ORAL HYPOGLYCAEMIC DRUGS

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INTRODUCTION

Diabetes mellitus is a chronic disease which derives its name from the fact that the urine is plentiful and sweet-tasting. About 1–2 per cent of the adult population of the United Kingdom suffer from the condition which develops either when the production of insulin (the internal secretion of the pancreas) is inadequate or, alternatively, when the action of insulin on the tissues is antagonized. Diabetes insipidus is a different condition due to a pituitary or renal lesion; in this condition, large quantities of a dilute, tasteless urine are produced.

von Mering and Minkowski in 1889 were the first to show that removal of the pancreas caused diabetes mellitus. In 1909 the name insulin was suggested by de Meyer for a hypothetical internal secretion of the pancreas as it seemed likely that the ductless Islets of Langerhans, which lie embedded in the body of the pancreas, were the source of this material. Despite suggestive results by other workers, it was not until 1922 that Banting and Best were able to obtain a preparation containing the antidiabetic hormone in a form which consistently alleviated all manifestations of diabetes in totally depancreatized dogs. Four years later crystals of insulin were isolated from pancreatic extracts.

Insulin is a polypeptide which is rapidly destroyed by enzymic action in the gastro-intestinal tract. Although attempts have been made to protect insulin from being digested, preparations giving adequate and consistent absorption when administered by mouth have so far been unsuccessful. Patients with severe diabetes mellitus therefore need daily injections in order to maintain good health.

The search for hypoglycaemic or antidiabetic drugs effective when given by mouth was stimulated by the isolation of insulin. In 1926, the hypoglycaemic effect of synthalin, a diguanide, was reported by Frank, Nothman and Wagner and this drug was studied later by Graham and Linder. It was soon abandoned, however, as it produced severe liver damage when given in therapeutic doses.

Extracts of plants have been used for a long time as traditional remedies for diabetes in many parts of the world. Blueberry leaf extracts (Myrtillin), for example, were investigated by Allen in 1927, and extracts of periwinkle, mistletoe and the nicker berry (long popular as ‘doctor’ bush teas in Jamaica) were studied by Hugh-Jones in 1955. Many other plant derivatives have been tested, and some have been found to possess marked hypoglycaemic properties. For example, the two alkaloids, galegine (from the seeds of Galega officinalis) and lupanine (from the seeds of Lupinus albus) reduce the blood-sugar of normal individuals and diabetic patients, and other material...
ORAL HYPOGLYCAEMIC DRUGS

with hypoglycaemic properties has been extracted from a wide variety of plant tissues ranging from cabbage and celery to yeasts\(^{10}\). Little, Levine and Best\(^{11}\) found an insulin-like substance in the disintegration products of killed bacteria, while Collip\(^{12}\) extracted from clams (and also many plant sources) a substance, glucokinin, which produced marked hypoglycaemia when injected subcutaneously into rabbits. These preparations were usually toxic to the liver and this action is probably the basis of the hypoglycaemic effect.

Recently, two polypeptides, hypoglycin A and B, have been isolated from the unripe 'ackee', the fruit of a plant, *Blighia sapida* which probably produces the vomiting-sickness sometimes seen among the poorer classes in Jamaica\(^{13}\). 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\(^{14}\) been confirmed as the following:

\[
\text{CH}_2\cdot\text{C}\cdot\text{CH}_2\cdot\text{CH}\cdot\text{CO}_2\hat{\text{O}}\quad \hat{\text{NH}}_3\quad \text{H}_2
\]

Recent work\(^{14a}\) has shown that hypoglycin B is a dipeptide with the following unit structure:

\[
\text{H}_2\quad \text{C}\quad \text{H}_2\cdot\text{C}\cdot\text{CH}_2\cdot\text{CH}\cdot\text{NH}\cdot\text{CO}\cdot(\text{CH}_2)_2\cdot\text{CH}\cdot\text{CO}_2\text{H}
\]

\[
\text{CO}_2\text{H} \quad \hat{\text{NH}}_2
\]

The structure and activity of related compounds has been reviewed recently\(^{14b}\).

There are many other compounds which have hypoglycaemic properties. For example, oestradiol benzoate in large doses may reduce the hyperglycaemia of post-menopausal women suffering from diabetes\(^{15}\). Injections of dimercaprol sometimes produce a fall of blood-sugar concentration in diabetic patients who are receiving large doses of insulin\(^{16}\). Recently there has been renewed interest in the hypoglycaemic action of salicylates. Since the end of the last century, salicylates have been known to reduce the blood and urine sugar concentrations in diabetic patients\(^{17}\) but adequate doses of acetylsalicylic acid produce troublesome side-effects and this limits their clinical usefulness for the treatment of diabetes\(^{18}\). They probably act by enhancing the peripheral utilization of glucose. A fall of blood-sugar concentration associated with depletion of liver glycogen may be produced by salicylates in alloxan-diabetic rats and in animals made hyperglycaemic with cortisone\(^{19}\). Further, they stimulate glucose uptake by the isolated rat diaphragm\(^{20}\), probably by uncoupling oxidative phosphorylation.

The present era of renewed interest in oral hypoglycaemic agents began in 1942 when Janbon, Chaptal, Vedel and Schaap\(^{21}\), while assessing the antibacterial properties of 5-isopropyl-2-sulphanilamido-1,3,4-thiadiazole (IPTD, RP 2254, *I*) in typhoid fever, discovered the dramatic hypoglycaemic action of the sulphonamide. A few of the patients died from hypoglycaemia.
Between 1942 and 1946, studies on the mode of action of IPTD and other thiadiazoles in dogs and rabbits were made by Loubatières at Montpellier. He showed that IPTD (a) does not produce hypoglycaemia if the whole pancreas is removed but may do so if as little as one-sixth of the gland remains, (b) is most effective when injected directly into the pancreatic artery or through the duct of Wirsung under pressure, (c) acts independently of the nervous system, (d) lowers the blood-sugar in inverse proportion to the blood-sulphonamide level, and (e) raises the respiratory quotient after glucose administration. He postulated a pancreaticotropic mechanism to explain these actions of IPTD.

In 1946 Chen, Anderson and Maze reported on the hypoglycaemic effect in intact rabbits of another thiadiazole, 5-cyclopropyl-2-sulphanilamido-1,3,4-thiadiazole. They showed that the compound was inactive or sometimes hyperglycaemic in animals with severe alloxan diabetes.

More recently the hypoglycaemic properties of many other sulphonamide derivatives have been discovered. This account is concerned chiefly with the new arylsulphonylurea compounds and the guanidine derivatives.

ARYLSULPHONYLUREA COMPOUNDS

Since 1955, when a group of German workers published clinical observations on the potent hypoglycaemic action of a sulphanilamide derivative, N-butyl-N'-sulphanilylurea (carbutamide, II) many hundreds of chemically related compounds have been studied for their hypoglycaemic activity.

The synthesis of arylsulphonamides, N-arylsulphonylcarbamates, N-aryl-N'-alkylureas and of alkyl and other substituted sulphonylureas has been described. The majority of these compounds are too toxic for human use but two analogues of carbutamide, namely tolbutamide, (III) and chlorpropamide (IV) have been subjected to extensive clinical and pharmacological studies in the past few years.

After large-scale laboratory investigation and clinical trial in the United States of America, Canada and the United Kingdom, carbutamide was...
withdrawn from clinical use owing to the high incidence of serious toxic side-effects. About 5 per cent of 7,193 patients developed skin rashes, gastro-intestinal disorders, severe leucopenia, liver damage and generalized sulphonamide sensitivity reactions; eight patients died 10–30 days after commencing treatment. However, tolbutamide (introduced in 1956) and more recently chlorpropamide, have been widely used without serious ill effects in the treatment of mild diabetes mellitus of the adult type. Doubtless other similar compounds will become available for clinical trial in the future, but their advantages and disadvantages may always have to be measured against the extensive data collected about tolbutamide and chlorpropamide. Already \( N \)-cyclohexyl-\( N' \)-(3-amino-4-toluenesulphonyl)-urea (metahexamide, Euglycin, \( V \)), \( N \)-(3-aminobenzenesulphonyl)-\( N' \)-n-butylurea (Sucrida Berna, SB 1, \( VI \)) and \( N \)-2-furfuryl-\( N' \)-p-toluenesulphonylurea, (furfuralurea, \( VII \)) have been used clinically although none of them offer any important new beneficial features.

\[
\begin{align*}
\text{(V)} & \quad \text{metahexamide} \\
\text{(VI)} & \quad \text{Sucrida Berna, SB 1} \\
\text{(VII)} & \quad \text{furfuralurea}
\end{align*}
\]

This account will therefore be chiefly concerned with the chemistry, metabolic features, mode of action and clinical applications of tolbutamide and chlorpropamide. Although carbutamide is not now used clinically, the data obtained from its use will be reviewed, as this compound was the first hypoglycaemic arylsulphonylurea to be discovered and many of the original investigations were carried out with it.

Chemistry and Metabolic Features

Carbutamide

Carbutamide (\( N \)-butyl-\( N' \)-sulphanilylurea, BZ55, U6987, Invenol or Nadisan, \( II \)) has physical properties similar to other \( N \)-monosubstituted sulphonamide derivatives, being a white crystalline substance which has weak acidic properties associated with the sulphonamoyl group. It forms salts with alkalis and the sodium salts are easily soluble in water. Estimation of the free compound may be carried out in blood and urine by diazotization and coupling reactions.

In 1956, Ridolfo and Kirtley showed that, as with the earlier sulphonamides, intestinal absorption of carbutamide after oral administration is rapid; the compound may be detected in the blood within 30 minutes
after taking 2.5 g by mouth. The peak blood-level (10–15 mg/100 ml. whole blood) is reached in 3–4 hours (at which time only 3–4 mg/100 ml. is acetylated) and after 6–7 hours the level begins to fall slowly. The decline of sulphonamide concentration in the blood behaves as a first-order exponential function, which gives a biological half-life of 30–60 hours. Within 2–3 hours there is a definite lowering of blood-sugar concentration, the effective blood-level of carbutamide being 6–8 mg/100 ml. The liver partially detoxifies carbutamide by acetylation and then the drug is slowly eliminated via the kidneys. The urine contains about 66 per cent of the free sulphonamide and 33 per cent of the acetylated derivative.

As with other sulphonamides containing a p-amino group, carbutamide has some antibacterial action, and thyroid function is temporarily impaired. MacKensie and MacKensie, for example, showed that various sulphanalamide derivatives may produce goitre in small experimental animals, and Anderson, Worth and Harris reported that this is also true of carbutamide. In human beings with mild diabetes mellitus, large doses of carbutamide (4 g daily) considerably depress thyroid uptake. Longer term studies in 39 patients using therapeutic doses (2 g daily) for 47 weeks showed that the thyroid uptake is depressed to about 20 per cent of the pretreatment value by the end of the third week of therapy, and remains below normal for about 9 weeks. According to Brown and Solomon, the mechanism of the antithyroid effects of carbutamide in rats is inhibition of the organic binding of thyroidal iodide—an effect similar to that of propylthiouracil but only about 1/200th as strong.

Tolbutamide

In tolbutamide (N-butyl-N'-p-toluenesulphonylurea, Rastinon, Orinase, D.860, U.2043, III) the p-amino group of carbutamide has been replaced by a methyl group and this change of chemical structure is responsible for many important biological differences. It is a white crystalline solid with a melting point of 128.5–129.5°C. It is practically insoluble in water although it forms soluble salts with alkalis. It dissolves readily in acetone, chloroform and alcohol.

Since tolbutamide lacks a p-amino group it cannot be estimated by the Bratton and Marshall procedure, but spectrophotometric methods have been developed for the determination of total tolbutamide in blood and urine, based on its intense ultra-violet absorption at 228 mµ. After acidification with 1/15 phosphoric acid, the serum is extracted with chloroform (which must be completely removed subsequently as chloroform also absorbs strongly at 228 mµ). The solvent-free extract is then taken up in 95 per cent ethanol and shaken with charcoal before being estimated spectrophotometrically. This forms a useful and sensitive method for the range 1–25 mg/100 ml. but it lacks specificity; recoveries are good (85–90 per cent). Difficulties may be encountered with variations in the plasma blank and even slight degrees of haemolysis lead to spuriously high readings. The method developed by Toolan and Wagner for chlorpropamide (see below) has recently been found to be suitable for tolbutamide and is unaffected by haemolysis. McDonald and Sawinski and Spingler have developed colorimetric procedures which are probably less satisfactory.
As with carbutamide, absorption of tolbutamide from the intestine is rapid and maximal blood levels are reached in 3–4 hours. Based on the blood levels (extrapolated to zero time) after a given oral dose, the volume of distribution of tolbutamide is found to approximate to that of the extracellular fluid space in man and in nephrectomized, eviserated rabbits. In contrast to carbutamide, the tolbutamide concentration begins to fall according to a first-order exponential curve as soon as the maximal blood level is reached, giving a biological half-life of about 4 hours, both in normal and diabetic subjects. There is considerable individual variation in the blood levels attained, but after 8–10 hours the concentration in most subjects has fallen below the minimum effective range of 6–10 mg/100 ml. after optimal therapeutic doses by mouth. Increasing the dose does not increase the blood level comparably and even with doses of 6 g the blood level 24 hours later is still below the minimum required. Some serum protein-binding probably occurs as tolbutamide does not penetrate into the cerebrospinal or oedema fluid.

In man, tolbutamide is converted into a freely soluble, non-toxic, carboxylic acid (N-butyl-N'-p-carboxyphenylsulphonylurea, VIII) by oxidation of the p-methyl group. This acid has no hypoglycaemic action but is responsible for a variable proportion (10–28 per cent in 24 hours after a single oral dose) of the total blood tolbutamide level. A small amount of N-butyl-N'-p-hydroxymethylphenylsulphonylurea (IX) has also been identified chromatographically in blood. From the urine of dogs given tolbutamide, Mohnike and Wittenhagen isolated a toxic metabolite, p-toluenesulphonamide (X) and also another compound which was later shown to be p-toluenesulphonylurea (XI). Tolbutamide can therefore be metabolized in at least three ways:

\[
\begin{align*}
\text{(XI)} & \quad \text{p-Toluenesulphonylurea} \\
\text{(VIII)} & \quad \text{N-Butyl-N'-p-carboxyphenylsulphonylurea} \\
\text{(III)} & \quad \text{Tolbutamide} \\
\text{(IX)} & \quad \text{N-Butyl-N'-p-hydroxymethylphenylsulphonylurea} \\
\text{(X)} & \quad \text{p-Toluenesulphonamide}
\end{align*}
\]

However, there may be other species differences in the pathways of metabolism of the sulphonylurea compounds.
Tolbutamide is rapidly eliminated by the human kidneys, mainly as the carboxylic acid. This metabolite is freely water-soluble within the physiological urinary pH range and the danger of crystalluria is negligible, but a white precipitate may develop on testing the urine with sulphosalicylic acid if large amounts of compound (VIII) are being excreted. Renal tubular transport is probably important in the excretion of the drug, as there is a much greater proportion of compound (VIII) in the urine compared with blood, and probenecid, which affects many other tubular transport mechanisms, delays the disappearance of tolbutamide from the serum.

The effective and optimal dose of tolbutamide for suitable diabetic patients is 1–3 g/day, but as it is rapidly excreted, the drug must be given in divided doses every 6–8 hours. The optimal blood level is variable and ranges from 8–18 mg/100 ml. Further increases in the dose are, paradoxically, associated with a diminishing hypoglycaemic effect.

Chlorpropamide

Chlorpropamide (N-propyl-N'-p-chlorobenzenesulphonylurea, Diabinese, P.607, IV) is a white crystalline compound melting at 127-5–128-5°C. In contrast to tolbutamide, there is a chlorine atom in the para position on the benzene ring and a propyl instead of a butyl group as the alkyl radical. It behaves as a monobasic acid in aqueous dioxan. Solubility in water is limited below pH 5 but it may readily be dissolved at room temperature in most organic solvents, particularly chloroform, acetone, ethanol and dioxan. Chlorpropamide absorbs strongly at 232.5 mp in 0.01 N hydrochloric acid, and this property forms the basis of its estimation in serum. The serum is acidified with dilute phosphoric acid (0.067 N), extracted with chloroform and then washed with 1 per cent sodium carbonate. Aliquots of the sodium carbonate layer are then neutralized with hydrochloric acid and the ultraviolet absorption measured in a spectrophotometer. The recovery, reproducibility and serum blank levels (about 1.3 mg/100 ml. of apparent chlorpropamide concentration) are good but the method lacks specificity. Toolan and Wagner have shown that β-hydroxybutyric acid, p-chlorobenzenesulphonamide, acetylsalicylic acid and salicylic acid may seriously interfere with the estimation but haemolysis does not. The method is not suitable for detecting chlorpropamide in urine.

The metabolic fate of chlorpropamide after oral absorption has been studied using the radioactive 35S-labelled drug. Absorption from the intestine is more rapid than with carbutamide or tolbutamide, as maximum radioactivity and optical density is reached in 2 hours. The decay of the radioactivity (confirmed by measurement of chloroform-soluble optical density) in the blood-plasma showed that, unlike carbutamide or tolbutamide, chlorpropamide disappeared at two separate rates, a rapid one with an uncorrected biological half-life of 32 hours and a slower component with a half-time of approximately 16 days. In diabetic patients, a comparable
initial rapid decline was seen, but the second component was much slower. The diabetic patients received chlorpropamide or tolbutamide therapeutically for some time before the study was made, so that this observation does not provide good evidence for the suggestion that diabetic and normal subjects handle chlorpropamide differently. The two-phase disappearance rates from the blood may be explained by slow serum protein-binding or by chemical alteration of the compound. Chlorpropamide may be made partially non-dialysable by the plasma proteins, and the degree of binding increases as the chlorpropamide concentration rises. Extrapolation of the slower component to zero time, however, suggests that about 20 per cent of chlorpropamide is represented as the slowly excreted component and this is three times greater than the values obtained from protein-binding experiments.

Urinary excretion of the radioactive chlorpropamide parallels its disappearance from the serum, but considerable variations are seen in diabetic patients pretreated with the drug. Nearly all the administered chlorpropamide is very slowly eliminated in the urine, 77 per cent being accounted for in 96 hours. The observation that salicylate increases the serum level of chlorpropamide and vice versa suggests that renal transport mechanisms are involved. Facial excretion of chlorpropamide is minimal.

In man, this compound in contrast to carbutamide or tolbutamide is probably not metabolized before excretion. No definite chromatographic differences have been found between the pure drug and that in serum or urine, and the ultra-violet absorption spectrum is identical in plasma and urine. However, paper chromatography of the urine of dogs treated with chlorpropamide labelled with $^{35}$S yields three different spots: the unchanged compound, p-chlorobenzenesulphonylurea and p-chlorobenzencesulphonamide, the three compounds accounting for about 30, 40 and 20 per cent respectively of the administered dose. In rabbits, 80 to 95 per cent of chlorpropamide is excreted unchanged.

On a weight for weight basis and using both duration of action and the degree of hypoglycaemia as criterion for evaluation, Roots showed that in rats and dogs chlorpropamide is more effective than tolbutamide. Similar comparisons were made in rhesus monkeys; at dose levels comparable to those used in man (5–10 mg/kg), a definite and prolonged hypoglycaemic action was obtained, whereas a dose of tolbutamide (10 mg/kg) had only minimal effect and 5 mg/kg produced a rise of blood-sugar level. These animal studies, showing that chlorpropamide has a greater and more prolonged hypoglycaemic effect than tolbutamide, have been fully confirmed in man.

The optimal therapeutic dose is between 100 and 500 mg daily, the effective blood chlorpropamide level ranging from 3–17.5 mg/100 ml. With doses of 500 mg or more, blood levels of up to 40 mg/ml. are frequently seen. There is a fairly good relationship between the dose and blood level attained both in normal human beings and in diabetic patients but the relationship between serum level and hypoglycaemic effect varies considerably from patient to patient.

The increased hypoglycaemic potency of chlorpropamide as compared with tolbutamide is probably due to the higher initial and more prolonged blood levels attained, and not to any inherent increased 'potency'.
although a greater hypoglycaemic effect has been obtained with equivalent blood levels\textsuperscript{56}. Studies of serum levels and blood-sugar concentration in human beings do not suggest a cumulative action in doses of 500 mg or less, either in normal\textsuperscript{64} or diabetic subjects\textsuperscript{63}.

As with tolbutamide, thyroid function as assessed by plasma protein-bound iodine levels or the thyroid uptake of radiiodine, is not impaired by chlorpropamide\textsuperscript{66} and the drug has no antibacterial action.

\textbf{Metahexamide}

Metahexamide (\textit{N}-cyclohexyl-\textit{N}'-(3-amino-4-toluenesulphonyl) urea, Euglycin, \textit{V}) is a very effective hypoglycaemic agent with a duration of action comparable to that of carbutamide and a potency which is rather higher than that of chlorpropamide. In rats it is more active than either tolbutamide or chlorpropamide on a weight for weight basis\textsuperscript{67}. In normal humans the hypoglycaemic potency of metahexamide is four times higher than chlorpropamide or tolbutamide when judged by the serum concentration attained or twice as active as chlorpropamide and fifteen times more potent than tolbutamide when assessed by weight\textsuperscript{68}. Absorption from the intestine is rapid, being complete in 2–3 hours\textsuperscript{68}. The biological half-time is 19–26 hours in normal people\textsuperscript{68,69} but it may be longer in diabetic patients\textsuperscript{70}. It is about half as effective as chloropropamide and about five times as effective as tolbutamide in producing sustained blood levels\textsuperscript{68}. Dogs and rabbits excrete 30–35 per cent of the administered metahexamide dose unchanged in the urine and 45–50 per cent as 3-amino-4-benzenesulphonamide\textsuperscript{57}. The aromatic amino group is resistant to acetylation even in rabbits, a species that acetylates carbutamide readily\textsuperscript{83}. In appropriate diabetic patients, a single daily dose of 50–300 mg gives an adequate hypoglycaemic response and it compares favourably with tolbutamide and chlorpropamide\textsuperscript{70–73}. Patients resistant to tolbutamide may respond to metahexamide but those resistant to chlorpropamide usually cannot be controlled with the drug\textsuperscript{69}. Gastro-intestinal and allergic side-effects are probably as frequent as with other arylsulphonyl compounds, particularly when high doses are used. No information about the effect of metahexamide on the bone marrow is available at present, but jaundice of the obstructive variety occurs\textsuperscript{74,74a}, and for this reason it is no longer used clinically.

\textit{SB 1}

This compound (\textit{N}-(3-aminobenzenesulphonyl)-\textit{N}'-n-butylurea, Sucrida Berna, \textit{VI}) differs from carbutamide by having the aromatic amino group in the meta instead of the para position on the benzene ring. It has no antibacterial activity\textsuperscript{75}. In divided doses of 0.5–1.5 g daily, \textit{SB 1} produces an adequate hypoglycaemic response in selected diabetic patients\textsuperscript{76}. In rabbits it is rather less toxic and somewhat more potent than tolbutamide on a weight for weight basis\textsuperscript{77}. Hypoglycaemia is maximal 6–9 hours after 250 mg/kg orally and lasts for 13–15 hours. Urinary excretion of an acetylated metabolite is rapid, 40–50 per cent being eliminated in 12 hours.
ORAL HYPOGLYCAEMIC DRUGS

Furfuralurea

Furfuralurea (N-2'-furfuryl-N'-p-toluenesulphonylurea, VII) is mentioned by Danowski and Mateer78. Its hypoglycaemic activity is comparable to that of chlorpropamide.

The Toxicity of Tolbutamide and Chlorpropamide in Animals and in Man

Toxicity studies in animals with chlorpropamide and tolbutamide indicate that with both compounds the acute and chronic toxicity is low69,67. For tolbutamide, the oral LD$_{50}$ (calculated according to the method of Litchfield and Wilcoxon79) in rats is 2.49 and in mice is 1.83 g/kg. By the intravenous route the corresponding figures are 0.77 and 0.70 g/kg respectively. Chlorpropamide is slightly more toxic, the oral and intravenous LD$_{50}$ for rats being 2.39 and 0.59 and for mice, 1.67 and 0.50 g/kg respectively. Similar results are reported by Root, Sigal and Anderson67. Chronic toxicity experiments with chlorpropamide indicated that in dogs oral doses of 150 mg/kg (corresponding to more than 20 times the recommended clinical dose in man) may be tolerated, the only symptoms being ataxia and muscular weakness in some of the animals. In higher doses (up to 200 mg/kg) rhesus monkeys remained well, apparently, except for some intermittent diarrhoea. No histological or functional changes were observed in the liver69.

This lack of hepatotoxic effect contrasts sharply with the effect of tolbutamide in depancreatized and partially depancreatized dogs and puppies60. In doses of 100–150 mg/kg, all the animals died in 3–6 weeks with severe liver damage; one out of three animals receiving 30 mg/kg (the amount usually recommended for humans) succumbed in a similar fashion. At all dose levels, the albumen and the protein-bound polysaccharide levels in the plasma declined, the serum alkaline phosphatase, glutamic-oxaloacetic and glutamic-pyruvic transaminase levels rose sharply, and in two animals the prothrombin time was prolonged terminally. This was unaffected by Vitamin K. The bromsulphalein excretion (often used as a more refined test of hepatic function in man) remained normal. These effects, which are seen in dogs, may be due to the formation of a toxic metabolite, possibly p-toluenesulphonamide61. Similar hepatotoxic effects in dogs have been described61,82.

When used therapeutically in man, tolbutamide, however, does not damage the liver. Repeated liver function studies have shown that changes of the serum bilirubin, flocculation tests and bromsulphalein excretion have not been seen over a period of 12–16 months83,84. A slight rise of serum alkaline phosphatase level has been reported85 but its significance is not clear. Dolger86 has given tolbutamide to several patients who recently recovered from hepatitis or obstructive jaundice, without evidence of further liver damage.

Chlorpropamide, however, may cause an intrahepatic obstructive jaundice of the type seen sometimes following chlorpromazine or methyltestosterone. This develops most commonly in patients given over 500 mg daily but it has been reported after smaller doses87,88. The condition subsides rapidly on stopping the drug and evidence of permanent liver-cell damage has not, as
yet, been reported. Liver biopsy material which showed histological evidence of a healing pericholangiolitis was found in one out of seven patients on chlorpropamide, and four out of six patients taking metahexamide. The hepatic histology was normal in all of six patients given tolbutamide.

With tolbutamide or chlorpropamide, most workers have been unable to detect any significant change in the quantity or appearance of the white blood-cells, but leucopenia has occasionally been reported. In contrast to the serious effects of carbutamide, the leucopenia is usually transient and no case of agranulocytosis has so far been recorded.

As far as important side-effects are concerned, tolbutamide and chlorpropamide are remarkably non-toxic when used in effective hypoglycaemic doses for appropriate diabetic patients. At the Joslin Clinic, since November 1957, only 1.1 per cent of 772 patients treated with tolbutamide have developed side-effects sufficiently severe to warrant stopping the drugs. Dolger has seen no serious toxic reactions among 500 private patients treated for 3–12 months. Chlorpropamide seems equally innocuous considering the large number of patients who do not suffer from any side-effects, but a curious ataxia with muscle weakness (not due to hypoglycaemia) has been described following large doses. Minor side-effects are however more frequently encountered. Both drugs may cause gastro-intestinal symptoms such as epigastric pain, nausea and diarrhoea; and, in patients with peptic ulcer, their dyspepsia may be aggravated. These features appear to be rather more common with tolbutamide than with chlorpropamide. Urticarial skin rashes are not uncommon and a single case of purpura has been reported by Sugar. One patient nearly died of anaphylactic shock following chlorpropamide. During treatment with tolbutamide, flushing of the face may occur if ethanol is taken, and an antabuse-like effect may occur with chlorpropamide. Most clinical reports describe abdominal symptoms and skin rashes in 1–7 per cent of patients.

THE RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND ACTIVITY OF THE HYPOGLYCAEMIC SULPHONAMIDES

Sulphanilamidothiadiazoles

Between 1942 and 1946, Loubatières and Bovet and Dubost tested many thiazole derivatives in dogs and rabbits during studies of the relationship between structure and hypoglycaemic activity in this series. When the alkyl group of the original compound, 5-isopropyl-2-sulphanilamido-1,3,4-thiadiazole, (I) was replaced by either methyl or ethyl radicals, the hypoglycaemic effect was reduced or even lost. Maximum activity was obtained when the alkyl chain in the 5-position was either an iso or a tertiarybutyl group but longer hydrocarbon chains, e.g. hexyl or heptyl, produced compounds with little hypoglycaemic activity (Table 5.1). In a clinical trial, 5-t-butyl-2-sulphanilamido-1,3,4-thiadiazole (RP 2259, Gli pasol) was shown to control the blood-sugar level in 65 per cent of the thirty-one patients but its use was not recommended because of its toxicity.
to the liver. Thiadiazole derivatives without a p-aminobenzenesulphonyl portion (e.g., 2-amino-5-propyl- or -5-isopropylthiadiazole, or 2-acetamido-5-isopropylthiadiazole) or without the p-amino group (2-benzenesulphonamido-5-isopropylthiadiazole) had no hypoglycaemic properties, but 2-(p-methoxybenzenesulphonamido)-5-isobutylthiadiazole (Stabinol) was recently reported to be active. Loubatier also found that alcohols corresponding to the hydrocarbon chains, particularly isopropyl, butyl and pentyl alcohols, themselves have some hypoglycaemic activity, and he postulated that it was the hydrocarbon moiety that conferred hypoglycaemic activity, although the sulphanilamido portion was nevertheless essential as a ‘reinforcing agent’ to the rest of the molecule.

### Arylsulphonylureas

Only a few of the many hundreds of sulphonylurea compounds which have now been synthesized possess sufficient hypoglycaemic potency and duration of action to justify more detailed investigation. Nevertheless, it is interesting to examine how variations in chemical structure modify hypoglycaemic activity. There is a close structural similarity between the early sulphanilamidothiadiazoles e.g. (I) and the later sulphonylureas e.g. (II). In view of this chemical resemblance, it is surprising that the hypoglycaemic effects of the sulphonylurea compounds were discovered so much later, and even then by chance. The discovery of the hypoglycaemic properties of carbutamide was soon followed by the discovery that the aromatic p-amino group was not necessary for hypoglycaemic activity and the potent p-methyl analogue, tolbutamide, was synthesized. This compound and all subsequent ones without a free aromatic p-amino group e.g. SB 1 (VI) have no antibacterial activity. Tolbutamide, however, is a short-lived drug within the body because of the metabolic oxidation of the methyl group, and a more potent, longer-acting drug was sought, with a similarly low degree of toxicity.

The effect of change in structure of a series of sulphonylureas, ArSO₂-NH-CNHR, on hypoglycaemic activity was systematically studied by giving fasting rats a single oral dose of 100–300 mg/kg of the compound. Modification of the urea portion led to loss of hypoglycaemic activity although
J. D. H. SLATER

weak action was retained when the NH-CO-NHBu group was replaced by a
NH-CS-NHBu, NH-CO-OBu or NH-CO-Ph group (Table 5.2). As with the

Table 5.2. Effect on hypoglycaemic activity of chemical changes in the urea
part of the molecule:*

<table>
<thead>
<tr>
<th>NHXSNHBu</th>
<th>Activity</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH-CS-NHBu</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>NH-CO-OBu</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>NH-CO-CH₃Me</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>NH-CO-CH₂Bu</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>NH-CO-Ph</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>NH-NH-CO-NHBu</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>NH-NH-CO-Pr</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>NMe-CO-NHBu</td>
<td>±</td>
<td>±</td>
</tr>
</tbody>
</table>

sulphanilamidothiadiazoles, changing the length and character of the alkyl
radical has, in most of the active compounds, a definite but relatively small
effect on hypoglycaemic activity (Table 5.3). Simple alkyl chains of three or
four carbon atoms endow maximal activity and homologues having a

Table 5.3. Effect on hypoglycaemic activity of changes in the substituents on
the terminal nitrogen atom of N-arylsulphonylurea compounds:

<table>
<thead>
<tr>
<th>Radical, R</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal alkyl, C₆H₅ or C₄H₉</td>
<td>+++, to ++++</td>
</tr>
<tr>
<td>Branched alkyl, C₆H₅ to C₆H₁₁</td>
<td>+++, to ++++</td>
</tr>
<tr>
<td>Alicyclic, C₅H₆ or C₆H₁₁</td>
<td>+++, to ++++</td>
</tr>
<tr>
<td>Aryl, -Cl</td>
<td>++ to +++</td>
</tr>
<tr>
<td>Dialkyl, N</td>
<td>++ to +++</td>
</tr>
</tbody>
</table>

branched alkyl chain (such as tertiary butyl) or a cyclic substituent have
good activity; in the latter case, a five to seven carbon atom radical seems
to give maximal effect e.g. metahexamide (V) and N-cyclohexyl-N'-p-
toluene sulphonylurea which is reported to be about as active orally and
intraperitoneally as tolbutamide but to have a lower toxicity. Comp-
ounds in which the alkyl-carrying nitrogen atom is di-substituted are
moderately active.

The greatest enhancement of hypoglycaemic effect is achieved, however,
by substitution in the aromatic ring, Ar (Table 5.4). Para-substitution
gives the most potent and longest-acting compounds, the halogens, especially
chlorine, being particularly active. The p-methoxy analogue of tolbutamide
has been reported to be about as active orally as tolbutamide in rabbits and
to be less toxic. But substitution of groups other than methyl, methoxy
or halogen in the p-position gives compounds of low activity, for example,
ORAL HYPOGLYCAEMIC DRUGS

$p$-isopropyl, $p$-carboxy, $p$-ethoxycarbonyl or $p$-hydrazinocarbonyl\textsuperscript{28}. Unsubstituted phenyl derivatives are rather less active, and ortho-substituted and di-substituted (2,4-, 2,5-, 3,4-) phenyl compounds are even weaker\textsuperscript{29}.

Table 5.4. Effect on hypoglycaemic activity of substitution in the aryl radical of the $N$-alkyl-$N'$-arylsulphonylurea compounds\textsuperscript{29,77-81}: $\text{ArSO}_2\text{NHCO-NHR}$

<table>
<thead>
<tr>
<th>Aryl radical, $\text{Ar}$</th>
<th>Alkyl radical, $\text{R}$</th>
<th>Hypoglycaemic action</th>
<th>Acute effect</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{NH}_2$</td>
<td>Butyl</td>
<td>++ + +</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>$\text{NH}_2$</td>
<td>Butyl</td>
<td>+ +</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>$\text{Me}$</td>
<td>Butyl</td>
<td>+ +</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>$\text{Me}$</td>
<td>Butyl</td>
<td>$\pm$</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>$\text{Me}$</td>
<td>Butyl</td>
<td>++ + +</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>$\text{Me}$</td>
<td>Propyl</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>$\text{Me}$</td>
<td>Propyl</td>
<td>+ + + +</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>$\text{Me}$</td>
<td>Isopropyl or ethyl</td>
<td>++ + +</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>$\text{Me}$</td>
<td>Butyl</td>
<td>++ + +</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>$\text{Me}$</td>
<td>Butyl</td>
<td>++ + +</td>
<td>++ + +</td>
<td></td>
</tr>
<tr>
<td>$\text{Cl}$</td>
<td>Propyl</td>
<td>++ + +</td>
<td>++ + +</td>
<td></td>
</tr>
<tr>
<td>$\text{Br}$</td>
<td>Propyl</td>
<td>+ + + +</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>$\text{F}$</td>
<td>Propyl</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Naphthylsulphonylureas are only feebly hypoglycaemic but compounds in which the aromatic ring is replaced by a heterocyclic group (for example,
2-thienyl, or 5-chloro-2-thienyl) are moderately active. A detailed study of the effect of chemical modification on hypoglycaemic activity may throw considerable light on the mechanism of action of these compounds.

MODE OF ACTION OF THE ARYLSULPHONYLUREA COMPOUNDS

Sulphonylurea compounds are now widely used in selected cases of diabetes mellitus. Probably over a million patients all over the world have already been treated with tolbutamide or chlorpropamide, or, to a much lesser extent, with metahexamide. Responsive diabetics may take sulphonylurea compounds continuously for considerable periods of time. Many publications on the mode of action of the sulphonylurea drugs, particularly carbutamide, have appeared since 1955, but the question of how the drugs lower the blood-sugar concentration still remains partly unsolved.

It is generally agreed that in mammals the sulphonylurea compounds only produce hypoglycaemia when some functioning pancreatic beta-cells are present. Houssay and Penhos, for example, showed that the presence of some pancreatic tissue is essential for the hypoglycaemic effect of these compounds in much the same way that Loubatières showed that this effect was true of the sulphanilamidothiadiazoles. The sulphonylureas reduce the fasting blood-sugar level of intact and partially pancreatectomized animals or animals made mildly diabetic with alloxan, but they do not do so after total pancreatectomy in dogs, rabbits, rats, cats, toads or man, nor in animals made severely diabetic with alloxan.

In human beings, the arylsulphonylurea compounds are effective only in individuals who have a pancreatic reserve of insulin. They reduce the fasting blood-sugar concentration in non-diabetics and mild diabetics whose illness started in adult life, but with few exceptions the drugs do not act in patients with severe diabetes of the juvenile type. This may be because the pancreas of diabetic adults contains over 30 per cent of the normal insulin content, while in juvenile diabetic patients little or no insulin may be extracted from the gland. In a similar way, insulin-like activity may be readily detected in the plasma of many diabetic adults but generally is not found in plasma of patients with the juvenile form of the disease.

After evisceration in dogs and rabbits, the drugs cease to produce hypoglycaemia. This suggests that the lowering of blood-sugar concentration is not produced by a direct action on peripheral tissues, although some studies suggest that the sulphonylurea compounds stimulate isolated excised muscle to take up glucose (see below).

However, the dependence of the hypoglycaemic action of the sulphonylurea compounds on the presence of some islet-containing pancreatic tissue only applies to mammals. Mirsky and Gitelson, for example, showed that a hypoglycaemic response to tolbutamide may be obtained in alloxanized chickens and in depancreatized or enterectomized ducks. Hazelwood has further shown that hepatectomy, with or without pancreatectomy does not prevent the hypoglycaemic response in domestic fowls. As the carbohydrate metabolism in avians is therefore complex, all further remarks in this review will be concerned with the mode of action of the sulphonylurea compounds in mammals.
The sulphonylurea drugs may produce hypoglycaemia in mammals by inhibition of the secretion of the pituitary or adrenal glands, but this is unlikely as removal of either or both of these glands does not prevent the hypoglycaemic effects. For example, hypophysectomy in dogs increases the degree of hypoglycaemia (thus simulating the effects of insulin) yet hypophysectomized cats are not more sensitive to the sulphonylurea drugs than are normal animals. Unlike insulin, however, adrenalectomy greatly enhances their hypoglycaemic effect in both cats and dogs. The drugs are effective in patients with pan-hypopituitarism and Addison's disease, both before and after replacement therapy with hydrocortisone, and the hyperglycaemic response to adrenaline is unaffected during the administration of these drugs.

The idea that the sulphonylurea compounds inhibit the release of glucagon from the alpha-cells of the pancreas was suggested in 1956 by Ferner and Runge who found histological evidence of damage to the alpha-cells following carbutamide. Similar changes had previously been reported after IPTD. The sulphonylureas however are ineffective in severe alloxan diabetes. The disappearance of labelled glucagon is unaffected by pretreatment with tolbutamide or carbutamide, and the hypoglycaemic response to injected glucagon is not inhibited in man. The possibility of tissue antagonism to glucagon is therefore unlikely. It appears that hypoglycaemia as such may alter the staining properties and decrease the granularity of the pancreatic alpha-cells.

From these results, the following conclusions may be drawn concerning the mechanism by which sulphonylurea compounds lower the blood-sugar level in mammals: (a) they do not possess a direct insulin-like action on peripheral tissues; (b) the presence of an insulin-containing pancreas is essential; (c) they do not act by reduction of glucagon secretion or by tissue antagonism to glucagon, and (d) inhibition of hormonal antagonists of insulin from the pituitary or adrenal glands is not involved.

Three ways of explaining the hypoglycaemic effect of the compounds therefore remain: (a) the pancreas is stimulated to release more insulin, (b) the rate of endogenous insulin destruction is diminished, and (c) hepatic glucose release and/or production is depressed.

**Increased Release of Insulin from the Pancreas**

This was first suggested by Loubatières to explain the hypoglycaemic effects of IPTD and other thiadiazolylsulphonamides. In cross circulation experiments, he showed that when IPTD was injected into a dog whose pancreatic-duodenal vein was anastomosed to the jugular vein of a depancreatized dog, the blood-sugar level of the diabetic animal was depressed. In similar experiments with intact animals previously given intravenous carbutamide, the blood-sugar level of the recipient animal is lowered by injections of the pancreatic venous blood of the donor, but not by injections of blood from the donor's mesenteric vein. Direct perfusion of the pancreas with tolbutamide via the right gastro-epiploic branch of the gastro-duodenal artery produced a greater reduction in the blood-sugar level than did injection into the femoral vein. But hypoglycaemia was not produced by
pancreatic injection in doses which were too small to be effective when given peripherally. Other workers found that the hypoglycaemic effect when metahexamide or chlorpropamide was injected into the pancreatic artery was no greater than when either drug was given into the femoral vein.

The response to tolbutamide is roughly proportional to the insulin content of the pancreas of the mammal. Mirsky, Perisutti and Gitelson showed that pretreatment of dogs with growth hormone from the pituitary gland, a hormone known to deplete the pancreas of insulin, reduces the acute hypoglycaemic effect of tolbutamide. Similarly, prolonged fasting, which also reduces the insulin content of the pancreas, diminishes the hypoglycaemic response. The onset of alloxan diabetes in cats is heralded by a phase of hypoglycaemia which is probably due to the release of preformed insulin from necrotic beta-cells in the pancreatic islets. Pretreatment with tolbutamide prevents this effect so the drug may reduce the amount of insulin within the islets. Four weeks after hypophysectomy in rats, the amount of insulin in the pancreas falls to about half the pre-operative level and sensitivity to tolbutamide diminishes correspondingly. In human beings, the rate at which the blood-sugar concentration is lowered following a single dose of tolbutamide (1 g intravenously) may be used as a diagnostic test for mild diabetes; the decrease is more rapid and more profound in non-diabetic patients than in patients with mild diabetes. Wrenshall and Hamilton have shown, using post-mortem material, that the levels of extractable pancreatic insulin are lower in diabetic men than women. Analysis of clinical data also indicates that adult males with mild diabetes are considerably less likely to respond to the sulphonylurea drugs than are women, a result which adds more evidence to the hypothesis that the hypoglycaemic effect of the sulphonylurea compounds is related to the reserve of pancreatic insulin.

Morphological studies of the pancreatic islet tissue in animals show that tolbutamide or carbutamide produce degranulation of the beta-cells with swelling of their nuclei; the islets increase in size and number and mitoses are more frequent. No significant morphological changes in the islet tissue have been reported as yet in diabetic patients who have died while receiving tolbutamide. Histological changes in the beta-cells, however, have been correlated with changes of blood-sugar level and pancreatic insulin content. In experiments with calves, oral tolbutamide was shown to produce a transient degranulation of the beta-cells, with increase in nuclear volume and a transient reduction of extractable insulin from the pancreas. The fall in blood-sugar concentration closely paralleled these changes. Carbutamide lowers the amount of insulin extractable from the pancreas of dogs but this does not persist. The suggestion that beta-cell degranulation is a specific response to the hypoglycaemic sulphonylurea compounds has been made by Creutzfeldt, Detering and Welte, who found that large doses of two other inactive sulphonylureas (N-p-toluene-sulphonyl-N'-methylurea and N-sulphanilyl-N'-ethylurea) failed to alter the histology of the beta-cells.

Perhaps, the most compelling evidence that sulphonylureas produce hypoglycaemia by the release of endogenous insulin is that the insulin-like
activity of pancreatic venous blood of dogs increases 'many times' after the administration of hypoglycaemic sulphonylureas. Goetz and Egdahl have confirmed this by assaying the ability of pancreatic venous blood of dogs given tolbutamide to lower the blood-sugar level in fasting, intact mice. In rats, a group of German workers measured the incorporation of glycogen into rat diaphragm muscle as a test for insulin and reported that the insulin-like activity of peripheral venous blood is increased by carbutamide and tolbutamide. Evidence concerning the effect of sulphonylureas on the plasma insulin-like substances in human beings, however, is conflicting. Venous blood taken during maximal hypoglycaemia following intravenous or oral tolbutamide fails to stimulate the uptake of glucose by the rat diaphragm

If the sulphonylurea compounds produce hypoglycaemia by stimulating insulin release from the pancreas, then it should be possible to demonstrate in man some of the metabolic effects of increased peripheral glucose utilization. After intra-arterial injections of insulin, the peripheral arterio-venous glucose differences of the injected limb increase, plasma potassium and phosphate concentrations decrease, lactate and pyruvate levels rise and the respiratory quotient increases. These changes have not generally been observed following tolbutamide, carbutamide or chlorpropamide. Such negative findings lose much of their importance, however, when it is realized that these indices of peripheral glucose utilization are relatively insensitive and liable to considerable experimental error.

Measurement of peripheral arterio-venous glucose differences are difficult to interpret, and this is particularly so when the blood-flow through the tissues, known to be increased by insulin, is not measured simultaneously. The decrease in the blood-sugar concentration following intravenous insulin injections (or following the rise after a glucose load) is associated with a narrowing of peripheral arterio-venous glucose concentration differences but the proportion of arterial glucose concentration taken up by the tissues

nevertheless widens, where \( A \) and \( V \) are the glucose levels in artery and vein respectively. This parameter was measured following large doses of intravenous tolbutamide during 5 or 10 per cent glucose infusions. In mildly diabetic and in normal subjects no change was detected in the ratio \( \frac{A - V}{A} \) when the results were analysed statistically. However, simultaneous blood-flow measurements were not performed, and closer scrutiny of the data shows that when definite hypoglycaemia was produced the ratio \( \frac{A - V}{A} \) increased considerably. A rise in the ratio \( \frac{A - V}{A} \) in fasting normal subjects following intravenous doses of tolbutamide has been described. Butterfield, Fry and Holling developed a method for
the simultaneous measurement of forearm blood-flow and found that there is a critical blood-sugar concentration below which glucose fails to enter cells. This threshold value is raised in diabetes and may be lowered by oral tolbutamide.

When the changes in glucose uptake are slight, there is no correlation between the change of glucose assimilation and the maximal decrease in serum inorganic phosphorus concentration. Likewise, alterations of serum pyruvate and lactate concentrations following the sulphonylureas are also insignificant. Moreover, difficulties in finding consistent and unequivocal evidence of increased peripheral glucose utilization also apply to the changes following insulin. When insulin is given intravenously in doses which mimic the fall of blood-sugar level produced by tolbutamide, evidence of increased glucose uptake by the tissues is also often inconclusive, especially if the injection is made slowly. Madison and Unger claim that when insulin is injected into the portal vein (thus simulating endogenous insulin secretion) little effect on peripheral arterio-venous glucose differences may be demonstrated, although equivalent doses of insulin injected into a peripheral vein are followed by an increase which is easily measured. No qualitative differences have however been observed in the serum potassium, pyruvate and lactate levels after injections of insulin into the portal vein and into the femoral veins of dogs, although different rates of insulin administration (rapidly, or 1.0 and 0.1 units/5 minutes) were employed at two different dose levels (0.1 or 1.0 units/kg). In intact animals, the sulphonylurea compounds increase the liver glycogen content but do not affect significantly that of muscle glycogen, whereas single injections of insulin have the reverse effect. When an intravenous infusion of insulin is given to fasting intact rats, the blood-sugar decreases although the muscle glycogen remains unchanged. This again emphasizes that the metabolic response is greatly affected by the route and the rate of insulin administration.

Nevertheless, there are two effects other than the hypoglycaemic action in which the sulphonylureas may mimic the metabolic effects of insulin. Firstly, tolbutamide reduces the blood amino acid level in normal subjects and in stable diabetic patients, and, in vitro, an increased incorporation of $^{14}$C-glycine into rat liver-slice protein has been observed. Secondly, serum unesterified fatty acid levels are reduced by about 30 per cent following tolbutamide and metahexamide. These two findings are important as they show that the sulphonylureas do not merely produce hypoglycaemia but may also simulate some of the other effects of insulin.

**Diminished Destruction of Insulin**

This mechanism has been suggested since insulinase in rat liver-slices is inhibited by the sulphonylurea drugs. It is of doubtful significance, however, since insulinase is not inhibited by the concentrations of tolbutamide reached in man following therapeutic doses. The rate of degradation of $^{131}$I-labelled insulin in rabbits is not altered by tolbutamide and the liver of rats pretreated with the drug destroys $^{131}$I-labelled insulin at the normal rate. Likewise most workers have not been able to show that the
ORAL HYPOGLYCAEMIC DRUGS

sulphonylureas potentiate the effect of exogenous insulin either in severe diabetic patients or following pancreatectomy in man. However, there is considerable evidence in depancreatized dogs that large doses of carbutamide or tolbutamide\textsuperscript{80-8,106,117} potentiate insulin. This effect, which is obtained only after prolonged administration, is probably related to the severe hepatic damage that these compounds produce in dogs. Diminished destruction of insulin therefore is not an important effect of the sulphonylureas, but, as Mirsky suggests\textsuperscript{123}, inactivation of insulinase may contribute to the slow rise in the blood-sugar level following tolbutamide-induced hypoglycaemia.

\textit{Reduction of Hepatic Glucose Output by a Direct Action on the Liver}

There is little doubt that tolbutamide reduces the output of glucose from the liver in human beings and in dogs. This occurs both during fasting and after fructose administration\textsuperscript{168,169} and may be measured directly by catheterization studies or indirectly by determining the specific activity of plasma glucose following injections of \textsuperscript{14}C-labelled glucose\textsuperscript{104,148,149,170,171}.

Tolbutamide interferes with the \textit{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\textsuperscript{172-4}. The increased glucose output induced by adrenalin is particularly affected\textsuperscript{164}. Weber and Canter\textsuperscript{175} found that tolbutamide inhibits glucose-6-phosphatase predominantly, slightly reduces phosphohexoisomerase activity but does not alter liver phosphoglucomutase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. This suggests that tolbutamide \textit{in vitro} has a specific effect on glucose-6-phosphatase but the concentrations necessary for this effect are 5-10 times higher than the peripheral blood levels which produce hypoglycaemia \textit{in vivo}. Liver tissue removed from rats during tolbutamide hypoglycaemia, however, fails to show reduced glucose-6-phosphatase activity\textsuperscript{178}, and many other sulphonamides or sulphonamide derivatives which do not lower the blood-sugar level may also inhibit liver glucogenic enzymes. This has recently been confirmed by Jasmin and Johnson\textsuperscript{177} who found that there is no relationship between the hypoglycaemic activity of 17 new 5-alkyl-2-benzenesulphonamido-1,3,4-thiadiazoles and their ability to inhibit glucose-6-phosphatase (using the method of Cori and Cori\textsuperscript{178}) in mice liver homogenates. Tolbutamide produced a 50 per cent inhibition at $5 \times 10^{-3}$M concentration, and a comparable degree of inhibition was obtained with concentrations of sulphaethylthiadiazole which have antibacterial, but no hypoglycaemic, activity. The presence of the liver is not essential for the hypoglycaemic action of the sulphonylurea compounds, since hepatectomy does not prevent the hypoglycaemic response to tolbutamide. Further, moderate doses of tolbutamide produce a similar decrease in the blood-sugar concentration in hepatectomized and intact dogs\textsuperscript{160,179}.

These findings make it difficult to believe that, in therapeutic doses, the sulphonylurea compounds reduce hepatic glucose output by a \textit{direct} action on the liver, and, as described above, the evidence points to the sulphonylurea compounds producing hypoglycaemia by reducing glucose release and/or
production from the liver. This apparent paradox can only be resolved if it is assumed that endogenous insulin, secreted into the portal vein, produces a diminution of hepatic glucose output. If this is not so, it is difficult to explain why the sulphonylureas are only effective in the presence of an insulin-containing pancreas, although it may be that the metabolic upset of alloxan diabetes, human juvenile diabetes and the diabetes following total pancreatectomy, masks in some way a primary hepatic effect of the drug. Our knowledge of the hepatic action of insulin is still inconclusive. An increase of hepatic glycogen under the influence of insulin can be demonstrated readily in intact animals provided the insulin is rigorously glucagon-free and no fall of blood-sugar is allowed to occur.

In man, the glucose output from the splanchnic area was shown by catheterization techniques to be reduced by insulin. Later, it was suggested that insulin increased the glucose uptake by hepatic as well as by peripheral cells, but these experiments did not distinguish between the relative contributions of the liver and the rest of the splanchnic tissues, and the problem remains unsolved. Comparing the intraportal and peripheral venous routes of insulin administration in anaesthetized dogs, Madison and Unger claim to have found that intraportal insulin produces a greater decrease of hepatic glucose production and a relatively smaller increase of peripheral glucose utilization than when insulin is injected into a foreleg vein. Their conclusions were based on glucose gradients without simultaneous blood-flow measurements. More recently, Shoemaker, Mahler and Ashmore using direct simultaneous measurements of glucose concentration gradients across the liver, the total splanchnic bed, and the non-hepatic splanchnic tissue bed, were unable to detect any decrease of hepatic glucose output following various doses of insulin. These studies were performed in unanaesthetized dogs and were combined with simultaneous measurements of hepatic blood-flow. Tarding and Schambye have also failed to show a reduction of hepatic glucose output following constant intraportal infusions of small amounts of insulin.

The rate of decay of the specific activity of plasma glucose after a single injection of $^{14}$C-labelled glucose has been used as an index of hepatic glucose output. After the intravenous administration of 10 units of insulin into unanaesthetized, intact dogs, a 'plateau' lasting 10–20 minutes was observed in the specific activity values. Similar effects have been reported in human beings. These observations are claimed to indicate suppression of the entry of glucose into the circulation, but similar experiments using constant infusions of $^{14}$C-labelled glucose to eliminate problems of equilibration failed to confirm this interpretation. However, during a prolonged infusion of insulin, the increased hepatic glucose output in response to hypoglycaemia was held in abeyance until the infusion was stopped, and then it rose sharply.

Although the evidence from animal experiments that the sulphonylureas produce hypoglycaemia by releasing insulin from the pancreas is convincing, the only unequivocal evidence of such a mechanism in man is that juvenile diabetic and pancreatectomized patients do not respond to the drugs. Reduction of glucose production in the liver appears to be the reason for the decrease in the blood-sugar level after the administration of sulphonylurea.
compounds. This may be produced pharmacologically by a direct, possibly enzymic, effect on the liver or physiologically by an increased output of endogenous insulin. The answer to this problem will probably not be found until our knowledge of the hepatic effect of insulin is more precise. Both mechanisms may operate, and a direct action on the liver-cells may allow endogenous insulin to reduce the hepatic glucose output.

**Extrapancreatic Effects of Sulphonylurea Compounds**

The *in vitro* effects of sulphonylureas on isolated tissues other than the liver are still largely unexplored. This type of study is important not so much to further our understanding of the mechanism of the fall in blood-sugar produced by the drugs, but to explore the possible ways in which insulin release from the pancreas is accomplished and to detect any secondary effects which may be harmful.

Most workers have been unable to detect any increase in the rate of glucose uptake by the rat hemidiaphragm or in the incorporation of glycogen into diaphragm muscle, when incubations are carried out in bicarbonate buffer with high concentrations of tolbutamide\(^{186,187}\) or carbutamide\(^{188}\). It has been further shown that the stimulating effect of insulin on glucose uptake by the diaphragm is unaffected by tolbutamide\(^{187}\).

However, Raphaelson\(^{189}\) found that both carbutamide and tolbutamide considerably increase the glucose uptake by the diaphragm at a comparable sulphonylurea concentration. He found considerable variation at a given drug concentration and there was no suggestion of a dose-response relationship. Similar findings have also been reported elsewhere\(^{190,191}\). These different results are difficult to explain at the present time and further work is required.

Tolbutamide and chlorpropamide stimulate the rate of oxidation of \(^{14}\)C-labelled glucose to carbon dioxide by the rat epididymal pad of fat and diminish the incorporation of glucose into lipid\(^{192}\). Further experiments with glucose-1-\(^{14}\)C and glucose-6-\(^{14}\)C show that most of the increased carbon dioxide production arises from carbon-1 of the glucose molecule, suggesting stimulation of the phosphogluconate oxidative pathway. Increased glucose oxidation is generally accompanied by increased synthesis of fatty acid from glucose carbon\(^{193}\) so the significance of this finding is uncertain.

Slices of liver tissue removed from rats fasted for 48 hours show considerable inhibition of ketogenesis when incubated with chlorpropamide or tolbutamide at concentrations within the expected therapeutic range, and this effect is not altered by using liver tissue from pancreatectomized or alloxanized animals\(^{192}\). The relationship of this finding to the production of hypoglycaemia is obscure. Bornstein\(^{194}\) has reported that hepatic alanine transaminase is inhibited *in vitro* by sulphonylureas.

These scattered, preliminary observations are compatible with the hypothesis that the sulphonylureas affect enzyme reactions which depend upon pyridine nucleotides as co-factors\(^{195}\).
CLINICAL CONSIDERATIONS OF THE SULPHONYLUREA COMPOUNDS

The sulphonylurea compounds are only effective in patients with mild diabetes of the late-onset or adult type; 60-70 per cent of the diabetic population belong to this group. They are not insulin-deficient (as defined above) and they have no tendency to develop diabetic ketosis. In contrast, the sulphonylureas are useless in patients with severe diabetes of the juvenile type, who are insulin-deficient and need daily injections of insulin to maintain good health. Sulphonylurea compounds do not usually allow a reduction of the insulin dose in these patients, and a labile or ‘brittle’ diabetic patient cannot be made more stable.

The majority of patients with mild diabetes of the adult type are obese, and weight reduction with adequate restriction of carbohydrate in the diet will often control their symptoms and hyperglycaemia. There is, however, a small group of middle-aged or elderly diabetic patients, constituting perhaps 5–10 per cent of the total diabetic population whose diabetes has developed late in life; they have little or no tendency to ketosis, they are often under-weight, and their hyperglycaemia cannot be adequately controlled by diet alone. These patients would otherwise require insulin and will benefit most from treatment with the sulphonylurea compounds.

Patients suitable for treatment with the sulphonylurea compounds can usually be selected on clinical grounds alone although this may be difficult if the patient is already taking insulin. Objective tests, therefore, have been devised to assess the likelihood of a reasonable therapeutic response to tolbutamide. At the Joslin Clinic a single 3-g oral dose of tolbutamide is given during fasting; after 4 hours, the blood-sugar in patients expected to respond to the drug falls to 100 mg/100 ml., or less (Somogyi-Nelson technique). Patients with a fasting blood-sugar of over 250 mg/100 ml. generally do not show the requisite reduction in the blood-sugar and are usually unsuitable for treatment with tolbutamide. Selection by this method is stringent and reasonably reliable, but some suitable patients may be missed. Duncan, Lee and Young used the development of ketoacidosis as an index of response to tolbutamide; if ketones appear in the urine 8 hours after the last insulin dose, oral therapy is contra-indicated, but if no ketones have appeared within 24 hours, treatment with tolbutamide will usually be successful. There are, however, some patients unresponsive to tolbutamide who do not easily develop ketosis. Thus, the best method of selection is a therapeutic trial. This is best carried out by using placebo tablets but great care must be taken with patients already receiving insulin, and the change-over is best performed in hospital if the patient is not of the maturity-onset type.

About 5–10 per cent of patients who initially respond well to tolbutamide cease to do so after some months of treatment. Dietary indiscretions may explain a few of these cases, but there are many who appear to develop a genuine resistance to the drug. When assessed in hospital, hyperglycaemia persists despite large doses and a rigid diet, and the drug may be stopped without any further rise of the blood-sugar level. An ‘exhaustion’ of the pancreatic beta-cells may be produced due to repeated stimulation, although
Pfeiffer has found that the dose of insulin needed afterwards is no higher than that needed before tolbutamide was begun, and chlorpropamide is often successful. Nevertheless, following a period of insulin treatment, many of these patients may again respond to tolbutamide.

Sulphonylureas should only be used when frequent and detailed observation of the patient is possible. The long-term toxicity is unknown so that sulphonylurea treatment is only justified if hyperglycaemia is adequately controlled. A high renal threshold for glucose makes urine sugar-testing a poor index of blood-sugar concentration in many of the diabetic patients who are suitable for the sulphonylureas, and, therefore, frequent blood-sugar estimations should be performed. Ketoacidosis may occur at the time of changing over from insulin or it may appear rapidly during the course of an acute infection. Even without ketonuria, serious hyperglycaemia due to acquired resistance may develop insidiously. Hypoglycaemia is rare with tolbutamide but prolonged hypoglycaemia is an important danger with chlorpropamide. Like the hypoglycaemia of the long-acting insulin preparations, it may only respond slowly to glucose administration. Obesity with its attendant dangers develops easily and excessive weight gain can only be avoided by careful supervision of the patient's diet. It is important that the sulphonylurea compounds directly inhibit enzyme systems in the liver (and possibly elsewhere) as any drug which chronically affects hepatic function may ultimately damage the liver cells. It is too early to say whether the incidence of 'degenerative' complications of diabetes mellitus will be affected by treatment with tolbutamide. Better control of hyperglycaemia tends to reduce the risk of complications but any drug which increases the tendency to haemorrhage must be used with great caution, particularly in patients with retinopathy. Carbutamide increases capillary fragility but careful studies have failed to reveal any difference in the incidence of haemostatic abnormalities between insulin-treated and tolbutamide-treated patients.

THE GUANIDINE DERIVATIVES

Synthalin (XII) was discovered in Minkowski's clinic in Breslau in 1926, after earlier scattered reports that administration of guanidine (XIII) lowers the blood-sugar concentration. Since guanidine is a highly toxic substance, particularly to the liver, attempts were made to produce compounds which were less toxic but still exerted a hypoglycaemic action. As the substituent side-chains were made longer, the compounds became less toxic and more effective as hypoglycaemic agents. For example, agmatine (XIV) is less toxic and more hypoglycaemic than guanidine and the pentamethylene and hexamethylene homologues are even better. The two synthalins, (XII) are equally efficacious and show high hypoglycaemic activity.
de Bod6 and Marks802 showed that synthalin inhibits tissue respiration but increases the glucose uptake into muscle with a concomitant increase of lactic acid production, thus simulating some of the effects of anaerobic glycolysis.

\[ H_2N-C-NH-(CH_2)_4-NH_2 \]
\[ \text{NH} \]
\[ (XIV) \text{ Agmatine} \]

Synthalin was used extensively in diabetic patients during the late 1920’s but following reports by Bertram5 in 1927 that large doses may produce histological changes in the liver and kidney of animals within a few days, the drug fell into disfavour, although it was still used sporadically throughout the 1930’s.

\[ \text{(XV) Phenethylguanylguanidine} \]

In 1957 the hypoglycaemic effect of \( N^1-\beta\)-phenethylformamidinyliminoura hydrochloride or phenethylguanylguanidine (DBI, phenformin, Dibotin, XV) was described by Ungar, Freedman and Shapiro203. As will be seen from formulae (XII) and (XV), the drug has only a general structural resemblance to synthalin. The chemical properties of phenformin are given by Shapiro, Parrino and Freedman204. Hydrolyses suggested that it is stable in strongly acidic solutions and that it can be degraded in hot alkaline solutions to \( \beta\)-phenethylguanidine, \( \beta\)-phenethylurea and \( \beta\)-phenethylamine. Attempts at alkylation with alkyl halides yielded the corresponding hydrohalide salts. The same worker205 synthesized a large number of arylbiguanides of the type:

\[
\text{R} \quad \text{N'NH.C.NH}_2
\]

where \( R \) represented mono-, di- or tri-substitution with alkyl, alkoxy, amino, arylamino, halogen, hydroxy, or \( R \) and \( R' \) combined to give a heterocyclic ring, or where \( R' \) was hydrogen, methyl or ethyl. None of these compounds, however, showed oral hypoglycaemic activity approaching that of phenformin. A very large number of \( N'\)-alkyl- and aralkylbiguanides of the type

\[
\text{R} \quad \text{N'NH.C.NH}_2
\]

were then prepared206 and many of them showed good oral hypoglycaemic activity. In the series \( R = \text{alkyl} \), the activity reached a peak with \( n\)-pentyl, then diminished through \( n\)-octyl and disappeared with \( n\)-decyl. In comparison, branched or cyclic structures showed less activity. The most effective variant of \( R' \) was hydrogen. In
the aralkyl series, good activity occurred with \( R = \text{benzyl} \) and peak effects were obtained with the \( p \)-chlorobenzyl and \( \beta \)-phenethyl (phenformin, \( XV \)) compounds. Lengthening of or substitution on the alkylene chain diminished or abolished activity. In a series of \( N^1, N^5 \)-substituted biguanides (\( XVI \)) good hypoglycaemic activity was attained, particularly when \( N^5 \)-methyl or \( N^5 \)-dimethyl substituents were introduced in physiologically active \( N^1 \)-substituted biguanides\(^{507} \). However hypoglycaemic \( N^1, N^5 \)-substituted biguanides were found to be less easily absorbed from the gastro-intestinal tract than the corresponding \( N^4 \)-substituted compounds.

The most active compounds had \( R^2 = H, R^3 = R^4 = \text{Me} \) and \( R^1 = \text{Bu} \) or \( \text{PhCH}_2 \). Generally, the most active of these compounds also produced the most side-effects.

In 1929, Slotta and Tschesche\(^{208} \) synthesized a series of biguanides and examined them for hypoglycaemic activity. The most active compound was \( N^1, N^5 \)-dimethylguanylguanidine, \( (N^1, N^5 \)-dimethylbiguanide, metformin, Glucophage, \( XVII \)) and this has recently reappeared for clinical trial. It produces a fall of blood-sugar concentration but gastro-intestinal side-effects were seen in five out of eight diabetic patients\(^{70} \). Other workers claim less gastro-intestinal disorder when it is compared with phenformin\(^{208a} \).

Up to the time of writing, no means of estimating the biguanides in biological fluids have been developed, so that there is little or no information about their intestinal absorption or metabolic fate. Based on the doses necessary to lower the blood-sugar level, it seems that phenformin is absorbed to a greater extent than metformin. One study using \(^{14}\text{C}\)-labelled phenformin has shown that radioactivity becomes concentrated in the stomach and liver with less in muscle\(^{208b} \).

The mode of action of these compounds\(^{208c} \) is different from that of the sulphonylureas as the drugs produce hypoglycaemia in pancreatectomized animals\(^{209} \), in severe alloxan diabetes\(^{503} \) and in patients with the juvenile or insulin-deficient type of diabetes\(^{510} \). In spite of the differences of detailed chemical structure, the recently synthesized diguanides affect biological tissues in much the same way as synthalin.

Phenformin increases the glucose uptake by the rat diaphragm \textit{in vitro}, but the muscle glycogen content decreases\(^{511} \); there is also a marked increase in lactic acid production and a decrease in oxygen consumption. This suggests that phenformin stimulates anaerobic glycolysis by inhibiting oxidative enzyme systems, thereby mimicking the metabolic effect of severe muscular effort. Similar \textit{in vitro} effects have been described with rat or guinea-pig liver slices\(^{512} \); glucose output is not reduced and there is no inhibition of glucose-6-phosphatase. Using adipose tissue, Wick, Larson and Serf\(^{213} \)
have also shown that phenformin in vitro inhibits the oxidation of glucose, acetate and succinate by adipose tissue and also considerably reduces fat synthesis. Similar results have been obtained in vivo. Hepatic glycogen is reduced and there is a decreased, hyperglycaemic response to glucagon and adrenaline. Muscle glycogen is also reduced and blood lactate levels rise. Diaphragm muscle, adipose tissue and liver slices from phenformin-treated animals do not incorporate $^{14}$C into protein or fat when incubated with $^{14}$C-glucose. In man, the blood pyruvate and lactate levels are raised and the alkali reserve is lowered. In diabetic patients treated with phenformin, this may lead to a dangerous acidosis with ketonuria, although there is a normal blood-sugar concentration. Hypoglycaemia cannot be obtained in normal humans although with intact animals a decrease in the blood-sugar concentration is easily produced. In man, there is no change in hepatic vein glucose, urea, pyruvate or lactate nor in the oxygen consumption of the liver. Lactate levels in the blood from the femoral artery also remain unchanged. These results confirm that the effects of phenformin are largely mediated on the peripheral tissues.

The evidence therefore suggests that tissue anoxia is produced but how this is brought about is unknown. It may be attributed to inhibition of cytochrome oxidase and succinc dehydrogenase, but a decrease in oxidative phosphorylation would have the same result. Many factors which inhibit oxidative phosphorylation increase the glucose uptake in the rat diaphragm. Later work suggests that biguanides do not reduce oxygen uptake by liver mitochondria but inhibit the transfer of energy-rich phosphate bonds to adenosine diphosphate.

Clinically, the biguanides are important because, if tolerated, they would be useful in controlling the hyperglycaemia of all types of diabetes. However, the therapeutic dose of phenformin is too near the toxic one, and produces severe anorexia, nausea and vomiting in about 40 to 50 per cent of patients. Weakness, lethargy and weight loss may develop later. Despite the fact that toxic damage to the renal tubules has been reported in the rabbit and in the guinea-pig after large doses of phenformin, no serious toxic effects have been reported in humans during the past 2 years and repeated liver function studies have shown that phenformin, in contrast to synthalin, does not produce any hepatic abnormality. The apparent lack of hepato-toxic effects may be related to the fact that biguanides are able to form stable chelate rings. Krall, White and Bradley have shown, however, that phenformin combined with insulin may make a labile or 'brittle' diabetic patient more stable, and this may prove to be its main clinical application unless a less toxic derivative is discovered.

**EVALUATION OF HYPOGLYCAEMIC ACTIVITY**

The preliminary testing of compounds for hypoglycaemic activity may be carried out on rats, from which food has been withdrawn for 18 hours. For general screening purposes, the compound is given orally at a dose level of 100–300 mg/kg and the blood glucose concentration is determined on samples taken from the tail vein at 1, 2, 3, 5, and 7 hours after administration. A measure of the degree and duration of the hypoglycaemia is then
calculated, and used to plot dose–response curves\(^{57,67}\). Rabbits, cats, dogs, guinea-pigs and monkeys have been used for extending the scope of these preliminary tests\(^{203}\).

Compounds such as tolbutamide and chlorpropamide also exert their hypoglycaemic effect when given intravenously, after they have been dissolved in water containing sufficient 0.1\(\text{N}\) sodium hydroxide solution to bring the pH to 8–9\(^{67}\). Other compounds such as phenformin produce hypoglycaemia on subcutaneous injection into guinea-pigs but fail to lower the blood glucose concentration in dogs\(^{203}\).

Animals in which diabetes is artificially induced are used in further studies. This may be achieved either by means of alloxan injections which destroy the insulin-producing beta-cells of the pancreas or by total pancreatectomy. Rats become diabetic when given alloxan monohydrate intravenously (40 mg/kg)\(^{53}\) or intraperitoneally (250 mg/kg)\(^{203}\), but rabbits and monkeys need a higher intravenous dose (150–200 mg/kg)\(^{53,203}\). Dogs may be made diabetic by an intravenous injection of 75 mg/kg of alloxan monohydrate or by removal of the pancreas\(^{53}\). After injection or pancreatectomy, the animals are kept in metabolism cages so that a regular check may be kept on urine volume, urine sugar excretion, and food consumption. It is of interest that injections of alloxan do not produce diabetes in the guinea-pig\(^{228}\).

Variation in the effect of a compound within an animal species is often high and it is essential to have a sufficient number of animals for each dose level for the results to be analysed statistically\(^{53,203}\). Compounds such as the sulphonamides do not generally produce hypoglycaemia in severe alloxan-diabetic animals or after total removal of the pancreas, whereas other compounds such as phenformin do so in these conditions. When highly potent hypoglycaemic drugs are undergoing toxicity studies, the possibility that death is the result of a hypoglycaemic convulsion must be borne in mind.

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214
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218
J. D. H. SLATER

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