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Compounds Inhibiting Insulin Degradation

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Since insulin is rapidly degraded in the body,[1,2] any safe measure employed to prevent this in diabetic patients might be of distinct advantage, particularly since it would probably increase the effectiveness of the body's homeostatic mechanisms. Using an in vitro system previously described,[3] many compounds have been tested for their capacity to inhibit the degradation of insulin. Since most of these studies have been published recently in this *Journal*[4] only a very brief account is given here.

In table 1 is presented the activity of a few representative compounds. It is noted that a wide variety of chemicals cause inhibition of insulin degradation in vitro. Whereas bis (carboxyaminopropionylphenyl) disulfide is highly active, relatively high concentrations are required for some of the other compounds, including carbutamide (butylaminobenzenesulfonylurea). Despite the high in vitro activity of the disulfide compound, it has been found to cause no hypoglycemia in rats. Moreover, carbutamide causes marked hypoglycemia in doses smaller than are apparently required to cause significant inhibition of insulin degradation.[5] Other compounds that are active by the in vitro test, but which have been found by my collaborators and me to cause little or no hypoglycemia in rats, include cystine, dithiouracil 6-ethoxybenzothiazolesulfonamide, 2-acetylamino-1, 3, 4-thiadiazole (Diamox), arbutin, ergothioneine, hydroxyindoleacetic acid, dihydroxyphenylalanine, thiolhistidine, dithioaniline, and bis (morpholinothiocarbamoyl) disulfide.

The fact that there are relatively many compounds which inhibit the degradation of insulin in vitro, many of which do not exert a hypoglycemic effect, poses some important questions: (a) What are the mechanisms by which insulin degradation are accomplished? (b) What are the best procedures for evaluating potential hypoglycemic agents?

The in vitro inhibitors may react in the following ways: (a) Oxidize the SH-groups of the liver enzyme preparation, (b) combine with an enzyme at "non-key" or "key" sites, (c) combine with insulin, or (d) combine with an insulin-enzyme complex. Enlightenment relative to the types of reaction of different compounds occurring in the liver enzyme-insulin system might

TABLE 1

Inhibition of insulin degradation in vitro

	mM/liter causing 50 per cent inhibition
Disulfides	
Bis-β(2-carboxyaminopropionylphenyl) disulfide	.002
Bis-(dimethylcarbamyl) disulfide	.011
Cystine	.1
Lipoic acid	1.1
Thioureas	
2, 4 Dithiouracil	0.5
1-p-Methoxylbenzyl-3-thiosemicarbazide	10
Thiazoles	
2-Amino-5-(p-methoxyphenyl)-1, 3, 4-thiadiazole	0.8
Thiamine	15
Sulfonamides	
6-Ethoxy-2-benzothiazolesulfonamide	0.5
2-Acetylamino-1, 3, 4-thiadiazole	6
2-Isopropyl-5-p-aminobenzene-sulfonamido-1, 3, 4-thiadiazole	11.5
1-n-Butyl-3-p-aminobenzene sulfonylurea	15
Nonsulfur Compounds	
Decamethylenediguanidine	5
Indole-3-propionic acid	7
Arbutin	9
Indole	11
3-Phenylpropionic acid	22

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significantly aid the search for orally effective antidiabetic compounds.

Most of our hypoglycemic tests have been conducted with intact adrenalectomized rats, some of which were primed with glucose during the test. Pertinent questions relative to this type of screening procedure are: (a) Is the rat the best species? (b) Are intact or adrenalectomized animals better? (c) Is it preferable to administer glucose during the test with the objective of stimulating the secretion of insulin? (d) Should alloxanized animals be tested? (e) What are the intervals at which blood glucose should be measured? Presumably a variety of these tests should be employed since they test different phases and since some compounds can be expected to act through different mechanisms.

The clinical results with the sulfonylureas demonstrate that oral therapy can be effective in many diabetic patients. However, the association of the sulfonylureas with significant untoward reaction in some subjects prompts search for still more useful compounds.

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Effects of Substituted Sulfonylureas on Rat Diaphragm and Liver Tissue

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A previous communication from this laboratory[1] reported hepatic changes following administration of the substituted arylsulfonylureas both in vivo and in vitro.

Since glucose-6-phosphatase appears to play a significant role in hepatic glucose production (elevated in diabetes and fasting and deficient to absent in the common form of glycogen storage disease), this enzyme was studied in rat liver. It was found that addition of both compounds in 10^{-2} molar concentration [N-Sulfanilyl-N^1-butyl-urea and N-(4-Methyl-benzolsulfonyl)-N^1-butyl-urea)] to experimental liver preparations in vitro resulted in 25 per cent inhibition of enzyme activity. No inhibition was observed at lower concentrations. This inhibition occurred in both crude homogenates and the separated microsomal fraction. Since inhibition was also observed with sulfanilamide in the same concentration, it was concluded that the sulfonylureas had no direct physiological effect on glucose-6-phosphatase.

Rat liver slices incubated in the presence of these agents showed no change in either the degree of glycogenolysis or the rate of glucose production, nor were any alterations noted in the distribution of labeled products after incubation with uniformly labeled fructose-C^{14} or pyruvate-2-C^{14}.

To study the effects in vivo, rats were fed a single dose of sulfonylurea by stomach tube, killed two to four hours later during the hypoglycemic episode, and the livers assayed for glucose-6-phosphatase activity. No changes were found in the level of this enzyme although blood glucose fell 40 per cent. If the drugs were given every twelve hours for forty-eight hours, however, a marked decrease in hepatic glucose-6-phosphatase activity was noted similar to that found in normal animals treated with protamine zinc insulin over the same period of time (table 1).

From other studies[2] it has become apparent that the level of glucose-6-phosphatase merely mirrors the degree of sustained hepatic glucose production. The enzyme is present in considerable excess when determined

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