COMPOUNDS ADMINISTERED ORALLY IN THE TREATMENT OF DIABETES MELLITUS

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INTRODUCTION

In the years before the discovery of insulin many substances and preparations were traditionally used for the relief of diabetic symptoms. Few, however, had appreciable hypoglycaemic properties and many had undesirable toxic effects (240).

The discovery of insulin in 1922 appeared at first to have solved the problem of diabetes mellitus: the disorder was due to deficiency of insulin and all its metabolic and other consequences could be avoided by administration of this hormone. The continuous search since then for alternative methods of treatment has had two sources of inspiration; firstly, the inconvenience resulting from the necessity to administer insulin by injection, and secondly, the growing realisation that idiopathic diabetes may not necessarily be due primarily to insulin deficiency.

In 1918 guanidine was shown to have a considerable hypoglycaemic effect, but the neurotoxic properties of this compound precluded its clinical use. In 1926 Synthalin "A" (decamethylenediguanidine) and later Synthalin "B" (dodecamethylenediguanidine) were introduced as oral forms of treatment for diabetic patients since they were found to be free from these neurotoxic effects. The belief that the compounds were hepatotoxic in man led to their general disuse in the early 1930's, although the relationship between the liver damage and the drugs now seems less certain. Nevertheless their clinical use was impracticable on account of other factors discussed later.

The hypoglycaemic effect of several sulphonamide compounds was first reported in 1930 by Ruiz *et al.* (349) but was not systematically studied until 1942 when Janbon *et al.* (201) observed the occurrence of grave, sometimes fatal, neurological disturbances in some patients suffering from typhoid fever who were being treated with a chemotherapeutic thiodiazole derivative of sulphanilamide, IPTD (PASIT, 2254 RP., *p*-amino-benzenesulphonyl-isopropyl-thiodiazole). These disturbances were found to be due to prolonged hypoglycaemia. During the next two years the mechanism of the hypoglycaemic action of IPTD and related derivatives was very extensively studied in animals by Loubatières (242) and to a lesser extent by a few other workers (51, 73, 225). Although Loubatières then stressed the possible therapeutic application of these compounds in diabetes mellitus (242) almost ten years passed before they and related thiodiazole compounds were so used (243).

In 1954 Franke and Fuchs recognised the hypoglycaemic properties, in man, of a new antibacterial sulphonamide, carbutamide [BZ55, Aleutin, Glucidoral, Invenol, Nadisan, N-(*p*-aminobenzenesulphonyl)=N′-(*n*-butyl) urea]. A year later they (139) and other German clinicians (2, 39) reported its successful substitution for insulin in a number of middle-aged or elderly patients suffering from mild stable diabetes. Extensive clinical trials confirmed the hypoglycaemic effect of orally administered carbutamide in such patients but evidence accumulated which suggested that it had toxic properties sufficiently serious to preclude its general clinical use.

Numerous sulphonyl compounds have since been investigated for hypoglycaemic activity, and several have been employed therapeutically (373). Of these tolbutamide [D860, Artosin, Dolipol, Orinase, Rastinon, N-(*p*-toluenesulphonyl) =N′-(*n*-butyl) urea] was the most extensively studied and many thousands of diabetic patients are now being treated with it; to date few untoward effects have been reported. More recently chlorpropamide [P607, Diabinese, N-(*p*-chlorobenzenesulphonyl)=N′-(*n*-propyl) urea] has undergone extensive clinical trial.

The historical aspects of the development and clinical use of these oral hypoglycaemic agents have been previously reviewed (163, 244).

Although the place of the sulphonyl compounds in the treatment of diabetes mellitus is limited, a tremendous impetus to diabetic research has resulted from their discovery. New light has been shed on the physiological function and mode of action of insulin and on the metabolic abnormalities in the varied syndrome of diabetes mellitus. Moreover, interest has been renewed in many other compounds possessing hypoglycaemic properties and their mode of action has been increasingly studied (163, 326). A new synthetic hypoglycaemic diguanide, DBI (phenethylbiguanide, N′-*beta*-phenethylformamidinyliminourea hydrochloride), is at the moment undergoing widespread clinical study.

This review will deal with the mechanism of hypoglycaemic action of the sulphonylureas and the guanidine derivatives, and the rationale and principles of their therapeutic use in the treatment of diabetes mellitus.

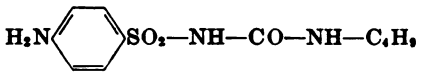
I. THE SULPHONYLUREAS

A. Pharmacology

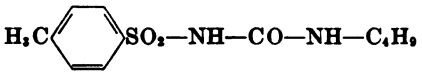
When given orally or parenterally hypoglycaemic thiodiazole and urea derivatives, collectively termed the sulphonylureas, reduce the blood glucose concentration of intact or incompletely alloxanised laboratory animals, all non-diabetic persons and a proportion of diabetic persons. The magnitude and duration of the hypoglycaemic response is mainly determined by the hypoglycaemic potency of the drug; by species and individual sensitivity to its action; and within limits by the concentration of the active compound in the plasma. This last factor depends on the dose administered and on the rate at which it is inactivated and excreted.

This section will deal with the pharmacology of the three sulphonylureas which have been most generally used in the treatment of diabetic patients: carbutamide, tolbutamide and chlorpropamide.

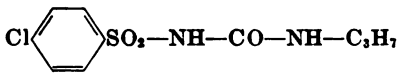
Carbutamide: N-(*p*-aminobenzenesulphonyl)═N′-(*n*-butyl) urea



Tolbutamide: N-(*p*-toluenesulphonyl)═N′-(*n*-butyl) urea



Chlorpropamide: N-(*p*-chlorobenzenesulphonyl)═N′-(*n*-propyl) urea



1. Relationship of hypoglycaemic activity to chemical structure. Numerous sulphonyl compounds with the general formula $R_1—SO_2—NH—CO—NH—R_2$ have been examined by many investigators (158, 175, 202, 270, 350). Linkage of a sulphone radical to a ureide or similar chemical group seems essential for hypoglycaemic activity but the extent, duration and even direction of the change in blood glucose concentration is determined specifically by the the nature of groups R_1 and R_2. Weight for weight, chlorpropamide has a greater hypoglycaemic effect than carbutamide, which in turn is more potent than tolbutamide.

2. Absorption from the alimentary tract. Carbutamide and tolbutamide are relatively insoluble in water but form soluble alkaline salts. Rodents have little intestinal alkali so that if the drugs are given orally to rats in acid form absorption is slow and the blood glucose concentration falls only transiently by about 10%. However, if the same dose is given with bicarbonate or as the sodium salt rapid absorption from both stomach and intestine occurs (279) resulting in a significant concentration in the blood within half an hour and a prolonged fall in blood glucose concentration of 40 to 50% (347). In the intestine of man and carnivora the acid is quickly converted to the sodium salt which is rapidly absorbed (19). Although in a few persons absorption may be delayed for several hours (17, 314), maximum blood concentrations of both drugs are usually achieved within 4 hours (17, 19, 332). When introduced rectally in dogs and man (159, 165) both compounds are absorbed and have considerable hypoglycaemic effect.

Studies with orally administered S^{35} labelled sodium tolbutamide have shown its absorption from the gut to be complete in rats (279), rabbits and dogs (20). Although both drugs are excreted in the bile they are completely reabsorbed so that none appears in the faeces (20, 328, 390).

Chlorpropamide is also rapidly absorbed from the intestine in man and dogs (206, 357).

3. Chemical methods of determination in blood and urine. The concentration of carbutamide in whole blood, plasma, serum or urine can be determined by the method of Bratton and Marshall (52) or modifications of it (398). This procedure is simple and reliable and can be used to determine either unchanged carbutamide only, or its inactive metabolite may be included to give the total concentration of

carbutamide. As neither enters the erythrocytes (389) the carbutamide concentration in plasma exceeds that in the whole blood.

Spectrophotometric procedures have been devised for the measurement of plasma tolbutamide (42, 137, 379). They can be adapted to measure either total plasma tolbutamide, or the unchanged compound and its carboxylated derivative separately. These methods are tedious and unsatisfactory (17, 112) and only one (379) can be used for determinations in urine.

The concentration of chlorpropamide in serum can be determined by another spectrophotometric method developed by Toolan and Wagner (401). This technique may also prove to be the most satisfactory method of estimating tolbutamide. It is not, however, suitable for determinations in urine.

4. Distribution in the body. Studies in intact dogs, rabbits and rats (19, 20), eviscerated nephrectomised rabbits (429), and man (206, 388, 389), have shown that the volume of distribution of tolbutamide and chlorpropamide is approximately that of the extracellular fluid, whereas carbutamide is apparently distributed in a volume about twice as large (328, 388). Some entry of both tolbutamide and carbutamide into cells is indicated by their presence in considerable concentration in the bile (328, 390), by the reported concentration of S^{35} carbutamide in the liver (173), and by the fact that both drugs are probably metabolised in that organ. These two compounds have been shown to pass very slowly into the cerebro-spinal fluid (328). Administration of insulin does not alter the distribution of tolbutamide in eviscerated nephrectomised rabbits (429).

About 50 to 60 % of the concentration of all three drugs in the plasma is bound to protein (206, 308, 388).

5. Metabolic fate in the body. Carbutamide and tolbutamide are metabolised in the body to derivatives which have no hypoglycaemic activity (2, 112, 127); in fact it has recently been suggested that the metabolic derivative of tolbutamide may have a hyperglycaemic effect (3). Carbutamide is acetylated (2), and tolbutamide carboxylated (112), in the *p*-position; the proportion of the administered dose which is metabolised varies considerably with the animal species (347). More information is required regarding the sites where these conversions occur. Although liver or kidney homogenates from rats have been shown to carboxylate tolbutamide in appropriate conditions *in vitro* (20), considerable breakdown of carbutamide has also been reported to occur in the pancreas, testicles and adrenals (308).

There is no evidence that chlorpropamide undergoes comparable metabolic change in man (206, 388); the presence of the chlorine group in the *p*-position rules out carboxylation.

The dog differs from other species in its handling of tolbutamide and chlorpropamide; a proportion of tolbutamide is inactivated by removal of the butyl group to give *p*-tolylsulphonyl urea (112) and chlorpropamide is partly metabolised in a manner not yet elucidated.

6. Excretion. Both the unchanged active and metabolised forms of the drugs are entirely eliminated from the body by the kidney in all species studied (127, 173, 206, 214, 233, 291). The proportion of each form appearing in the urine

varies considerably, and depends on the drug given, the dose administered, and on individual and species differences.

Quattrin *et al.* (328) and Lee *et al.* (233) showed that carbutamide did not affect the glomerular filtration rate or maximal rate of tubular reabsorption of glucose in dogs or man, and that in these species 90 to 97 % of the filtered load of unchanged or acetylated carbutamide was reabsorbed by the tubules so that excretion was slow. The percentage of urinary carbutamide present as acetylated derivative was found to vary from 30 to 50 % in human subjects given the same daily dose of the drug (328, 436).

Little information is available about the renal excretion of tolbutamide. Fajans *et al.* (127) reported that when daily doses of 5–6 g, 3–4 g and 1–2 g were given to normal and diabetic persons the percentage of administered tolbutamide excreted as the carboxylated form was respectively 75, 24–67 and 2–17; Mohnike and Wittenhagen (291), however, found a mean value of 80 % in twenty-five diabetics receiving 3 g daily. Renal tubular transport is probably important in the excretion of tolbutamide. This is suggested by the reported inability to detect the carboxylated derivative in glomerular filtrate (379); by the fact that the proportion of tolbutamide to carboxyltolbutamide is several times greater in plasma than in urine; and by the marked reduction in the rate of disappearance of tolbutamide from the blood produced by giving probenecid BP (389), in contrast to the insignificant effect of severe impairment of glomerular filtration rate (389) or alterations in daily urine volume (291). A point of practical importance is that carboxyltolbutamide gives a floccular precipitate in urine at pH 5.2 or less, which may lead to confusion when the urine is tested for protein by methods depending on acid precipitation (127).

All three sulphonylureas and the metabolic products of carbutamide and tolbutamide are readily soluble in urine over a wide range of pH so that crystalluria is not a problem (401).

The rate of disappearance of the sulphonylureas from the blood reflects their rate of renal excretion since they are eliminated from the body only by this route. When administration of a sulphonylurea is discontinued its concentration in the blood falls exponentially with time (17, 389), at a rate which is determined chiefly by the chemical structure of the sulphonylurea given and the species of animal used; there are, however, considerable individual differences in rate of excretion within the same species.

7. *Relationship of hypoglycaemic response and concentration of sulphonylurea in the blood.* The magnitude and duration of the reduction in blood glucose in man and animals is, within certain limits, related to the plasma concentration of active sulphonylureas. This relationship is relatively direct for carbutamide and chlorpropamide (2, 121, 217, 275, 328) but is difficult to define with larger doses of tolbutamide since the same absolute reduction of blood glucose concentration occurs at different plasma drug concentrations in the same subject (17). It is likely that the hypoglycaemic response increases as the plasma tolbutamide concentration rises from its minimal effective level—about 8 mg/100 ml—to an upper limit of about 20 mg/100 ml. Plasma values above this limit, resulting

from administration of single doses in excess of 2.0–3.0 g in man, do not increase and may even decrease the hypoglycaemic response (17, 19, 69, 177). The diminished response may be due to the greater concentration of carboxyltolbutamide which has been reported to have a hyperglycaemic action (3) or to adrenomedullary stimulation (18, 118).

Carbutamide is rapidly inactivated by the rabbit. The acetylated derivative forms about 80% of the total plasma sulphonylurea concentration and accounts for almost all the carbutamide in the urine. Since excretion is also rapid the level of active carbutamide in the plasma falls quickly and the hypoglycaemic effect is short lived (347). In contrast acetylation in the dog is minimal so that the drug is present in plasma and urine almost exclusively in the active form, excretion is slow and the fall in blood glucose prolonged (347). Man occupies an intermediate position: about 10 to 30% of the carbutamide in plasma is in the acetylated form (213, 328); its biological half-life ranges from 30 to 60 hours (17, 332, 389), but the value is very constant for any one subject.

Less is known about the rates of inactivation and excretion of tolbutamide in animals but examination of the limited data available suggests that its half-life in the rabbit is 4 to 6 hours, and in the dog 10 to 20 hours (19) which accord with the duration of the observed hypoglycaemic effect in these animals (19). In man, the carboxyl ester accounts for 10 to 30% of the total plasma tolbutamide (389) and the tolbutamide plasma half-life is 4 to 8 hours (17, 379, 389).

Chlorpropamide shows the same species differences as carbutamide in respect of its excretion rate and biological half-life in plasma; excretion is rapid in rabbits, and slow in dogs (348) and the half-life is about 35 hours (206, 217, 389).

Thus in man, chlorpropamide is probably the most active hypoglycaemic agent of the three: it is more potent weight for weight; is rapidly absorbed from the gut; is not inactivated in the body, and is slowly excreted, so that its plasma concentration is sustained and the hypoglycaemic response prolonged.

8. Antibacterial activity. The antibacterial properties of the sulphonylureas have been studied fully (2, 239, 302). Carbutamide is bacteriostatic to gram-negative bacilli—particularly the dysentery, coliform and salmonella organisms; staphylococci and streptococci are also inhibited. Tolbutamide has minimal activity *in vivo* but has some bacteriostatic effect on gram-positive cocci *in vitro*. The metabolic products of both have no antibacterial effect. Chlorpropamide has no significant antibacterial activity either *in vitro* or *in vivo*.

9. Acute and chronic toxicity in animals. The acute toxicity of the sulphonylureas in clinical use has been studied in several species of laboratory animals (2, 213, 287, 309, 357). The dose required to produce profound hypoglycaemia varies with the route of its administration, the species, age, size and endocrine status of the animal. In intact animals rendered hypoglycaemic by administration of sulphonylureas, death can usually be prevented by giving glucose, but very large doses result in the development of neurological changes and death of the animal in a euglycaemic or hyperglycaemic state (192, 213, 287). The hyperglycaemic response seems to depend on adrenal stimulation (18, 118). Adrenalectomised (118, 168, 194, 227, 357) and to a lesser extent adrenal-

demedullated (118) animals are extremely sensitive to the hypoglycaemic action of the sulphonylureas; even moderate doses cause a profound fall in blood glucose and neurological disturbances leading to death. Early administration of corticotrophin or corticosteroids may prevent death and the occurrence of neurological changes, without, however, affecting the fall in blood glucose significantly. The injection of adrenaline, glucagon or glucose has no such protective action (118, 194). It is possible that the neurological manifestations in both normal and adrenalectomised animals are not entirely due to prolonged hypoglycaemia but result, in part at least, from a direct effect of the sulphonylureas on the nervous system (157, 357).

Chronic toxicity has been studied in monkeys, dogs, rabbits and rats (106, 213, 254, 285, 357) given daily doses just short of those provoking hypoglycaemic signs, for periods of up to nine months. No consistent degenerative histological or biochemical abnormalities were observed. In intact or partially depancreatised or alloxanised, but not diabetic, animals, hyperglycaemia and glycosuria, impairment of glucose tolerance or decreased insulin sensitivity occasionally occurred after several weeks of treatment (92, 106, 155, 232, 295, 359). The sulphonylureas, especially carbutamide, have a moderate antithyroid effect in animals, particularly rats (56, 441), a species very sensitive to goitrogens. The rise in plasma cholesterol occasionally, but not always (176, 295), observed in rats given carbutamide in large daily doses was shown to be due to thyroid inhibition (176).

When carbutamide was given daily to intact (8) or to depancreatised or mildly alloxan-diabetic dogs (195, 213, 344) for periods usually exceeding four weeks, the animals often became anorexic and weak, developed neurological disturbances, such as paresis and convulsions, and showed alterations in prothrombin time, blood albumin, transaminase and alkaline phosphatase values. Death frequently resulted and at necropsy widespread haemorrhages and hepatic and renal damage were found (354, 374). Although the same changes have occasionally occurred in such animals given tolbutamide (375) or chlorpropamide (348) it would seem that under such circumstances these two sulphonylureas are less toxic.

The growth of young animals was not impaired by daily administration of any of the three sulphonylureas (8, 213, 357, 405). When carbutamide was given to rats in daily doses of from 150 to 200 mg from the first week of pregnancy onwards a significant increase in abortion, foetal resorption and congenital anomalies was noted (108, 402); these changes were not related to hypoglycaemia. The metamorphosis of tadpoles was much retarded by both these sulphonylureas (11), but although they have a limited antimitotic effect in the cells of the intestinal mucosa of rats (156), no inhibition of spermatogenesis was noted in male animals (310).

The toxicity of these drugs in man is considered in the clinical section.

B. Mechanism of hypoglycaemic action

The various hypoglycaemic sulphonylureas do not seem to differ significantly in their mode of action. In spite of the many observations made in laboratory

animals, diabetic and non-diabetic persons, this mechanism has not yet been clearly defined. There are several reasons for this: 1) like most pharmacological agents the sulphonylureas probably have secondary effects which may be complementary, antagonistic or unrelated to their hypoglycaemic action; 2) results obtained in laboratory animals are not necessarily valid for man since, quite apart from species differences, the metabolic changes in experimentally induced diabetes differ in so many respects from those in the naturally occurring syndromes in man; 3) the factors which influence the blood glucose concentration are numerous, complex, interdependent and inadequately understood, and insufficient is known of the sites and mechanism of the action of insulin; 4) similar and apparently carefully conducted studies have often yielded conflicting results; 5) and finally, it is probable that several mechanisms are involved. It is against this most confusing background that the problem, which has been previously reviewed by others (148, 228, 245, 246), will be considered.

The various ways in which the hypoglycaemic action of the sulphonylureas might be effected will be discussed in turn.

1. Effect on alimentary absorption and renal excretion of glucose. Although carbutamide may delay slightly the absorption of glucose from the gut in rats (141) and dogs (233), tolbutamide has been shown to have no such effect (216, 234), and the increase in the concentration of glucose in the blood which follows the ingestion of a meal (60, 122, 436) or glucose (16, 60) is not reduced in subjects receiving these drugs. Moreover an inhibitory effect on absorption could not account for the hypoglycaemic action of these compounds in fasting animals and man. Similarly their hypoglycaemic effect is not due to a reduction of the renal threshold for glucose, as the fall in blood glucose in responsive diabetics is accompanied by decreased glycosuria.

2. Acceleration of glycolysis in blood. The sulphonylureas do not influence glycolysis in blood (245, 389).

3. Effect on endocrine glands other than the pancreas. Although the hypoglycaemic effect of the sulphonylureas may be modified by the functional state of endocrine glands other than the pancreas, it does not depend on their suppression or stimulation, since the blood glucose concentration of animals deprived of the pituitary, adrenal, thyroid, parathyroid glands and gonads was reduced by administration of the compounds (246). A hypoglycaemic response has been observed also in patients suffering from acromegaly (30, 53, 109, 290), panhypopituitarism (30, 162), Addison's disease (30, 162, 184), Cushing's disease (53, 290), and myxoedema (122).

4. Direct action on extrapancreatic tissues. The initial fall in blood glucose concentration might be due to the more rapid removal of glucose from the blood by the tissues, as a result of a direct effect of the sulphonylureas on the extrapancreatic tissues. However, the following observations virtually exclude such an explanation.

a. In vivo studies. i. Depancreatised animals and man. The sulphonylureas have never been shown to reduce the blood glucose concentration when given, in single or repeated doses, to depancreatised humans (127, 162, 292, 327), cats (168), dogs (20, 68, 142, 245, 275, 347), or toads (192), who have not recently

been given exogenous insulin. For example, sodium tolbutamide was found to have no effect on the blood glucose concentration when given intravenously to dogs which had been depancreatised either three hours (409) or eight days (20) previously and maintained without insulin. In the latter animals endogenous insulin was unlikely to have been present in significant amounts although this was possible in the former.

Moreover, pancreatectomised-adrenalectomised animals were shown to be refractory to the hypoglycaemic action of the sulphonylureas (192), although adrenalectomised animals are extremely sensitive to it. Similarly the administration of sulphonylureas in single or repeated doses to pancreatectomised-hypophysectomised animals, not recently given insulin, evoked no hypoglycaemic response (168, 192). Such Houssay animals are very responsive to insulin and it might have been expected that, if the sulphonylureas promoted glucose assimilation by acting directly on the tissues in the same way as insulin, their hypoglycaemic effect would be readily apparent in these animals. Loubatières (248), in a different type of experiment, showed that an adrenalectomised-hypophysectomised dog, extremely responsive to the hypoglycaemic action of the sulphonylureas, could be made unresponsive by pancreatectomy. When the pancreas was removed in the early hypoglycaemic phase following administration of the drug, the blood glucose concentration which would normally have remained depressed for many hours, rose rapidly to levels above those present before the experiment. Although no control observations were made on the effect of surgical trauma or anaesthesia, such a marked hyperglycaemic response might not have been expected in such an animal.

ii. Eviscerated animals. Unlike an injection of insulin, the intravenous administration of a sulphonylurea did not accelerate the removal of glucose (142, 227, 273, 429) or galactose (142, 234) from the blood of eviscerated animals receiving a constant infusion of glucose. The distribution of injected U-C^{14} glucose (uniformly labelled) was similarly unaltered (67).

iii. Depancreatised ducks and chickens. Mirsky and Gitelson (282) reported that tolbutamide, given orally or intravenously, had as great a hypoglycaemic effect in fasting ducks and chickens depancreatised 16 days before, as in intact birds. The presence of accessory pancreatic tissue was excluded. In these domestic fowls the sulphonylureas must act directly on the liver or on other extrapancreatic tissues and their hypoglycaemic effect does not appear to depend on the presence of insulin. It must be emphasised, however, that pancreatectomy or complete alloxanisation does not alter significantly the blood glucose concentration of these birds.

b. In vitro studies. The effect of the sulphonylureas on the *in vitro* metabolism of insulin-sensitive tissues has been studied by many investigators. These studies have yielded varied and often conflicting results, and their critical comparison and evaluation is precluded by the considerable differences in the experimental techniques employed—particularly the duration of incubation—and by the very limited data provided in many of the reports.

The presence of carbutamide or tolbutamide in balanced salt incubation

medium in concentrations ranging from 0.5 mg to 270 mg/100 ml has been reported to increase (65, 148, 149, 305, 329), inhibit (289), and to have no effect on (62, 110, 144, 342, 411), the glucose uptake of the diaphragm removed from intact rats. Similarly glycogen synthesis has been said to be increased (148), and reduced (76, 289, 320). Some workers found that glycogen synthesis was increased by carbutamide in concentrations of up to 100 mg/100 ml; but in higher concentrations this effect was lost (148). An increase of (289, 320, 321), and a biphasic effect on (305), oxygen consumption were reported, and incorporation of C^{14} from labelled glucose into glycogen and CO_2 has been said to be increased (65, 148, 150). Some workers who obtained a positive response using diaphragms from intact rats, could not demonstrate this when diaphragms from fully alloxanised animals were used; however, the response was restored by previous treatment of the alloxanised donor rats with insulin (148, 149, 150).

The glucose uptake by fat (219) and brain (304) tissue has not been found to be affected, but it has been claimed that the incorporation of C^{14} from isotopic glucose into fatty acids was inhibited by sulphonylureas (325). Gourlay (170) reported that, although both carbutamide and tolbutamide altered the metabolism of isolated frog muscles *in vitro*, their effects were not qualitatively similar.

In summary, *in vivo* studies, except those in domestic fowls, provide unanimous evidence either a) that the immediate hypoglycaemic effect of the sulphonylureas is not primarily due to their direct action on the extrapancreatic tissues; or b) that the hypoglycaemic consequence of such an extrapancreatic action occurs only when some functioning pancreatic tissue or hormone is present.

The *in vitro* studies provide conflicting evidence on these points. However, with few exceptions, control observations with non-hypoglycaemic sulphonamides were not made and alterations in the pH of the medium and in the oxygen supply were not considered. These criticisms are pertinent to almost all the *in vitro* studies referred to in this review.

5. Role of the pancreas. Loubatières' (245) early observation that animals deprived of all but one-tenth of their pancreas exhibited a hypoglycaemic response to IPTD has been amply confirmed by several workers using other sulphonylureas (26, 192). The single or daily administration of a sulphonylurea has also been shown to reduce the blood glucose of patients with diabetes produced by subtotal pancreatectomy (290).

Since the presence of pancreatic tissue seems to be indispensable in almost all species for the hypoglycaemic effect of the sulphonylureas to occur, the question arises as to which pancreatic cells are involved.

a. Effect on pancreatic alpha-cells. The alpha-cells almost certainly produce and liberate glucagon (229). The physiological function of this hormone is still controversial and has been recently reviewed (135). The sustained hyperglycaemia and glycosuria in animals or man given glucagon for some time are probably not due to impairment of peripheral assimilation of glucose but result from increased hepatic glycogenolysis (6). Ferner (131), however, considers that in permanent diabetes in man the alpha-cells are disproportionately increased in number, the normal alpha:beta cell ratio of 1:4 is upset, and that the resulting

hormonal imbalance may be responsible for the metabolic disturbance. Both the histological observations and the hypothesis derived from them are, however, disputed (31, 97, 365).

In 1954 Holt *et al.* (187) confirmed Davis' original observation (103) that alpha-cell damage resulted from the administration of Synthalin, and also reported that similar changes followed the administration of IPTD in very large doses. On the basis of this and other observations (188), these workers attributed the hypoglycaemic effect of IPTD to a cytotoxic action on the alpha-cells. It was, therefore, not surprising that the same mode of action was suggested for carbutamide by those who first reported its successful therapeutic use in some diabetic patients (39, 139). This hypothesis gained further support when Ferner (132) reported alpha-cell damage in rabbits and rats given very large doses of carbutamide—up to 3.5 g/kg. There is now much evidence against this explanation. Several authors either did not observe any changes, or found only minimal and inconstant alterations in the alpha-cell histology in animals given large, single or repeated doses of IPTD (154, 247, 254), and such changes were unrelated to the alteration in blood glucose concentration. Moreover, alpha-cell histology was only rarely altered by the similar administration of carbutamide, tolbutamide or other sulphonylureas to animals of several species (64, 154, 179, 254, 357, 359). For example, these cells were undamaged in rabbits, a species very sensitive to alpha-cell cytotoxins, given very large doses (1 to 2 g/kg) of tolbutamide, and killed when severely hypoglycaemic; or in partially-alloxanised rabbits treated with tolbutamide for several weeks (89, 91). In addition, no alpha-cell abnormalities were found in diabetic patients treated for several months with a sulphonylurea (88, 133).

No reduction in the extractable glucagon content of the pancreas was found in rabbits shortly after administration of hypoglycaemic doses of IPTD (187), carbutamide or tolbutamide (37, 99) or in dogs given the latter two compounds for three days (99). There is a single report of diminution of pancreatic glucagon in rats given IPTD (187). The activity of alpha-cell phosphatase was not altered in any of the three species by administration of sulphonylurea (93).

The effect of the administration of cobaltous chloride on the response to sulphonylurea has been tested on the assumption that cobalt specifically damages alpha-cells. This view is not generally accepted, and experiments where both these substances were used gave contradictory results (148, 276).

The above studies of alpha-cell histology and of pancreatic glucagon content provide no definite evidence that the hypoglycaemic effect of the sulphonylureas is due primarily to their damage to alpha-cells. They do not, however, exclude the possibility that changes in alpha-cell function may contribute to their hypoglycaemic action.

b. Effect in alloxanised animals. The sulphonylureas have been shown never to reduce the blood glucose of severely alloxan-diabetic rats (73, 75, 227, 282), rabbits (32, 89, 213, 386), or dogs (20, 250, 344) not receiving insulin. Holt *et al.* (186), however, reported that carbutamide lowered the blood glucose concentration of fully alloxanised adrenalectomised rats, a study which should be repeated.

On the other hand, the sulphonylureas always reduced the blood glucose concentration of incompletely alloxanised animals with mild or moderate diabetes (89, 187, 225, 247, 284). The response seemed to depend on the presence of a sufficient number of beta-cells and could be abolished if the animals were given a further dose of alloxan or were subjected to pancreatectomy (89, 247). Withdrawal of the drug was usually followed by metabolic deterioration (89, 247) but occasionally the diabetic state did not recur, although as far as we know this happened only in animals treated with a thiodiazole derivative (247, 250). This metabolic remission is believed to have been due to the beta-cell regeneration observed in the animals so treated (250). Loubatières (245, 250) considers that the latter resulted from the action of the sulphonylurea, but it may have been due to the inherent capacity of beta-cells for spontaneous regeneration which varies with the animal species. No new beta-cell formation has been observed in diabetic patients treated with the sulphonylureas (88, 133).

c. Effect on diabetes due to haemochromatosis. It is surprising that the sulphonylureas have, so far, been found to be ineffective in all patients suffering from diabetes due to haemochromatosis (94, 199). A proportion of those with mild diabetes might have been expected to respond.

The fact that the sulphonylureas reduce the blood glucose concentration only in subjects, except domestic fowls, possessing some functioning beta-cells, suggests that their action might be to increase the effective activity of endogenous insulin, and/or to stimulate the secretion of increased amounts of the hormone. The former could result from 1) inhibition of insulin destruction; 2) release of insulin from inactive linkages in blood or tissues; 3) inhibition of "anti-insulin" factors; or 4) direct sensitisation of a tissue or tissues to the action of the hormone. However, it does not exclude the possibility that the sulphonylureas might alter the metabolism of extrapancreatic tissue or tissues directly, the hypoglycaemic consequence of this action being obscured in the altered metabolic environment which results from the action of hyperglycaemic hormones unopposed by a minimum of insulin activity. The observations of Holt *et al.* (186) support such a hypothesis. A more remote possibility is that some as yet unknown hormonal product of the pancreas is involved.

d. Increased liberation of insulin. The hypoglycaemic effect of the sulphonylureas was originally attributed by Loubatières (242) to their stimulating the increased liberation of insulin. This, the most generally accepted hypothesis, is derived from the seemingly obligatory presence of pancreatic beta-cells and is supported by the following observations.

i. Cross-circulation studies. In these experiments either the pancreatico-duodenal or a mesenteric vein of a donor dog was anastomosed with a femoral vein of a recipient dog, and a return circulation obtained by establishing a limited flow of blood from a femoral artery of the recipient to a femoral vein of the donor. After the blood glucose level had become steady, 50 mg/kg of carbutamide was injected into a peripheral vein of the donor dog. In seven experiments with pancreatico-femoral preparations, Pozza *et al.* (322) found that the blood glucose of both dogs fell to an equal degree, ranging from 25 to 70 % of the control value. After interruption of the anastomoses, the blood glucose concentration of the

recipient rose slowly to pre-injection values, whereas that of the donor remained reduced or continued to decline. In five experiments using mesenteric-femoral preparations, the injection of carbutamide caused a 30 to 60% reduction in the blood glucose concentration of the donor but no fall in that of the recipient. In both types of experiments the blood carbutamide concentration in the recipient was less than 2 mg/100 ml,—insufficient to influence the blood glucose concentration and about a fifth of that in the donor. These experiments were conducted by investigators who had considerable experience with the technique and the results are clear and convincing. Moreover, Loubatières (241) had made similar studies, except that the recipient dog of pancreatico-jugular preparations had previously been fully alloxanised. In these experiments IPTD was injected into the pancreatic artery of the donor; a fall in the blood glucose level of both animals resulted and only that of the recipient rose after interruption of the anastomosis. The failure of La Barre and Reuse (225) to obtain a hypoglycaemic response in the recipient dog was probably due to the pancreatico-femoral anastomoses being made two hours after injection of the IPTD. The metabolic consequences of the operation may have prevented the development of hypoglycaemia but it may also be—as discussed later—that the increased liberation of insulin is of shorter duration than two hours.

ii. Changes in plasma "insulin-like" activity. Several workers have compared the "insulin-like" activity of peripheral venous blood plasma withdrawn from subjects before the oral or intravenous administration of a sulphonylurea with that obtained during the resultant hypoglycaemic phase. Their results have been contradictory. Thus Renold *et al.* (338), Seltzer (367) and Cugadda *et al.* (98) were unable to demonstrate any change in normal (338, 367) and sulphonylurea-responsive diabetic (98, 367) persons. The rat hemidiaphragm bioassay systems used were shown by these workers to be sufficiently sensitive to detect and measure the rise in plasma activity following the intravenous administration of 0.1 unit/kg insulin (338) or oral glucose (98, 367) to the same subjects. In diabetic patients unresponsive to the sulphonylureas, administration of the drug did not alter the already minimal "insulin-like" activity of the plasma (367, 411). Weaver *et al.* (424) also observed no increase in the plasma "insulin-like" activity of newly diagnosed sulphonylurea-responsive diabetics given 3 g of tolbutamide by mouth two hours before; however, their assay system was probably insufficiently sensitive to detect small increases.

In experiments using dogs, no rise in the "insulin-like" activity of peripheral blood could be detected when about 50 mg/kg tolbutamide was given (312, 334), which accords with results of the cross-circulation studies.

In contrast, Aiman and Kulkarni (5) found that the "insulin-like" activity of the peripheral blood plasma in ten non-diabetic subjects was probably increased ($P > 0.05$) after four days' treatment with carbutamide. More recently Vallance-Owen (411) demonstrated a highly significant increase in the "insulin-like" activity of peripheral vein blood plasma obtained two and a half hours after oral administration of 2 g tolbutamide to normal persons and to diabetic patients responsive to the drug. It is interesting to note that in this study the mean "in-

sulin-like" activity of fasting blood obtained before administration of the drug was greater in the diabetics than in the non-diabetics, and that the increase in both was of equal magnitude. Similar results were obtained by Gambassi and Pirelli (148). Pfeiffer (312) has also recently reported that he obtained an increase in the plasma "insulin-like" activity of peripheral blood when 100 mg/kg of tolbutamide was given intravenously to dogs but this assertion was preliminary and derived from very limited data. An increase in "insulin-like" activity of peripheral blood of rats given carbutamide has been reported (218), but the extraordinarily high values obtained cast doubt on the validity of the assay system employed.

In contrast to the results for peripheral blood, there is unanimous agreement that the intravenous injection of tolbutamide to dogs is rapidly followed by a very considerable increase in the "insulin-like" activity of pancreatic vein blood. Goetz and Egdahl (161) assayed this activity by the hypoglycaemic response of mice, Pfeiffer *et al.* (312) by the *in vitro* incorporation of C^{14} from C_1^{14} glucose into CO_2 by rat adipose tissue, and Recant (334) by the glucose uptake of the isolated rat hemidiaphragm. The full data of these studies have not yet been published but those made available to the reviewers fully substantiate the conclusion that the "insulin-like" activity was considerably and rapidly increased. The increase determined by Recant was relatively transient, being maximal at 10 to 30 minutes and then declining rapidly; in Goetz's studies the rise appeared to be sustained over the two-hour period of observation. The duration of the rise in activity merits further study as the prolonged hypoglycaemic effect of a single dose of sulphonylurea could hardly be accounted for entirely by a transient increase in insulin release.

These experiments and the cross-circulation studies show that the sulphonylureas rapidly and markedly increase the "insulin-like" hypoglycaemic activity of blood from the pancreatic vein in dogs. The duration of this increase requires further study. Whether or not the "insulin-like" activity of peripheral blood is simultaneously increased is still unresolved; in dogs it may depend on the dose of drug given, and although some workers have found no increase in man, the positive results cannot be dismissed, despite the limitations of the bioassay technique employed.

iii. Changes in insulin content of pancreas. Pfeiffer *et al.* (316) found that three hours after administration of a single dose of tolbutamide to calves the extractable insulin content of the pancreas was reduced about 75 %; this was estimated to represent the discharge of about 200 units of the hormone. The content returned to normal twenty-four hours after administration of the drug. Root (346) observed a similar but more prolonged cycle in dogs given carbutamide. Both investigators showed that this phenomenon continued to occur each day in animals given a single daily dose for several weeks, although the absolute reductions in insulin content became smaller with time. Despite the limitations of the techniques used, the results suggest that the administration of a sulphonylurea is followed by a fall in the pancreatic content of extractable insulin. This deduction is supported by the recent work of Grodsky and Forsham (172) using

a more specific assay system. They found that there was a considerable reduction in the extractable pancreatic insulin content in about 50% of a large number of mice given tolbutamide by gavage two hours previously; however in the remainder no reduction was detected.

iv. Effect of intrapancreatic injection of the sulphonylureas. In studies using dogs, Loubatières (241, 242) found that the blood glucose concentration was markedly decreased by the injection of small amounts of IPTD into the artery, uncinate process or duct of Wirsung of the pancreas. Similar results were obtained by Colwell *et al.* (80) who infused carbutamide or tolbutamide, 7 to 12 mg in 0.1 N NaOH at pH 7.4, into the pancreatico-duodenal artery of anaesthetised dogs. This caused a substantial fall in the peripheral blood glucose concentration, although the plasma sulphonylurea level was less than 4 mg/100 ml; a smaller reduction followed the infusion of the same dose into the femoral or portal vein and injection of 0.1 N NaOH alone into the pancreatic artery did not significantly affect the blood glucose concentration. Definitive interpretation of these earlier studies was precluded by their limited number and the marked changes in blood glucose concentration which occurred post-operatively; however, these investigators have now obtained identical results in studies conducted in 59 dogs (81). Capelli *et al.* (66) reported similar changes in a single dog studied.

However, Houssay *et al.* (195) obtained quite contrary results in virtually identical experiments in 65 dogs. IPTD, carbutamide, or tolbutamide in doses ranging from 2.5 to 100 mg/kg in 0.1 N NaOH at pH 7.3, did not have any greater hypoglycaemic effect when infused into the pancreatic artery than when injected into a peripheral or the portal vein. They concluded that it was not possible to say definitely that the sulphonylureas specifically acted on the pancreas.

The discrepancy in the results of these studies cannot be adequately explained at present.

v. Histological changes in the beta-cells. Many investigators have observed degranulation of the beta-cells following administration of a sulphonylurea to animals of all species studied (91, 155, 247, 316, 359). For instance Bänder *et al.* (19) examined the pancreas of intact fasting rats killed at intervals after oral administration of tolbutamide 50 mg/kg. Incipient beta-cell degranulation was noted half an hour after the administration of the drug; between the third and sixth hours 75% degranulation had occurred; after twenty-four hours only 50% degranulation was present; and by the forty-eighth hour the granulation had returned to normal again. After tolbutamide was given in large doses—2 g/kg—degranulation occurred to the same degree and with the same speed but regranulation took place more slowly—ninety-two hours being required for it to return to normal. Rats given tolbutamide for several weeks and killed twenty-four hours after the last dose, showed only moderate degranulation of their beta-cells but full granulation did not return until six days after withdrawal of the drug. Beta-cell degranulation occurred more slowly in the rabbit (132, 230). Other investigators, however, were unable to find degranulation of the beta-cells in rats (37, 254, 318), guinea-pigs (64, 254), dogs (254, 347) or rabbits (37, 254,

318) given IPTD, carbutamide or tolbutamide in single or repeated doses; and no significant difference has been observed between the beta-cells of responsive diabetic patients treated with sulphonylureas and those of untreated responsive patients (88, 133).

Other histological changes observed in the islets of animals treated for some time with a sulphonylurea were: an increase in islet cell volume (186, 242), enlargement of the beta-cell nucleus (188, 218, 316), occasional mitoses (155, 218) and new formation (92, 154, 250) of beta-cells.

The belief that the degranulation induced by administration of sulphonylureas indicates increased liberation of insulin has been criticised on several grounds: the first is a legitimate criticism, the others may be refuted. 1) Beta-cell degranulation may occur in circumstances associated with either increased or decreased insulin demands, and may thus result from either increased or reduced liberation of insulin. 2) It has been pointed out that the fall in blood glucose elicited by the sulphonylureas precedes the beta-cell changes by several hours in the rat and by several days in the rabbit. A similar delay in degranulation is, however, seen after administration of cortisone or somatotropin (415). It may be that the immediate fall in blood glucose is due to the liberation of free insulin and that degranulation represents the later mobilisation of bound insulin. 3) No change in the zinc content of beta-cells has been demonstrated histochemically (268); this criticism cannot be sustained until more is known of the relationship of zinc content to insulin content. 4) It has been suggested that the degranulation is due to the fall in blood glucose *per se*. This is improbable since beta-cell degranulation occurs in the rabbit and the rat only after a reduced blood glucose concentration has been maintained by insulin administration for 7 and 35 days respectively (415).

Nevertheless, because of the lack of agreement about their occurrence, and some doubt regarding their interpretation, the beta-cell changes *per se* do not provide sufficient evidence to sustain the hypothesis of increased release or production of insulin.

vi. Relation of beta-cell reserve to hypoglycaemic response. As indicated above, the hypoglycaemic response to the sulphonylureas of alloxanised rats with mild or moderate diabetes seems to be related to the number and functional capacity of the remaining beta-cells. Mirsky (284) has also shown that the fall in blood glucose concentration following administration of a single dose of a sulphonylurea to such animals occurs more gradually than in intact animals.

Little or no insulin can usually be extracted from the pancreas of patients suffering from the juvenile, labile type of diabetes (437). Such patients are almost always unresponsive to the hypoglycaemic action of the sulphonylureas except a few who have recently developed the disorder and are presumably still capable of elaborating some endogenous insulin (63, 215, 236, 299, 353). In contrast most mild stable diabetics have significant, though somewhat diminished, amounts of insulin extractable from the pancreas (437) and demonstrable in the peripheral blood (411). They are almost always responsive to the sulphonylureas, but the fall in blood glucose is more gradual than that which occurs in non-

diabetic persons (408). Both these mildly diabetic patients and the incompletely alloxanised animals have a demonstrable reduction of beta-cell reserve function (120). Moreover, animals subjected to a prolonged fast, which reduces their pancreatic insulin content but does not diminish their response to exogenous insulin, also respond with a slower fall in blood glucose than fed animals (19, 284).

Although the diabetogenic effect of the corticosteroids and somatotrophin is mainly due to their influence on extrapancreatic tissues, the development of hyperglycaemia due to their administration is accompanied and often preceded by a reduction in the pancreatic insulin content and reserve function of the beta-cells (415). Animals with mild idiohypophyseal diabetes show a gradual fall in blood glucose when given a sulphonylurea (284), and with few exceptions (438) metahypophyseal-diabetic animals are refractory to these compounds (230, 245). The reported positive (30, 53, 109, 292) and negative (127, 290) responses of patients with acromegaly and diabetes may similarly reflect the beta-cell reserve function. Thus Mohnike and Stötter (290) found that one such patient who did not require insulin was responsive, whilst two with long-standing acromegaly who required insulin were refractory. The reported positive (292) and negative (30, 127) response of animals or man with idiosteroid diabetes may be similarly explained. Those with metasteroid diabetes are refractory.

The recent studies of Lazarus and Volk (230) support the view that the occurrence and character of the hypoglycaemic response is related to the functional capacity of beta-cells to discharge insulin. They found that in rabbits treated with somatotrophin or corticosteroids, the magnitude and speed of the hypoglycaemic response to a single dose of a sulphonylurea were inversely related to the duration and severity of the diabetic state and to the degree of beta-cell degranulation.

There is thus considerable evidence that the initial administration of a sulphonylurea to a responsive animal increases the liberation of a hypoglycaemic substance, almost certainly derived from the beta-cells, into the pancreatic vein blood. Whether this substance is insulin and whether the fall in blood glucose can be ascribed to its action is more conveniently considered later.

The mechanisms by which a sulphonylurea might stimulate the liberation of insulin are unknown. The obligatory presence of the higher nerve centres seems to be excluded by the facts that the hypoglycaemic response was reported to be unimpaired in vagotomised or fully atropinised animals, in those receiving ganglion-blocking agents, in decerebrate animals and in those in whom the diencephalic portions of the brain had been destroyed or the carotid arteries ligated (19, 245, 247). Capelli *et al.* (66), however, found that tolbutamide had a considerable hypoglycaemic effect when injected into the carotid artery in quantities which had no effect when given intravenously.

The disputed hypoglycaemic effect of very small quantities of tolbutamide injected into the pancreatic artery suggests a direct pancreatrophic action. However, the incorporation of labelled cysteine into endogenous insulin is not increased in intact animals given tolbutamide (20), nor is the radioactivity of S^{35} tolbutamide incorporated into the insulin molecule (67).

The several mechanisms proposed so far to account for the stimulation by sulphonylureas of insulin release (19, 92, 316, 423) are purely speculative since the fundamental mechanisms of insulin elaboration and discharge remain obscure (229). The problem is not merely of academic interest, as prolonged stimulation by glucose infusion, high carbohydrate diet, corticosteroids or somatotrophin has been shown to result in eventual failure of insulin secretion especially when pancreatic reserve was initially decreased. In these circumstances, however, the liberation of insulin is increased in response to a greater demand for the hormone, a situation which probably differs from that resulting from sulphonylurea administration. It may be that these compounds facilitate insulin formation and/or release by activating some step in these processes which in turn may be impaired in patients with stable diabetes. There is certainly little evidence of beta-cell exhaustion in patients treated for up to four years with these compounds.

6. "Potentiation" of insulin activity. Those experiments, already cited, in which the sulphonylureas were shown to be without effect when given to animals shortly after pancreatectomy or evisceration suggest that the compounds do not activate endogenous insulin bound to the tissues. The possibility remains, however, that the sulphonylureas "potentiate" the effective activity of insulin as it is secreted, or of injected exogenous insulin.

a. Exogenous insulin. i. Depancreatised, alloxan-diabetic and eviscerated animals. Many workers have demonstrated that depancreatised or severely alloxan-diabetic dogs receiving exogenous insulin show a hypoglycaemic response to the administration of a sulphonylurea. For example, administration of a sulphonylurea to depancreatised dogs within eighteen hours of an injection of soluble (344, 347), or eighteen to twenty-four hours of a depot (26, 354, 397) insulin, caused a 40 to 50% fall in blood glucose concentration; however, when the insulin was given sixty-two hours before the sulphonylurea, the latter had no effect on the blood glucose concentration (347). Caren and Carbo (68), on the other hand, found that intravenous tolbutamide had no effect when given to depancreatised dogs deprived of insulin for eighteen hours.

These workers (68) and Schambye (354) showed that administration of a sulphonylurea to depancreatised dogs shortly before (68), or after, the single intravenous injection of 0.01–0.25 units/kg of soluble insulin (68), or the subcutaneous injection of 10 units insulin zinc suspension, resulted in a fall in blood glucose which was greater in degree and especially in duration, than that observed when the insulin was given alone. Although some workers (143, 275) have been unable to detect any hypoglycaemic effect resulting from the injection of a sulphonylurea in depancreatised or fully alloxanised dogs receiving a constant infusion of small amounts of insulin into a peripheral or the portal vein, others (191, 409) demonstrated a considerable fall in blood glucose concentration.

Loubatières and his associates (249) recently reported that the oral administration of 10 units/kg of insulin in glycerol to depancreatised dogs caused a small but definite fall in the blood glucose level; when IPTD, carbutamide or tolbutamide was given orally three hours before the insulin the magnitude and especially the duration of the blood glucose reduction were increased.

The "potentiation" by the sulphonylureas of the blood glucose fall following insulin administration to partially depancreatised or incompletely alloxanised dogs was, however, very much greater (50) than that in totally depancreatised animals. This increased effect was presumably due to the action of the sulphonylurea on the pancreas.

More prolonged studies have been made in depancreatised or fully alloxanised dogs given suboptimal daily doses of insulin for periods of several weeks. A variable but definite improvement in glycaemia and glycosuria occurred when a sulphonylurea was given in addition (195, 347, 432) although during this period the animals usually became weak and anorexic. Nevertheless the data obtained from these animals in which the studies were completed, particularly when control periods were interspersed between those of administration of sulphonylurea, support the conclusion that the change in glycaemia was at least partly due to a hypoglycaemic action of the sulphonylureas.

The uniformity of the results obtained in dogs contrasts with the often contradictory findings in animals of other species. Thus in severely alloxan-diabetic rats and rabbits very slight "potentiation" of insulin was reported (287) and denied (32, 116, 227); tolbutamide administration did not, however, accelerate the incorporation of C^{14} from isotopic glucose into CO_2 in fully alloxanised rats given insulin (278, 399). The blood glucose of depancreatised-hypophysectomised cats receiving insulin was not influenced by the daily administration of tolbutamide (168), and there is not any evidence of a hypoglycaemic effect of the compounds when given to depancreatised patients receiving insulin (127, 162, 327).

Insulin "potentiation" has also been reported (195) and denied (142) in eviscerated dogs receiving a constant infusion of insulin and glucose; no sulphonylurea effect has, however, been shown in eviscerated rats or rabbits similarly infused (116, 168, 227).

ii. Intact animals and man. No significant increase in the degree or duration of the hypoglycaemic response to injected insulin has generally been observed in animals in whom the blood glucose level had been reduced for several days or weeks by sustained administration of a sulphonylurea (106, 227, 294, 357, 386). In these circumstances any material potentiation of activity of the insulin given should not have been obscured by a primary pancreatotrophic action of the compounds. Marigo and Panelli (266) and several other groups of workers (23, 60, 127) also showed that the insulin sensitivity of normal and diabetic subjects responsive to the sulphonylureas was not increased by prolonged administration of the compounds. Danowski *et al.* (101) could not demonstrate any evidence of increased insulin sensitivity, in terms of fall in blood glucose or inorganic phosphorus concentration, in diabetics unresponsive to the sulphonylureas who were, however, receiving chlorpropamide; this accords with the almost unanimous clinical observation that the insulin requirements of these unresponsive diabetics are very rarely reduced by sulphonylurea administration (184, 236, 353, 391). Mirsky and Diengott (281) have, however, presented evidence which suggests that the sulphonylureas do to some extent potentiate the effect of insulin in juvenile-type diabetics.

iii. In vitro studies. The effect of insulin, added to the medium in either effective or sub-threshold amounts, on the glucose uptake and glycogen synthesis of diaphragm from intact rats has been claimed to be increased (148), uninfluenced (62, 76, 110, 144, 320) and slightly inhibited (286) by the addition of a sulphonylurea. The response of diaphragms from alloxanised rats to insulin added *in vitro* was reported to be unaffected by either the addition of the sulphonylurea to the medium (148) or by their previous administration to the donor animals (77, 144, 343).

These studies strongly suggest that the sulphonylureas do not appreciably increase the hypoglycaemic activity of exogenous insulin except in depancreatised dogs. Even in these animals the hypoglycaemic effect of the sulphonylureas need not necessarily be due to the compounds' increasing or "potentiating" the activity of exogenous insulin; it could result from a direct action on extrapancreatic tissues leading to a hypoglycaemic response which, however, occurs only in a metabolic environment provided by the action of insulin. That this may be so is suggested by the observation of Tarding and Schambye (355, 397) who demonstrated by isotope techniques that the sustained fall in blood glucose of depancreatised dogs, given a sulphonylurea eighteen hours after the last injection of a depot insulin, was entirely due to suppression of hepatic glucose release. As discussed later, the initial hypoglycaemic effect of the sulphonylureas in intact subjects results from this same inhibition of glucose output from the liver. Although Tarding and Schambye, again using isotope techniques, demonstrated that the hepatic release of glucose in depancreatised dogs was not inhibited by the intraportal infusion of insulin, there is other evidence (see p. 130) that the hormone may have such an effect. If this latter view is accepted then the fall in blood glucose of the depancreatised dogs receiving insulin and given a sulphonylurea could be due to the increased action of the hormone. However, the recent studies of Wildberger and Ricketts (432) seem to contradict this hypothesis. These workers found that the daily administration of a sulphonylurea to depancreatised dogs receiving suboptimal doses of insulin did not cause nitrogen retention whereas such retention occurred when larger doses of insulin, which produced the same reduction in glycaemia and glycosuria, were given alone. There is then no unequivocal evidence that the sulphonylureas "potentiate" or increase the activity of exogenous insulin in depancreatised dogs.

b. Endogenous insulin. Krahl (219) states that when rat diaphragm or adipose tissue was incubated in serum from a normal person the addition of a sulphonylurea caused a "marginal stimulation" of glucose uptake. This did not occur when a balanced salt incubation medium was used. Gambassi and Pirelli (148) studied the effect on the glucose uptake by isolated rat diaphragm, of blood plasma obtained from normal subjects before and shortly after the administration of a sulphonylurea. The effective "insulin-like" activity of plasma withdrawn after administration of a sulphonylurea was greater than that which was obtained before it was given. Addition of a sulphonylurea to control plasma, in the same concentration as that produced by its administration *in vivo*, resulted in a smaller increase in the glucose uptake by the diaphragm than that effected by plasma

withdrawn after the patients had received the drug. From these findings the authors concluded that the sulphonylurea increased both the liberation of and the activity of endogenous insulin. Aiman and Kulkarni (5) conducted somewhat similar studies and concluded that the sulphonylureas increased the activity of both endogenous and exogenous insulin, that the increase in insulin-like activity of normal persons given the sulphonylurea could thus be explained, and that an increased liberation of endogenous insulin need not necessarily be postulated.

Loubatières (251) has recently shown that the transfusion of blood from an intact to a pancreatectomised dog causes a transient fall in the blood glucose concentration of the latter. He also demonstrated that the degree and still more the duration of the fall was increased by the previous administration of a sulphonylurea to the recipient. Blood from a depancreatised donor had no effect in either circumstance. These experiments demonstrate the hypoglycaemic effect of a sulphonylurea in the depancreatised dog in the presence of natural endogenous insulin from another dog which, as discussed previously, may be the result of increased action of the hormone or a direct effect of the drug in its presence.

It has also been shown that tolbutamide *in vitro* does not alter the "anti-insulin" effect of *beta*-1-lipoprotein from responsive diabetic patients (148) or inhibit the insulin-binding properties of "antibody-like protein" in sera from insulin-resistant diabetic patients (171).

There is thus no indisputable evidence that the sulphonylureas increase the activity of endogenous insulin.

c. Inhibition of insulinase activity. Insulinase is the term applied to an enzyme system which catalyses the hydrolysis of insulin and is present mainly in mammalian liver and to a lesser extent in other tissues. At first thought to be specific for insulin, it is now considered to affect other compounds such as corticotrophin, glucagon, somatotrophin and casein (433). The role of insulinase in diabetes mellitus is, however, not fully defined although Mirsky has for several years postulated that its excessive activity may be of aetiological significance in mild stable diabetes (280).

Mirsky *et al.* (283), and Tamiya and Kizima (396) reported a reduction of 20 to 30% in the insulinase activity of livers obtained from rats and chickens given a hypoglycaemic dose of tolbutamide within the previous hour. They also reported that tolbutamide in relatively high concentration inhibited the insulinase activity of fresh liver extracts *in vitro*. They deduced from these observations that the hypoglycaemic effect of the sulphonylureas was due to their non-competitive inhibition of insulinase, although their hypoglycaemic effect in chickens, in which species reduction of insulinase activity was demonstrated, occurs in the absence of insulin.

The observations of these investigators have not, however, been generally confirmed. Thus Cova (84) found that the hepatic insulinase activity of rats and guinea pigs was uninfluenced by the single or daily administration of tolbutamide in hypoglycaemic doses. Although many workers have shown that the insulinase activity of various liver preparations was inhibited by the sulphonyl-

ureas *in vitro*, the concentration of the drug required always exceeded 100 mg/100 ml medium (36, 321, 413, 433). This concentration is about ten times that required in the plasma to lower the blood glucose of intact animals. Moreover, at these high concentrations corticotrophinase and glucagonase are also inhibited, and compounds which inhibit insulinase *in vitro* do not always have hypoglycaemic properties *in vivo*. Mirsky (284) has offered an explanation for the apparent excessive concentration required. He suggests that the heat-stable factor in liver extracts which deiodinates I^{131} insulin, used in many of the studies, is not appreciably affected by the sulphonylureas, whereas the heat-labile system responsible for the destruction of insulin by fresh liver extracts can be inhibited *in vitro* by tolbutamide present in concentrations as low as 13.5 mg/100 ml medium. However, other workers (321) were unable to demonstrate an inhibition of either the heat labile or the stable insulinase systems *in vitro* by tolbutamide in concentrations of 50–60 mg/100 ml.

Moreover, administration of tolbutamide in large dosage two hours previously to the donor animals did not affect the degradation of I^{131} insulin by rat liver homogenates *in vitro*; nor was the rate of degradation of injected labelled insulin, as measured by whole carcase analysis, altered *in vivo* (435). Administration of the compounds to intact animals (36, 435), eviscerated or eviscerated-nephrectomised preparations (430), and responsive and unresponsive diabetics (45, 424) given I^{131} insulin did not slow the decline in plasma radioactivity as measured by trichloracetic acid (TCA) precipitation (36, 45, 424, 430, 435) or electrophoresis (424).

The sulphonylureas do not significantly alter the blood glutathione level when given to diabetic and non-diabetic patients or animals (148, 295, 366) nor do they affect the inactivation of insulin by glutathione *in vitro* (321), although they may increase the insulinase-inhibiting power of blood in normal and diabetic subjects (148).

While Mirsky now attributes the early fall in blood glucose concentration following sulphonylurea administration to the increased liberation of insulin, he ascribes the more sustained phase to inhibition of insulinase (284). This explanation would accord with much of the experimental data if the enzyme system inactivates endogenous insulin to a greater degree than the exogenous hormone, and if insulin directly influences the hepatic release of glucose. Until these assumptions are disproved this hypothesis, which has been reviewed recently by Marigo and Panelli (266), cannot be rejected.

The experimental evidence reviewed so far suggests that the presence of some pancreatic tissue, almost certainly beta-cells, is necessary for the hypoglycaemic response to the sulphonylureas to occur *in vivo* in all species studied except domestic fowls and dogs. Their hypoglycaemic action could then result from one of the following:

1) Increased liberation of insulin. In this connection it must be remembered that it is possible that endogenous and exogenous insulin may differ in their metabolic effects since the latter has undergone a complex chemical extraction from the pancreas and is usually heterologous for the species tested; moreover,

if insulin is liberated by the action of the sulphonylureas, it may in turn have altered properties.

2) Increased activity of endogenous insulin.

3) A direct action of the sulphonylureas on hepatic or peripheral tissues the hypoglycaemic manifestation of which occurs only in a metabolic environment provided by the action of some secreted product of the beta-cell, presumably insulin.

4) Increased secretion of some unknown hypoglycaemic material, or decreased secretion of some unidentified hyperglycaemic substance from the pancreatic beta-cells.

Both endogenous and exogenous insulin promote the uptake of glucose by the cells of certain peripheral tissues, particularly those of muscle and fat, *in vivo* and *in vitro*. If the hypoglycaemic effect of the sulphonylureas is due to an increase in the normal action of insulin throughout the body it ought to be accompanied by those metabolic changes which occur when the hormone is injected. If the release of endogenous insulin is increased, similar changes should occur unless the hormone fails to traverse the liver in effective amounts, or is altered in regard to its metabolic effects.

7. Metabolic consequences of the action of insulin on peripheral tissues. a. Arterio-venous difference in blood glucose concentration. Bell and Burns (27) showed that the fall in blood glucose concentration following the rapid injection of insulin (0.012 units/kg) into a femoral artery of normal persons was associated with an increase in the arterio-venous (A-V) blood glucose concentration difference across the injected limb. Craig *et al.* (87) confirmed this observation and demonstrated in addition that no alteration in the A-V glucose difference across the limb occurred when 200–400 mg of sodium tolbutamide was given intra-arterially, although the resultant fall in blood glucose concentration was much greater than that elicited by the insulin. This suggests that the sulphonylureas, unlike insulin, do not immediately promote glucose uptake by skeletal muscle tissue *in vivo* even when endogenous insulin is present.

In Bell's experiments an increase in the femoral A-V glucose difference across the uninjected limb occurred only when the hypoglycaemic effect was maximal and during the subsequent rise in blood glucose level. The A-V glucose differences actually decreased as the blood glucose fell which suggests a diminution of glucose entry into the blood rather than an overall acceleration of its removal. This discrepancy in A-V glucose difference changes in the two limbs might be due to rapid fixation of insulin in the injected limb. However, when insulin was given intravenously in the same (27) or similar dosage (85, 162, 257, 258), the A-V glucose difference also showed a small but significant initial decrease, and later an increase. A similar decrease and delayed increase in A-V difference and A-V:A ratio occurred when tolbutamide was given by mouth or intravenously to dogs (257), normal persons (85, 162, 258) and responsive diabetics (85, 424). Thus, these studies suggest that the reduction in blood glucose concentration elicited by administration of sulphonylureas is not accompanied by accelerated glucose assimilation by skeletal muscles, or that if it is, the increase occurs at

a rate which cannot be detected by this experimental method; moreover they suggest that the fall effected by small doses of insulin may also be unaccompanied by such increased uptake. They cannot, however, be considered to have shown that the fall in blood glucose was unaccompanied by accelerated removal of glucose from the blood as no measurements were made across the very insulin-sensitive adipose tissues in the splanchnic area.

b. Changes in muscle or adipose tissue in vivo. Although the muscle glycogen content of fasted animals increased markedly within two hours of the intravenous, subcutaneous, intraperitoneal, or intrasplenic injection of a single small dose of insulin (95, 116, 237), no increase occurred in the glycogen content of diaphragm or skeletal muscle of fasted or fed intact (14, 95, 144, 346, 386) hypophysectomised (95), or adrenalectomised (167) animals given a single dose of a sulphonylurea up to ten hours previously. In hypophysectomised animals, whose peripheral tissues are extremely sensitive to insulin, any such effect of increased insulin activity consequent on sulphonylurea administration should have been demonstrable. On the other hand, the administration of insulin may not necessarily be associated with an increase in muscle glycogen, since it has been reported, on the basis of limited data, that the muscle glycogen content of intact rats was not significantly altered by the intravenous infusion of 1 unit/kg insulin over four hours, although the blood glucose was much reduced (116); moreover animals treated with a sulphonylurea for several weeks have a normal muscle glycogen content (77, 95).

Isotopic glucose studies have provided conflicting results. Ashmore *et al.* (14) found in fasting rats that the incorporation of C^{14} from U-C^{14} glucose into striated muscle glycogen and peripheral fatty acids was considerably increased by insulin given subcutaneously one hour previously, but was not altered by tolbutamide given intravenously in a dose which caused the blood glucose to fall at the same rate and to the same degree. In contrast Miller *et al.* (278) found that tolbutamide, given five hours previously, increased the specific activity of muscle glycogen in fasting rats given a high-activity tracer dose of C_6^{14} glucose, but the degree of incorporation was less than that following insulin. As expected both tolbutamide and insulin were without this effect in animals which had been previously fed.

The dorsal fat pad of rats is very sensitive to insulin. Bressler and Engel (54) reported that insulin administered to fasting rats markedly increased the glycogen content of this tissue, whereas tolbutamide given in effective hypoglycaemic doses had no such action. In fed animals given glucose, however, both insulin and sulphonylureas increased the glycogen deposition but the former had much the greater effect. On the other hand, incorporation of C^{14} from labelled glucose into peripheral fatty acids in mice has been reported to be reduced by both single and repeated administration of a sulphonylurea (325).

It has been suggested that because the pancreatic insulin content is reduced by fasting, the extra insulin liberated in response to sulphonylureas in fasting animals could be insufficient to traverse the liver and exert a detectable peripheral effect which might perhaps be demonstrable in fed animals liberating greater

amounts of insulin (54). The fact that hepatic insulinase activity is considerably less in fasting than in fed animals (284) is against this explanation.

c. In vitro studies. Insulin given to the donor animal *in vivo* increases the glucose uptake of rat diaphragm tissue *in vitro* (335); the uptake of diaphragms removed from rats given a single hypoglycaemic dose of a sulphonylurea has been variously reported to be slightly greater than (65, 329, 343), and the same as (144, 335) that of untreated animals. Daily administration of a sulphonylurea for two weeks to the donor animal did not alter the glucose uptake or glycogen synthesis by the diaphragm *in vitro* (77, 343); moreover the effect of insulin added *in vitro* was usually diminished (77, 144, 343).

d. Plasma inorganic phosphorus. The fall in plasma inorganic phosphorus which follows the injection of insulin or the administration of glucose is generally thought—though not by all—to reflect the utilisation of glucose by anaerobic glycolysis in the extrahepatic tissues (101). Although the oral or intravenous administration of a sulphonylurea has been reported to reduce the plasma inorganic phosphorus in animals and in non-diabetic and responsive diabetic persons (162, 414), most observers noted only inconstant and small changes (327, 351, 389, 395). Moreover the fall in inorganic phosphorus in response to glucose given orally or intravenously to normal or sulphonylurea-responsive diabetic persons was not altered during their long-term treatment with the compounds (152, 204, 327). However, the impaired ability of mildly alloxan-diabetic rats to incorporate administered P^{32} disodium hydrogen phosphate into muscle tissue was restored to the same extent by their treatment for five days with either insulin or a sulphonylurea (83).

e. Effect on distribution of pentoses. D-Xylose and L-arabinose do not stimulate the release of endogenous insulin (323), but their volume of distribution in the body is increased by insulin which accelerates their entry into the cells of peripheral tissues. Segal *et al.* (363) showed that the rate of removal of administered D-xylose and L-arabinose from the blood of normal persons was increased threefold by the rapid intravenous injection of 0.05–0.1 unit/kg of insulin, but was not significantly altered by 2.0 g of tolbutamide given orally, although the hypoglycaemic effects of both were almost identical.

f. Effect of 2-deoxyglucose on the hypoglycaemic response. 2-Deoxyglucose, by inhibiting oxidation of glucose in peripheral tissue cells, retards the uptake of glucose by these cells even when this is maximally stimulated by exogenous insulin; it also stimulates the adrenal medulla in the absence of hypoglycaemia. Preliminary studies (55) have shown that although 2-deoxyglucose also markedly inhibited glucose oxidation in adrenal-demedullated rats, the hypoglycaemic response of such animals to tolbutamide was not modified by previous loading with 2-deoxyglucose.

g. Effect of hypophysectomy or adrenalectomy on the hypoglycaemic response. Hypophysectomised animals are extremely sensitive to exogenous insulin. This is due mainly to the greatly exaggerated rate of glucose removal from the blood by the tissues and partly to the failure of the liver to discharge adequate amounts of glucose in response to hypoglycaemia (422). Hypophysectomised animals are, however, no more responsive to the hypoglycaemic effect of the sulphonylureas

than are intact animals (20, 168, 192), although animals hypophysectomised not more than four days previously have been reported to be unduly sensitive (117).

Dulin and Miller (117) attributed these differences in hypoglycaemic response to a fall in the extractable insulin content of the pancreas to an amount half that of intact animals during the first few days after hypophysectomy, so that the insulin then available for discharge in response to administration of sulphonylurea is considerably reduced. This explanation is open to several objections: other investigators have found only minimal reduction of pancreatic insulin in hypophysectomised animals; beta-cell degranulation after sulphonylurea administration is as obvious in hypophysectomised as in intact animals (90); and the escape of even one unit of insulin through the hepatic barrier should cause a profound hypoglycaemia in hypophysectomised cats (168).

Moreover, although adrenalectomy greatly increases the sensitivity of hypophysectomised animals to the hypoglycaemic and toxic effects of the sulphonylureas it does not increase their response to exogenous insulin (117). The greater response to sulphonylureas may be partly explained if the compounds initially stimulate the adrenal medulla (18, 118)—a possibility supported by the greater sensitivity to the sulphonylureas of adrenalectomised or adrenomedullated but otherwise intact animals.

The studies on pentose distribution, those using 2-deoxyglucose and the observations on hypophysectomised and adrenalectomised animals strongly support the hypothesis that the initial fall in blood glucose level following administration of sulphonylurea is neither accompanied by accelerated outflow of glucose from the blood into peripheral tissue cells, nor associated with any increased action of insulin on these tissues.

8. Metabolic effects not exclusively due to alteration in the metabolism of extra-hepatic tissues. a. Respiratory quotient, oxygen consumption and basal metabolic rate. The R.Q. of normal and diabetic subjects receiving a sulphonylurea has been variously reported to be increased (387) and unaltered (160, 338). The B.M.R. has been stated to be decreased in such patients without alteration of thyroid function (264). The diminution in the oxygen consumption of rats treated with carbutamide is, however, probably due to inhibition of thyroid function (69).

b. CO_2 production from glucose. Miller *et al.* (278) studied the effect of tolbutamide given intravenously and of insulin injected subcutaneously on the oxidation of C_6^{14} glucose to $C^{14}O_2$. In fed rats, both accelerated oxidation of glucose although the effect of sulphonylurea was more prolonged; in fasted rats insulin had a greater effect than tolbutamide; in severely alloxan-diabetic rats and in eviscerated preparations (429) tolbutamide influenced neither the concentration of blood glucose nor glucose oxidation. In general these findings accord with those reported by Tolbert and Kirk (399) in extensive, fully documented studies on the C^{14} respiratory pattern of normal and alloxanised rats. However, the latter investigators observed that the early phase of glucose-to-CO_2 production which followed insulin injection was much less when the hormone and carbutamide were given simultaneously.

c. Blood constituents. The changes in the concentration of pyruvate and lactate

in the peripheral blood reported to have occurred after sulphonylurea administration were inconstant and very variable (147, 181, 292, 338, 351, 382). Miller *et al.* (277) showed, however, that the early changes in pyruvate and lactate were equally variable following insulin injection and were affected by the route and rate of the administration. Valid deductions regarding the site and mode of action of the sulphonylureas cannot, therefore, be made from changes in these constituents. The changes reported in other blood constituents, such as potassium, have been equally inconsistent and contradictory (60, 162, 181, 286, 382, 389, 395). However, the hypoglycaemic effect of the sulphonylureas was, like that of insulin, accompanied by a rapid reduction in the non-esterified fatty acids (41, 338) and amino acids (107) in plasma. The reduction in the former may have been due to the direct inhibition of fatty-acid synthesis by adipose tissue—a property demonstrated *in vitro* (339).

d. Glucose tolerance. Glucose tolerance is the capacity to limit the magnitude and duration of the rise in blood glucose concentration which follows the administration of exogenous glucose. It depends not only on the speed with which the peripheral tissues can assimilate glucose, but also on the ability of the liver to reduce its net output of glucose.

Intact rats (92, 186, 232, 295), rabbits (91, 386), and dogs (285) given a sulphonylurea daily for up to nine months showed no improvement in tolerance to glucose administered orally or intravenously and indeed an impairment of tolerance often occurred in intact (186, 232, 285) and partially depancreatised (92) animals. This might have been due either to increased adrenocortical activity in response to the sutained hypoglycaemic effect of the sulphonylureas or to exhaustion of beta-cell reserve.

Although a small improvement in tolerance is stated to have occurred in responsive diabetics treated for a few days with a sulphonylurea (23, 204), their continued treatment almost always resulted in no improvement in tolerance to oral (16, 23, 60, 292) or intravenous glucose (16, 60, 129, 264, 389). The shape of the oral glucose tolerance curve was not altered, but since it rose from a lower fasting base line the absolute values were less. The "total index" of intravenous glucose tolerance (119) was therefore improved (16, 60, 197, 204) but the "increment index" (119) remained unchanged (16, 60, 192). Moreover the fall in plasma inorganic phosphorus (101, 152, 204) and the change in A-V blood glucose differences (389) following administration of glucose were not altered by treatment with a sulphonylurea, which also suggests that the rate of removal of glucose from the blood is not increased.

Butterfield *et al.* (61) have obtained evidence suggesting that the assimilation of blood glucose by the muscle tissues of the forearm of normal and diabetic persons occurred only when the arterial blood glucose concentration exceeded a certain value. This threshold level closely approximated the fasting blood glucose concentration, and the rate of glucose uptake by the tissues appeared to be directly related to the arterial glucose level in excess of the threshold. Although the elevated threshold of mildly diabetic patients was lowered by several days' treatment with a sulphonylurea or insulin, this relationship between the tissue

glucose uptake and the rise in arterial blood glucose, following slow glucose infusion, was unaltered. These observations accord with the view that no improvement in glucose tolerance, as defined, results from the sustained administration of a sulphonylurea and that the rate of removal of exogenous glucose from the blood is unaltered although it occurs at lower absolute blood glucose concentrations.

The question is crucial whether the initial hypoglycaemic response to the sulphonylureas is accompanied by metabolic changes in the peripheral extrahepatic tissues—particularly by an accelerated glucose uptake—which would indicate increased insulin action on these tissues; but, as stated recently by Stadie (380), the question cannot be definitely answered from the available evidence.

Reported changes in A-V glucose difference cannot be ignored but their significance and interpretation is in doubt. The changes in muscle and peripheral fat *in vivo*, the *in vitro* behaviour of muscle, the studies using the insulin responsive pentoses and 2-deoxyglucose, and the observations on hypophysectomised and adrenalectomised animals provide strong evidence that the initial fall in blood glucose caused by the sulphonylureas is not accompanied by accelerated assimilation of glucose by the extrahepatic peripheral tissue or by increased insulin action on these tissues. This agrees with the failure to detect increased "insulin-like" hypoglycaemic activity in peripheral blood shortly after administration of sulphonylurea either by cross-circulation studies or, in most instances, by *in vitro* bio-assay.

If the fall in blood glucose is not due to accelerated assimilation of glucose by extrahepatic tissues then it must result from a reduction in the entry of endogenously derived glucose into the blood. This is suggested particularly by the pentose studies, and almost conclusively confirmed by the following investigations.

9. Reduction of net hepatic glucose release. a. Studies using isotopic glucose. Observations have been made on the effect of a single dose of a sulphonylurea on the decline in plasma concentration of administered U-C^{14} glucose (35), and on the fall in the specific activity of blood glucose (14, 200, 259, 260, 355, 397). Tests were made in rats (14, 35), dogs (259, 260, 355, 397), and non-diabetic and mildly diabetic persons (200). The data obtained showed that the fall in blood glucose was not accompanied by an acceleration in the rate of removal of glucose from the blood (blood glucose outflow), and that it resulted entirely from diminished entry of glucose into the blood (blood glucose inflow).

Nevertheless, it must be stressed that the inhibited entry of unlabelled glucose into the blood, as indicated by the cessation of the decline in the specific activity of blood glucose, does not necessarily represent complete inhibition of hepatic glucose release. This point will be discussed later (see p. 128).

b. Hepatic vein catheterisation studies. Several groups of investigators have demonstrated a rapid and sustained inhibition of net splanchnic glucose production (N.S.G.P.) in dogs (7, 327, 397), non-diabetic (85, 209, 335) and responsive diabetic (209) persons given a single dose of a sulphonylurea. For ex-

ample, the careful experiments of Craig *et al.* (85) clearly showed that the fall in blood glucose concentration of normal persons could be entirely accounted for by the reduction in N.S.G.P.

The N.S.G.P. is, however, not necessarily synonymous with net hepatic glucose release (N.H.G.R.) which must be more directly determined.

c. Transhepatic measurement of hepatic glucose release. Ashmore and his co-workers (14, 259, 260) made direct transhepatic observations in fasting unanesthetised dogs from which portal, hepatic venous and aortic arterial blood could be withdrawn through permanently inserted catheters. Injection of 0.5 g tolbutamide into the portal or a peripheral vein always caused a rapid and very prolonged reduction in N.H.G.R., often amounting to complete inhibition; moreover the N.H.G.R. remained suppressed even when the blood glucose concentration had fallen to very low values. The N.H.G.R. was calculated from formulae which allowed for the contribution of the hepatic artery to the hepatic blood inflow; hepatic blood flow was unchanged.

The very considerable fluctuations in hepatic glucose release and blood flow, and the influence of even minimal errors in the determination of the blood glucose concentration make it undesirable to draw definite conclusions from single experiments. However, these studies leave little doubt that the administration of a sulphonylurea was rapidly followed by a very considerable and sustained suppression of N.H.G.R.

d. Indirect determination of hepatic glucose release. Bastenie *et al.* (23), using an indirect technique, found that the fasting hepatic glucose release of responsive diabetic patients was considerably reduced by their treatment with a sulphonylurea for several days; moreover, the rate of tissue assimilation of glucose was found to be unchanged.

Inhibition of hepatic glucose release may also be investigated in subjects in whom the rate of this release has been altered by one of a variety of techniques.

The administration of exogenous glucose suppresses the release of glucose by the liver. Purnell *et al.* (327) gave tolbutamide intravenously to dogs, to non-diabetic and mildly diabetic persons, while they were receiving a constant infusion of glucose; no hypoglycaemic effect of the sulphonylurea was demonstrable. Moorhouse and Kark (292) found that mildly diabetic persons continuously tube fed with a balanced synthetic fluid diet of which glucose was the only carbohydrate constituent showed no fall in blood glucose in response to a single dose of 4.0 g tolbutamide or its administration for several days. As it is considered that the administration of glucose also stimulates insulin release (323), the absence of a hypoglycaemic response could, however, be due to inability of the beta-cells to respond further to the sulphonylurea. This explanation appears unlikely, as a considerable response occurred in hepatectomised animals maintained at hyperglycaemic levels by glucose infusion (116).

If fructose, which does not stimulate insulin release (323), is substituted for glucose as the only source of exogenous carbohydrate, the liver is compelled to synthesise and release glucose. The single or daily administration of tolbutamide very greatly reduced the blood glucose level of mildly diabetic patients continu-

ously fed with a glucose-free fructose diet (292). Moreover, the characteristic rise in blood glucose concentration which follows the administration of fructose or galactose to mild diabetics was significantly diminished when they had been treated with a sulphonylurea for several days (46, 153). As the treatment did not accelerate the removal of glucose, galactose or fructose from the blood in the patients studied, the reduction in the rise of blood glucose was almost certainly due to inhibition of the increased hepatic glucose release; this has been directly demonstrated by hepatic catheterisation studies in subjects given fructose (277).

e. In vitro studies. Recant and Fischer (335) found that the spontaneous release of glucose by liver slices obtained from intact rats was greatly diminished by the intraperitoneal injection of tolbutamide to the donor animals within the previous hour. The reduction was related quantitatively to the fall in blood glucose concentration when the animals were killed. The glucose release of liver slices from animals treated with tolbutamide for four days was also much reduced (404), which demonstrates that the diminution is sustained.

These studies substantiate the hypothesis that in intact animals given a sulphonylurea, the initial and possibly also the sustained fall in blood glucose is almost entirely due to the diminution of hepatic release of glucose into the blood.

10. Mechanism of the reduction in hepatic glucose release. a. Reduction of glycogenolysis. Many investigators have confirmed Loubatières' (242) original observation that a two- to threefold increase in hepatic glycogen content very rapidly followed the administration of a sulphonylurea to fasted intact animals (20, 32, 95, 144, 169, 287, 404). The injection of tolbutamide also accelerated the incorporation of C^{14} from tracer doses of isotopic glucose into liver glycogen and fatty acids in fasted rats (14, 278). Neither of these effects, however, accompanies the hypoglycaemic response to insulin (14, 32, 95, 237, 278) even when it is given intraportally (95). These observations support the earlier view of Berringer *et al.* (32), that the sulphonylureas reduce the blood glucose concentration by increasing the uptake of glucose by the liver, which then converts it to glycogen.

No increase in hepatic glycogen, however, followed the administration of the same drugs in effective hypoglycaemic dosage to fed animals (20, 71, 404), and to fasted hypophysectomised (20, 95) or adrenalectomised (20, 167, 182) animals. In fasted adrenalectomised animals maintained with both cortisone and adrenaline an increase in hepatic glycogen was, however, found (182).

Although the sulphonylureas neither lowered the blood glucose nor altered the hepatic glycogen content of fully alloxanised animals (32, 89, 182), their administration in a single dose to such animals maintained with depot insulin did result in a rapid increase in hepatic glycogen although there was no significant reduction in the blood glucose concentration.

These observations show that the hypoglycaemic effect of the sulphonylureas is not necessarily accompanied by an increase in hepatic glycogen and, conversely, that in appropriate metabolic conditions the compounds can increase hepatic glycogen without influencing the concentration of blood glucose.

If inhibition of hepatic glycogenolysis was responsible for the reduction in hepatic glucose release, then the inhibition could be due either to a direct effect of the sulphonylureas on an enzyme system in the liver, or to interference with the release of, or to inactivation of, either glucagon or adrenaline, or to their diminishing the hepatic response to these hormones.

Inhibition of glucagon or adrenaline release is unlikely as the drugs do not invariably damage alpha-cells and adrenal-demedullated animals are certainly responsive to their hypoglycaemic action. It is also improbable that the compounds increase the inactivation of glucagon because: 1) they do not affect the activity of glucagonase *in vitro* (36); 2) they do not alter the rate of disappearance from the blood of labelled glucagon (36); and 3) the glycogenolytic activity of serum extracted for glucagon was stated to be no less in animals receiving tolbutamide than in controls (404). The sulphonylureas might reduce the hepatic response to the hormones. The intravenous injection, however, of 2–20 μg of glucagon into intact dogs (7) and rabbits (36, 71, 276) given a single or daily dose of sulphonylurea caused the same rise in blood glucose concentration as in untreated animals; similarly, the hyperglycaemic response of normal and sulphonylurea-responsive diabetic persons to 0.1–2.0 mg glucagon was not altered by their treatment with the compounds (60, 127, 292, 336); some workers have, however, reported a small diminution in response (12, 164). The hyperglycaemic effect of injected adrenaline was usually uninfluenced (12, 151). Moreover, Bulgarelli (57) found that carbutamide had a hypoglycaemic effect in a patient suffering from Von Gierke's disease who was refractory to the hyperglycaemic effect of glucagon. On the above evidence any inhibition of hepatic glycogenolysis could scarcely be attributed to the altered influence of glucagon or adrenaline.

That inhibition of glycogenolysis is not necessarily the cause of the initial or sustained hypoglycaemic effect of the sulphonylureas is suggested by the observations of Lang and Sherry (227). They found that the hepatic glycogen content of fed rats was 2.5 to 3.0 g %. When these animals were given effective hypoglycaemic doses of tolbutamide at four hourly intervals during a twenty-four hour fast, inhibition of hepatic glycogenolysis occurred only during the first six hours. After 24 hours the hepatic glycogen had fallen to 0.1–0.2 g %—the same value as that in control animals given only saline. Considerable glycogen breakdown had thus occurred during the sustained reduction in the blood glucose concentration. Moreover, the hepatic glycogen content of animals given tolbutamide for more than two to three days (20, 77, 95, 346), and of mild diabetics treated for several months (146), was within normal limits.

The above evidence suggests that the glycogenolytic pathways remain open in animals and man given a sulphonylurea, that the secretion and activity of the glycogenolytic hormones is not inhibited, and that neither the initial nor the sustained hypoglycaemic effect of the compounds can be accounted for by inhibition of hepatic glycogenolysis. Nevertheless the data collectively suggest that the compounds cause a net retention of glucose by the liver.

b. Inhibition of gluconeogenesis. Hepatic gluconeogenesis can be reduced by

inhibition of adrenocortical activity. However, the hypoglycaemic effect of the sulphonylureas cannot be due to such an action as they cause an extremely marked fall in blood glucose concentration in adrenalectomised animals. Moreover no change has generally been reported in the plasma concentration or urinary excretion of corticosteroids (72, 127, 313) nor in the response to corticotrophin (174, 428) of patients treated with the compounds.

If gluconeogenesis were diminished, a reduction in the nitrogen excretion might be expected. The daily urinary nitrogen excretion of diabetic patients successfully treated with the compounds has been found to be unchanged (127, 160) or reduced (30, 122); the daily urinary nitrogen loss of such patients is, however, rarely excessive so that a marked reduction would not have been expected. A consistent reduction in creatinine excretion occurred in responsive diabetics, but not in those unresponsive, given tolbutamide (105).

c. In vitro enzyme studies. i. Spontaneous glucose release. Liver slices from animals given a sulphonylurea showed a marked reduction in spontaneous glucose release *in vitro* (335). In contrast the presence of a sulphonylurea in concentrations of from 40 to 100 mg/100 ml medium did not inhibit the glucose release of incubated liver slices from intact animals (62, 413); higher concentrations, however, were reported to produce a 10 to 30% reduction (37, 76, 288).

ii. Glucose-6-phosphatase activity. Several workers have reported that the addition of a sulphonylurea in concentrations of up to 100 mg/100 ml medium did not alter significantly the glucose-6-phosphatase activity of rat and rabbit liver homogenates or microsomal preparations (62, 222, 413). However, other investigators have observed reductions of 15 to 25% by the addition of sulphonylurea to a concentration of 40 to 50 mg/100 ml (289, 423, 425), of 10 to 30% at 80 mg/100 ml (37, 144), and of 60% at 400 mg/100 ml (289, 425); at concentrations within the therapeutic range a maximum inhibition of only 10% was noted. Moreover, the activity in preparations of liver from fully alloxanised animals was equally reduced (289).

In contrast, a considerable reduction in glucose-6-phosphatase activity was found, *in vitro*, in liver preparations of intact (62, 178, 223) or incompletely alloxanised (78) animals and responsive diabetics (307) given a sulphonylurea for the previous two or more days. The reduction in glucose-6-phosphatase activity took place slowly, no effect being seen within three hours of administration of sulphonylurea (62, 144, 223, 335), whereas the reduction in hepatic glucose release was shown to occur much more rapidly both *in vivo* and *in vitro*. When given for 8 days, tolbutamide reduced neither the blood glucose concentration nor the hepatic glucose-6-phosphatase activity of completely alloxanised animals, whereas insulin reduced both (178).

Glucose-6-phosphatase is present, however, in considerable excess relative to other glycogenolytic enzymes, it is increased in fasted and in alloxan-diabetic animals and in diabetic patients, and decreases only slowly in response to insulin administration. Its altered level is thus thought to reflect sustained changes in the hepatic release of glucose, and the studies reviewed strongly suggest that the alterations in glucose-6-phosphatase activity are the consequence of, rather than

the cause of, the rapid reduction of hepatic glucose release effected by the sulphonylureas.

iii. Glycogenolysis. Spontaneous glycogen breakdown by liver slices is not altered by the presence of sulphonylureas in concentrations of from 40 to 100 mg/100 ml medium or by treatment of the donor animal with the compounds for two days prior to death (37), despite the fact that the spontaneous glucose release is much reduced in the latter circumstance (335, 404). The glucose released by liver slices in response to adrenaline or glucagon added *in vitro* is, however, reduced by addition of tolbutamide to the medium. Tolbutamide in concentration of 13.5 mg/100 ml medium was stated to cause a 50% reduction in glucose release in response to added adrenaline and at 140 mg/100 ml almost to abolish the response to glucagon (413), but the data of other investigators showed a reduction of only 10% in the glycogenolysis effected by either hormone at tolbutamide concentrations of 50 mg/100 ml. Moreover, although tolbutamide in a concentration of 30 to 150 mg/100 ml inhibited the reactivation of liver phosphorylase (37), this effect was also obtained when non-hypoglycaemic sulphonamides were used (37).

Weber and Cantero (425) observed a 45% inhibition of glucose-6-phosphatase and 21% inhibition of phosphohexoseisomerase in the presence of tolbutamide, 160 mg/100 ml medium, and a 16 and 12% reduction respectively at 44 mg/100 ml. Phosphoglucomutase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase activity were quite uninfluenced by tolbutamide in concentrations not greater than 440 mg/100 ml. There is thus some degree of specificity in the effect of tolbutamide on the enzyme systems. These investigators considered that the altered ratio of the activity of the hepatic enzymes, rather than their absolute degree of inhibition, could account for the diminished glucose release and, if the rate of glucose entry into the liver were unchanged, would lead to either increased glycogenesis and/or increased utilisation of glucose by way of the hexose monophosphate shunt. The observations of Wallenfels *et al.* (423) generally accord with this hypothesis.

iv. Gluconeogenesis. A number of studies of hepatic transaminase activity have been reported. Bornstein (47) observed a 70 to 80% reduction of alanine transaminase activity by tolbutamide in a concentration of 270 mg/100 ml medium, and a 10% reduction at 27 mg/100 ml; non-hypoglycaemic sulphonamides had no effect. Several workers have shown that the administration of a sulphonylurea for a number of days to rats considerably reduced the activity of hepatic glutamic-oxaloacetic (352), aspartic and alanine-ketoglutaric (79, 439) transaminases; the increased activity of these enzymes in animals receiving cortisone was also inhibited by the sulphonylureas (352). However, the reduction of transaminase activity developed slowly being demonstrable only twenty-four hours after a single dose of the sulphonylurea (352, 439), and is therefore unlikely to have been directly responsible for the rapid fall in blood glucose concentration.

Downie *et al.* (114) found that the oxidation of the keto-acid moiety of C^{14} labelled amino acids by liver slices was reduced by sulphonylurea present in a concentration of 30 mg/100 ml medium. The apparent inhibition of transami-

nase activity, demonstrated in the other studies, might therefore have resulted from accelerated oxidation of their substrates in the Krebs cycle—which in turn would diminish gluconeogenesis—rather than from inhibited formation of the enzymes.

Barnabei *et al.* (21) studied the effect of tolbutamide and of insulin on the formation of *p*-aminohippuric acid from *p*-aminobenzoic acid and glycine by liver homogenates from incompletely alloxanised rats in which this synthesis was reduced. Insulin restored the synthesis to normal when added *in vitro* to the medium and when injected to the donor animal. In contrast, sulphonylurea added *in vitro* inhibited both the spontaneous synthesis and considerably reduced the increase effected by insulin. This *in vitro* action is reflected by the effect of treating responsive diabetics with a sulphonylurea, which reduces their ability to synthesise *p*-aminohippuric acid from administered glycine and *p*-aminobenzoic acid. This is not due to inhibition of glycine incorporation into the liver, as this function is increased, *in vitro*, by daily treatment of the donor animal with tolbutamide (335).

v. Ketogenesis. The reduction by the sulphonylureas of the ketonaemia of fasting animals (54, 228), of human subjects given a ketogenic diet (211), and of mild diabetics (70, 203) could be due to increased oxidation of acetate, but Kinsell *et al.* (210) found no increased utilisation of C^{14} carboxyl-labelled acetate. Renold *et al.* (339) have, however, shown that the ketogenesis of liver slices from fasted or diabetic rats was substantially reduced by the presence of carbutamide or tolbutamide in concentration of 20 mg/100 ml medium; insulin 0.1 unit/ml medium had no such effect. The combination of reduction in blood glucose and ketones with increase in hepatic glycogen suggests increased utilisation of glucose by the liver.

vi. Glycolysis. Lamprecht and Trautschold (226) showed that carbutamide could partly eliminate the block in hepatic glycolysis at the triosephosphate level which occurs when starved rats are given glucose. Carbutamide completely restored glycolysis when a small dose of insulin—about one-fiftieth of that effective *per se*—was given in addition. This suggests a hepatic action of the sulphonylurea, considerably greater in the presence of insulin, in a metabolic situation similar to that occurring in mild stable diabetes.

vii. Other studies. The presence of a sulphonylurea in the medium did not alter the succinodehydrogenase activity (76), or the distribution of labelled products of C^{14} fructose or pyruvate in (62) liver slices from fasted rats; oxygen consumption and cytochrome oxidase activity were reduced (289), but there was no inhibition of pyruvic dehydrogenase activity or of oxygen consumption when pyruvate was added to the medium (76). The hepatic uptake of injected P^{32} disodium hydrogen phosphate in incompletely alloxanised animals was restored to normal by their treatment with insulin or tolbutamide (83). Wallenfels *et al.* (423) concluded from their extensive studies that the sulphonylureas affected the activity of several dehydrogenase systems dependent on pyridine nucleotide.

From these *in vitro* studies it appears that the sulphonylureas can modify hepatic metabolism at several sites. Nevertheless the changes develop slowly

after administration of the compounds to the intact animal, and are unlikely therefore to account, *per se*, for the rapid inhibition of hepatic glucose release. Moreover, the changes observed are, with some few exceptions, the same as those following insulin administration.

11. Hypoglycaemic effect in hepatectomised animals. It has been unequivocally demonstrated that the sulphonylureas have a hypoglycaemic effect in hepatectomised animals.

Dulin and Johnston (116) used fasted rats in which porto-caval anastomoses had been established by operation four weeks previously. Following hepatectomy a continuous infusion of glucose was begun and tolbutamide 400 mg/kg was injected into the test animals, each of which was paired with a control. When killed four hours later the mean blood glucose of the control animals was 161 mg/100 ml and of the treated group 126 mg/100 ml.

The effect of tolbutamide was also studied by these investigators (116) and by Sobel *et al.* (377) in dogs hepatectomised by the one stage technique of Markowitz and maintained with a constant infusion of glucose. After a control period, the test animals were given tolbutamide [50 mg/kg intraperitoneally (116) or 125 mg/kg intravenously]; control animals were given saline. The blood glucose concentration was determined at intervals for 4 hours; that of the control animals remained unchanged or was increased by 10 to 40 mg/100 ml; that of the test animals fell by 20 to 30 mg/100 ml.

Richter (340) has also shown that tolbutamide reduced the blood glucose of hepatectomised rabbits receiving a glucose infusion.

Although these experiments show that the presence of the liver is not essential for the sulphonylureas to cause a reduction in blood glucose, there is no doubt that in intact animals the immediate hypoglycaemic effect of these compounds is almost entirely the consequence of inhibition of hepatic glucose release. The failure of the compounds to reduce the blood glucose concentration in intact man or animals receiving glucose by infusion (292, 327) is probably due to the fact that the hepatic release of glucose is already inhibited by the glucose infusion itself. In hepatectomised animals no such inhibition is possible; moreover, in these hepatectomised animals the hypoglycaemic substance, presumably insulin, discharged by the pancreas in response to the drugs, passes directly through the porto-caval anastomoses into the vena cava and thus reaches the peripheral tissues without prior passage through the liver. The increased action of insulin on the peripheral tissues could thus cause the fall in blood glucose concentration. In the intact animal the additional insulin released presumably fails to traverse the liver in quantities sufficient to have a demonstrable action at the periphery.

12. The release of additional insulin. It is now widely thought that the hypoglycaemic effect of the sulphonylureas is due to the action of insulin liberated in increased amounts in response to administration of these compounds.

Cross-circulation and other studies have established that the hypoglycaemic activity of blood from the pancreatic vein increases rapidly after the administration of a single dose of a sulphonylurea to intact dogs. The histological changes in the beta-cells, the direct relationship between the reserve function of these

cells and the character of the hypoglycaemic response, and the failure of the compounds to reduce the blood glucose of fully alloxanised animals, suggest that the hypoglycaemic material is liberated by the beta-cells. The reduction in the extractable insulin content of the pancreas after administration of a sulphonylurea supports the view that the hypoglycaemic substance liberated by the beta-cells is insulin, as does the increase in the "insulin-like" activity of the pancreatic vein blood. The latter is demonstrated by the influence of pancreatic vein blood on the metabolism of isolated rat diaphragm and adipose tissue *in vitro*, its hypoglycaemic effect when injected into mice or when transfused into alloxanised dogs in cross-circulation studies, and the reduction of the blood glucose of hepatectomised animals when given a sulphonylurea.

It also seems to be established that the immediate fall in blood glucose concentration following administration of a sulphonylurea to intact animals is not accompanied by a demonstrable acceleration in removal of glucose from the blood, but is almost entirely due to a reduction in hepatic glucose release.

Thus if the initial hypoglycaemic response of intact animals is due to the normal action of the additional insulin released, the hormone must be able to effect an immediate reduction in the release of glucose by the liver. This possibility will be examined in some detail, since much new evidence pointing towards such a direct action of insulin on the liver has been presented since the subject was last reviewed (104, 238).

Using hepatic catheterisation, Bearn *et al.* (24) showed that the rapid injection of glucagon-free insulin, 0.1 unit/kg into a peripheral vein was quickly followed by a variable, but often considerable, reduction in N.S.G.P. in normal persons, a finding recently confirmed by Craig *et al.* (85), and in juvenile-type diabetics who were not ketotic. Patients suffering from stable diabetes generally showed a much smaller reduction. Bearn's deduction that insulin inhibited N.H.G.R. was, however, unwarranted since the fall in N.S.G.P. could have been due to increased glucose uptake by extrahepatic tissues—particularly fat—drained by the portal venous system. The fact that the removal of glucose from peripheral blood was shown to be accelerated makes this possibility all the more likely, although Craig *et al.* found no evidence of increased glucose uptake by forearm muscle. However, Ashmore and co-workers (14, 259, 260), in direct transhepatic studies in dogs, found that the rapid injection of glucagon-free insulin, 0.1 to 1.0 unit/kg, into a peripheral vein did not reduce the N.H.G.R. and that the observed reduction in N.S.G.P. could be accounted for by increased glucose uptake by the extrahepatic splanchnic tissues.

The kinetics of the initial fall in blood glucose caused by insulin have been studied by radioisotope techniques in depancreatised and intact dogs (125, 260, 355, 297, 421) and in diabetic and non-diabetic persons (200, 361, 397). Wall *et al.* (421) rapidly injected insulin, 0.025 to 0.25 units/kg, into a peripheral vein of dogs receiving a constant infusion of tracer doses of U-C^{14} glucose. Although an immediate two- to threefold increase in the rate of blood glucose outflow accounted for at least 80% of the fall in blood glucose concentration, a transient suppression of unlabelled glucose entry was noted within five minutes of in-

jecting the larger, but not the smaller dose. These observations accord with those of Tarding and Schambye (355, 397) who found that only doses greater than 0.3 units of insulin/kg suppressed entry of unlabelled glucose; this effect was, moreover, very transient. However, Dunn *et al.* (125) observed that the rapid injection of 3 to 10 units of insulin into a peripheral vein of intact dogs was quickly followed by a complete inhibition of unlabelled glucose entry, as indicated by cessation of the decline in the specific activity of blood glucose, which coincided in time with the fall in blood glucose concentration. Jacobs *et al.* (200) and Searle *et al.* (361) observed the same phenomenon in normal and diabetic patients given 3 to 20 units of insulin intravenously.

In all these experiments, however, it was calculated that the fall in blood glucose effected by the rapid intravenous injection of insulin resulted mainly from accelerated blood glucose outflow, and inhibited entry of labelled glucose was assumed to represent complete suppression of inflow of glucose into the blood, and thus to indicate cessation of hepatic glucose release. This need not necessarily be true as the unlabelled glucose discharged prior to the insulin injection could be partly or wholly replaced by labelled glucose. Thus the very rapid assimilation and utilisation of labelled glucose by muscle and adipose tissue which follows the peripheral injection of insulin (237) could lead to the formation and release of labelled metabolic products which might then be resynthesised by the liver and discharged as labelled glucose of the same or higher specific activity as that in the plasma at that time. This possibility is supported by evidence from recent studies showing that the injection of insulin 1) rapidly increases the fixation of $C^{14}O_2$ by the liver (259) and 2) causes C_1^{14} glucose to appear rapidly in the plasma of dogs given a tracer dose of C_6^{14} glucose, in increasing amounts which parallel the uptake of labelled lactate by the liver (259).

The same criticism might be levelled at the interpretation of isotopic studies taken to demonstrate inhibition of hepatic glucose release by the sulphonylureas. However, there are important differences between the two: in all the studies with insulin a considerable acceleration of blood glucose outflow occurred, whereas no such acceleration was observed to accompany the sulphonylurea-induced fall in blood glucose, so that in the latter case increased amounts of labelled metabolic products of isotopic glucose would not be available for resynthesis by the liver. Moreover, tolbutamide, unlike insulin, did not increase $C^{14}O_2$ fixation by the liver of intact animals *in vivo* (259). The complete and sustained inhibition of unlabelled glucose entry following sulphonylurea administration can thus confidently be assumed to indicate cessation of hepatic glucose release, but the more transient inhibition following peripheral intravenous insulin injection cannot be so interpreted with certainty. There is, moreover, the possibility that changes in the size of the glucose pool may partly account for the cessation in the decline in the specific activity of blood glucose.

Consideration of these criticisms in conjunction with the results of the transhepatic studies shows that there is no unequivocal evidence that insulin effects a reduction in hepatic glucose release.

Nevertheless, these same isotopic studies, even if differences in interpretation

are allowed for, show that the processes of blood glucose outflow and the hepatic release of glucose are disproportionately influenced by the sulphonylureas and by the single injection of insulin into a peripheral vein. It may be, however, that the route of insulin administration and the dose and rate at which it is given, could account for these quantitative differences.

Thus, the slow absorption of a small dose of insulin from a peripheral site may cause a fall in blood glucose concentration which is not accompanied by an accelerated flow of glucose from the blood. Jacobs *et al.* (200), in studies using isotopic glucose, found that the fall in blood glucose following subcutaneous injection of 0.16 unit of soluble insulin/kg to normal or mildly diabetic subjects was accompanied by a sustained cessation of the fall in the specific activity of blood glucose. This was interpreted to indicate cessation of hepatic glucose release which in turn was calculated to be wholly responsible for the observed fall in the concentration of blood glucose. This absence of increased glucose uptake by the tissues would accord with the observed changes in blood pyruvate and lactate (277) and muscle glycogen (116) following the subcutaneous or slow intravenous injection of insulin. These studies, and those of Madison *et al.* (258) to be considered later, suggest that the hypoglycaemic effect of insulin released slowly into the peripheral blood may be exclusively due to inhibition of hepatic glucose release. Nevertheless, in rabbits, the subcutaneous injection of smaller doses of insulin, weight for weight, than used by Jacobs *et al.* in human subjects, greatly accelerated the flow of glucose outflow from the blood without reducing inflow demonstrably (34).

The liberation of endogenous insulin into the portal circulation, so that it reaches the liver in greater amounts and concentration than other tissues, may have considerable physiological significance. If the fall in blood glucose induced by the sulphonylureas resulted from the normal action of the additional insulin liberated, then it should be possible to demonstrate that endoportally introduced insulin can suppress hepatic glucose release without increasing the peripheral tissue assimilation of glucose or that endogenous and exogenous insulin differ quantitatively in their effect on these two processes.

Studies using portal infusions have provided conflicting results. Insulin, 0.07 units/kg, was found in dogs to have a smaller effect on peripheral glucose uptake, as measured by femoral A-V blood glucose differences, when injected over a period of two minutes into the portal vein than when injected into a peripheral vein (256). As the curves of blood glucose reduction following administration by either route were almost identical, the intraportally injected insulin was considered to have had the greater inhibiting effect on hepatic glucose release. D'Amico *et al.* (100) also found no significant difference in the hypoglycaemic effect of insulin given rapidly intraportally and into a peripheral vein, but other workers found that insulin had a smaller effect when given by the former route (397, 426). However, studies in dogs, using isotopic glucose and direct transhepatic measurements, showed that the rapid intraportal injection of insulin, 0.08 to 1.0 unit/kg, did not diminish blood glucose inflow or hepatic glucose release but did considerably accelerate blood glucose outflow (14, 259,

260, 355); indeed, hepatic glucose release increased greatly when the blood glucose concentration was much reduced. This contrasts completely with the prolonged sulphonylurea-induced inhibition of hepatic glucose release which is maintained even at very low blood glucose concentrations and is also difficult to reconcile with the view that the hypoglycaemic effect of the sulphonylureas is due solely to the action of the additional insulin liberated. Nevertheless the insulin was injected rapidly in these studies so that a possible explanation might be that the increased liberation of insulin following sulphonylurea administration continues for a considerable period of time.

Several investigators have therefore studied, in dogs, the effect of the slow infusion—for periods of up to two hours—of insulin, 0.04 to 1.0 units/kg, into the portal or into a peripheral vein (100, 256, 259, 260, 355, 397). The fall in blood glucose resulting from the intraportal infusion of insulin was 1) only 40 to 60 % of that elicited by the same dose given by peripheral intravenous infusion (100, 256, 355, 397), this being probably due to the greater hepatic binding of insulin given intraportally; 2) not accompanied by a reduction in hepatic glucose release as determined by isotope techniques and transhepatic measurements (259, 260, 355, 397); 3) accompanied by an increased peripheral assimilation of glucose as demonstrated by widening of the femoral A-V blood glucose differences (256, 355); 4) followed by a 30 to 80 % rise in blood pyruvate and a doubling of blood lactate (100); this contrasts with the reduction in both following sulphonylurea administration (100). These studies therefore suggest that insulin, even when given intraportally in doses just sufficient to reduce the blood glucose, does not inhibit hepatic release of glucose.

A study which provides strong evidence for a direct effect of insulin on hepatic glucose release has, however, been very recently reported by Madison *et al.* (258). These workers used dogs in which complete porto-caval shunts had been established by previous operation; in these animals all the blood traversing the liver comes from the arterial circulation so that alterations in the glucose concentration differences between arterial and hepatic venous blood truly reflect changes in net hepatic—as opposed to splanchnic—glucose release. The slow infusion of insulin, 1.5 units/hour, into a peripheral vein of these animals caused a 50 % reduction in hepatic glucose release which was calculated to account entirely for the observed decline in blood glucose concentration.

There is therefore a considerable amount of apparently valid evidence derived from carefully conducted experiments both for and against the hypothesis that exogenous insulin can reduce, although perhaps not entirely suppress, hepatic glucose release. Although the more recent studies strongly favour a direct action on the liver, it would be premature and unjustified to be dogmatic on this still unresolved problem.

Even if it is accepted that insulin may suppress glucose release by the liver an explanation is still required for the fact that few or no demonstrable consequences of increased insulin action on peripheral tissues accompany the immediate hypoglycaemic response to the sulphonylureas.

It is unlikely that the action of insulin on peripheral tissues is inhibited by

the presence of a sulphonylurea in the blood, since the hypoglycaemic response of hepatectomised animals to the drugs is presumably due to the action of insulin on these tissues.

The amount of additional insulin released might be so small that it failed to traverse the liver; calculations from the above experiments suggest that about 0.1 to 0.05 unit/kg/hour would be sufficient to cause the initial blood glucose fall elicited by the compounds in man and dogs. However this explanation seems improbable as: 1) the great increase in the "insulin-like" activity of pancreatic venous blood and the large reduction in pancreatic insulin content observed in animals given a sulphonylurea suggest that the amount of insulin released is considerable; and 2) the slow intraportal infusion of insulin, 0.04 unit/kg/hour, to dogs increased the peripheral assimilation of glucose (355).

The failure of insulin to traverse the liver cannot be attributed to the presence of the sulphonylureas as the distribution and rate of degradation of I^{131} insulin was not altered in animals given the compounds (36, 435). It is possible, however, that the sulphonylureas might promote the liberation of insulin in a form more readily arrested by the liver.

Hyperglycaemia due to exogenous glucose-loading both increases the liberation of insulin and rapidly suppresses hepatic glucose release (337, 378). The latter effect, however, is not necessarily dependent on the additional release of insulin, since the liver of a depancreatised dog, maintained at normal blood glucose levels by a constant infusion of insulin, can autonomously reduce its release of glucose in response to a glucose load (378). The additional insulin, moreover, apparently released by non-diabetic or sulphonylurea-responsive mildly diabetic persons in response to a glucose load traverses the liver sufficiently to cause a five- to tenfold increase in the "insulin-like" activity of the peripheral blood plasma (367).

Recent studies by Madison *et al.* (255) suggest that when insulin secretion in man is continuously stimulated by sustained carbohydrate loading, the hepatic insulin binding capacity becomes progressively saturated so that the amount of insulin reaching the periphery may increase with time. It might be that the insulin immediately liberated in response to sulphonylurea administration is arrested by the liver and possibly reduces its release of glucose, and that later some traverses that organ and reaches the periphery. This would account for the failure to demonstrate increased insulin action at the periphery during the early acute hypoglycaemic response. It would also explain the occurrence, in partially alloxanised animals or mildly diabetic persons treated for some days with a sulphonylurea, of changes in peripheral tissue metabolism which are also elicited by insulin—for example, the uptake of disodium hydrogen phosphate by skeletal muscle, increase in muscle glycogen, glucose assimilation by muscle at low blood glucose levels, and changes in blood constituents such as pyruvate, phosphate and lipids. The lack of improvement in glucose tolerance could be due to the failure of the sulphonylureas to increase the ability of the beta-cells to release insulin in response to glucose loading, since it is probable that glucose and sulphonylureas stimulate insulin release by different mechanisms.

There are, however, several objections to the hypothesis that the sulphonylureas act solely by releasing additional endogenous insulin. The following are examples. 1) It presupposes that insulin directly inhibits hepatic glucose release—a still controversial assumption, although it is increasingly supported by recent evidence. 2) Diabetic patients responsive to the sulphonylureas often have normal or even elevated "insulin-like" activity in their peripheral blood plasma and it seems unlikely that the increased liberation of small amounts of insulin would effect such a considerable hypoglycaemic effect. Moreover, insulin is relatively ineffective in some of these patients who may be poorly controlled by its daily administration in large doses, although there is no evidence of "anti-insulin" factors; daily treatment with a sulphonylurea may greatly reduce the blood glucose of these stable, relatively insulin-resistant diabetics. The diabetes of these patients may well not be due to insulin deficiency but to a primary abnormality of hepatic metabolism. 3) Other pharmacological effects of the sulphonylureas have been demonstrated which may contribute significantly to their hypoglycaemic action.

The hypothesis that the hypoglycaemic effect of the sulphonylureas in intact subjects is, at least partly, due to the action of the additional insulin liberated is certainly that which at present accords best with the often conflicting data. Nevertheless the Scottish juridical verdict of "not proven," which permits reconsideration of the question should new relevant evidence become available, is that which is most applicable to this hypothesis.

13. Other possible mechanisms of action. It is clear that the sulphonylureas do not directly act on the peripheral extrahepatic tissues to promote their uptake of glucose from the blood in all species tested with the possible exception of domestic fowls. It is also very improbable that the sulphonylureas increase the effect of either endogenous or exogenous insulin on these tissues by potentiating their activity, releasing them from inactive linkage or by inhibiting their destruction.

Although the hypoglycaemic effect of the sulphonylureas in hepatectomised animals is presumably due to the action of the additional insulin liberated on peripheral tissues, there is no doubt that the initial—and possibly the sustained—hypoglycaemic response of intact subjects to the compounds is almost exclusively due to suppression of glucose release by the liver. This inhibition of hepatic glucose release might, as already discussed, be due to the action of the additional insulin liberated, but it might equally well be due to the direct effect of the sulphonylureas themselves on hepatic metabolism. The sulphonylureas inhibit hepatic ketogenesis *in vitro*; and the administration of a single dose of a sulphonylurea to intact animals rapidly causes an increase in hepatic glycogen, accelerates incorporation of C^{14} from labelled glucose into hepatic glycogen and fatty acids, and produces a sustained inhibition of hepatic glucose output in the presence of profound hypoglycaemia. Increased C^{14} O_2 fixation by the liver does not occur, nor does the plasma lactate or pyruvate concentration increase. These effects contrast with those observed following insulin given intraportally, so that it is unlikely that the sulphonylureas render the liver more sensitive to

the action of insulin even if it is conceded that insulin has a direct hepatic action.

The mechanism by which the sulphonylureas might themselves directly inhibit hepatic glucose release is, however, unknown. Except in dogs, in which species the presence of exogenous insulin suffices, endogenous insulin—or as a remote possibility, an unknown product of the beta-cells—is necessary for the hypoglycaemic consequence of such an action on the liver to be manifest. It is possible that endogenous insulin has a permissive role in that it provides, directly or by its effect on peripheral tissues, a hepatic metabolism responsive to the direct hypoglycaemic action of the compounds.

However, although the sulphonylureas have a hypoglycaemic effect when given to depancreatised or fully alloxanised dogs receiving insulin the fall in blood glucose is much less and of shorter duration than that seen in partially alloxanised dogs given a sulphonylurea in the same experimental conditions. This indicates either that endogenous insulin has a greater permissive effect than exogenous insulin or—more likely—that the additional insulin liberated in response to sulphonylureas contributes to the reduction of hepatic glucose release.

It is thus very probable that the hypoglycaemic effect of the sulphonylureas is due to their action on several tissues and metabolic processes. The problem of the mechanisms by which they produce a hypoglycaemic effect will remain disputed and unresolved until the sites, mode of action, and physiological functions of endogenous insulin have been more precisely defined, and until the nature and underlying causes of the metabolic abnormalities in the varying diseases embraced by the term "diabetes mellitus" are clarified.

C. Therapeutic use

There is general agreement about the type of patient who is likely to be responsive to the hypoglycaemic action of the sulphonylureas, and a variable proportion of the diabetic population in almost all countries is now being treated with these compounds. Patients initially responsive to one sulphonylurea are usually responsive to the others, but those developing secondary resistance to tolbutamide may sometimes respond to chlorpropamide (392). Because of its toxicity, carbutamide is now seldom used therapeutically.

1. Character of the hypoglycaemic response. The one undisputed effect of maintenance treatment with a sulphonylurea is reduction of the fasting blood glucose level (17, 60, 121, 436). Since glucose tolerance is not improved, the range over which the blood glucose swings in the course of the day is not narrowed, although the absolute values are much reduced. In fact, in a responsive patient the pre-treatment pattern of glycaemia is reproduced—at a lower level—when the drugs are properly administered (17, 267). Thus patients most suitable for treatment with a sulphonylurea are those who combine relatively good glucose tolerance with fasting hyperglycaemia. Although individually variable, the response is limited, and little or no increase in the hypoglycaemic effect occurs if doses greater than the optimal therapeutic range are given (17, 271). In this respect the sulphonylureas are unlike insulin.

2. Type of patients responsive to maintenance treatment with sulphonylureas, and their identification. On the basis of their response to maintenance treatment with sulphonylureas, diabetic patients again roughly fall into the two main groups long recognised on purely clinical grounds: those who are completely insulin-dependent, quickly develop severe ketoacidosis on the withdrawal of insulin, and do not respond to sulphonylureas; and those with stable diabetes, who in the absence of infection or other stress, do not develop ketoacidosis and marked nitrogen loss on the withdrawal of insulin, and are responsive to sulphonylureas.

From 70 to 80% of patients who develop diabetes after the age of forty suffer from the latter type of the disease; the remaining 20 to 30% and almost all those who become diabetic before the age of thirty-five, have insulin-dependent diabetes. Thus age of onset of clinical diabetes is the most important single factor determining responsiveness to long-term administration of sulphonylureas in diabetic patients (391). Although young patients, particularly those whose insulin requirement is small and whose diabetes is of very recent origin, sometimes respond to the sulphonylureas for a time, responsive patients are rare among those who develop diabetes before the age of thirty-five (63, 215, 236, 299, 353). In contrast, about 80% of those who develop diabetes after this age do respond (184, 228, 291, 372). Amongst those unresponsive are some who are completely insulin-dependent although they are well controlled by less than 30 units of insulin daily (122, 271, 391); and unexpectedly others who appear to have a type of diabetes precisely the same as those most responsive, are completely refractory to the hypoglycaemic action of the sulphonylureas. These two groups cannot be distinguished on any clinical count; nor does there seem to be any significant difference in the blood levels of sulphonylurea, or in the way in which it is metabolised (436).

Owing to the difficulty of anticipating individual response on clinical grounds, attempts have been made to predict the response to long-term maintenance therapy, by the effect of a single oral or intravenous dose of a sulphonylurea. There is wide disagreement about the value of this test: some clinicians consider it a reliable guide to clinical success or failure (10, 263), while others have said it is of no (189) or limited (122, 184, 271, 301, 314) value.

The only certain method of identifying responsive patients is by careful clinical trial. Many factors, however, make the clinical evaluation of a hypoglycaemic compound difficult. The concentration of glucose in the blood is affected by changes in dietary intake, exercise, weight, hormonal balance, emotion and many other factors. In addition, the severity of the diabetic state may fluctuate spontaneously. Evaluation of a hypoglycaemic agent should demonstrate, by discontinuing therapy or by the use of placebo tablets, that the substance being tested is solely responsible for the hypoglycaemic effect (122, 224, 263, 271).

3. Scope of therapeutic application. On the basis of careful clinical trial there is general agreement that 60 to 65% of the total diabetic population would respond to long-term therapy with sulphonylureas. Not all potentially responsive patients

really require this treatment, however, and the estimated percentage of the total diabetic population who ought to be treated by sulphonylureas varies widely.

This variation is due 1) to national and individual differences in attitude towards diet and obesity and 2) to different standards and criteria of control.

In the United Kingdom generally, and in many clinics in America (25, 122), it is felt that additional therapy should not be substituted for dietary restriction and reduction in weight in patients in whom it is possible to control the diabetes satisfactorily by this means alone. In the United Kingdom it is estimated that such patients represent about 40 to 50% of the total diabetic population; that about 35 to 40% require treatment with insulin; and that only the remaining 10 to 25% should be treated with a sulphonylurea (124, 296). In Germany the estimated number of insulin-dependent patients is the same as in Britain but only 15 to 20% are treated by diet alone, so that 40 to 50% receive sulphonylureas (391). It is clear that many of the latter would have been treated by dietary restriction only in the United Kingdom. The practice of other countries falls in between these two extremes (184, 373).

The discrepancy between German and British estimates of the therapeutic usefulness of the sulphonylureas may be accounted for to some extent also by differences in standards of control and in the criteria used to assess it. Since the hypoglycaemic response is limited, and affects the fasting blood glucose only, even fully responsive patients may not be adequately controlled by a sulphonylurea alone if their fasting blood glucose values are too high or their glucose tolerance is too much impaired (17, 121). On the other hand, some patients thought by British clinicians to be well controlled by diet alone may really require additional therapy.

4. Clinical management. This aspect of sulphonylurea therapy has been fully discussed in many recent and extensive reviews from several countries (184, 228, 269, 373, 391, 392). Only those points which require emphasis or are not generally appreciated, and those which are controversial, will be considered.

a. Diet. Since the sulphonylureas do not improve glucose tolerance, some degree of dietary restriction is essential if hyperglycaemia is to be avoided, quite apart from the necessity to prevent gain in weight.

b. Method of starting treatment. A control period of observation is always desirable to ensure, as far as possible, that the prescribed diet is being adhered to, that the diabetic state is relatively steady, and to assess control of the disorder on present treatment. Patients who are not taking insulin should be given placebo tablets. A surprisingly large number thereupon make renewed efforts to adhere to their prescribed diets "to give the tablets a chance" satisfactorily to control results. In those not so controlled the active drug is substituted.

Numerous workers have emphasised the caution required in transferring insulin-taking diabetic patients to a sulphonylurea. The withdrawal or reduction of even a moderate daily dose of insulin from patients who appear to be in every way suitable for therapy with sulphonylureas, will result in a small number developing severe ketoacidosis with alarming rapidity.

Some clinicians effect the change-over by abruptly stopping the administration

of insulin; others gradually reduce the dose of insulin and simultaneously increase the amount of sulphonylurea. The latter method increases the danger of hypoglycaemic episodes, and the assessment of response is more difficult and prolonged. Whichever method is used patients should ideally be seen every two or three days for the first week. If this is impracticable they must at least test their urine daily for sugar and acetone and report at once to their physician should ketonuria develop. Placebo tablets should be given for a period either immediately after insulin is withdrawn or when successful control is established. Complete substitution of more than 40 units of insulin daily by a sulphonylurea is usually not possible; trial of sulphonylurea therapy in suitable patients requiring more insulin than this is probably best conducted in the hospital.

The development of ketosis in patients whose diabetes is well controlled by a sulphonylurea will usually require treatment with insulin since increasing the dose of sulphonylurea is ineffective in such circumstances. Similarly, patients exposed to stress, for example when undergoing a surgical operation, must be carefully supervised and given insulin at the first sign of metabolic decompensation.

c. Dose of sulphonylurea required, and frequency of its administration. The minimal plasma concentration of the drug at which a hypoglycaemic response occurs varies from patient to patient, but is approximately 2.5 to 5.0 mg/100 ml for chlorpropamide (392), 4.0 to 10.0 mg/100 ml for carbutamide (122, 436), and 6.0 to 10.0 mg/100 ml for tolbutamide (17, 389).

The size or number of daily maintenance doses required to achieve these levels throughout a twenty-four hour period depends mainly on the plasma half-life of the drug. Carbutamide and chlorpropamide have plasma half-lives of more than thirty hours, and therefore need only be given once daily in doses of 0.5–2.0 g, and 0.1–1.0 g respectively. However, single doses of chlorpropamide greater than 0.5 g commonly give rise to gastrointestinal side effects (392), and for this reason a total daily dose of 1.0 g is better divided. Tolbutamide on the other hand, has a plasma half-life of only 4 to 8 hours. The hypoglycaemic effect is thus relatively short and almost all patients require at least two daily doses to achieve good control (17, 82, 207, 269), although a few can be satisfactorily controlled by a single daily dose. No single dose should exceed 1.5 to 2.0 g since neither the magnitude nor the duration of the hypoglycaemic response is significantly increased by doses greater than this (17).

The "loading-dose" technique of starting therapy used to be frequently employed but is now considered unnecessary.

Although the maximal hypoglycaemic response usually occurs on the first day of treatment (391, 392) and subsequent doses are generally less effective than the first (17), some patients do not show a full response for several days. An accurate assessment of response is usually possible in one to two weeks (373, 391, 392), but final regulation may take several weeks, and in the aged even months (371).

d. Assessment of control. Diabetic control should be assessed by appropriately timed determinations of the blood glucose concentration. Fasting values alone

may be misleading (17, 121, 205), and post-prandial determinations are necessary. Provided the renal threshold for glucose is relatively normal, the twenty-four hour urinary glucose loss or the testing of appropriate urine specimens gives useful additional information.

e. Intermittent treatment. Although intermittent treatment has been advocated, long-term studies indicate that maintenance therapy with sulphonylureas must be continuous in the vast majority of patients, if it is confined to those who really require therapy in addition to dietary restriction (122, 373, 391). As with insulin, remissions of varying degree and duration may occur following the initial treatment of diabetic patients with a sulphonylurea, particularly in those who have recently developed the disease, but there is no evidence that these compounds differ significantly from insulin in their ability to effect remissions.

f. Secondary failure. Long-term studies with carbutamide and tolbutamide have shown a relapse or "secondary failure" rate of as high as 65% (74, 113, 184, 315, 364, 373, 391), where secondary failure is defined as that occurring after thirty days of therapy, regardless of cause. Many are undoubtedly due to disregard of the prescribed diet and to other exogenous factors, but studies in the hospital have shown the development of a true resistance to the hypoglycaemic action of these drugs in about 5 to 10% of all those initially well controlled. Such resistance is usually not seen before at least four months of treatment and is not necessarily associated either with deterioration in glucose tolerance or with increased insulin requirements. It has been suggested that true secondary failure occurs most frequently in those whose initial response was only fair, but this has not been the experience of many clinicians. Treatment with insulin may sometimes restore the response to the sulphonylureas (391), and diabetic patients resistant to tolbutamide and carbutamide may sometimes be treated successfully with chlorpropamide (392). The "secondary failure" rate in patients given chlorpropamide is not yet known.

g. Other uses. Although orally administered sulphonylureas have their main therapeutic use as complete substitutes for parenteral insulin in mild and stable, and hence mainly elderly, diabetic patients who are not obese, they may have a subsidiary use when combined with insulin therapy, in rendering labile diabetics more stable (126, 392). This is difficult to assess, and more carefully designed studies in the hospital will be required before an accurate assessment of their value in this field can be made. Diabetic control is, however, often improved in patients suffering from stable diabetes who require relatively large amounts of insulin, by the replacement of part of the daily dose of insulin by a sulphonylurea (123, 126, 417). Patients with true "insulin resistance" may sometimes be helped in the same way (94, 140, 392). Temporary treatment may be justified to relieve troublesome symptoms such as pruritus in the obese (364).

5. Toxic and side effects. Although theoretically, all three drugs are potentially haematotoxic, only carbutamide has caused an appreciable incidence of serious toxic effects. Some degree of neutropaenia and thrombocytopaenia commonly occurs in the early stages of treatment with carbutamide (115, 317, 364); although

this is usually transient, most clinicians consider that sufficient numbers of cases of marked leucopaenia (9, 48, 115, 211, 212, 317, 419), agranulocytosis (1, 40, 180, 198, 212, 298, 303, 324, 341, 368, 370, 400, 403, 412, 418), thrombocytopaenic purpura (49, 212, 303, 317, 345, 403) and pancytopaenia (1, 212, 317, 403), have been reported to preclude the clinical use of this drug. Carbutamide not infrequently has other toxic sensitisation effects similar to those produced by other sulphonamides. These include increased capillary fragility (317); anaemia (212); interstitial myocarditis (134); skin allergies, including exfoliative dermatitis and photo-allergy (58, 212, 360, 384, 391, 440); drug fever (212, 391); and a syndrome comprising malaise, lethargy and depression frequently accompanied by nausea and vomiting (122). Other serious effects reported have been anuria accompanying a generalised reaction (303, 412); jaundice (102); nephrosis (356); and a profound anaphylactic reaction (130, 261). Carbutamide may also occasionally cause a significant reduction in thyroid function (361); polyneuritis (111, 166); encephalitis; and myositis (22). Of the allergic manifestations, those in the skin occur most commonly.

Tolbutamide and probably chlorpropamide are very much less toxic than carbutamide (373, 391, 392) and, as far as we know, no fatalities have been reported which can definitely be attributed to either. Tests of hepatic and renal function (373, 391, 392) and haematological (184, 265, 373, 385, 391, 392) studies have been made extensively and few changes have been noted, although blood dyscrasias (145, 317, 373, 391, 392, 420) have occasionally been observed with both drugs. They may revert to normal spontaneously even when treatment with sulphonylurea is continued, and all are quickly reversible when therapy is discontinued. Jaundice has, however, occurred in a number of patients given chlorpropamide in large but not excessive doses (392); liver biopsy in these cases has shown intracanalicular biliary stasis. Allergic manifestations in the skin occur in about 5% of patients treated with either of the drugs (74, 184, 358, 373, 391, 392), and other side effects observed include intolerance to alcohol, and gastrointestinal upsets (373, 391, 392).

Some patients receiving chlorpropamide experience lethargy, muscular weakness, ataxia and dizziness; these symptoms are not caused by hypoglycaemia but seem to be due to the direct action of the drug on the central nervous system (392).

Hypoglycaemia accompanied by unconsciousness is a relatively uncommon complication of sulphonylurea therapy as compared with insulin. When hypoglycaemic symptoms do occur they tend to be less severe and less abrupt in onset (306, 364, 373, 391). They are usually easily relieved by carbohydrate or by reduction in sulphonylurea dosage. More profound hypoglycaemia, however, accompanied by loss of consciousness, may be very refractory to treatment, and the development of severe, sometimes fatal hypoglycaemic coma in patients treated with carbutamide has been reported surprisingly often (28, 59, 235, 252, 274, 297, 306, 311, 427). There are fewer reports of severe hypoglycaemia with tolbutamide (74, 269, 319, 373, 391).

Patients exhibiting an allergic response to one drug do not necessarily do so to the others (392).

6. Rationale of long-term treatment of diabetic patients with sulphonylureas. The one undisputed effect of long-term therapy with sulphonylureas is reduction of the fasting blood glucose level with abolition or diminution of glycosuria and its symptomatic manifestations. However, the fate of the glucose previously lost in the urine remains unknown, as do the other metabolic consequences of its retention. Studies of total body utilisation of carbohydrate, protein and fat in patients before and during treatment with these compounds and with insulin would be valuable.

Diabetic ketoacidosis rarely occurs in patients with the stable "adult-onset" type of diabetes which can be controlled by the sulphonylureas. To them the only serious implication of the disease is the development of the so-called complications, particularly vascular disease, retinopathy and nephropathy. These result in considerable disability and often in premature mortality. Their aetiological relationship to the metabolic disease of diabetes is unknown, although their greater incidence in patients with diabetes of long duration and those poorly regulated suggests that they are due to some aspect of the metabolic disturbance which itself is complex and not confined to carbohydrates. Biochemical abnormalities in the lesions themselves have been described, and alterations in plasma lipids, lipoproteins, glycoproteins and mucopolysaccharides noted which may be related to their development (15, 231). There are a few, somewhat contradictory, reports of the effect of treatment with a sulphonylurea on these plasma constituents (33, 196, 262, 373). The influence of the best possible diabetic control by insulin, the sulphonylureas and dietetic measures on the plasma abnormalities and the development of the complications demands investigation by meticulously controlled and prolonged studies.

The outstanding clinical problem is the prevention of these complications, and enthusiasm for the convenience of orally effective hypoglycaemic agents which can only be substituted for insulin in a limited proportion of diabetics, should not obscure this fact.

II. THE GUANIDINE DERIVATIVES

Although several groups of hypoglycaemic compounds are conveniently considered under this heading, the considerable structural differences between the substituted monoguanidines, the alkylated diguanidines of which Synthalin "A" and "B" are the best known, and the more recently studied diguanides (biguanides, guanylguanidines) exemplified by DBI, require emphasis. Although the three groups may have a similar mechanism of hypoglycaemic action, they differ very markedly in toxicity.

Methylguanidine:

$$CH_3HN—CNH—NH_2$$

Synthalin "A": decamethylenediguanidine

$$H_2N—CNH—NH(CH_2)_{10}—NH—CNH—NH_2$$

Synthalin "B": dodecamethylenediguanidine

$$H_2N—CNH—NH(CH_2)_{12}—NH—CNH—NH_2$$

DBI: phenethyl-formamidinyliminourea

$$(CH_2)_2—NH—CNH—NH—CNH—NH_2$$

A. The diguanidines: Synthalin "A" and "B"

1. Mechanism of hypoglycaemic action. The blood glucose concentration of intact laboratory animals, including the dog, of non-diabetic and of most diabetic persons was shown to be reduced by orally or parenterally administered Synthalin (44, 138). The fall commenced two to four hours after administration of the drug and was usually preceded by a rise in the blood glucose level (103, 331) which, however, could be prevented by ergotamine (381). Both the rise in the concentration of blood glucose and the failure of glucagon or adrenaline to influence the subsequent fall in blood glucose (44, 96) were probably due to hepatic glycogenolysis which caused the observed depletion of liver glycogen (44, 331, 382) and which occurred even if glucose was given (103). The hypoglycaemia was accompanied by an increased R.Q. (253) and elevation of blood and urinary lactate (44, 372, 381). Synthalin was also shown to reduce the blood glucose concentration of depancreatised and completely alloxanised animals (183, 187, 333); in alloxanised, but not in intact, dogs it markedly inhibited the intestinal absorption of glucose (333).

Synthalin had a considerable hypoglycaemic effect when given to eviscerated decerebrate animals receiving glucose infusions; there was very little delay in the fall of the blood glucose concentration and this was not preceded by a rise in glycaemia (44, 333). The glucose decline was accompanied by a reduction in oxygen consumption and an elevation of the R.Q.; lactic and citric acid accumulated in both blood and muscle (44, 253, 372), and the skeletal muscles showed a loss of glycogen (44, 253). Similar changes were observed in perfusion studies of isolated muscle preparations (44).

Although histological changes were observed in the pancreatic beta- and alpha-cells some time after administration of Synthalin (96, 103), its hypoglycaemic action in depancreatised animals and its effect on the metabolism of isolated muscle indicate an extrapancreatic site of action. The available data are considered to support the view that the hypoglycaemic and metabolic changes induced by Synthalin are due to a reduction in the oxidative activity of mitochondria resulting from inhibition of the mechanisms which simultaneously promote phosphorylation of adenosinediphosphate (ADP) and stimulate oxidation by diphosphopyridine nucleotide (DPN) in the citric acid cycle (185).

2. Toxicity in animals. Following the administration of 5 to 10 mg of Synthalin per kg, incipient hepatic necrosis and marked proximal renal tubular damage were observed in laboratory animals which died or were killed during the ensuing hypoglycaemic phase (44). Similar damage occurred when the drug was given in moderate daily doses for a few days or weeks (43, 96, 208). The constancy and early development of these changes are to be emphasised.

3. Clinical use. Synthalin was used for several years as an oral form of treatment for patients suffering from mild or moderate, but not completely insulin-dependent, diabetes. Its subsequent disuse is generally attributed to the occurrence of serious liver damage in many of the patients so treated. However, the reports of jaundice (4, 293, 330, 394), hepatic necrosis (38) or abnormal liver function tests (190) in patients receiving the drug are few, ill-documented, and do not prove a causal relationship; moreover, other clinicians did not observe such changes (138). Factors which more probably led to its abandonment may have been its evident toxicity in animals, the belief that it was a respiratory poison, its failure to control all diabetic patients, the frequency of gastrointestinal side effects, and particularly its introduction at an inopportune time when insulin was regarded as the physiological treatment for a disease accepted to be solely due to deficiency of this hormone.

B. The diguanides: DBI

1. Pharmacology. Recently developed techniques for the determination of DBI in biological fluids and tissues should permit investigations of its absorption, distribution, and fate about which little is known at the present time (369).

When given orally or subcutaneously, DBI reduced the blood glucose concentration of intact mice, rats, toads, rabbits, pigeons, cats, guinea-pigs and rhesus monkeys (193, 300, 406, 407); incompletely alloxanised animals were also responsive (406, 407). Species differences in sensitivity were, however, considerable, the responsiveness increasing in the order given above. Dogs showed no consistent hypoglycaemic response, but the amount of DBI which could be given to the animals was limited as doses greater than 60–80 mg/kg caused hypocalcaemia and eventually death (193, 406).

Most diabetic patients show a hypoglycaemic response to 50–200 mg DBI given orally. In non-diabetic persons, however, a hypoglycaemic effect of DBI could not be shown even by the administration of daily doses up to 400 mg (86, 128). Doses larger than this could not be given because of vomiting.

The speed, magnitude and duration of the fall in blood glucose concentration is determined by the dose of DBI given and the route of its administration. Fasted animals are more sensitive to the hypoglycaemic action of DBI than those who have free access to food (406).

2. Toxicity in animals. The administration of an excessive dose of DBI by oral or subcutaneous route results in fatal hypoglycaemia which can, however, usually be prevented by glucose (193, 406). It has been shown that hypophysectomy and adrenalectomy intensify the hypoglycaemic response and increase the mortality, while glucose, adrenaline and corticosteroids exert protective effects; the species differences are again considerable (193). Intravenously injected DBI may cause a transient elevation of the blood pressure (13, 221, 406), but large doses so given cause fatal hypotension (13).

No hepatic or renal damage was detected in animals dying in or killed during the hypoglycaemic phase induced by a single excessive dose of DBI (193, 300, 406, 416). Nor was there any biochemical or histological evidence of toxicity in guinea-pigs, rats, rabbits, monkeys, or dogs (300, 406) given DBI for periods up

to six months in daily doses which were, weight for weight, greatly in excess of the therapeutic dose for diabetic patients, who are much more sensitive to the hypoglycaemic action of the drug than these animals (406). The growth of young animals similarly treated was unimpaired (300, 406).

3. Mechanism of hypoglycaemic action. The hypoglycaemic effect of DBI is not due to a reduction in the intestinal absorption (128), or increase in the urinary excretion (220) of glucose; nor is it dependent on suppression of pituitary (193), adrenal (29, 128, 193, 376), or thyroid activity (376).

a. Role of insulin. DBI has been shown to reduce the blood glucose concentration of depancreatised monkeys (406) and young patients with insulin-dependent diabetes (220, 393) receiving exogenous insulin. No histological changes were noted in the beta-cells of animals given a single or daily dose of DBI (416). Although DBI significantly inhibited hepatic insulinase activity *in vitro* its administration to animals did not appreciably retard the degradation of exogenous I^{131} insulin (434); nor did the daily administration of DBI to normal and diabetic subjects alter their sensitivity to exogenous insulin (128). It is therefore unlikely that the hypoglycaemic effect of DBI is due either to its increasing the liberation of endogenous insulin, or to its potentiating the activity of endogenous or exogenous insulin, although the presence of insulin seems to be necessary for the hypoglycaemic effect of DBI to be manifest *in vivo*.

b. Influence on hepatic metabolism. The initial fall in blood glucose induced by DBI in intact guinea-pigs, may be accompanied by a reduction in hepatic glucose release which is not antagonised by glucagon or adrenaline (300). Although the oxygen consumption of liver slices obtained from animals given DBI two to three hours previously was found to be reduced (383), no observations on the spontaneous release of glucose were reported. Nevertheless, no diminution in hepatic glucose-6-phosphatase activity was noted in slices obtained from animals treated with DBI over a period of 48 hours (434), and in these circumstances a reduction, such as that following insulin or sulphonylurea administration, would have been expected if glucose output had been significantly suppressed. Although there is some evidence that DBI diminishes gluconeogenesis in guinea-pigs (434), limited studies in diabetic patients receiving the compound did not reveal this effect (128).

Changes in hepatic glycogen content have been reported. In rabbits considerable depletion was noted twenty-four hours after administration of DBI (221); in guinea-pigs some reduction occurred four hours after dosage, but the content was restored twenty hours later (406). However, DBI inhibited the glycogen deposition which normally follows the administration of glucose or alanine (434).

The metabolism of liver preparations *in vitro* is considerably altered by the addition of DBI to the medium. Although the spontaneous release of glucose by liver slices was not diminished, the glycogen content was reduced and production of lactic acid much increased (406, 434). The oxidation of succinate and ascorbate by liver homogenates and mitochondrial preparations supplemented with cytochrome C was found to be inhibited by DBI (383); the diminution of

succinate oxidation in the presence or absence of cytochrome C has been confirmed, but not that of ascorbate (431).

c. Action on extrahepatic tissues. The addition of DBI to the balanced salt incubation medium decreased the O_2 consumption, the CO_2 production and glycogen content, and increased the lactic acid and phosphate loss to the medium, and the glucose uptake of the isolated rat hemi-diaphragm (136, 383). However, Steiner and Williams (383) have suggested that the increased glucose uptake and lactate production are the consequences of inhibition of oxidative activity, and that it is unlikely that the aerobic energy deficit could be compensated by the modicum of energy derived from glycolysis of the extra glucose assimilated. The muscle glycogen content, however, is not significantly reduced in intact animals given single or repeated doses of DBI (221, 406).

The presence of DBI in the medium reduced the oxidation by rat adipose tissue of glucose, acetate, succinate and, to a lesser degree, of citrate and fumarate; the conversion of glucose to glycogen or total lipid was also inhibited (431).

d. Metabolic changes in intact subjects. The fall in the blood glucose concentration of fasting animals given a single dose of DBI was not accompanied by an increased production of CO_2, or by incorporation of C^{14} from universally labelled glucose into CO_2, but the R.Q. (434), venous blood lactate and inorganic phosphorus concentration increased (383, 406).

The daily treatment of responsive diabetic patients with DBI did not alter their sensitivity to insulin or to glucagon (128); the shape of the glucose tolerance curve remained the same (128) although the threshold of glucose uptake by muscle tissue was lowered (61); the fasting venous blood lactate concentration and daily urinary loss of lactate increased as did the rise in blood lactate and pyruvate (86, 128) following glucose loading.

The *in vitro* studies demonstrate the ability of DBI to inhibit electron transport and enzymatic oxidation at the succinic dehydrogenase and/or cytochrome oxidase level in muscle, adipose tissue and liver. This inhibition is held to account for the hypoglycaemic effect of DBI (383, 431) and is compatible with the other metabolic changes observed in the intact animal. Which tissues are affected is unknown. Ungar (406, 407), however, has recently observed that 1) some diguanides which have a hypoglycaemic effect *in vivo* have little or no inhibitory effect on tissue oxidation *in vitro*, and conversely, that others which had no hypoglycaemic action *in vivo* inhibited oxidation markedly *in vitro*. 2) DBI reduced the blood glucose concentration of pigeons but did not inhibit the oxygen uptake of homogenates of pigeon tissue. 3) The concentration of DBI required to inhibit tissue oxidation *in vitro*—0.1 mg/ml—greatly exceeded that which would be obtained by the administration of effective hypoglycaemic doses *in vivo*. 4) The oxygen uptake by homogenates of tissues from animals given DBI in doses which caused profound hypoglycaemia was only slightly and inconsistently less than that of tissues from control animals. 5) Many non-diguanide compounds which are not hypoglycaemic inhibit oxidative metabolism in the Krebs' cycle *in vitro* and this inhibition does not necessarily cause an acceleration of anaerobic glycolysis as measured by tissue lactate production.

These observations have led Ungar to question the hypothesis that the hypoglycaemic action of DBI is due to inhibition of tissue oxidation.

Thus, the mechanism of the hypoglycaemic action of DBI is still unknown.

4. Clinical use. The majority of diabetics, irrespective of the type of disorder from which they suffer, are responsive to the hypoglycaemic action of DBI. Cautious clinical trials have clarified partially the therapeutic potentialities of DBI and some of its related analogues (123, 220, 272, 393, 434).

Its general use is much restricted by the occurrence of gastro-intestinal symptoms. If a sufficient dosage could be given, then most stable diabetics could be regulated by DBI alone, but the frequent gastro-intestinal effects too often preclude the administration of effective amounts of DBI. However, the combined administration of a small daily dose of DBI together with a sulphonylurea, to patients who are resistant to or inadequately controlled by the latter compound, promises to be a very effective form of treatment (25, 123, 272). Many patients with labile diabetes, readily developing ketosis on withdrawal of insulin, are also responsive to the hypoglycaemic action of DBI; however, such insulin-dependent diabetics almost always continue to require insulin in addition, although in considerably reduced dosage. It has been asserted that very unstable diabetic patients, in whom careful control is made difficult by the frequent occurrence of inexplicable hypoglycaemic episodes, can be more smoothly regulated by treatment with DBI and more moderate doses of insulin than they previously required; such an effect would be therapeutically valuable but requires confirmation by more detailed and carefully controlled studies. However, DBI has been found to reduce markedly the insulin requirement of insulin-resistant patients (123).

The fall in blood glucose concentration begins about 2 hours after oral administration of DBI, and lasts for approximately six to fourteen hours; DBI is therefore best given twice or thrice daily. The daily dose required differs from patient to patient; many mild stable diabetics may be controlled by 50 to 100 mg daily though the majority usually require about 150 mg. A very considerable reduction in the daily insulin requirement of patients suffering from a "juvenile" type of diabetes may be obtained by similar doses of DBI. Anorexia, nausea, vomiting and diarrhoea occur early in treatment in many patients. Such side effects can generally be avoided by not exceeding a daily dose of 150 mg; in some persons smaller quantities elicit these untoward effects but others may tolerate as much as 400 mg. Although gastrointestinal disturbances occur in some 40 to 60% of patients, they are sufficiently severe to preclude continued treatment in less than 20 to 30% (393).

No untoward effects in terms of haematopoiesis, blood chemistry, and hepatic and renal function tests have been detected in several hundreds of patients treated with DBI for one to two years (376, 393). This, combined with failure to demonstrate toxicity in animals, should dispel any prejudice cast on the diguanides by experience with Synthalin.

III. SUMMARY

The way in which the sulphonylureas and the guanidine derivatives exert their hypoglycaemic effect is still unknown.

With regard to the sulphonylureas it is certain that 1) with the exception of domestic fowls, the presence of endogenous insulin, or exogenous insulin in the dog, is essential for their hypoglycaemic effect; 2) they stimulate, at least initially, the release of insulin from the pancreas; and 3) they reduce hepatic glucose release.

Consideration of these facts has given rise to two main hypotheses which differ particularly in the role they assign to insulin:

1. It is held that the hypoglycaemia results from the direct influence of the sulphonylureas on the metabolism of the extrapancreatic tissues, in particular that of the liver and possibly that of other peripheral tissues. Insulin is considered to act in a purely "permissive" capacity in that it provides that metabolic environment necessary either for the action of the sulphonylureas on these structures or for the manifestation of the hypoglycaemic consequence of that action.

2. On the other hand it is postulated that the hypoglycaemic effect is directly due to the action of the increased amounts of insulin liberated from the pancreas on the liver, or on the peripheral tissues, or on both.

The second hypothesis is that most generally favoured, but its validity is not established and depends largely on the answers to these three problems. 1) Does insulin directly influence hepatic glucose release? 2) Is the sulphonylurea-induced reduction in blood glucose accompanied by an increase in the insulin-like activity of peripheral blood and by effects attributable to the increased action of insulin on peripheral extrahepatic tissues? 3) What is the correct interpretation of experiments using isotopic glucose designed to determine blood glucose outflow and inflow?

During the year since this review was completed much new evidence on these problems has been published (442, 443, 444, 445, 446, 447, 450, 451); nevertheless these questions are still unanswered and the mode or modes of hypoglycaemic action of the sulphonylureas remain unresolved.

The indications for the therapeutic use of the sulphonylureas remain unaltered (449) but there is still considerable disagreement regarding the incidence of "secondary failure" (448).

The mode of action of the guanidine derivatives is still unknown and their place in the treatment of diabetic patients remains to be established.

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