diabetic rats showed good reductions in glucose-6-phosphatase activity. The rise in glucose-6-phosphatase activity after alloxan, previously reported by other groups,[6, 7] was observed also. The results of this experiment indicate that insulin is required for the effect of BZ-55 on the glucose-6-phosphatase activity of liver, but whether or not an increased amount of insulin is necessary for this effect is not known at present.

Another effect of BZ-55 which has been noted previously is its influence on the thyroid. The administration of BZ-55 was observed to cause a reduction in I^{131} uptake by the thyroid gland and, over a period of time, an increase in its size. Dr. J. Logothetopoulos has compared BZ-55 and Orinase with respect to I^{131} uptake and goitrogenic effect. He found the reduction in I^{131} uptake to be much more pronounced in rats treated with BZ-55 than in those treated with equimolecular quantities of Orinase. The goitrogenic effect of BZ-55 was high also, whereas Orinase in similar doses did not have a goitrogenic effect. In his series too, the hypoglycemic effects of these materials appeared to be unrelated to their effect on the thyroid gland.

It is difficult to interpret the various results. We can say, however, that the results presented seem to be consonant with a stimulation of insulin secretion by BZ-55. It is evident too that the presence of insulin is required for at least one of the changes in the activity of the liver, namely the effect on glucose-6-phosphatase. However, the fact that BZ-55 does not appear to stimulate glucose uptake or glycogen formation in muscle is against this view unless this drug, in addition to stimulating insulin secretion, also has an effect which masks the action of insulin on muscle.

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Studies of the Effect of Carbutamide on Glucose-6-Phosphatase

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In a previous publication[1] it was shown that addition of carbutamide (Amino-phenurobutan, Lilly) to a rat liver microsome preparation in vitro had no significant effect on the glucose-6-phosphatase activity of the microsomes. At the time this work was completed the report of Hawkins and others[2] appeared, showing that oral administration of carbutamide to rats over a period of three weeks led to a decreased glucose-6-phosphatase activity in the liver. The above studies were extended therefore to include the effect of oral administration of carbutamide on the glucose-6-phosphatase activity of the liver of fed and fasted normal and alloxan diabetic rats. This report is concerned with these findings.

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MATERIALS AND METHODS

Male, 150 to 200 gm. albino rats were made diabetic by the intravenous injection of 40 mg. per kg. of alloxan after a forty-eight hour fast. One week following the injection, all of the animals used for the experiments had blood glucose concentrations (not fasted) of 290 mg. per 100 ml. or more.

Incubations and analytical procedures were performed as described previously[1] except that whole liver homogenate was used as the source of glucose-6-phosphatase.

A group of four, sixteen hour fasted animals was

TABLE 1

Summary of results

	Time min.	Weight of rat gm.	Weight liver gm.	Blood sugar change mg. per cent	Blood carbutamide γ/ml.	Liver carbutamide γ/gm.	Liver glucose-6-phosphatase mg.P/gm./15 min.
Group I	0	180	6.2	—	0	0	4.15
Fasted	30	155	5.9	+15	149	96	4.10
normal	60	164	6.1	−10	187	110	4.21
rats	90	170	6.6	−14	194	124	4.23
	120	163	5.9	− 2	144	102	4.72
							4.28±.11*
Group II	0	165	7.5	—	0	0	3.19
Fed	30	155	6.8	−24	144	35	2.97
normal	60	145	5.9	−43	207	48	2.87
rats	90	155	6.9	−59	162	43	3.45
	120	145	5.7	−60	273	62	3.29
							3.15±.10*
Group III	0	150	6.5	—	0	0	4.51
Fasted	30	126	5.1	− 2	115	94	4.63
alloxan	60	145	6.6	+16	125	58	4.68
rats	90	157	6.9	−18	120	52	4.97
	120	162	8.4	−30	156	76	4.27
							4.61±.11*
Group IV	0	180	8.8	—	0	0	4.75
Fed	30	188	7.9	+112	77	28	5.18
alloxan	60	190	9.6	−24	121	54	4.84
rats	90	188	9.4	0	117	40	4.80
	120	182	8.8	+156	96	49	4.49
							4.81±.11*

* Mean and standard error of mean.

given, by stomach tube, 100 mg. per kg. of carbutamide as a 1 per cent aqueous solution. A control animal that had received distilled water instead of carbutamide was killed immediately after dosing; other animals were killed after 30, 60, 90, and 120 minutes. Blood sugar concentrations were determined before dosing and just before killing. Blood and liver concentrations of carbutamide were measured at the time the animals were killed.

RESULTS AND DISCUSSION

The results are summarized in table 1. It will be seen that the dose of carbutamide was sufficient to produce a significant concentration of the drug in the blood and in the liver at 30 to 120 minutes after dosing.

The glucose-6-phosphatase activity of the liver did not change significantly during the first 120 minutes following administration of the drug in any of the four groups of animals. In the group of fed normal rats (Group II) there was a significant drop in blood glucose during this time. The blood sugar changes observed in the diabetic rats were inconsistent, as were those of the fasted normal rats. There does not appear to be any correlation between blood sugar changes and liver glucose-6-phosphatase activity following oral administration of carbutamide.

Mean values of the glucose-6-phosphatase activity were calculated for each group of animals (table 1). In Group II (fed normal rats), the activity was significantly lower than that observed in the other groups. The P value for the difference from each of the other means individually is less than 0.001, indicating a high degree of significance. This is in agreement with the findings of Ashmore and others[3] that fasting and alloxan diabetes both increase glucose-6-phosphatase activity.

SUMMARY AND CONCLUSIONS

Fed and fasted normal and alloxan diabetic rats were given carbutamide in doses of 100 mg. per kg. orally, and were killed after 0, 30, 60, 90, and 120 minutes. At no time was there a significant change in the glucose-6-phosphatase activity of a liver homogenate even though there were significant changes in blood glucose concentrations within this time. Change in liver glucose-6-phosphatase activity is not the cause of the change in blood glucose concentration following oral administration of carbutamide.

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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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