DISCUSSION
Several hundred of new sulphonylurea compounds have so far been synthesized and tested. Despite the fact that only a few of them have been found to possess sufficient hypoglycaemic potency and duration of action, a study of their structure and hypoglycaemic activity is of considerable pharmacological interest. Such data is very useful in the synthesis of new compounds.

While studying the effects of substituents at R in general sulphonylurea structure, $R_1$-$SO_2.NH.CO.NH.R$, different workers (McLamore et al, 1959; Hokfelt et al, 1962) arrived at the conclusion that simple alkyl chains of 3 to 4 carbon atoms lead to peak activity. Replacement of alkyl substituent by aryl also provided some compounds of good activity (McLamore et al, 1959). This statement is well substantiated by the exhaustive investigations of Holland and co-workers (1961). They reported that $1$-(4-chlorobenzenesulphonyl)-3-(4-dimethylaminophenyl)-urea to be the most effective compound comparable in activity to chlorpropamide. The present investigations deal with a new series of sulphonylurea derivatives (Trivedi and Pathan, 1964) synthesized by substituting benzyl substituents in place of phenyl substituents.
In the initial screening these compounds were studied for their hypoglycaemic action on the normal blood sugar level of healthy adult male albino rats. These experiments have revealed that all the compounds have significant hypoglycaemic activity though there are quantitative differences amongst the individual compounds. Compound 1. (1-m-methylbenzyl)-3-(p-bromobenzenesulphonyl)-urea) and compound 4. (1-(p-chlorobenzyl)-3-(p-bromobenzenesulphonyl)-urea) were found to be most effective in group 1, amongst which the later is more effective (p= 0.05) and comparable to chlorpropamide. Compounds 5, 6 and 7, though potent are not as effective as compounds 1 and 4. Superiority of compound 4 over the reference drug chlorpropamide was particularly noticed after 24 hours of its administration since it showed longer duration of action as compared to chlorpropamide. Johnson et al (1959) explained the longer duration of action with chlorpropamide due to presence of chlorine radicle in para position. Present work shows that bromo and chloro substitution and benzyl ring at R also increases the duration of action. In the present series, compounds without having a benzyl ring have shown shorter duration of action, while o-methyl and 2:5 dimethyl substitution at R slightly decreases the activity. The activity
Further decreases by substituents like m-methyl, 2:4 dimethyl, p-methoxy, o-methoxy and p-methyl.

In the second group of compounds (10 to 22) those having different substituents at position R₁ and R have been studied. The results show that compound 13 (1-(p-chlorobenzyl)-3-(p-chlorobenzenesulphonyl)-urea) and compound 22 (1-(p-methoxyphenyl)-3-(p-chlorobenzenesulphonyl)-urea) are most effective but the later has shown short duration of action. p-bromo, o-chloro, p-methyl and 3:4 dimethyl substituents at R with respect to p-chloro at R₁ have shown slightly lesser activity as compared to above two compounds while compounds having 2:5 dimethyl, m-methyl, o-methyl, 2:4 dimethyl, o-methoxy, o-methyl and p-chloro at R with respect to p-chloro at R₁ have shown still lesser activity as compared to above compounds.

Most of the compounds possessing a benzyl ring at position R have shown longer duration of action though there are certain exceptions viz. compounds 15, 16, 20, 21 and 22 which though lacking in a benzyl group at position R have shown longer duration of hypoglycaemic action extending upto 48 hours. On the other hand compound 20 having a benzyl ring has only a shorter duration of action.
In the third group of compounds comprising of compounds numbering from 23 to 39, compound 32, \(1-(p\text{-chlorobenzyl})-3-p\text{-}(\text{methoxybenzenesulphonyl})\text{-urea}\) and compound 33 \(1-(m\text{-methylbenzyl})-3-p\text{-}(\text{methoxybenzenesulphonyl})\text{-urea}\) have shown the maximum hypoglycaemic activity of which the former is more potent. Both the compounds 32 and 33 have p-methoxyl group at \(R_1\) but compound 32 has para chloro at \(R\) and compound 33 has m-methyl at \(R\). Compound 32 has para chloro at \(R\), which might be responsible for its enhanced activity. Compounds having no substitution in the phenyl ring at \(R_1\) and different substituents at \(R\) are generally less effective. All the compounds in this group showed the hypoglycaemic action upto 48 hours while compound 39 has shown this effect extending upto 60 hours which differs from other compounds in having a para chloro grouping at position \(R\).

In the fourth group comprising of compounds numbering from 40 to 45, compound 41, \(1-(p\text{-chlorobenzyl})-3-p\text{-}(\text{methylbenzenesulphonyl})\text{-urea}\) and compound 43, \(1-(p\text{-bromobenzyl})-3-p\text{-}(\text{methylbenzenesulphonyl})\text{-urea}\) have shown good hypoglycaemic activity as compared to other compounds of this group. Both these compounds have a p-methyl substitution at \(R_1\) and para chloro and para bromo respectively at \(R\).
Remaining compounds having para methyl at R₁ and para methyl, 2:4 dimethyl, 2:5 dimethyl or 3:4 dimethyl at R are comparatively less effective.

On statistical analysis it was found that compound 4 and chlorpropamide were more potent than the rest. Compound 4 though comparable to chlorpropamide in its hypoglycaemic activity has a longer duration of action lasting for 60 hours while the effect of chlorpropamide ended within 48 hours.

Compounds which were found to be more potent in each group viz. 4, 13, 28, 32 and 41 were subjected to further pharmacological investigations. Compound 13 was included since it had shown longer duration of action. In order to investigate whether the hypoglycaemic activity of these compounds had any relationship with their plasma levels, such levels were determined spectrophotometrically after oral administration of these compounds in a dosage of 100 mg/kg. Plasma concentration of sulphonylurea compound were determined upto 60 hours. The peak level was reached within 5 hours but varying amounts were detectable upto 60 hours. Considering 5 hour samples, chlorpropamide produced maximum concentration but 48 hour and 60 hour samples showed maximum concentration with compounds 41 and 4
followed by 28, 13 and 32. The blood concentration values followed a pattern resembling closely with their hypoglycaemic values. This shows that though chlorpropamide produced greater hypoglycaemic action initially, which is consistent with its plasma levels, it is lagging in persistence of action which is shown by the new compounds.

Since these compounds had significant hypoglycaemic activity on F.B.S., it was of interest to determine their capability to limit the magnitude levels which follow on administration of glucose. Analysis of the data presented in Table 12 shows a significant difference in the glucose tolerance of rats during control and treatment period. Looking to the mean values, compound 41 was most effective in case of oral glucose tolerance tests. However, there was no statistically significant difference between chlorpropamide and compound 41. In case of I.V. glucose tolerance test, compound 13 was found to be more potent than the rest including chlorpropamide. The effect of sulphonylurea compounds on glucose tolerance curves may be accounted for by two mechanisms, one, retardation of glucose absorption from the gut for which there is no evidence and the other which seems more probable, is due to enhanced
activity of the beta cells of pancreas, thus secreting larger amounts of insulin which are otherwise sensitised in response to glucose (Best and Taylor, 1959). Another possibility is inhibition of adrenocortical activity, since the adrenocortical steroids are known to antagonise insulin effect on hexokinase reaction in glucose utilization by the tissue (Colowick et al, 1947). This seems to be further substantiated by the fact that they have slight inhibiting effect on epinephrine induced hyperglycaemia, which is also known to be partly affected through inhibition of glucose utilization (Cori et al, 1956), as well as through secretion of corticoids (Vogt, 1944). The beneficial effects seen with sulphonylurea compounds on glucose tolerance may have an important bearing in mild adolescent obese diabetes where the mechanism of glucose tolerance is known to be deranged.

Sub-maximal doses of alloxan were used to produce partial destruction of beta cells of islets, resulting in a condition simulating mild diabetes of adulthood. Sulphonylurea compounds showed significant effect in these experiments. Compounds 28, 32 and 41 showed statistically significant effect after 20 days treatment while compounds 4 and 13 showed significant effect after 15 days treatment only. The later compounds could thus be considered better than the rest but there was no significant
difference between these and the reference drug chlorpropamide. Chemical structure of compounds 4 and 13 and chlorpropamide shows the presence of a halogen substituent at position R₁ which seems to be essential for the optimum activity in alloxinised induced diabetes. These results strongly support the theory that the hypoglycaemic action of sulphonylurea compounds is chiefly through the release of insulin from the beta cells which should be present in sufficient number since the hypoglycaemic action is abolished on administering further doses of alloxan.

The anterior pituitary through its corticotrophin and somatotrophic hormones is known to affect the activity of glucagon and adrenal cortex. It is therefore responsible for maintaining the hyperglycaemic state through rapid gluconeogenesis and elevated phosphorylase activity. Administration of anterior pituitary extract in experimental animals fed on high carbohydrate diet induces greater hyperglycaemia than normally which has been attributed to inhibition of glucose utilisation by the tissues. While investigating the mechanism of action of the present series of sulphonylurea derivatives, it was considered desirable to assess their value in antagonising the effect of anterior pituitary induced hyperglycaemia in experimental animals.
In one set of experiments where the effect of sulphonylurea compounds was investigated for 24 hours, it was observed that the hyperglycaemic response was considerably less than in the controlled animals. The blood sugar level, in each of the control sets, increased gradually after the third hour of administration of pituitary extract. This appears to be due to a direct effect of anterior pituitary extract on the glucose absorbed from the gastro-intestinal tract, since within this period it did not cause any significant change in blood sugar level of fasting animals in the initial screening. Thus the 3rd hour blood glucose values (one and half hours after glucose administration) seem to be indicative of the glucose tolerance in the treated and untreated groups. Analysis of the data presented in table 12 shows that the present series of sulphonylurea derivatives has a favourable influence on glucose tolerance since the blood glucose level did not rise much in the treated animals as compared to controls. The increased glucose tolerance after administering the compounds, seems to be in conformity with the results reported by other workers (Holt and Holt, 1956; Lenderer and de Myer, 1957; Mohnike, 1957; Mukherjee et al, 1958). The blood sugar level was significantly raised in control animals
by the sixth hour of administration of anterior pituitary extract. This hyperglycaemic action seems to be related to the increased gluconeogenesis (Houssay, 1936; Long, 1937) as well as to the inhibition of peripheral utilisation of glucose affected by the anterior pituitary hormones (Young, loc. cit.).

The hyperglycaemic effect of anterior pituitary extract was well marked by the 12th hour and seems to be related to inhibition of oxidation of the 2nd dose of glucose administered after the sixth hour. On administration of sulphonylurea compounds, there was a significant inhibition of hyperglycaemic response caused by anterior pituitary extract at this hour. The persistent hyperglycaemic response of the anterior pituitary has been demonstrated to be due to inhibition of glucose uptake by the tissues (Park, 1952; Kato, 1956), it is therefore likely that the sulphonylureas compounds might be exerting a direct influence on glucose utilisation in the hexokinase reaction, which is known to be inhibited by the anterior pituitary hormones (Price et al, 1946). However, an indirect effect of the drugs through stimulation of pancreatic insulin secretion is also likely.
In another set of experiments, weekly fasting blood sugar levels in rats given anterior pituitary extract alone (Group I) and those given sulphonylurea in addition (Groups II to VII) were compared. Analysis of the data shows that the blood sugar level of the control group of rats (without any treatment) remained more or less constant (varying within the Fiducial limits of 84.68 to 92.92), while those given daily doses of anterior pituitary extract (40 mg/kg) showed gradual increase in blood sugar level every week, attaining a maximum percentage increase (131.0 mg per 100 cc of blood) by the end of fourth week. The difference between the blood sugar levels of the normal and those treated with anterior pituitary extract was highly significant. On the other hand, the weekly rise in blood sugar level after similar doses of anterior pituitary extract in animals simultaneously treated with 100 mg/kg daily doses of compounds was found to be less and the difference between the two groups was highly significant. The percentage increase in blood sugar level by the end of fourth week was found to be 100, 102, 121 and 109 mg with chlorpropamide and compounds 4, 13, 28 and 41 respectively as against 132 mg in the control group. Blood sugar levels in
these animals however, remained unchanged during the subsequent week even after stoppage of drug treatment indicating cumulative effect of the compounds. Some reduction in blood sugar level was seen in control animals after stopping injections of anterior pituitary extract. Since hyperglycaemia due to anterior pituitary extract is known to be mediated through gluconeogenesis from proteins (Houssay, 1936), it is likely that the sulphonylurea derivatives might be acting by inhibiting this catabolic effect. This hypothesis is supported by an observation that tolbutamide counteracts reduction in body weight of rats induced by anterior pituitary extract (Gupta, 1961).

Partial pancreatectomy in experimental animals simulates human diabetes. There is characteristic rise of blood sugar level on account of diminished secretion of insulin which is due to the lesser number of beta cells available in the pancreas. In the present study most of the compounds showed significant effect in reducing hyperglycaemia caused by partial pancreatectomy.

Many workers (Rodriguez-Minon, et al, 1957; Root, 1957; Becker et al, 1956; Schambye, 1957; Tarding et al, 1958) have demonstrated that depancreatized or severely alloxinised dogs receiving exogenous
insulin show additional hypoglycaemic response on administration of sulphonylurea compounds. Schambye (1957) and other workers (Caren et al, 1937) showed that administration of sulphonylurea compounds to depancreatised dogs shortly before and after the single intravenous injection of soluble insulin results in a greater fall in blood glucose levels lasting for a longer duration as compared to the effects of insulin alone.

Thus increased secretion of insulin by stimulation of beta cells of islets does not seem to be the sole mechanism of hypoglycaemic action by these compounds. It was therefore postulated that these compounds might be having some peripheral sites of action. Accordingly experiments were conducted on rat diaphragms for glucose uptake and liver slices for glycogenolysis. The results obtained have been discussed in the following paragraphs.

All the compounds (8 mg/ml) under investigation showed significant effect on the glucose uptake by the diaphragm of rats as indicated by the 95% fiducial limits of these compounds (Table 26). Chlorpropamide and compound 13 showed the maximum effect but amongst themselves chlorpropamide was
more effective at a concentration of 4 mg and 8 mg/ml. None of the compounds including chlorpropamide had any significant effect on glucose uptake of the alloxanised diaphragms as compared to normal diaphragms.

Experiments with liver slices indicate that the compounds possess inhibitory effect against conversion of liver glycogen into blood glucose as compared to control group. Chlorpropamide, compound 4, compound 28, and compound 32 showed glucose uptake of 2.7, 3.2, 2.9 and 4.8 mg respectively per 100 cc of buffer though compounds 13 and 41 showed glucose transfer to the extent of 2.2 and 5.25 mg respectively per 100 cc of buffer. These results are in confirmation with the results obtained by other workers (Anderson et al, 1956; Anderson et al, 1957). Tolbutamide has been shown to interfere with in vitro conversion of liver glycogen to glucose. Large amounts added to the incubating medium prevent glucose release from both rat and rabbit liver slices (Clark et al, 1956, Vaughan, 1956; Berthat et al, 1956).

Weber and Cantero (1958) found that tolbutamide inhibits glucose-6-phosphatase predominantly, slightly reduces phosphoehexoisomerase activity but
does not alter liver phosphoglucomutase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. This suggests that tolbutamide in vitro has a specific effect on glucose-6-phosphatase but the concentrations necessary for this effect is 5-10 times higher than required to produce hypoglycaemia in vivo. These findings make it difficult to believe that in therapeutic doses, sulphonylurea compounds reduce hepatic glucose output by a direct action on the liver.

There are conflicting reports regarding the uptake of glucose by rat hemidiaphragms. Pleščech and Gey (1957) and Leijnse et al. (1959) showed that carbutamide and tolbutamide considerably increase the glucose uptake by the rat hemidiaphragms when incubation was carried out in bicarbonate with high concentration of sulphonylurea compounds. On the other hand Fry and Wright (1957) and Field and Woodson (1956) could not detect any increase in the rate of glucose uptake by rat hemi-diaphragms in their experiments. The influence of chlorpropanide in vitro on certain tissues was studied by Miller et al (1956), Loubatieres (1955) and Tyberglein et al (1956). The results obtained by these workers show that the glucose output of liver slices from normally fed rats is decreased when the compound was
added to the incubation medium in different concentrations. These observations thus confirm our findings that the sulphonylurea compounds produce their hypoglycaemic action partly by their peripheral action i.e. increased uptake of glucose by the tissues and diminished glycogenolysis.

General pharmacological study of the sulphonylurea compounds under test was carried out on cardiovascular system, respiratory system, skeletal and smooth musculature and central nervous system. Some rise in blood pressure was observed with a dose as high as 250 mg/kg body weight with most of the compounds including chlorpropamide. The rise in blood pressure was antagonised by priscoline (8 mg/kg) indicating a sympathomimetic effect of these compounds in larger doses. There was no effect on isolated perfused heart of frog and rabbit, neither there was any effect on blood vessels of frog, rat and dog. The smooth muscle preparations employed were isolated intestine of rat, rabbit and guinea-pig. None of the compounds including chlorpropamide had any effect on smooth muscle preparations. The effect on skeletal muscles was investigated; in vitro, on rectus abdominis muscle of frog and in vivo, on gastrocnemius-scatic preparation of cat. None of the compounds with any of the doses
employed produced any effect on rectus abdominis muscle of frog, however, with larger doses such as 250-300 mg/kg they produced partial blockade of the contractions of the gastrocnemius muscle when stimulated indirectly through the nerve.

The effect of these compounds on central nervous system was investigated regarding their (i) general sedative, hypnotic or stimulant action in intact animals such as mice and rats (ii) anticonvulsant action against electrically induced convulsions in mice and rats (iii) Analgesic action by the radiant heat method in rats.

None of the compounds including chlorproamide had any sedative, hypnotic, stimulant, anticonvulsant and analgesic properties with any of the doses employed.

Acute toxicity studies indicate that no toxic symptoms were observed even with very high doses such as 3-5 G/kg orally. LD50 and lethal dose could not be determined for any of the compounds.

Chronic toxicity studies were carried out in rats fed on sulphonylurea compounds for six months. None of the animals showed any adverse reaction or mortality in any of the groups. The average gain in weight of the treated animals was comparable to the control group. The histopathological examination of
pancreas, liver, intestine and stomach, did not reveal any changes as compared to control animals. Haematological examination of the treated animals was very near to normal.

General pharmacological studies have thus revealed that the present series of sulphonylurea compounds tested have no pharmacodynamic actions other than their potent hypoglycaemic properties. These compounds were also found to be non-toxic when given in doses far in excess than required for their hypoglycaemic action.