PER OS TREATMENT OF EXPERIMENTAL DIABETES MELLITUS

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At the beginning of 1957, in the Ukrainian Institute of Experimental Endocrinology, was synthesized 4-methylbenzenesulfanilylbutyl urea (T. F. Sysoeva and N. I. Makhnenko). In the Soviet Union this preparation is called butamid; abroad it is called D-860, orinase or tolbutamide (in the USA), rastinon or artosine (in Germany) and dolipol (in France). Like the preparation B-55 (nadisan, invenol, glucidoral or carbutamide) it possesses a hypoglycemic action on per os administration, but in contrast to nadisan it is practically without any antibacterial properties. Treatment of patients with diabetes mellitus lasts throughout life, and so the absence of antibacterial properties is an important advantage of butamid. It is, in addition, less toxic than nadisan.

As previous investigations (1957) have shown, butamide in a dose of 0.5 g per 1 kg body weight lowered the blood sugar of normal rabbits for 24 hours by 33-52%, while the blood sugar level in control animals fell by 8-10% and in dogs (in a dose of 100 mg per 1 kg body weight) – by 23 and 36% compared with 15 and 10% in control experiments. Butamid acted differently on the blood sugar of dogs with alloxan diabetes. In some animals the hypoglycemic sulfonamides lowered the blood sugar and in others its level was not altered. The same thing was observed in dogs with alloxan diabetes after injection of these animals with butamid. This effect evidently depends on the amount of residual islet tissue: the more it is damaged, the weaker the hypoglycemic action of the sulfonamides will appear.

We subsequently set out to discover whether butamid reinforces the action of insulin when injected from outside.

EXPERIMENTAL METHOD

Experiments were carried out on dogs with alloxan diabetes and subjected to total pancreatectomy, requiring considerable amounts of insulin. The animals spent the whole time in metabolism cages. Because of this, their daily dietary intake could be calculated and the excreted carbohydrate estimated, from which the utilized carbohydrate could be determined. The effectiveness of butamid was judged by the intensity of fall of hyperglycemia and glycosuria, by the value of utilized carbohydrate and by the amount of insulin which could be dispensed with by means of butamid. The blood sugar was determined by the Hagedorn-Jensen method, and the sugar in the urine polarimetrically. The investigation was carried out on 4 dogs with alloxan diabetes and receiving large amounts of insulin: 16, 24, 32, and 40 units per day. This quantity of insulin did not abolish the hyperglycemia and glycosuria. Butamid was injected into all the dogs in a dose of 100 mg per 1 kg body weight. 84 experiments were performed on each dog. In some experiments (control) the effects were studied on hyperglycemia, glycosuria and assimilation of carbohydrate of insulin alone, and in others – of insulin and butamid. Experiments of a similar type were carried out also on dogs subjected to total pancreatectomy. These received daily 150 g of raw pancreas in addition to their usual diet. We performed 134 experiments on the dog Mal'chik, and 66 on Pushok. Insulin was injected twice daily into the animals – at 9 a.m. and 4 p. m., and butamid – at 9 a.m. (except in a few experiments when it was injected at 2 p.m.).

EXPERIMENTAL RESULTS

The results in Table 1 demonstrate the considerable reinforcement by butamid of the insulin effect. The same results were obtained in comparable experiments on all 4 dogs.

Later on we attempted to discover the intensity of action of butamid – the amount of insulin which could be replaced by butamid. For this purpose the doses of insulin injected into the dogs were gradually reduced to values which, together with butamid, would give the same effect as large doses of insulin alone.

TABLE 1

The Effect of Butamid on Alloxan-Diabetic Dogs

Dogs	Sivka				Torba			
Data of experiments	13—22/VII	23—27/VII	14—20/IX	21—30/IX	13—22/VII	23—27/VII	11—15/IX	16—26/IX
Number of experiments	10	5	7	10	10	5	5	11
Dose of insulin, units	24	24+B *	24	24+B	24	24+B	36	36+B
Hyperglycemia, in mg%	349	211	302	313	348	229	418	277
Glycosuria, g	45	5	68	44	32	3	37	4
Assimilation of carbohydrate, g	119	148	94	154	164	192	161	194

* In this and the subsequent tables, B – butamid.

The figures in Table 2 show that butamid reinforces the action of insulin by 25, 33 and 50%. This means that with the aid of butamid it is possible to reduce the insulin requirement of alloxan-diabetic dogs by 25-50% respectively.

What is the mechanism of reinforcement of the insulin effect by butamid?

The sulfanilamides are known not to influence the blood sugar level of animals from which the pancreas has been removed. On this basis Loubatières put forward the hypothesis that the sulfanilamides stimulated the β-cells of the insulin apparatus. This hypothesis was supported by several authors [1, 4, 5, 6, 9].

Although in the lowering of the blood sugar by sulphanilamides, depression of the glucose-6-phosphatase activity of the liver may possibly have some importance, together with a reduction in the absorption of glucose from the intestine and other factors, Holt et al. [1] nevertheless assert that the main role in this activity belongs to stimulation of the β-cells and not to extrapancreatic reinforcement of the insulin effect. Some authors have tried to prove the importance of suppression of the α-cells in the hypoglycemic effect of the sulfanilamides. However investigation of the factors quoted as evidence in favor of this action of the sulfanilamides failed to confirm them.

Nevertheless there appeared recently a paper by Schmidt and Blobel [10], which showed that under the influence of D-860, rats always showed a reduction (statistically significant) in the nuclear volume of the α-cells of the insulin apparatus.

If depression of the α-cells is really of importance in the hypoglycemic effect of the sulfanilamides, it is not clear why butamid, like nadisan, does not affect the blood sugar level in animals with severe alloxan diabetes. It is not clear why the effect of sulfanilamides is not shown in patients with severe diabetes mellitus. From the point of view of the importance of the β-cells in the hypoglycemic effect of these preparations, its absence in some of them may be due to the small number of β-cells which are left or to severe weakening of their function.

But how do we explain the reinforcement of the insulin effect in all the alloxan-diabetic animals? It seemed to us that this effect in the dogs with severe alloxan diabetes depends not on the action of butamid on the pancreas but on its action on the insulin effect itself. We verified this hypothesis on totally depancreatized dogs receiving a definite amount of insulin which did not abolish the hyperglycemia and glycosuria (Table 3).

TABLE 2

Intensity of the Action of Butamid on Alloxan-Diabetic Dogs

Dogs	Aza			Kutska			Sivka		Torba	
Date of experiments	13—18/VII	20—22/VII	23—27/VII	13—21/VII	16—20/IX	21—30/IX	13—22/VII	28/VII—3/VIII	11—15/IX	13—22/VII
Number of experiments	6	3	5	9	5	10	10	7	5	5
Dose of insulin, units	32	16	16+B	16	12	12+B	24	16+B	36	24+B
Hyperglycemia, mg%	209	284	195	207	342	244	349	175	418	229
Glycosuria, g	8	71	8	6	63	10	45	4	37	3
Assimilation of carbohydrate, g	141	105	127	141	130	165	119	128	161	192

The results in Table 3 show that additional injections of butamid considerably reinforce the insulin effect in totally depancreatized animals. Thus, after injection of 40 units of insulin with butamid to the dog Mal'chik, the hyperglycemia fell from 298 to 123 mg%, the glycosuria from 9 to 0 g, and the carbohydrate assimilation increased from 92 to 114 g. After injection of butamid with 20 units of insulin the hyperglycemia fell from 339 to 233 mg% and the glycosuria from 33 to 8 g, and the carbohydrate assimilation rose from 71 to 136 g. From Table 3 it can also be seen that butamid sharply reinforced the insulin effect on the dog Pushok also. Table 3 further shows that butamid in the dog Mal'chik can reduce the insulin requirement by 20 units. Butamid will replace the same amount of insulin in the dog Pushok also.

After the conclusion of the present investigation we became aware of the papers of Sirek and Sirek [11] and by Campbell. These workers also found it possible to replace a considerable amount of insulin in totally depancreatized animals by nadisan — 50-75%, and in one dog they reduced the insulin intake to 1 unit for a period of 8-10 days. On the other hand, Sirek and Sirek [11] were unable to obtain any reaction to injection of nadisan (in addition to insulin) in one dog after total removal of the pancreas.

Considering the large number of experiments which we performed on 6 diabetic dogs, and also the findings of other workers, it may be concluded that nadisan and butamid considerably reinforce the insulin effect after total removal of the pancreas.

Attention is drawn to the fact that in our experiments butamid reinforces the insulin effect in alloxan-diabetic and totally depancreatized dogs to almost the same intensity — by 25-50%. On this basis it can be assumed that the action of butamid on alloxan-diabetic dogs is mainly expressed as reinforcement of the insulin effect and not as stimulation of the β-cells of the pancreas. It is possible also that the effect of butamid on the blood sugar level in normal animals depends not only on the stimulation of the secretion of insulin by the pancreas but also on reinforcement of the action of the insulin. Although the ability of butamid to stimulate the activity of the β-cells has been convincingly proved, the almost identical intensity of reinforcement of the insulin effect in alloxan-diabetic and totally depancreatized dogs gives rise to doubt of the geat importance in the mechanism of action of butamid of stimulation of the β-cells by the drug.

TABLE 3

The Effect of Butamid on the Metabolism of Totally Depancreatized Dogs

Dogs	Mal'chik				Pushok	
Duration of diabetes mellitus	over 8 years				over 4 years	
Date of experiments	28/V—12/VI	13—21/VI	6—18/VII	19—21/VII	4—17/VIII	18—28/VIII
Number of experiments	15	9	13	3	14	11
Dose of insulin, units	40	40+B	20+B	20	26	12+B
Hyperglycemia, mg%	298	123	233	339	363	322
Glycosuria, g	9	0	8	33	21	30
Assimilation of carbohydrate	92	114	136	71	82	81

How may the effect of butamid on insulin activity be represented in the absence of the pancreas? Even recently attempts were made to explain the action of butamid as weakening the activity of insulinase [7, 8]. However it was quickly shown that the action of butamid on insulin is apparent only at very high concentrations, and in therapeutic doses it does not affect the rate of destruction of insulin in the body to any great extent. The possibility is not excluded that in the presence of butamid, glycogen formation is intensified under the influence of insulin. It follows from the experiments discussed that the main action of butamid is to reinforce the insulin effect.

SUMMARY

It was shown by experiments on dogs with alloxan diabetes and those completely depancreatized that the amount of insulin required for the maintenance of a definite condition of diabetes mellitus is considerbly decreased (by 25-50% under the effect of butamide, synthetized in the Ukrainian Institute of Experimental Endocrinology).

This definite condition of diabetes mellitus was evalutated by the level of hyperglycemia, glucosurea and the quantity of the absorbed carbohydrates. There was almost an equal potentiation of insulin action by butamide in both groups of animals. Thus, a conclusion was drawn that the main effect of butamide consists in the potentiation of the insulin effect.

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