Résumé

Le fait que le fructose disparaît de la semence incubée *in vitro* est utilisé par plusieurs chercheurs pour déterminer l'activité métabolique des spermatozoaires. La quantité du fructose est dosée par le Résorcinol. Ce dosage est très pratique pour mesurer la teneur en fructose et la fructolyse dans le cas de la semence du Bœuf. Mais, puis-que la semence humaine contient d'autres produits aussi réductibles que le fructose, cette méthode ne convient pas au sperme humain.

Nous avons essayé de séparer le fructose de la semence humaine par la chromatographie circulaire, et de le doser ensuite quantitativement en utilisant le tetrazoliumchloride du triphényl. Cette méthode donne des résultats précis pour déterminer la teneur en fructose et la fructolyse dans la semence humaine.

Studies on the Swelling of Rat-Liver Mitochondria in Relation to Tumor Incidence During Feeding of Aminoazo Dyes

It was reported previously by one of us[1] that the swelling ability of rat liver microsomes decreases during feeding of 3'-methyl-*p*-dimethylaminoazobenzene (3'-Me-DAB), reaching a minimum level at 4 weeks. Even though the feeding of the dye is continued, this swelling ability eventually recovers and reaches a normal level at about 20 weeks. The microsomes from hepatoma, induced with 3'-Me-DAB, swell to an extent comparable to those of the liver after 4 weeks of feeding of this same dye. The non-carcinogenic isomer, 2-Me-DAB, however, does not produce these effects.

This report[2] describes a correlation that has now been established between microsomal swelling and the appearance of gross tumors. Table I shows that if the feeding of 3'-Me-DAB to rats is continued beyond 4 weeks under our experimental conditions, the animals rapidly reach a point of no return, since the percentage of tumor incidence in the groups shows a sudden steep rise at about 4 weeks.

The fine structural alterations in the cell, that can be detected by the study of swelling, during chemical carcinogenesis are, however, not restricted to the endoplasmic reticulum, origin of the 'microsome' fraction. Of more particular interest in this respect are the findings of EMMELOT and Bos, that the thyroxine-induced swelling of rat liver mitochondria decreases after feeding DAB for 5 months[3] or by incubating these particulates *in vitro* with carcinogens[4].

Table II shows alterations of the mitochondrial swelling, essentially similar to those of microsomal swelling during feeding 3'-Me-DAB[1]. There is a minimum swelling with the liver at about 4 weeks, and low values have been observed with the hepatoma. As with the microsomes[1], no appreciable change in mitochondrial swelling was observed during feeding 0·06% of the non-carcinogenic dye, 2-Me-DAB, for 6 weeks.

Table I

Groups, of 16 male Sprague-Dawley rats each, were fed a riboflavin-low semi-synthetic diet[1], containing 0·06% 3'-Me-DAB, for periods varying from 1 to 12 weeks. After the respective periods of dye feeding the animals were continued to be fed the same diet without dye, and were sacrificed after a total of 7 months[5].

Weeks of feeding	No. of tumor bearers over total survivors	Percentage of tumor incidence
1	0/14	0 %
2	0/15	0 %
3	1/13	7·5%
4	4/16	25 %
5	8/16	50 %
6	11/17	65 %
8	16/16	100 %
10	16/16	100 %

The differences between the macromolecular organization of liver mitochondria and of hepatoma mitochondria are evident not only from the extent of swelling, but also from its pH dependence (cf. [1]). Swelling was studied in 0·17 *M* sucrose, and in 0·30 *M* sucrose in presence of 1×10^{-5} *M*/l thyroxine or of 5×10^{-3} *M*/l $CaCl_2$. The pH range was from 5·0 to 9·6. All curves obtained with liver mitochondria show relatively sharp maxima around pH 7·4, while the curves obtained with the hepatoma were rather flat with no clear-cut maxima.

Table II

Animals and diet were identical to those described in Table I. The method of isolation of the mitochondria and the swelling test (40 min) were essentially those described by TAPLEY[6]. Percentage swelling was calculated as in previous work[1].

'Percentage Swelling'

Weeks of dye feeding	0·17 *M* sucrose	0·30 *M* sucrose 1×10^{-5} thyroxine	0·30 *M* sucrose 5×10^{-3} $CaCl_2$	0·30 *M* sucrose 1×10^{-5} $HgCl_2$
0	50·2	37·2	38·0	65·1
2	44·1	24·1	46·5	55·8
3	—	28·1	41·4	50·2
4	35·0	13·8	29·9	29·4
5	—	9·3	32·3	30·9
8	—	19·2	36·2	35·9
10	38·6	21·2	37·1	—
12	—	29·8	41·4	47·6
Tumor	18·7	12·1	19·7	21·3

The influence of various compounds on the swelling on normal mitochondria was studied with particular emphasis on the role of sulfhydryl groups. Since carbonyl compounds are known to interact with sulfhydryl groups of proteins, the effect of such agents was investigated. The fact, that at the same molar concentration of 1×10^{-2} only alloxan and diacetyl are inhibitors of swelling, while acetone, chloroacetone, acetylacetone, and acetonylacetone are inactive, seems to indicate that at least two vicinal carbonyl groups in the molecule are required for the inhibition of mitochondrial swelling by these com-

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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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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.

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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].

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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-

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