THE EFFECT OF BZ 55 ON THE PANCREATIC ISLETS

By

H. F. L. Schöler and J. H. Gaarenstroom

In normal subjects and in experimental animals BZ 55 lowers the blood sugar level (Achelis et al., 1955, and many others) and is therefore used for this purpose in diabetes. There is no agreement as to the mechanism of action of BZ 55. In the hope of throwing more light on this mechanism, the effect of prolonged treatment with BZ 55 on the blood sugar level and the histology of the pancreas was investigated. For comparison other substances were used which are known:

1. to damage the β-cells of the islets of Langerhans,
2. to inactivate the β-cells and
3. to stimulate the β-cells.

MATERIAL AND METHODS

Rats of a T.N.O.-strain were used weighing 150–200 gm. Males and females were used without discrimination, since it was previously found that the results were independent of sex.

I. Six rats were given a single injection in the tail vein, consisting of 40 mg./kg. of alloxan as a 1% solution. To the four rats of this group which during the following 10 days showed a constant glycosuria as measured by Benedict’s qualitative test, 500 mg./kg. of BZ 55 (the sodium salt in 20% solution) were administered by stomach tube. Immediately before administration and thereafter at hourly intervals for 5 hours, blood was taken from the tail vein and the blood sugar content determined by the Hagedorn-Jensen micromethod.

An equal dose of BZ 55 was administered to four normal rats in a similar manner and their blood sugar concentration determined at the same intervals.

Immediately after the sixth blood sample had been taken, the rats were decapitated and the pancreas prepared for histology (for the method see later).

The object of experiments II to VI was to study the effect of prolonged treatment with various substances on the blood sugar concentration. The compounds were ad-
ministered once a day. The fasting blood sugar levels were determined twice a week. When the results of the determinations had been obtained, the animals were sacrificed and the pancreas examined histologically.

II. Six rats were injected with 0.5 U. of protamine zinc insulin (Roxane) subcutaneously twice a day. Owing to the high mortality the experiment was discontinued after 3 weeks. The experiment was repeated in a somewhat modified form: 18 rats were injected subcutaneously four times a day with 1/4 I. U. of insulin. The blood sugar values of these animals were not determined. After thirty days only 7 rats had survived; these received single doses as follows: 2 rats 500 mg./kg. of BZ 55 by stomach tube; 2 rats 500 mg./kg. of glucose by stomach tube; 2 rats 40 mg./kg. of alloxan by intravenous injection and 1 rat 0.5 ml. of 0.85% NaCl solution by stomach tube.

III. Six rats received 500 mg./kg. of BZ 55 daily by stomach tube for 13 weeks. The experiments were repeated with 15 rats, the injections lasting in this case for 9 weeks.

IV. Six rats received 5 ml./kg. of 0.85% NaCl solution daily by stomach tube for 13 weeks. A second group of 15 rats received saline for 9 weeks.

The experiments III and IV were performed a third time with rats of another strain (UL), 15 rats receiving BZ 55 and 15 saline for 10 weeks (Addendum).

V. Six rats received 10 mg./kg. of alloxan (1% solution) daily by intravenous injection in the tail vein for 7 weeks.

VI. Six rats received 500 mg./kg. of glucose (50% solution) daily by stomach tube for 13 weeks.

A few additional experiments were also performed:

Four normal rats were given 4 I. U. of insulin by subcutaneous injection; 2 hours later the rats were decapitated and the pancreas removed and prepared histologically.

Four rats were given 500 mg./kg. of glucose by stomach tube; 2 hours later the rats were decapitated and the pancreas similarly prepared for histology.

Four rats were given 1 gm./kg. of isopropyl sulphathiazole (IPTD) dissolved in 2 N NaOH (pH 7.6–8.0); 48 hours later the animals were sacrificed and the pancreas again prepared for histology.

Histology of the pancreas: The pancreas was fixed in freshly prepared Bouin’s fluid. 3 µ sections were cut and stained with aldehyde-fuchsin solution of Gomori (1950) as modified by Scott (1952); differential staining was performed by Masson's trichrome method.

Statistical analyses of all experiments were made with a confidence coefficient of 0.95.

RESULTS

I. A single high dose of BZ 55 given to rats was found to cause a similar fall in the blood sugar concentration in accordance with the literature. The same dose of BZ 55 had no effect on rats that had previously been rendered diabetic with alloxan (Fig. 1). It is remarkable that in non-diabetic rats which were injected with BZ 55, degranulation of the β-cells was found (Plate I No. 1 and Plate I No. 2). In the alloxan diabetic rats treated with BZ 55, there were no further changes in the β-cells, other than the usual degeneration which occurs after a diabetogenic dose of alloxan: the α-cells remained unaltered (Plate I No. 3 and Plate I No. 4).
Fig. 1.
To the left: Effect of 500 mg./kg. BZ 55 on the blood sugar level of normal rats.
To the right: Effect of 500 mg./kg. BZ 55 on the blood sugar level of alloxan diabetic rats.

Fig. 2.
Fasting blood sugar level after daily administration of protamine-zinc-insulin (PZI).
The horizontal lines is the mean level of the control animals and the levels of the standard error in the controls.
II. It is known from the literature that prolonged treatment of rats with insulin causes a degranulation of the \( \beta \)-cells, which Nerenberg (1953) regards as an »inactivation degranulation«, since regranulation commences six days after the insulin injections have been discontinued. It appears that glucose markedly promotes this regranulation process.

In our experiment with PZI the expected hypoglycaemia did occur (Fig. 2). The mortality was so high that only two of the six rats were still alive after 28 days and the \( \beta \)-cells were found to be totally degranulated (Plate II No. 5).

Repetition of the experiment with a larger number of animals gave similar
Plate II.
Pancreatic islet of normal rat after: (5) long term insulin treatment; (6) prolonged PZI treatment followed by glucose administration; (7) prolonged insulin treatment followed by BZ55 administration; (8) chronic insulin treatment followed by alloxan administration; (9) a single injection of insulin.
Fig. 3.
Fasting blood sugar level after daily administration of BZ 55 (6 rats) + urine sugar positive.

Fig. 4.
Fasting blood sugar level after daily administration of BZ 55 (15 rats).
results. Seven rats survived out of the initial eighteen. Attempts were made
to bring about the regranulation of the $\beta$-cells of these animals. The small
number of animals used did not allow us to attach much significance to the
results obtained in this section of the experiment. From Plate II No. 6 it seems
that regranulation actually occurs 24 hours after glucose administration, as
described by Nerenberg (1953). On the other hand neither with BZ 55 nor
with alloxan could regranulation be initiated (Plate II No. 7 and 8).

It is noteworthy that alloxan did not further impair $\beta$-cells degranulated
by insulin, whereas a similar (diabetogenic) dose of alloxan caused complete
degeneration of the $\beta$-cells in animals which had not previously been treated.

A single dose of insulin did not bring about degranulation, on the contrary
it seemed that the $\beta$-cells were granulated more markedly than normal (Plate II
No. 9).

III. Prolonged treatment with BZ 55 caused a very gradual, but unmistakable
increase in the fasting blood sugar level. As can be seen from Figs. 3 and 4.
the first glycosuria ensued after about 7-9 weeks at a fasting blood sugar level
of about 170 mg. %o. On the subsequent days the fasting blood sugar levels rose
to almost 180-200 mg. %o and the glycosuria persisted.

Degranulation of the $\beta$-cells was again the main feature of the histological
picture of the pancreas after treatment with BZ 55 (Plate III No. 10). The cell
nuclei were perhaps slightly enlarged, but no other signs of degeneration were
found. The $\alpha$-cells were likewise found to be completely normal after pro-
longed treatment with BZ 55.

IV. The trend of the fasting blood sugar levels after prolonged treatment with
saline is shown in Figs. 5 and 6. The pancreas of this group of animals served
as control (Plate I No. 2 and Plate III No. 14).

V. Prolonged treatment with low doses of alloxan which were not immediately
diabetogenic likewise gave a gradual though a more rapid rise in the fasting
Plate III.
Pancreatic islet of normal rat after: (10) chronic treatment with BZ 55; (11) chronic treatment with non-diabetic doses of alloxan; (12) chronic treatment with glucose; (13) a single dose of glucose; (14) no treatment; (15) a single dose of IPTD.
blood sugar levels (Fig. 7). Glycosuria was noted 4 weeks after the first administration, and after 7 weeks the fasting blood sugar levels were 200 mg. %.

At this time the rats were sacrificed. As in chronic treatment with BZ 55, histological examination revealed total degranulation of the $\beta$-cells (Plate III
No. 11). It is important to note that by this form of treatment no degeneration occurred unlike that observed after a single diabetogenic dose of alloxan (Plate I No. 4).

VI. Glucose was chosen as the stimulus for the $\beta$-cells. Apart from a few incidental increases, no abnormalities were found in the fasting blood sugar levels. These rats were also sacrificed after 13 weeks. In the pancreas an increase in granulation of $\beta$-cells was noted (Plate III No. 12). These cells seemed to show more granules than those in the normal animals.

In contrast to what is observed after prolonged treatment, a single dose of glucose administered by stomach tube gives degranulated $\beta$-cells (Plate III No. 13).

Plate III No. 15 shows a section of pancreas after administration of IPTD. This picture is given in order to show that the histological staining method applied to the islets of Langerhans demonstrates any changes in the $\alpha$-cells, like the damage to these cells caused by the latter substance.

**DISCUSSION**

The absence of any changes in the $\alpha$-cells of the islets of Langerhans in the present experiments lends no support to the opinion expressed by Ferner & Runge (1956) that BZ 55 damages the $\alpha$-cells.

As these experiments have again confirmed, the fall in blood sugar caused by BZ 55 does not occur in alloxan diabetic rats and thus differs from the similar effect of substances such as IPTD and synthaline (Von Holt et al., 1955) which are known to damage the $\alpha$-cells. Further this indicates the importance of functioning $\beta$-cells for the effective action of BZ 55, a suggestion already put forward by Loubatière (1955). It is possible therefore, that BZ 55 either:

1. reinforces the action of endogenous insulin;
2. stimulates the production of endogenous insulin, or
3. promotes the release of endogenous insulin.

Let us examine these three possibilities in greater detail.

1. In their investigations with insulinase and insulinase inhibitors, Mirsky et al. (1956) observed that BZ 55 shows an «insulinase inhibitor» action. However, the amount of BZ 55 needed for inhibition of insulinase is far in excess of the doses usually given to patients (Bernson et al., 1957). Further it is questionable if BZ 55 has an insulin sparing action. According to Fritz et al. (1956) it does not reinforce the effect of exogenously administered insulin in depancreatized dogs. Caren & Corbo (1957) on the other hand, found a potentiation of small doses of insulin by tolbutamide.

2. In contrast to the assumption that BZ 55 stimulates the production of endogenous insulin is the fact that even a single dose of BZ 55 brings about de-
granulation of the β-cells. Another point against the stimulation theory is the observation that the insulin content of the pancreas in dogs does not increase after the administration of «therapeutic» doses of BZ 55 and actually decreases when toxic doses are given (Root, 1957). Further, if stimulation occurred, it would be expected that the administration of BZ 55 would promote regranulation, when the β-cells were in a state of «inactivation degranulation» as a result of insulin administration. In the few animals used for these experiments the administration of glucose (which can be assumed to bring about insulin production) did lead to regranulation, whereas this was not the case with BZ 55.

3. In our opinion the fall in the blood sugar level and the concurrent degranulation of the β-cells after the administration of a single dose of BZ 55 are more probably an indication that the insulin that was already present in these cells is released by the administration of BZ 55. Something analogous to what happens with alloxan can be postulated. The latter substance is assumed to bring about a discharge of the insulin present in the pancreas (Wrenshall et al., 1950, Maske, 1956). In some respects the effect of BZ 55 is the same as that of alloxan. Both alloxan and BZ 55 may cause hypoglycaemia. As is shown by our experiments, the prolonged administration of either of these substances is followed by a gradual rise of the fasting blood sugar level, the histological picture of the pancreas showing the same alteration. The β-cells probably gradually enter into a phase of exhaustion on administration of either of these substances. In this phase the animals react by showing glycosuria and diabetic blood sugar levels. Alloxan can be considered as promoting degeneration of the β-cells, whereas with BZ 55 this process is stopped at the degranulation stage.

Support for the present experiments was given in a paper read by Von Holt (1955) at the Symposium on «Les drogues Hypoglycémiantes» held at Brussels on 8–9 December, 1956 (cited by Querido, 1957). Von Holt stated that rats and dogs showed diabetic blood sugar curves after long-term treatment with BZ 55.

A final elucidation of the action mechanism of BZ 55 cannot be given on the basis of the data so far available.

ADDENDUM

Chronic administration of the same amount of BZ 55 to an other strain of rats (UL-strain) did not result in a definite increase of the blood sugar content to diabetic levels. However, a tendency to rise is obviously present and significant, as shown in Figs. 8 and 9. The difference between these data and those obtained from the rats belonging to the TNO-strain suggests that in rats at least there exists considerable variation in susceptibility towards BZ 55.
**SUMMARY**

A single injection of 500 mg./kg. of BZ 55 caused a fall in the blood sugar level and degranulation of the $\beta$-cells of the pancreatic islets in rats.

Daily administration of the same amount of BZ 55 for 9–13 weeks was followed by a gradual increase in the fasting blood sugar. After several weeks a diabetic level was reached. In this case, the $\beta$-cells were found to be degranulated.

Daily treatment for about 6 weeks with one fourth of the amount of alloxan
which causes acute diabetes gave similar results to those obtained with BZ 55, i.e. a diabetic blood sugar level after treatment for a few weeks and degranulation (not degeneration) of the cells.

These results are discussed. Evidence is put forward which suggests that BZ 55 causes a release of the insulin content of the \( \beta \)-cells.

REFERENCES

Ferner, H.: Vortrag. Deutsche Ges. Pharm. (ref.).