# ANTIDIABETIC DRUGS AFTER THE UNIVERSITY GROUP DIABETES PROGRAM (UGDP)

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At the 30th Annual Meeting of the American Diabetes Association in June 1970, 7 of the 57 abstracts on the scientific program mentioned oral hypoglycemic agents in their titles. The last of these consisted of the UGDP report, whose findings "suggest that tolbutamide plus diet may be less effective than diet alone insofar as cardiovascular mortality is concerned" (1). Four years later, in June 1974, at the 34th Annual Meeting, not one title on a scientific program of 97 abstracts referred to an oral hypoglycemic agent (2). This review comments on the impact of the UGDP study on the direction of pharmacologic research regarding antidiabetic drugs.

## BACKGROUND OF THE UGDP REPORT

From 1922 until 1955, various preparations of animal insulins were the only acceptable pharmacologic agents for the treatment of diabetes mellitus. Their proven efficacy in correcting hyperglycemia and ketoacidosis proved life saving in many diabetic patients; however, it soon became apparent that chronic vascular complications were not necessarily prevented or reversed by insulin therapy as commonly employed.

In 1955, sulfonylurea drugs became widely available for the treatment of nonketosis-prone mild diabetics. At that time the bulk of evidence suggested that their hypoglycemic effect, which depended on the presence of beta cells, resulted from discharge of insulin into the portal system. It was initially hoped that this form of therapy, in addition to the convenience of its oral route, might prove superior to the administration of exogenous immunogenic insulin into subcutaneous depots that drain into the systemic rather than the portal circulation.

In 1959, because of the increasing use of sulfonylureas and the paucity of data regarding the relationship of blood glucose control to diabetic complications, a

long-term prospective cooperative clinical study was initiated between a number of university clinics and termed the University Group Diabetes Program (UGDP). Their stated aims were to study the natural history of vascular disease in maturity-onset, non-insulin-dependent, mild diabetics and to evaluate the efficacy of hypoglycemic treatments in the prevention of vascular complications.

Although phenformin had been available since 1957, it had not come into widespread use as an antidiabetic drug until after the initial UGDP study protocol had been finalized. However, in 1962 phenformin was added to the study and, with the addition of five more clinics and modification of treatment group assignments, recruitment of phenformin-treated patients caught up with the other groups.

In 1970, a comprehensive report on the design, methods, and base-line characteristics of the UGDP study was published (3), which included mortality results in all groups except the phenformin-treated group. Later, a preliminary report on this group's mortality appeared (4). The study had not been designed to investigate mortality, and it was totally unexpected to all concerned when cardiovascular mortality in the tolbutamide-treated group exceeded that of the other treatment groups. Likewise, cardiovascular mortality in the phenformin-treated group was greater than in a combined group of insulin-treated and placebo-treated patients. Because of these findings, tolbutamide therapy was discontinued from the study in 1969 and phenformin was similarly terminated in the spring of 1971.

It is not the purpose of this review to detail the many important contributions of this extensive study to our understanding of the epidemiology of maturity-onset non-insulin-dependent diabetes, nor to reiterate the pros (5, 6) and cons (7, 8) of the prolonged and emotional controversy that arose concerning the validity of the conclusions regarding cardiovascular mortality. Rather, it is our intention to record some of the directions in pharmacologic research resulting from the UGDP study. These include questions as to the mechanism of actions of antidiabetic drugs and whether they might have a direct cardiovascular effect apart from their hypoglycemic action. Could interaction with other medications have contributed to undesirable side effects from therapeutic doses of antidiabetic agents? The remainder of this review focuses on current concepts of the mechanism of action of oral antidiabetic agents in regard to these questions.

## SULFONYLUREAS

### *Mechanism of Hypoglycemic Action*

Franke & Fuchs demonstrated in 1955 (9) that, in selected patients with maturity-onset diabetes, sulfonylureas could successfully reduce their hyperglycemia. Yet 20 years later, controversy persists as to how these agents bring about this reduction in blood glucose. Certain mechanisms have been suggested and are discussed below.

EFFECT OF SULFONYLUREAS ON THE PANCREATIC BETA CELL The principal explanation for the hypoglycemic effects of sulfonylureas rests on the elegant and inspired work of Loubatieres (10, 11), who was the first to suggest an insulinotropic mechanism with its implied therapeutic potential in diabetics. With the advent of

specific insulin assays, a rise of plasma insulin after acute administration of tolbutamide was noted (12) and in vitro studies in the rat pancreas showed this to be a direct effect (13). The response of the perfused rat pancreas to tolbutamide is virtually instantaneous (14, 15) and does not require glucose (14). It differs from glucose stimulation in that doses of tolbutamide alone produce primarily a first phase of preformed insulin release, whereas with high glucose stimulation alone the second phase, involving at least some degree of insulin synthesis, predominates (16). This is in accord with reports that perifused islets do not incorporate $^{14}C$ leucine in response to tolbutamide (17, 18), as contrasted with glucose stimulation. Also, in vitro perfusion of tolbutamide *alone* has produced beta cell degranulation with proliferation of golgi apparatus and vesicles devoid of granules, compatible with defective insulin biosynthesis (19).

Fortunately, however, in the clinical situation, glucose is always circulating. An important observation relevant to therapy is the capability of tolbutamide in vitro to restore second-phase insulin release in the presence of a subthreshold level of glucose (20). This implies that sulfonylureas not only stimulate insulin secretion themselves, but also facilitate the effect of glucose on the pancreas (20). Thus, with sulfonylureas sensitizing insulin responsiveness of the beta cell to glucose variations, the blood glucose level can once again become a more efficient regulator of insulin secretion. This has obvious pharmacologic advantages in therapy in that it allows a drug to influence regulation of a biological system without requiring fluctuations in the blood level of the drug itself.

Despite intensive investigation, the mechanism by which sulfonylureas induce insulin release remains elusive. The bulk of evidence suggests that sulfonylureas produce their insulinotropic effect without entering the beta cell (21). It has been suggested that the phosphodiesterase inhibition shown to occur in islet cells exposed to sulfonylureas might account for the insulin release (22). This is unlikely, because this enzyme inhibition requires a high dose of sulfonylurea (22), and the transient "spike" response of sulfonylurea-induced insulin release is markedly different from the "square wave" produced by theophylline (23), a known inhibitor of phosphodiesterase. Also, one would not expect the observed marked potentiation of maximal tolbutamide-induced insulin release by theophylline (24) if both were acting at the same site.

The insulin-releasing effect of sulfonylureas is not reduced by a variety of agents such as beta adrenergic blockers (24), 2-deoxyglucose (25), or mannoheptulose (24), but can be inhibited by diazoxide (24) or epinephrine (26). Beta cells exposed to sulfonylureas show a reduction in their ATP content (27). They also accumulate calcium (28). This latter effect of ionic flux may relate to the electrical action potentials which are generated in beta cells by tolbutamide, but not by non-hypoglycemic metabolites such as carboxytolbutamide (29).

In a series of experiments with obvious implications for the treatment of diabetes, Loubatieres has reported that chronic administration of sulfonylureas to mice has resulted in increased weight of the islets of Langerhans and that electron microscopy confirmed the generation of new beta cells (24). However, other workers could not confirm an increase in beta cell volume (30) or islet size (31) after chronic tolbuta-

mide therapy. In fact, evaluation of beta cells in normal mice, rats, and hamsters after chronic administration of sulfonylureas showed functional impairment of insulin release in vitro (18, 32) associated with impaired glucose tolerance in vivo (18, 30). Thus, a "beta-cytotrophic" effect of sulfonylureas remains unsettled.

The above investigations on insulinotropic mechanisms have extended to a new "second generation" of highly potent sulfonylureas (Figure 1). However, there is no indication that any new insight into the fundamental mechanism of action has been acquired, because most differences have been essentially quantitative. All of these agents depend on the presence of glucose for their major insulinotropic effects in vitro, producing predominantly an early phase of insulin release in the absence of glucose and a maximal biphasic response in the presence of 150 mg/dl of glucose; they seldom increase the effect of a maximal glucose stimulus of 300 mg/dl or more (33). These common properties suggest that the site and mode of action on the beta cell are shared by all of the sulfonylureas, despite widely different potencies.

EXTRA-BETA CELL HYPOGLYCEMIC ACTION It had initially been presumed that the successful correction of hyperglycemia during therapy with sulfonylureas in maturity-onset diabetics was due to their insulinogenic action. In support of this are observations that during the first few weeks of oral therapy with acetohexamide (34) or chlorpropamide (35, 36), improved glucose tolerance is accompanied by

"SECOND GENERATION" SULFONYLUREAS

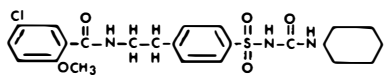
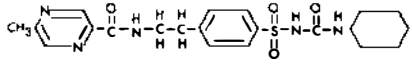
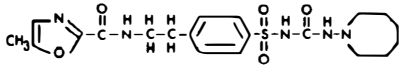
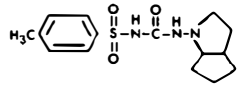
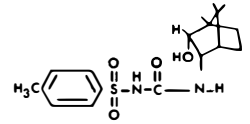
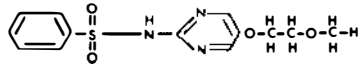
| GENERIC NAME | STRUCTURE | $ED_{50}^{*}$ | TRADE NAME |
|---|---|---|---|
| 1.) GLIBENCLAMIDE (GLIBURIDE) (GLIBENZCYCLAMIDE) |  | .01 μM | DAONIL, DIABETA EUGLUCON, MICRONASE HB 419 ; U 26452 |
| 2.) GLIPIZIDE |  | .04 μM | GLIBENESE, K—4024 |
| 3.) GLISOXEPIDE |  | .03 μM | B. S. 4231 |
| 4.) GLICLAZIDE |  | 1.2 μM | DIAMICRON, S 1702 |
| 5.) GLIBORNURIDE |  | —— | RO —6 —4563 |
| 6.) GLICODIAZINE (GLIMIDINE) |  | 10.4 μM | REDUL, LYCANOL GONDAFON, SH 71 |

*Figure 1* 1. Hoechst-Boehringer, Upjohn; 2. Pfizer Inc.; 3. Bayer and Schering Inc.; 4. Servier, Paris; 5. Hoffman-LaRoche Inc.; 6. A sulfonamide rather than a sulfonylurea, Schering Inc.

increased insulin levels. In one report in which urinary insulin was measured, it was suggested that chronic tolbutamide therapy acted by a continued insulinotropic action (37). However, the majority of reports have consistently refuted this concept based on the observation during *prolonged* chronic therapy with either chlorpropamide (35, 38–41), tolbutamide (41), acetohexamide (34), tolazamide (42), or gliburide (43, 44) that improvement in oral glucose tolerance was accompanied by either unchanged or decreased circulating insulin. It was documented that any possible rise in biologically active insulin was not obscured by a falling level of inactive proinsulin to account for the unchanged or even lower total immunoreactive insulin measurement during long-term therapy (44). Accordingly, the beneficial effect of chronic sulfonylurea therapy on glucose tolerance in diabetics has generally been attributed to effects of these agents apart from the beta cell, such as a direct action on the liver (45) or peripheral potentiation of the glucose-lowering effectiveness of insulin (46). These extra-pancreatic effects of sulfonylureas have been the subject of several thorough reviews (47, 48).

However, further consideration of the above clinical data, in light of certain new concepts regarding the kinetics of insulin release, need not necessarily exclude the beta cell as prime responder to explain this improved effectiveness of endogenous insulin. A very elegant, recent study using an artificial endocrine pancreas (49, 50) demonstrated the importance of an initial early burst of insulin to better control hyperglycemia during a glucose load. The effectiveness of exogenous insulin was increased almost tenfold in pancreatectomized dogs (49), and in three juvenile diabetic subjects post-prandial hyperglycemia was essentially normalized (50). This suggests the possibility that agents capable of correcting the pattern of diminished early phase insulin release characteristic of diabetes mellitus (51–53) might improve glucose tolerance.

Regarding three of the chronic sulfonylurea studies (41, 43, 44), note is made (43, 44) that the improvement in glucose tolerance could not be explained by any correction of the delay in insulin release. However, in these studies the first measurements were made 30 min after an *oral* glucose load. In another study, a correction of the delay in insulin release after chronic tolazamide therapy was reported (42). However, when their data are evaluated, chronic therapy did not increase the concentration of insulin when measured at 15 and 30 min after an oral glucose load, but rather markedly diminished the previously exaggerated insulin levels after 60 min. Whether this relative shift of peak insulin response can be appropriately termed a correction in the delayed insulin release or not points out the problems inherent in attempting to characterize the kinetics of insulin release with the standard oral glucose tolerance test alone. The oral glucose tolerance test was not designed to measure insulin kinetics, and its predominant reflection of the late phase of insulin release does not give reliable information about the transient first phase. As suggested by the recent observations with the artificial endocrine pancreas (49, 50), an absent or reduced early phase would reduce the effectiveness of insulin, while a restored first phase could reduce the subsequent hyperglycemia after a glucose load without necessarily increasing the late phase of insulin release. It is of interest that in those published reports in which a rapid *intravenous* glucose load was used

to evaluate the effect of sulfonylurea therapy for 1 week (42) or for as long as 3 months (54), a highly significant rise in the early-phase insulin release was measured at 5 min after the infusion. Thus, further studies of early-phase insulin release during chronic sulfonylurea therapy deserve clarification before attributing their effectiveness to other actions apart from the beta cell.

*Effect of sulfonylureas on glucagon release* With the recent availability of specific immunoassays for pancreatic glucagon, it has been possible to examine the postulate mentioned in the first clinical study of sulfonylureas (9) that these agents exert some of their hypoglycemic effect by reducing circulating glucagon. The recently discovered inhibitor of glucagon secretion, somatostatin, has been used to demonstrate clearly that reduction of circulating glucagon levels produces hypoglycemia in diabetics (55). In vitro studies using mice (56) and rat islets (57) and rat pancreatic slices (58) suggested no effect from sulfonylureas on glucagon release, whereas in the perfused pancreas of the rat (59) and dog (60) an inhibition has been reported. In vivo studies likewise are not definitive. In ducks, a clear-cut suppression of plasma pancreatic glucagon has been reported (61, 62), whereas in dogs this was not observed (62–64). In normal humans, the acute administration of tolbutamide did not suppress plasma glucagon levels (65), nor did oral administration of chlorpropamide (65), glipizide (66), or gliburide (66), whereas, in the only reported study in diabetics, therapy with chlorpropamide for 12 days in 6 maturity-onset diabetics was noted to reduce levels of circulating glucagon (67). At the present time, experience is too limited to establish whether the conflicting data are due to species differences, the nature of the sulfonylurea used, or the presence or absence of diabetes.

Thus, before pursuing "extra-pancreatic" causes of hypoglycemia during chronic sulfonylurea therapy, two "pancreatic" actions require further evaluation. These are the observations that early-phase insulin release in maturity-onset diabetics remains significantly increased after three months of sulfonylurea therapy (54) and that circulating glucagon levels are suppressed in maturity-onset diabetics (67) receiving sulfonylureas.

## *Non-Hypoglycemic Action of Sulfonylureas*

CARDIOVASCULAR EFFECT A great deal of basic research into the cardiovascular effects of sulfonylureas has been a direct result of the UGDP report. These have been extensively reviewed recently (68), and it seems clear that tolbutamide, at concentrations within the therapeutic range of the unbound active drug, as well as other sulfonylureas, increased contractility of isolated rabbit atria (69) and cat papillary muscle (70) but not the dog heart (71), and that this inotropic effect was associated with activation by sulfonylureas of adenyl cyclase (69). In the dog, whose heart tissue does not show this inotropic response to sulfonylureas, it has been said that the extent and magnitude of acute myocardial ischemic injury following acute coronary occlusion is not altered by pretreatment with tolbutamide (72).

It appears that, while inotropic effects may be beneficial in the failing heart by increasing efficiency of contraction and thereby reducing oxygen requirements, its effect on the non-failing heart increases the work load and oxygen need. This could

be detrimental and possibly arrhythmogenic in a myocardium already handicapped by ischemic heart disease.

In addition, there has been a report of increased automaticity in isolated Purkinje fibers of dogs, but not of rabbits, in response to sulfonylureas (69). Since inotropic and chronotropic stimulation can be deleterious in some clinical conditions, it is tempting to apply these findings in animals to humans, especially in view of the UGDP conclusions. However, the data in man are less conclusive. In isolated human papillary muscle there was no inotropic effect (48), and in intact humans, while a mild inotropic effect was reported with an acute bolus of intravenous tolbutamide during cardiac catheterization (73), another group reported no inotropic effect using a non-invasive method that measures systolic time intervals (74).

Thus, since the inotropic and chronotropic effects of sulfonylureas seem to be species specific, more data are required before this property of tolbutamide in certain animal tissues can be related to the outcome of the UGDP mortality results.



OTHER PHARMACOLOGIC EFFECTS OF SULFONYLUREAS *Dilutional hyponatremia* has been recognized as a complication of chlorpropamide therapy for patients with diabetes mellitus (75), but this is quite rare with tolbutamide therapy (76) and was not noted as a complication during the UGDP study. Chlorpropamide exerts its antidiuretic action by increasing release of ADH (77), as well as by potentiating vasopressin's effect on the renal tubule (78). This latter effect could explain chlorpropamide's superiority over tolbutamide as an antidiuretic, because relatively large quantities of *active* chlorpropamide are excreted in the urine and reabsorbed in the renal tubule, as opposed to the predominantly *inactive* metabolites of tolbutamide excreted in the urine (79).

The antidiuretic effect of chlorpropamide appears not to be a function of the sulfonylurea part of its structure, because three other sulfonylureas (acetohexamide, tolazamide, and glibenclamide) were found to have diuretic effects in man (80). Glibenclamide has been reported to inhibit clofibrate-induced antidiuresis in patients with diabetes insipidus (81), although its diuretic action involved neither suppression of vasopressin release nor interference with its peripheral action (80).

## *Drug Interactions of Sulfonylureas*

A renewed interest in the basic pharmacology of antidiabetic agents has been stimulated by the UGDP report on mortality. Tolbutamide, which is confined to the extracellular space, is at least 95% bound by serum proteins (82). Its metabolism within the liver involves hydroxylation of its methyl group as a preliminary step to formation of its carboxy derivative (83). Chlorpropamide, whose half-life of 32 hr is much longer than all the others (83), was once believed to be excreted unchanged in the urine. It is now known to be approximately 80% metabolized by either hydrolysis into sulfonamide metabolites or hydroxylation of its side chain (84). These compounds are prototypes for metabolism of the "second generation" sulfonylureas: Glibornuride is handled similarly to tolbutamide, remaining intact with formation of a carboxybenzene metabolite (85), while glibenclamide is metabolized

like chlorpropamide with cleavage into a sulfonamide fragment and hydroxylation of its side chain (86).

The interaction of sulfonylureas with other agents has been reviewed recently (87, 88), and adverse effects can occur in a variety of ways:

I. Interaction on Glucose Kinetics
   A. Cooperative
      1. *Direct:* Salicylates and alcohol have inherent hypoglycemic activity and have been reported to be responsible for precipitating severe hypoglycemic coma in sulfonylurea-treated diabetics (89).
      2. *Indirect:* Propranolol interferes with counter-regulatory mechanisms that respond to hypoglycemia, such as glycogenolysis, lipolysis, or glucagon release (90).
   B. Antagonistic
      Thiazide diuretics, oral contraceptives, and glucocorticoids are examples of pharmacologic agents that oppose the hypoglycemic effect of sulfonylureas.

II. Pharmacokinetic Drug Interactions (91)

This refers to the situation where one or more drugs affect the *absorption, distribution, metabolism,* or *excretion* of the sulfonylureas themselves. Severe hypoglycemic coma has been recorded in 17 patients in whom sulfonylurea therapy had been combined with certain sulfonamides, dicoumarol, or phenylbutazone (89, 92). The mechanism of these interactions has been reviewed (83, 88), and it has been clearly shown that all of these compounds increase the circulating concentration of tolbutamide in man, as well as prolong its half-life (88).

In vitro studies document the effectiveness of phenylbutazone, dicoumarol, certain sulfonamides, and salicylates in displacing sulfonylureas from human serum albumin (93); however, the relationship of this to their observed in vivo effects, which *raise* (rather than lower) blood levels of the sulfonylureas and *prolong* (rather than shorten) their half-life, is far from clear. In addition, other sulfonamides displace sulfonylureas to the same degree, yet do not affect half-life or enhance their hypoglycemic effects (87).

As regards metabolic interactions, it has been clearly shown for sulfaphenazole that it inhibits the hydroxylation of tolbutamide. Because phenylbutazone and dicoumarol, as well as a muscle relaxant, phenylramidol, also prolong the half-life of sulfonylureas (88), they probably act similarly by inhibiting the rate-limiting hydroxylation step.

An excretory mechanism for pharmacokinetic interaction is phenylbutazone's enhancement of hypoglycemic effects of acetohexamide by interference with renal excretion of the active metabolite, hydroxyhexamide (94).

The UGDP report did not include data on the nature of other medications being taken by patients in the various treatment groups. Adverse reactions, due to the coadministration of two or more drugs, could conceivably have influenced the mortality data. In any clinical situation where sulfonylureas are being administered, current knowledge of drug interactions should be considered. Because hydroxylation may be an obligatory step in the metabolism of these compounds, the use of

drugs that are known to inhibit hydroxylation reactions (e.g. certain sulfonamides) should be avoided.

## BIGUANIDES

Phenformin (N-β-phenethylformamidinyliminourea) is presently the only biguanide available in the United States for treatment of diabetes mellitus. Ever since its introduction to clinical use in 1957, a satisfactory explanation for its mechanism of action has remained elusive despite considerable investigative efforts. A higher incidence of cardiovascular mortality in the UGDP among phenformin-treated patients than in a combined group of placebo- and insulin-treated patients (4) renewed interest in the basic pharmacology of phenformin. These areas in particular are reviewed, including its mechanism of action in reducing blood glucose in diabetics, and whether this action or others may account for the adverse effects observed, such as a reported increase in cardiovascular mortality or the production of lactic acidosis.

### *Mechanism of Action of Phenformin in Reducing Hyperglycemia in Diabetics*

Several comprehensive reviews (95–98) have summarized current knowledge and conflicting views on the mechanism of action of biguanides in reducing hyperglycemia in diabetics. From a great deal of experiments there is general agreement on the following observations:

I. Experimental Data in Humans
   - A. Phenformin does not reduce fasting blood glucose in normal humans, but does so in diabetics (97) or normal subjects after a three-day fast (99).
   - B. Phenformin reduces hyperglycemia after an oral glucose tolerance test in both nondiabetics (100) and diabetics (101), but has little effect on hyperglycemia in either after a rapid intravenous glucose load (100, 101).
   - C. Its glucose-lowering effect after oral glucose is associated with reduced, rather than increased, circulating insulin levels (102).
   - D. Therapeutic doses of phenformin do not cause hypoglycemia in diabetics (89). It thus might be more appropriately termed a "euglycemic," rather than a "hypoglycemic," agent.
   - E. Phenformin significantly influences the metabolism of glucose in subjects in whom it exerts no hypoglycemic effect (103, 104). Increased glucose turnover in nondiabetic subjects in the face of a constant blood glucose concentration indicates that therapeutic doses of phenformin do not inhibit gluconeogenesis in nondiabetic subjects (103–105). At present, similar data in diabetics are not available.

II. Animal Experiments

Interpretation of in vitro and in vivo experimental data has been limited by the great species differences in sensitivity to the hypoglycemic action of biguanides, being high in man, monkey, and guinea pig, and low in the rat and the dog (98). However, a consistent finding in all species is the observa-

tion that doses of phenformin, several hundred times the level achieved therapeutically in man, are capable of interfering with oxidative metabolism of glucose in muscle, liver, and gut (97). Associated with this effect is an increased glucose uptake by muscle with lactic acid production, a decrease in hepatic gluconeogenesis, and reduced absorption by the gut of glucose. Because all of these mechanisms could contribute to the glucose-lowering effect in man, they have been considered as possible factors in the therapeutic response to phenformin in diabetics. However, it has been difficult to reconcile the proposed mechanisms involving inhibition of oxidative processes with the absence of adverse symptoms in diabetics responding to phenformin therapy.

III. Proposed Mechanism of Action in Man

A recent review (95) summarized four pieces of evidence against the concept that the clinical response in man is related to an inhibition of aerobic metabolism.

A. Circulating blood levels of phenformin in man (106, 107) are much lower than those required in animals to block oxidation in muscle (108).

B. There is no correlation between respiratory inhibition and hypoglycemic properties of various biguanides. Some of the least hypoglycemic produce the greatest respiratory inhibition (95).

C. Elevations in blood lactate in diabetics receiving the usual therapeutic doses are slight and not always present (95).

D. From glucose-kinetic studies, it appears that neither glucose oxidation nor gluconeogenesis is blocked in humans receiving therapeutic doses of phenformin (104, 105).

These differences were reconciled by proposing that phenformin has two qualitatively different effects on carbohydrate metabolism: a *"high dose,"* capable of producing respiratory inhibition; and a *"low dose,"* which does not inhibit oxidation and requires the presence of insulin (95). This was based on a report that, whereas high-dose biguanide effects were achieved in both normal and alloxan-diabetic animals, low doses affected glucose metabolism only in normal animals or in diabetic animals receiving insulin (109).

Others favor the explanation that a major effect on gut (101) and liver (110) would be due to high *tissue* concentrations in those organs probably approaching levels high enough to interfere slightly with aerobic oxidation in those tissues. This would reduce absorption of glucose and inhibit hepatic gluconeogenesis in diabetics.

A gas chromatographic method was recently developed (110a) which is sensitive enough to measure levels of phenformin achieved during therapy. In patients whose hyperglycemic responses to oral glucose loads were demonstrably reduced toward euglycemic levels, circulating phenformin concentrations were found to be 102–241 ng/ml (107). This is far below the 300,000 ng/ml or more required to

promote glucose uptake and inhibit oxidation in the rat diaphragm (108). Because there was no effect on glucose disappearance rate in these patients after a rapid intravenous load, it was felt that reduced glucose absorption due to possible high tissue levels of phenformin in the gut might explain the euglycemic effect of phenformin after oral glucose.

Changes in serum insulin levels do not account for the hypoglycemic action of phenformin, because these are reduced during the oral glucose test as compared with a pre-phenformin control test. Although direct inhibition of insulin release had been reported in rat islets, a concentration of phenformin as high as 100,000 ng/ml was required (111). However, levels in the therapeutic range (200 ng/ml), and even as high as 2000 ng/ml, had no effect on beta cell function in the perfused rat pancreas (107). These data indicate that the reduction in serum insulin after phenformin therapy is due more to a reduced stimulation of insulin secretion subsequent to the lessened hyperglycemia than to any toxic effect on oxidative metabolism of beta cells. Consistent with this is the absence of inhibition of the early insulin response to a rapid intravenous glucose load during phenformin therapy, as compared with pretreatment values (100, 107).

Recently, another mechanism for the insulin-sparing effect of phenformin has been suggested by the observation that secretin release may be impaired by phenformin therapy (112). This implies that insulinogenic intestinal hormones such as enteroglucagon or secretin, which are apparently released by oral glucose (113), may be reduced during phenformin therapy.

Studies of phenformin's effect on pancreatic alpha cell function are quite limited. Degranulation and hydropic degeneration of alpha cells have been reported after treatment of guinea pigs with high doses of phenformin (97). However, it is unlikely that biguanides lower blood sugar by a suppression of alpha cell functions, because normal subjects had no reduction of plasma glucagon and in fact had an elevated glucagon response to arginine infusion after dimethylbiguanide, as compared with a similar infusion before treatment (114).

OTHER ACTIONS OF PHENFORMIN Intestinal absorption of amino acids have been shown to be diminished in vitro (115) and also to a slight degree in diabetic patients (116). This suggests that biguanides do not act as specific inhibitors for glucose transport only, but rather affect active, energy-requiring, intestinal transport mechanisms in general.

A recent editorial summarized literature regarding the anti-inflammatory actions of phenformin in laboratory animals (117). Adjuvant-induced polyarthritis was either reduced or its development inhibited by phenformin treatment in rats. In addition, phenformin combined with ethylestrenol activates blood fibrinolytic activity in humans and has been reported to be useful in the treatment of recurrent venous thrombosis in cases where fibrinolytic activity was reported to be low, and

in rheumatoid arthritis where this combination of drugs reduced the inflammation (117).

Adult-onset diabetics showed a reduction in the ability to excrete an ammonium-chloride-induced acid load after phenformin therapy (118). Because oxidative phosphorylation and ATP production in the renal cortex are reduced by this drug (119), and because acid secretion by the kidney is energy-dependent, it is possible that accumulation of phenformin in the renal tubules during its excretion achieves concentrations high enough to produce respiratory and secretory inhibition.



### *Cardiovascular Effects of Phenformin*

In contrast to the reported inotropic effects of sulfonylureas on the heart, there was no comparable effect from phenformin on rabbit atria (68), nor was there activation of adenylcyclase by phenformin in either the rabbit or human heart (68). At present there is no satisfactory pharmacologic or cardiovascular explanation for the observation of the UGDP that 27 cardiovascular deaths occurred in the phenformin-treated group, as opposed to 12 deaths in a combined placebo-insulin–treated group (120).

Heart rate monitored on electrocardiogram records taken at each follow-up visit showed a slight increase above baseline measurement in phenformin-treated subjects. At the twenty-second follow-up visit, there was an average increase of 3.9 beats per min for placebo, 8.0 beats per min for phenformin, 1.1 beat per min for insulin-standard, and 1.6 beats per min for insulin-variable treatment groups (120).

Base-line levels of blood pressure were recorded on all patients entering the UGDP, and repeat measurements were monitored at three month intervals during the study. A progressive increase in systolic blood pressure was noted for phenformin-treated patients, and by the twenty-second follow-up exam the mean systolic blood pressure for this cohort of patients was +7.8%, whereas the percent change from base-line in the other groups was –3.0% for placebo, +0.9% for insulin-standard, and +2.7% for the insulin-variable groups (120).

Changes in diastolic blood pressure were less dramatic yet consistent in that the phenformin-treated group had diastolic blood pressure +3.6% above base-line values, whereas in the other three treatment groups blood pressure remained below base line (120).

Among those patients who died from all causes, a higher proportion of patients were classified as hypertensive or reported to be receiving treatment for hypertension during the course of the study. However, blood pressure findings for the patients in the phenformin-treated group who died during the study did not differ significantly from those observed for the entire phenformin treatment group.

Thus, whatever contribution to cardiovascular mortality resulted from the hypertensive effect of phenformin, it was the conclusion of the UGDP that this factor alone could not account for all the excess mortality observed for phenformin-treated patients (120).

### *Metabolism and Drug Interaction of Phenformin*

Phenformin is metabolized by parahydroxylation of its benzene ring, and thereby rendered biologically inactive (106). Experiments demonstrate that the relative

inactivity of phenformin in reducing blood glucose in rats can be reversed by agents that inhibit hepatic microsomal hydroxylating enzymes (121). In contrast, reduced effectiveness of phenformin occurs when agents that enhance hydroxylation mechanisms are administered to guinea pigs (121). These findings suggest that the hydroxylation of phenformin is a major controlling factor in the differential response of rats and guinea pigs to this agent. In man, only 33% of excreted phenformin is an inactive hydroxylated metabolite, in contrast to rats, in whom 100% of the compound appears in the hydroxylated form (106).

While the half-life and metabolism of sulfonylureas have been shown to be markedly affected by drugs that interfere with hydroxylation in man, this does not appear to have been investigated to any extent with phenformin. Ethanol-phenformin synergism in the production of hyperlacticacidemia in man has been reported (122), and consists of both an increase in glucose production and decreased lactate utilization.



### *Phenformin and Lactic Acidosis*

The UGDP reported one death ascribed to lactic acidosis and two nonfatal cases in the group of 204 patients receiving phenformin (120). There is no information regarding specific contributing factors in these 3 patients, such as impaired renal or hepatic function, circulatory failure, or ethanol ingestion. However, absence of any reported cases of lactic acidosis in the other 823 UGDP patients not receiving phenformin underscores its association with lactic acidosis as stressed in a number of reviews (123–126). In a recent comprehensive survey of the relationship of biguanides to lactate metabolism (96), it is suggested that hyperlactatemia and lactic acidosis represent a risk of phenformin therapy, especially, and perhaps only, when other predisposing factors are present. Because about two thirds of the phenformin excreted in the urine is unmetabolized (106), and because phenformin-induced effects are dose dependent, this probably accounts for the high incidence of impaired renal function in most cases of lactic acidosis associated with phenformin (123–125). In our laboratory, phenformin levels of 900 ng/ml were measured in an 86-year-old azotemic diabetic who died of severe lactic acidosis while on phenformin therapy. This is in contrast to therapeutic levels of 102–241 ng/ml (107), and is comparable to a level of 2000 ng/ml in a 41-year-old male diabetic who survived severe lactic acidosis precipitated by attempted suicide with phenformin (107). The development of lactic acidosis in previously healthy persons during self-administration of an overdose of phenformin demonstrates the ability of phenformin to precipitate lactic acidosis (127). However, it also implicates the dose-dependent effects of phenformin in explaining the high association of lactic acidosis in patients with renal impairment, and the relatively low association in those without predisposing causes (96).

## CONCLUSIONS

The UGDP report of increased cardiovascular mortality in a group of mild maturity-onset diabetics treated with either tolbutamide or phenformin was unexpected and quite perplexing to the medical profession. Its impact on basic pharmacologic research has been considerable. It has emphasized the paucity of knowledge avail-

able as to the mechanisms of action of these agents, despite their wide usage. Interest has been awakened in the field of pharmacokinetic drug interactions and its possible applicability in interpreting the UGDP. The clinician is reminded that multiple tissues may respond to drugs in addition to those specific organ systems whose pharmacologic response is being monitored. Renewed investigations into the basic pharmacology of sulfonylureas and biguanides have resulted in a plethora of data, some of which are contradictory and confusing at present. However, once proper distinction is made between *high-* and *low*-dose effects, and between species differences, it is hoped that this information will be useful in attempts to dissociate antidiabetic effects from any potentially toxic effects on the cardiovascular system. At the present time, it is not clear whether the "second generation" of sulfonylurea agents administered in one three-hundredths of the dose of tolbutamide would have produced comparable results in a long-term clinical trial. Further knowledge in these areas is critical for resolution of the question as to the ultimate safety of these drugs and for establishing finally their rationale in the treatment of diabetes mellitus.



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