglycaemic activity of a biguanide viz. N'-betaphenethyl formamidinylimino urea hydrochloride (DBI; Phenformin; Dibolin) was described by Unger in 1957. The other analogous are the normal amyl (DBB) or normal butyl (DBV) biguanides. The biguanide structure seems to be necessary for hypoglycaemic activity. A large number of compounds with substitution in the amino group on the biguanide molecule have been tested. The maximum activity is obtained by substitution of one of the hydrogen atoms of the amino group with methyl radical. Activity is lost progressively with substitution of longer alkyl chains.

Unlike the biguanides, the biguanides appear to be less toxic. In support of this it is quoted that paludrine, another biguanide, has been used extensively as an anti-malarial agent and without any significant adverse effect. Regarding the mechanism of action of this group of compounds some work has been done although not so extensively as in the case of sulphonylureas. This can be put in a nutshell as follows. Blood sugar level is reduced in normal and alloxan diabetic animals (Unger et al, 1957). Ability to lower blood sugar level in diabetic human (Krall et al, 1959) but
not in normal humans, was observed (Fajan et al., 1958). Although, glucose is not oxidised, effectively, increased uptake of glucose by rat liver and rat diaphragm has been reported. Muscle glycogen is neither formed nor depleted, (Unger et al., 1957). Absence of hyperglycaemic response to epinephrine and glucagon was seen. In diabetics these compounds elevate lactate, pyruvate and citrate (Steiner et al., 1959). Increased anaerobic glycolysis at unknown sites in the body is claimed to be the mechanism of biguanides.

Clinically it has been reported that biguanides are useful in controlling all types of diabetes (Pomerance et al., 1957). Contrary to the early reports, biguanide therapy alone is effective in some group of patients who are responsive to sulphonylureas (Creutzfeldt, 1961). However, the therapeutic dose of DBI is very near to the toxic dose. Occurrence of gastrointestinal side effects have been reported. In about 40 to 50% of patients, anorexia, nausea and vomiting has also been reported. Weakness, lethargy and weight loss may develop later (Odell et al., 1958). In brittle and unstable diabetic patients when carefully employed, biguanides have been found to be of considerable value.
SULPHANILYL DERIVATIVES AND SULPHONYLUREAS IN DIABETES

With the clinical use of oral hypoglycaemic compounds especially sulphonylureas, it may be said a new era in the treatment and management of diabetes has been ushered in. The tremendous impetus that these discoveries have given to diabetic research is evident from the voluminous literature reported in different scientific publications during the past decade. The mechanism of action and physiological role of insulin have been re-investigated and new data obtained.

Although the hypoglycaemic effect of sulphonamide derivative was reported by Ruiz et al, (1930), a regular first clinical and experimental observations that formed the background of the utilization of certain sulphonamide derivatives in the treatment of diabetes were made during the first half of 1942. At that time Janbon and his co-workers (1942) were investigating the therapeutic effect of isopropylthiadiazole derivative of sulphanilamide in typhoid fever. They found that this drug produced in some patients signs of hypoglycaemia. In 1946 he reported that "The sulphonamide compounds that depress the blood sugar seem to form a group of thiadiazole derivatives."
Among them the tertiary butyl and iso-butyl are most active, then in order of decreasing activity are butyl, amyl, iso-amyl, propyl and iso-propyl compounds.

In 1951 and 1952, they had studied the possible role of endogenous alloxan in the pathogenesis of diabetes and established that it seemed to inhibit the formation of endogenous alloxan. In 1954 and 1955 Von Holt named IPTD. They confirmed the hypoglycaemic action of this substance and also observed the phenomenon of attenuation of alloxan diabetes in the rabbit and cure in certain cases.

In 1955, a group of German investigators published their first experimental and clinical results on the sulphonamide BZ 55 (Franke and Fuchs, 1955) and it had the same action as IPTD. It appears, therefore, that IPTD can be considered as the first pharmacological and therapeutic group of agents of the sulphonamide variety that are hypoglycaemic and anti-diabetic.

In 1954, Frank and Fuchs recognised the hypoglycaemic properties in man, of a new antibacterial sulphonamide carbutamide (BZ 55, Glucidoral, Invenol, Nadisan, N-\(p\)-aminobenzene-sulphonyl) = \(N'-(n\text{-butyl})\text{-urea}\). A year later they
and other German clinicians (Achelis, J.D. and Hardebeck, K., 1955; Bertram F. et al, 1955) reported its successful substitution for insulin in number of middle-aged or elderly patients suffering from mild stable diabetes. Extensive clinical trials confirmed the hypoglycaemic effect of orally administered carbutamide in such patients but evidence accumulated which suggested that it had toxic properties sufficiently serious to preclude its general clinical use. Though these substances proved to be unsatisfactory for the treatment of diabetes mellitus, they deserve all the credit for providing the necessary impetus for the search of newer anti-diabetic drugs. A very large number of sulphonylurea derivatives have since been synthesized and tested for their hypoglycaemic action.

The following table shows a list of sulphonylurea derivatives in chronological order of their development.

**SULPHONYLUREA DERIVATIVES.**

<table>
<thead>
<tr>
<th>R1</th>
<th>SO2- NH - CO - NH- R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2N</td>
<td>Carbutamide</td>
</tr>
<tr>
<td>CH3</td>
<td>Tolbutamide</td>
</tr>
<tr>
<td>Cl</td>
<td>Chlorpropamide</td>
</tr>
<tr>
<td>CH3-</td>
<td>Acetohexamide</td>
</tr>
<tr>
<td>CH3- NH2</td>
<td>Metahexamide</td>
</tr>
</tbody>
</table>

\[
\text{Sulphonylurea Derivatives} = \begin{align*}
\text{Carbutamide} & : \text{R1} = \text{H2N} \\
\text{Tolbutamide} & : \text{R1} = \text{CH3} \\
\text{Chlorpropamide} & : \text{R1} = \text{Cl} \\
\text{Acetohexamide} & : \text{R1} = \text{CH3} \end{align*}
\]
At the present time, the safest and most effective are tolbutamide (Orinase, Rastinon, N-\((p\text{-toluenesulphonyl})\) = \(N'\text{-}(n\text{-butyl})\text{-urea}\) and chlorpropamide (Diabinese, \(N\text{-}(p\text{-chlorobenzenesulphonyl}) = N'\text{-}(n\text{-propyl})\text{-urea}\)). As they do not possess an amino-group attached to the benzene ring, they are without the antibacterial properties of the sulphonamides; but, probably for the same reason; they do not have the toxic actions of the sulphonamides on the bone marrow. Tolbutamide is metabolized in the body to an inactive compound by carboxylation in para position. Chlorpropamide, however, because of its chlorine group in that position, cannot be inactivated in the same way. Elimination from the body is entirely by renal excretion, both of the active and inactive forms. Acetohexamide is a new sulphonylurea derivative which has come to clinical use (Anderson et al, 1961). It disappears from the blood more slowly than tolbutamide, though in other respects it resembles tolbutamide.

Metahexamide was discovered by joint research programme with Boehringer and Mannheimer in Germany. In human beings the potency of drug is about 10 times more than that of tolbutamide and therapeutic dose is 2.5 mg/kg only. Though the acute toxicity of metahexamide is within the
range of tolbutamide, the chronic tolerability decreases with its increased potency. A dose as low as 100 mg/kg is not without any damage in rats. In dosage of 300 mg or higher it produces epigastric distress, jaundice and abnormal liver function test in patients.

Although the place of the sulphonyl compounds in the treatment of diabetes mellitus is limited, a tremendous impetus to diabetic research has resulted from their discovery. New light has been shed on the physiological function and mode of action of insulin and on the metabolic abnormalities in the varies syndrome of diabetes mellitus.

MECHANISM OF ACTION OF SULPHONYLUREAS

The problem has been tackled from various angles and extensive work has been carried out in different laboratory animals, diabetic patients and in normal beings. Yet this important problem of mechanism of action bristles with controversy and is still undecided. This is mainly so because of the complexity of the factors involved in blood sugar regulation and secondly our knowledge concerning the role of insulin especially its site of action is not fully known.
Various theories have been proposed to explain the hypoglycemic activity of sulphonylureas and the chief among which are follows.

1. **Stimulation of beta cells of pancreas.**

   A good deal of evidence has accumulated to show that beta cells of pancreas are stimulated to liberate insulin or insulin like substance. That pancreatic tissue is essential for sulphonylurea action has been amply demonstrated. In completely depancreatized laboratory animals and human beings with the exception of ducks and chickens sulphonylurea do not show any hypoglycemic activity. The alpha cells of pancreas do not play any role and this has been demonstrated by the absence of change in alpha cells after sulphonylurea treatment (Gepts, 1956). The hyperglycaemic principle glucagon content of pancreas is also not reduced by sulphonylureas. The beta cells of pancreas are stimulated by these drugs to liberate hyperglycaemic principle. Cross circulation experiments (Pozza et al, 1958) and increase in insulin like activity in blood plasma in normal subjects after treatment with carbutamide (Aiman et al, 1957) or with tolbutamide (Vallance et al, 1959) and in dogs in the blood of pancreatic vein after intra-venous injection of tolbutamide (Goetz et al, 1958) support this view.
Further evidence is obtained from the fact that in completely alloxanised animals sulphonylureas have no hypoglycaemic activity, whereas in partially alloxanised animals some activity though delayed could be obtained.

2. **Insulin like action in tissues.**

The hypoglycaemic activity may be due to increased uptake of glucose by tissues due to the action of sulphonylureas. Both in vivo and in vitro studies have been carried out.

Pancreatectomised hypophysectomised animals are extremely sensitive to insulin and in experiments carried out with such animals, sulphonylureas show no hypoglycaemic activity. In vitro studies on glucose metabolism have been carried out with rat diaphragm and conflicting results have been obtained with sulphonylureas. Similarly, no effect on glucose uptake by fat and brain tissue could be demonstrated.

3. **Potentiation of insulin action.**

Here again controversial reports have been obtained with different species. Sulphonylureas have been found to "potentiate" insulin activity in fully pancreactomised or alloxanised dogs, but negative results have been obtained with other species of animals. In unresponsive cases of
diabetes, clinical trials show that insulin requirements are not reduced by the simultaneous administration of sulphonylureas. In vitro studies which have been referred to earlier do not lend much support to the theory although some workers have found that sulphonylureas increased the activity of both endogenous and exogenous insulin as they have found increased insulin-like activity in plasma of persons given sulphonylureas (Aiman et al, 1957 and 1959).

4. Inhibition of insulinase activity.

Inhibition of insulinase activity has been suggested by some as they cause hypoglycaemic activity of sulphonylureas. Liver which is rich in insulinase appears to be non-specific enzyme beside insulin is able to hydrolyse glucagon, somatotropin and casein. In animals given hypoglycaemic dose of tolbutamide, liver insulinase has been found to be significantly reduced (Mirsky et al, 1957 and 1956; Tamiya et al, 1958). But in vitro studies, the dose required to inhibit insulinase to the same extent was to be about 10 times the optimum hypoglycaemic dose.

5. Inhibition of liver glycogenolysis.

There seems some unanimity of opinion regarding the increase in glycogen content of liver in fasted animals after administration of
sulphonylureas. These experiments have been confirmed by isotopic studies. However no such increase in hepatic glycogen could be observed in well fed animals or fasted hypophysectomised or adrenolectomised animals.

If hypoglycaemic activity is due to reduction of hepatic glycogen breakdown, then such a reaction can be brought about by inhibition, by sulphonylureas of some liver enzyme system interference in reactivation of glucagon or adrenaline. Experiments of hepatic response to hormones of glucose-6-phosphatase activity and other enzyme studies do not lend much support to this theory.
In the course of the 19th century, many natural products were isolated and purified and their chemical structure was elucidated. It soon became apparent that certain structural units of the molecules of biologically active compounds are to be found also in other substances having similar properties, and this observation was seized upon as the only available guiding thought for further researches. The modification of the structure of a promising compound is still the main line of approach to new drugs. It holds forth the hope that suitable structural variation may increase the therapeutic usefulness of a given type of compound by widening the difference between the desirable action and the toxicity syndrome. Structural variations bring about new physical properties and apart from the possibility of changes in the toxicity of a compound, improvements in solubility, volatility, colour, odour etc. may widen the utility of a given drug. Moreover, even a very minor change in the chemical structure may uncover physiological properties which had been dormant or overshadowed by the side effect in the parent compound.
The idea about relations of structure and activity underwent gradual changes as knowledge of the chemical and physical properties of matter expanded.

Even the most advanced and most carefully considered theories have not always revealed regularities in the relation of chemical structure to physiological action which could be used indiscriminately in the series of compounds after proving their value in another one. Sulphonamide derivatives which have been synthesised and tested till now is no exception to the general theory stated above.

Interest in hypoglycaemic agents began in 1942 with chance observation of Janbon while testing the antibacterial properties of IPTD in patients with typhoid fever. Since then, a large number of compounds have been synthesised and tested upon both in experimental animals and clinical cases.

Bovet and Dubost (1944) carried out tests on dogs while Loubatieres (1944) conducted tests both on dogs and rabbits. The study of compounds structurally similar to p-aminobenzene sulphonamido-isopropyl-thiadiazoles was done by Loubatieres (1956) to confirm the following facts:
1. Among the non-sulphonamide, none had hypoglycaemic action in dogs or rabbits. So he concluded that the sulphonamide group was necessary to reduce blood sugar level.

2. Among the benzene sulphonamide thiadiazoles the p-benzene-sulphonamido-isopropyl-thiadiazole produced a moderate hypoglycaemic effect in dogs.

3. Among the p-amino-benzene-sulphonamide thiiazoles p-acetyl-aminobenzene-sulfonamido-undecyl thiazole and p-aminobenzene-sulphonamido-undecylthiazole were slightly hypoglycaemic in dogs (Bovet and Dubost, 1944) but the later compound had a hypoglycaemic effect in rabbits (Loubatieres, 1944).

Loubatieres (1956) studied 12 p-amino benzene sulphonamidothiazoles in dogs and rabbits and concluded that the tertiary butyl or iso-butyl derivatives were the most active followed in order of decreasing activity by the butyl, amyl, isoamyl and finally propyl and iso-propyl derivatives, methyl and ethyl derivatives were inactive as were heptyl, hexyl and amino derivatives.

Janbon who investigated 5 of these products (iso-propyl, methyl, ethyl, propyl and iso-amyl) in human beings and Bovet and Dubost (1944) who tested 8 compounds in dogs
reached almost the same conclusion. Among the
p-aminobenzene-sulphonamido-iso-propyl thiadiazole
derivatives that were studied systematically in
rabbits by Loubatieres, some were immediately
hypoglycaemic while others had biphasic action,
initially hyperglycaemic and subsequently
hypoglycaemic.

It was clear that both the portions of
the molecule containing thiadiazole nucleus and
the nature of the hydrocarbon side chain in
position 5 of this nucleus played an important
part in the mechanism of hypoglycaemic action.
Later, work of Loubatieres (1946) was confirmed
by more recent research by Loubatieres and
co-workers (1955) who have shown that compounds
having a structure similar to that of thiadiazole
side chain had a depressant action on the sugar
level of the blood. Certain hydrocarbons such
as ethyl and methyl alcohol when administered
sub-cutaneously in doses of 1 mg/kg have been
shown to produce hypoglycaemia in experimental
animals. The blood sugar curve produced by these
alcohols were similar to the curves produced from
the corresponding sulphonamide compounds.
In 1955 German workers (Frank et al, Achelis et al; Bertram et al, 1955) reported clinical observations on the hypoglycaemic action of a potent sulphonamide derivative N-butyl-N'-sulphanylurea (carbutamide).

In extensive clinical trials both in U.K. and U.S.A. this compound has been found to be toxic. Several cases of bone marrow depression and jaundice have been reported and it was finally discarded in October, 1956.

Since then closely related compounds have been studied for their hypoglycaemic activity. Marshall et al (1958) and Cassody et al (1958) have synthesised arylsulphonamides, N-sulphonyl carbamates, N-aryl-N'-alkylureas and of alkyl and other substituted sulphonyl-ureas. Most of the compounds were found to be toxic to human beings but two of the analogues viz. tolbutamide and chlorpropamide have been subjected to extensive clinical and pharmacological studies.

Report on tolbutamide was first published by German workers in 1955. Soon after the discovery of tolbutamide another new compound having more potent activity than tolbutamide has been announced; using both
duration of action and the hypoglycaemic potency as criterion for evaluation. Root in 1958 reported that in rats and dogs chlorpropamide is more potent than tolbutamide. Similar results have also been obtained in rhesus monkeys by Schneider in 1959. Subsequently these studies in animals have been corroborated in man in extensive clinical trials (Stowers et al, 1959; West et al and Forsham et al, 1959).

Chlorpropamide although more effective (a single oral dose may be sufficient) cannot be said to be non-toxic as tolbutamide, as assessed by the toxic reactions reported both in experimental animals and clinical cases. Regarding the clinical effectiveness of the synthetic oral hypoglycaemic agents, it has been established that these drugs are useful only in certain selected cases of diabetes mellitus. They have been found to be suitable for elderly diabetics who have high blood sugar level but minimum symptoms. They are of no value in the growth-onset type of diabetes in which the pancreas has lost nearly all its capacity to secrete insulin. The presently known sulphonylurea derivatives are not free from toxic effects e.g. haematological, cutaneous and gastro-intestinal
Toxicity has been reported in 3.2% of the patients taking tolbutamide and nearly 6% for chlorpropamide (Goodman and Gilman, 1966). In addition, there has been increasing incidence of secondary failure with tolbutamide and chlorpropamide causing serious problems. If it continues it is likely that there will be only few diabetics satisfactorily responding after 5 years treatment with tolbutamide.

The biguanides are also used in the treatment of maturity onset type of diabetes, according to principles applied for sulphonylureas. The role of biguanides in the treatment of diabetes is mainly in cases of unstable diabetic. In the unstable insulin dependent diabetic, biguanide is not to be used as substitute for insulin but only as a supplementary part on the regime. Biguanides have also been utilised as a supplementary therapy along with sulphonylureas. Since the two drugs act by different mechanisms, their combined use, has been found to be superior in certain cases.

From the above discussion, it would be clear that none of the synthetic drugs so far discovered can effectively cure all types of diabetes or provide even an alternative for substitution therapy like insulin on long term basis. The search for better and more effective
drug is being continued in several laboratories for investigating newer compounds with greater activity and lesser toxicity.

With this aim in view Trivedi and Pathan (1964) synthesised a new series of sulphonylurea derivatives. These have been subjected to extensive pharmacological investigations and forms subject matter of this thesis.
Chemical structure of various substituted compounds have been shown in Table 1. The substitution has been done at two points 'R' and 'R₁', in the basic chemical structure given at the top of the table. The basic aim behind the synthesis of these compounds was to obtain compounds with greater potency and lesser toxicity. Earlier workers (Cardani et al., 1957) had shown that the basic substance without the substituents is less potent. Some of the workers have also tried series of substituent compounds in order to synthesise more potent compounds, but these attempts have generally failed. In the present series entirely different substituent groups have been used in order to explore possibility of synthesising compounds with greater potency.

Sulphonylureas have been prepared by desulphurisation of thioureas by alkaline hydrogen peroxide. (Trivedi et al., 1964).

Preparation of Thioureas:

Thioureas have been prepared by the interaction of arylsulphonylamides with aryl/benzyl/isothiocynates.

\[ R SO₂NH₂ + R₁NCS → R₁SO₂.NH.CS.NHR₁ \]

\[ R = \text{p-CH}_3\text{C}_6\text{H}_4; \text{p-CH}_3\text{O.C}_6\text{H}_4; \text{p-Cl.C}_6\text{H}_4; \text{p-Br.C}_6\text{H}_4; \text{C}_6\text{H}_5; \text{p-CH}_3\text{CO.NH.C}_6\text{H}_4; \text{p-NH}_2\text{C}_6\text{H}_4; \text{p-C}_10\text{H}_7. \]
Desulphorization of sulphonothiocureas to get corresponding sulphonylureas -

They were prepared as shown below in the case of 1-butyl-3-p-methylphenyl sulphonylurea. Me.C₆H₄.SO₂NHCSNHBu(2.5 G) in water (20 ml) and NaOH (1.1 G) was treated dropwise with 30% (3.7 G) in water (20 ml) at 20 - 30°C. Allowed to remain one hour at room temperature. Finally the solution was acidified with HCl and the product filtered gave 2.2 G of the corresponding urea derivative viz. 1-butyl-3-p-methylphenyl-sulphonylurea (Sadao Onishi, Yakuga Ku Zasshi, 1959). The sulphonylureas were crystallised from 70% methyl alcohol.
# TABLE - 1

SHOWING THE VARIOUS SUBSTITUENTS AT POSITIONS \( R \) AND \( R_1 \) IN THE BASIC STRUCTURE OF SULPHONYL-UREA SO AS TO FORM COMPOUNDS 1 TO 45.

\[
R_1-SO_2-NH.C-NH-R
\]

<table>
<thead>
<tr>
<th>Code number of the compound</th>
<th>Substitution at ( R_1 )</th>
<th>Substitution at ( R )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>( p-\text{Br-C}_6\text{H}_4 )</td>
<td>( m-\text{CH}_3.\text{C}_6\text{H}_4.\text{CH}_2 )</td>
</tr>
<tr>
<td>2.</td>
<td>( p-\text{Br-C}_6\text{H}_4 )</td>
<td>( 2:4(\text{CH}_3)_2.\text{C}_6\text{H}_3.\text{CH}_2 )</td>
</tr>
<tr>
<td>3.</td>
<td>( p-\text{Br-C}_6\text{H}_4 )</td>
<td>( p-\text{CH}_3.\text{C}_6\text{H}_4 )</td>
</tr>
<tr>
<td>4.</td>
<td>( p-\text{Br-C}_6\text{H}_4 )</td>
<td>( p-\text{Cl-C}_6\text{H}_4.\text{CH}_2 )</td>
</tr>
<tr>
<td>5.</td>
<td>( p-3r-C_6\text{H}_4 )</td>
<td>( o-\text{CH}_3.\text{C}_6\text{H}_4.\text{CH}_2 )</td>
</tr>
<tr>
<td>6.</td>
<td>( p-3r-C_6\text{H}_4 )</td>
<td>( 2:5(\text{CH}_3)_2.\text{C}_6\text{H}_3.\text{CH}_2 )</td>
</tr>
<tr>
<td>7.</td>
<td>( p-\text{Br-C}_6\text{H}_4 )</td>
<td>( 3:4(\text{CH}_3)_2.\text{C}_6\text{H}_3.\text{CH}_2 )</td>
</tr>
<tr>
<td>8.</td>
<td>( p-\text{Br-C}_6\text{H}_4 )</td>
<td>( o-\text{CH}_3.\text{C}_6\text{H}_4 )</td>
</tr>
<tr>
<td>9.</td>
<td>( p-\text{Br-C}_6\text{H}_4 )</td>
<td>( p-\text{CH}_3.\text{C}_6\text{H}_4 )</td>
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### TABLE 1 (CONTINUED)

<table>
<thead>
<tr>
<th>Code number</th>
<th>Substitution at the compound</th>
<th>Substitution at R₁</th>
<th>R₂</th>
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<tbody>
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<td>p-Cl·C₆H₄ - 2·5(CH₃)₂·C₆H₅·CH₂</td>
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<td>p-Cl·C₆H₄ - m-CH₃·C₆H₄·CH₂</td>
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<td>p-Cl·C₆H₄ - o-CH₃·C₆H₄·CH₂</td>
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<td>p-Cl·C₆H₄ - p-Cl·C₆H₄·CH₂</td>
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<td>p-Cl·C₆H₄ - 2·4(CH₃)₂·C₆H₅·CH₂</td>
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<tr>
<td>15</td>
<td>p-Cl·C₆H₄ - o-CH₃·C₆H₄</td>
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<tr>
<td>16</td>
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<td>p-Cl·C₆H₄ - 3·4(CH₃)₂·C₆H₅·CH₂</td>
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<td>p-Cl·C₆H₄ - p-Cl·C₆H₄</td>
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<tr>
<td>22</td>
<td>p-Cl·C₆H₄ - p-CH₃·C₆H₄</td>
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<tr>
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<td>C₃H₅ - 2·5(CH₃)₂·C₆H₅·CH₂</td>
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</tr>
<tr>
<td>Code number</td>
<td>Substitution at $R_1$</td>
<td>Substitution at $R_2$</td>
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<td>-------------</td>
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<td>26.</td>
<td>$C_6H_5 - 3:4(CH_3)_2 \cdot C_6H_3 \cdot CH_2^-$</td>
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### TABLE 1 (CONTINUED)

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<td>o-Cl.C₆H₄.CH₂⁻</td>
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<td>p-CH₃.O.C₆H₄.CH₂⁻</td>
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<td>p-Cl.C₆H₄.CH₂⁻</td>
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<td>p-CH³⁻.C₆H₅.CH₂⁻</td>
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<td>p-CH₃.C₆H₄</td>
<td>p-Br.C₆H₄.CH₂⁻</td>
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<td>2:5(CH₃)₂.C₆H₅.CH₂⁻</td>
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