pounds. The inhibition caused by diacetyl is abolished when the rats are fed 0·06% 3'-Me-DAB over 4 weeks.

Sulfhydryl compounds may have enhancing or inhibiting effect on mitochondrial swelling which suggests that it is not the mere presence of –SH groups or the reducing properties that are determining, but rather the general structural pattern of the whole molecule. In fact, at the same molar concentration of 1×10^{-2} reduced glutathione causes considerable enhancement of swelling (cf. [7]), sodium thiosulfate has no effect, while 2,3-dimercaptopropanol causes nearly total inhibition. α-Lipoic acid gives some enhancement at 1×10^{-3} and at 5×10^{-3} *M*/l, while the unsubstituted *n*-octanoic acid causes total inhibition at 5×10^{-3} *M*/l.

Tentatively the following mechanism is suggested for the action of azo-dyes at the level of the mitochondria: The many structurally unrelated biological agents, which affect the rate of metabolism, may do so by acting on reversible structural changes in the dynamic mitochondrial membrane (e.g. [8]). The azo-carcinogens act at this level by inhibiting swelling, possibly through cross-linking of the elastic membrane (cf. [9]). Thus, the membrane may escape certain metabolic regulatory stimuli because of an acquired greater structural rigidity, that is a new macromolecular pattern which, once established, is transmitted to the subsequent generations of cells. The large increase of cystine content of the mitochondria, when passing from the liver to the hepatoma [10] is not inconsistent with this concept.

A full account of these and related investigations will be given elsewhere.

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Cancer Research Laboratory, The J. Hillis Miller Health Center, University of Florida, Gainesville, April 9, 1959.

Résumé

Dans des groupes de rats nourris à un régime contenant du 3'-méthyl-*p*-diméthylaminoazobenzène, on constate, qu'il y a un accroissement soudain, à 4 semaines, de l'incidence de tumeurs hépatiques. Cette observation établit une correlation directe entre la cancérogenèse et le minimum des courbes de gonflement des microsomes et des mitochondries de foies de rats, nourris dans les mêmes conditions.

[7] A. L. Lehninger and M. Schneider, J. biophys. biochem. Cytol. *5*, 109 (1959).

[8] P. Siekevitz and M, Watson, J. biophys. biochem. Cytol. *2*, 639 (1956).

[9] M. Arcos and J. C. Arcos, Arzneimittelforschung *8*, 643 (1958).

[10] B. S. Schweigert, B. T. Guthneck, J. M. Price, J. A. Miller, and E. C. Miller, Proc. Soc. exp. Biol. Med., N.Y. *72*, 495 (1949).

Potentiating the Diabetogenic Effect of Alloxan by N-sulphonyl-N-butylurea (BZ-55)

Two important problems related to the use of recent hypoglycaemic sulphonamides (oral antidiabetics) await elucidation.

First, does their action in the intact animal depend on the presence of insulin or functioning pancreatic β-cells. Secondly, can these substances under certain circumstances exert an unfavourable effect on the metabolism of β-cells [1, 2].

[1] W. Creutzfeldt, Dtsch. med. Wschr. *1956*, 841.

[2] R. Levine, Ann. N.Y. Acad. Sci. *71*, 291 (1957).

In our previous work, we found that the blood level of reduced glutathione is not affected by the administration of BZ-55 [3], as it is the case with some known diabetogenic substances [4]. On the other hand, we observed that in rats the diabetogenic effect of alloxan after the administration of BZ-55 was increased [3]. In the present paper, we report observations of the same effect in mice and a time analysis of this phenomenon, i.e. we investigated the influence of the time of BZ-55 administration in acute and chronic experiments.

White mice (strain H), kept under standard conditions and fed on a Larsen mixture, were used. BZ-55 in suspension (Invenol Hoechst) was administered orally 1000 mg/kg in the acute and 500 mg/kg in the chronic experiment. Alloxan was prepared by oxidation of barbituric acid [5]. Blood sugar was estimated, using a modification of Somogyi-Nelson's method [6].

A total of 160 mice were divided into 8 groups. In the first group the animals received a dose of 1000 mg/kg of BZ-55 only, administered by tube. The second group received alloxan 10 min prior to the administration of BZ-55, the third, fourth, and fifth group alloxan 2, 6, and 24 h after the BZ-55. To the sixth and seventh group, we administered doses of BZ-55 of 500 mg/kg *per diem* for one or three weeks respectively. 24 h after the last dose of BZ-55, they were also given alloxan. The mice in the eighth group received alloxan only. The animals in all groups were fasted for 6 h before the experiment and were fed half an hour after the administration of alloxan. As a criterion of diabetogenic potency, we estimated the blood sugar level on the 3rd day after the administration of alloxan (the animals were fasted for 6 h before estimating the blood sugar), the percentage of diabetic animals (i.e. with a blood sugar level above 250 mg) and the mortality rate during ten days.

The administration of BZ-55 alone caused a drop of the blood sugar from the average value of 130 mg% to 100 mg% in 2 h, 95 mg% in 4 h, 85 mg% in 6 h, and to 115 mg% in 24 h.

The other results are summarized in the Table. The maximal, statistically highly significant, potentiation of the diabetogenic effect of alloxan was found during the 2nd and 6th hour after the administration of BZ-55. In the other time intervals, there was a tendency towards deterioration of the diabetes. In the group where BZ-55 was administered for 3 weeks, the blood sugar level was higher than in the control group, the difference being statistically significant.

It is known that alloxan acts as a selective poison on β-cells of the pancreas. Its toxicity, however, depends to a considerable degree on the functional state of the β-cells. Thus e.g. in fasting rats [7], in rats fed on a high-fat diet [8], or after the administration of exogenous insulin in mice [9], the diabetogenic effect of alloxan is enhanced. The β-cells degranulate under these conditions and the pancreas contains less extractable insulin. It is generally believed that under these circumstances the β-cells are less active [10]. Conversely, realimentation after fasting [4, 9] glucose ad-

[3] B. Mosinger, unpublished results.

[4] P. de Moor, *Le diabète alloxanique* (Masson et Cie, Paris 1953).

[5] R. Adams, Organic Synthesis *32*, 6 (1952).

[6] H. Frank und E. Kirberger, Biochem. Z. *320*, 359 (1950).

[7] E. H. Kass and B. A. Waisbren, Proc. Soc. exp. Biol. Med. *60*, 303 (1945).

[8] B. A. Houssay and C. Martinez, Science *105*, 548 (1947).

[9] B. Mosinger, *Alloxan diabetes* (Thesis, Prague 1958).

[10] C. H. Best and N. B. Taylor, *The Physiological Basis of Medical Practice* (The Williams and Wilkins Co., Baltimore 1945).

Changes in the diabetogenic effect of alloxan (250 mg/kg subcutaneously), administered to mice 10 min before, 2, 6, and 24 h after BZ-55 respectively, and 24 h following the daily administration of the same drug for 1 and 3 weeks

Alloxan administered after BZ-55	Number of animals	Average glycemia mg%	Percentage of diabetic animals *	Mortality rate in 10 days in %
−10 min	19	259±23	47·2	5·0
+2 h	20	467±56+	73·4+	65·0+
+6 h	7	510±48+	100·0+	57·0+
24 h	20	244±22	37·5	20·0
+24 h (after administration for one week)	15	231±36	35·7	20·0
+24 h (after administration for three weeks)	13	385±60+	69·2	21·4
Control—Alloxan alone	20	218±25	31·7	5·0

* glycemia above 250 mg%

\+ statistically significant difference as compared with control ($P = 0{\cdot}01 - 0{\cdot}02$)

ministration closely before alloxan injection in mice[9], glucagon in dogs[11], or adrenalin in rabbits[4], protect the β-cells from alloxan. Under these circumstances, β-cells are stimulated to increased activity.

Our experiments suggest that BZ-55 increases the diabetogenic effect of alloxan. We may thus conclude that this substance causes a state of *functional hypoactivity* of the β-cells. This may be due to the direct effect of BZ-55 or secondary to the decrease of the blood sugar or both factors. It will be necessary to make a more detailed analysis of these factors. So far we can only say that the factor of hypoglycemia could not play a role in the group of mice which were given alloxan 10 min prior the BZ-55 and 24 h after BZ-55 had been administered for three weeks. The tendency of potentiation of the diabetogenic effect of alloxan, however, exists. No signs of protection of β-cells were detected as might be expected if BZ-55 exerted a favorable effect on their metabolism and increased their functional activity.

This seems to be in disagreement with the hypothesis of some authors[2] that the hypoglycemic action of BZ-55 is due to stimulation of the β-cells. It cannot, however, be ruled out that after the administration of BZ-55 insulin is released in some unknown (more or less passive) manner. Our finding is in agreement with the views of other authors[12,13] who, as a result of histological changes of the β-cells in rabbits, assume that after the administration of BZ-55 these cells are in a quiescent stage similarly to that during fasting or after the administration of exogenous insulin.

B. MOSINGER and T. BRAUN

Institute for Human Nutrition, Physiological Department, Prague, April 9, 1959.

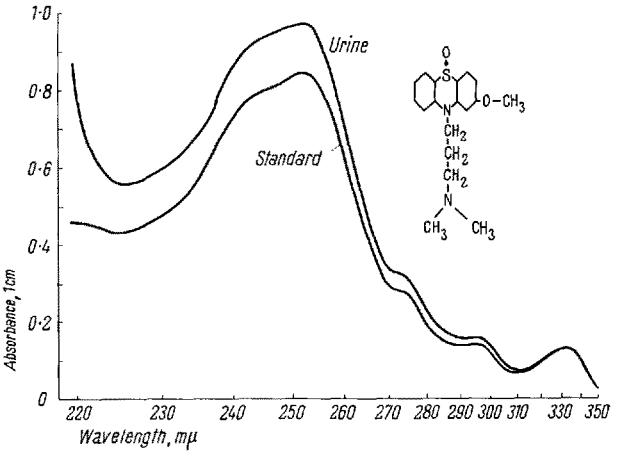
Zusammenfassung

Es wird festgestellt, dass im voraus verabreichtes BZ-55 die diabetogene Wirkung des Alloxans bei Mäusen potenziert.

[11] J. L. ARTETA and A. CARBALLIDO, J. Endocrin. *15*, 243 (1997).
[12] H. MASKE Dtsch. med. Wschr. *1956*, 899.
[13] W. CREUTZFELDT und H. FINTER, Dtsch. med. Wschr. *1956*, 892.

On the Urinary Elimination of Methoxypromazine in Man

Methoxypromazine[1] (MPZ) is a phenothiazine derivative, which has recently been introduced as a tranquillizer. Chemically it is closely related to chloropromazine (CPZ), although the 2-Cl group has been substituted for a 2-methoxy group. During a clinical trial of the drug it was thought of interest to determine the urinary output to check that the drug was taken by the patients and to get an idea of the metabolism.



U.V. absorption curves of methoxypromazine sulfoxide (standard) in aqueous solution (acetate buffer pH 5·6 acidified with sulfuric acid) and of the metabolite, extracted from urine according to SALZMAN and BRODIE[3], in the same solvent.

MPZ (maleate) in aqueous solution shows a characteristic ultraviolet absorption with a main maximum at 251 mμ and a low maximum at 302 mμ. This is closely analogous to that of CPZ[2,3] and other 10-aminoalkylphenothiazines[4]. The colour reaction characteristic of some phenothiazine compounds with concentrated sulfuric acid (DUBOST and PASCAL[5] and others) is also obtained. On addition of an equal volume of sulfuric acid to MPZ in aqueous solution, a red colour (absorption peak at 565 mμ) develops, which is fairly stable, but the absorbance is not very reproducible. Also this reaction is less sensitive than the U.V. absorption. Extraction of MPZ is easily performed from an alkaline aqueous solution with ether, and from ether to an acid aqueous phase, with recoveries around 90%. The acid aqueous extract is suitable for direct U.V. spectrophotometry or the sulfuric acid reaction. Absorption curves for the red colour obtained with such extracts from the urine of patients receiving 300–375 mg MPZ daily were similar to those for pure MPZ, but the U.V. absorption curves showed characteristic deviations indicating that metabolites might be present. In fact the curves were similar to those of CPZ sulfoxide[3], showing maxima at 252, 273, 296, and 332 mμ and a 'shoulder' at about 242 mμ (Fig.).

In order to establish whether the metabolite was identical with MPZ sulfoxide, this compound was synthesized

[1] Mopazine —, produced by A. B. Astra, Sweden, and Rhône-Poulenc S.A., France.
[2] N. P. SALZMAN, N. C. MORAN, and B. B. BRODIE, Nature *176*, 1122 (1955).
[3] N. P. SALZMAN and B. B. BRODIE, J. Pharmacol. exp. Therap. *118*, 46 (1956).
[4] R. DAHLBOM and T, EKSTRAND, Acta chem. scand. *5*, 102 (1951).
[5] P. M. DUBOST and S. PASCAL, Ann. pharm. franç. *11*, 615 (1953).