

## Cytotoxic Effects of Streptozotocin and *N*-Nitrosomethylurea on the Pancreatic B Cells with Special Regard to the Role of Nicotinamide-Adenine Dinucleotide

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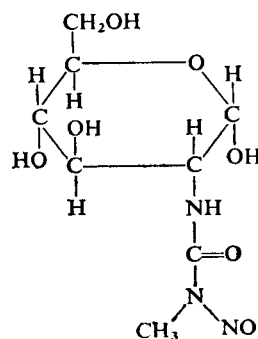
The effects on the pancreatic B cell of streptozotocin and its aglucone derivative *N*-nitrosomethylurea were investigated in obese-hyperglycaemic mice and their lean littermates. Both streptozotocin and *N*-nitrosomethylurea were found to be B-cytotoxic although *N*-nitrosomethylurea produced less islet damage. Both substances decreased the concentrations of NAD<sup>+</sup> in the islet cells to about 10% of the control values within 2h after injection. This NAD<sup>+</sup> depletion was prevented by injection of nicotinamide 10min after the administration of streptozotocin or *N*-nitrosomethylurea. In islets taken from animals 10min after injection of streptozotocin or *N*-nitrosomethylurea there was no stimulatory effect of glucose on the respiration or insulin release and the oxidation of glucose was markedly decreased. Addition of nicotinamide (10mM) to the incubated islets restored glucose stimulation of both the oxygen consumption and insulin release. It is concluded that islet NAD<sup>+</sup> depletion is probably important for the B-cytotoxic action of *N*-nitrosomethylurea and streptozotocin. The glucose residue in the streptozotocin molecule may potentiate the B-cytotoxic action of this drug in mice.

Chemically the diabetogenic agent streptozotocin consists of 2-deoxy-D-glucose with a *N*-nitrosomethylurea side chain at the second carbon atom [2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose] (Herr *et al.*, 1967) (I). Administration of streptozotocin brings about a selective destruction of the pancreatic B cells in several species, whereas *N*-nitrosomethylurea, which is an alkylating and strongly carcinogenic substance, has been claimed to be nondiabetogenic (cf. Rerup, 1970). The detailed mechanism for the streptozotocin-induced cell injury is so far not clear. Since injection of nicotinamide, a precursor to NAD, immediately before, or soon after, the administration of streptozotocin completely protects against the development of diabetes (Schein *et al.*, 1967; Dulin & Wyse, 1969a; Stauffacher *et al.*, 1970) it was postulated that streptozotocin acts on the B cell by depletion of islet NAD (Dulin & Wyse, 1969a). This notion has recently been confirmed by direct measurements that showed that streptozotocin caused a very large decrease of islet NAD<sup>+</sup> within 1 or 2h after injection or after addition *in vitro* to isolated islets (Ho & Hashim, 1972; Hinz *et al.*, 1973; Schein *et al.*, 1973).

Depletion of NAD<sup>+</sup> after streptozotocin administration is not specific for the islets, since it has been reported also in the liver (Schein & Loftus, 1968). *N*-Nitrosomethylurea also depresses the NAD<sup>+</sup> concentration in the liver cells (Schein & Loftus, 1968), but it is not known whether it also has a depressant effect on the islet-cell NAD<sup>+</sup>. This problem is of considerable interest, since a depletion of islet NAD<sup>+</sup>

without damage to the B cells would be contrary to the postulated mechanism of action of streptozotocin. In the present study streptozotocin and *N*-nitrosomethylurea have been investigated with regard both to their B-cytotoxic action and to their effects on the islet NAD<sup>+</sup>. To evaluate further and compare the acute metabolic effects of the two drugs on the B cells *in vitro*, oxygen consumption, glucose oxidation and insulin release of these cells were also measured after administration *in vivo*.

Since the B-cell-rich islets of obese-hyperglycaemic mice (Gepts *et al.*, 1960; Hellman, 1961) were used



(I) Streptozotocin [2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose]

the metabolic data can be considered representative of this cell type. The B cells of these animals have previously been shown to be damaged by streptozotocin administered *in vivo* (Brosky & Logothetopoulos, 1969).

## Materials and Methods

### Reagents

Streptozotocin was kindly given by Dr. W. E. Dulin, the Upjohn Company, Kalamazoo, Mich., U.S.A. *N*-Nitrosomethylurea was obtained from Pfaltz and Bauer, Flushing, N.Y., U.S.A., crude collagenase from Worthington Biochemical Corp., Freehold, N.J., U.S.A. and antimycin A from Sigma Chemical Co., St. Louis, Mo., U.S.A. D-[U-<sup>14</sup>C]-Glucose and the insulin assay kit were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. and bovine plasma albumin (fraction V) was from Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K. NAD<sup>+</sup>, NADH and alcohol dehydrogenase were purchased from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. Purified bacterial luciferase extract from *Achromobacter fischerii* and firefly lantern extract were obtained from Sigma. Semicarbazide hydrochloride was from Hopkin and Williams Ltd., Chadwell Heath, Essex, U.K. All other chemicals were of analytical grade.

### Animals

In all 82 female obese-hyperglycaemic mice (5–7 months old) and 42 of their lean littermates were used. This mouse strain was originally obtained from the Jackson Laboratory, Bar Harbor, Maine, U.S.A., and a colony has been bred at our department since 1959. The animals were reared on standard laboratory chow (Astra-Ewos AB, Södertälje, Sweden) and the obese-hyperglycaemic mice were starved overnight before the experiments involving isolation of islets.

### Administration of streptozotocin and *N*-nitrosomethylurea

Streptozotocin and *N*-nitrosomethylurea were dissolved in concentrations of 50 mg/ml and 19.5 mg/ml respectively in acidified saline (0.154 M-NaCl adjusted to pH 4.5 with 0.05 M-citric acid). The freshly prepared solutions were given intravenously in a tail vein in equimolar doses, i.e. 200 mg of streptozotocin (mol. wt. 265) per kg body wt. and 78 mg of *N*-nitrosomethylurea (mol. wt. 103) per kg. In some lean mice higher doses of *N*-nitrosomethylurea, 156 and 234 mg/kg, were also tested. Both streptozotocin and *N*-nitrosomethylurea are degraded rapidly in blood (Schein & Loftus, 1968; Swann, 1968) but are fairly stable in acid solution (Garrett, 1960; Druckrey *et al.*, 1965).

### Studies on blood sugar concentrations and islet morphology

The effects of streptozotocin and *N*-nitrosomethylurea on the islet morphology and the blood glucose concentrations were compared in 24 obese-hyperglycaemic mice and 42 lean mice. The doses of streptozotocin and *N*-nitrosomethylurea were 200 and 78 mg/kg respectively, but some lean mice also received higher doses of *N*-nitrosomethylurea, 156 and 234 mg/kg. Blood samples were taken from the orbital-vein plexus immediately before and at 2 and 7 days after injection. Blood glucose concentrations were measured with a glucose oxidase method (Hjelm & de Verdier, 1963). For the studies of the islet morphology animals were killed 24 h after administration of the drugs and the pancreatic glands were excised and fixed in Zenker formal solution (Romeis, 1968) and embedded in paraffin. Pancreatic sections were stained with chrome-haematoxylin ponceau fuchsin (Bencosme, 1952).

### Isolation of islets

Islet oxygen uptake, glucose oxidation and insulin release were determined *in vitro* with islets isolated from obese-hyperglycaemic mice killed within 10 min after the injection of streptozotocin (200 mg/kg) or *N*-nitrosomethylurea (78 mg/kg). In studies of the oxygen uptake, less than 20 islets were needed for each experiment and they were isolated by free-hand microdissection in Krebs-Ringer phosphate buffer (Umbreit *et al.*, 1964) at +4°C by the method described by Hellerström (1964). For the other experiments islets were prepared by collagenase treatment of the pancreas, mainly as described by Howell & Taylor (1968) except that islets were isolated with collagenase (6 mg/ml) at room temperature. In some experiments the buffer contained 10 mM-nicotinamide throughout the isolation procedure (see below).

### Determination of oxygen uptake

The oxygen consumption of the isolated islets was measured with the Cartesian-diver micro-respirometer (Holter & Linderström-Lang, 1943). Further details about the application of this technique to pancreatic islets are given by Hellerström (1967). The sensitivity of the system used was sufficient to detect changes in the gas volume of about 0.5 nl. This made it possible to measure the respiration of one to three islets from the obese-hyperglycaemic mouse. The islets were incubated for 2 h in Krebs-Ringer phosphate buffer with 16.7 mM-glucose, the gas phase being ambient air. After incubation the islets were recovered from the divers, dried and weighed on an ultramicro-balance (Mettler UM7) with a sensitivity of 0.1 µg. The oxygen uptake was expressed on the

basis of dry weight and calculated separately for the first and second hours of incubation.

#### Determination of glucose oxidation

Groups of five islets were incubated for 90 min at +37°C in 100 µl of Krebs-Ringer phosphate buffer containing 3.3 or 16.7 mM-D-glucose with D-[U-<sup>14</sup>C]-glucose (final specific radioactivity 1.0 mCi/mmol) in small glass vials which were inserted into liquid-scintillation flasks sealed with rubber caps (Keen *et al.*, 1963). Oxidation was stopped and CO<sub>2</sub> liberated by injection into the incubation vessels of 100 µl of a 0.05 mM-solution of antimycin A in 70% (v/v) ethanol immediately followed by 100 µl of 0.4 M-sodium phosphate buffer, pH 6.0. Hyamine hydroxide (0.25 ml of a 1 M-solution in methanol) was then injected into the scintillation vial outside the incubation vessel. After 2 h to allow complete CO<sub>2</sub> absorption the incubation vessels were removed and the radioactivity was determined in a liquid-scintillation spectrometer (Packard Model 3380) by using a toluene-based scintillator [toluene 1000 ml, 2,5-diphenyloxazole 5 g, 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene 0.05 g]. Blank values were obtained with samples of islet-free incubation medium, which were processed in the same way. Acid-labile impurities in the labelled glucose, which increased the blank values considerably, were removed before the experiments by treatment with weak acid as described by Snyder *et al.* (1970). After incubations islets were dried and weighed as described above.

#### Determination of insulin release

Insulin release from isolated islets was measured during an incubation period of 30 min at +37°C with groups of three to five islets in 250 µl of bicarbonate buffer (Gey & Gey, 1936) containing 0.2% (w/v) albumin and 3.3 or 16.7 mM-glucose. A preincubation period of 20 min in the low-glucose concentration was used as a routine. In some experiments 10 mM-nicotinamide was added to the buffer. Samples of the incubation medium were quickly frozen at the end of the incubation period and stored at -20°C until assay. Insulin was determined by a radioimmunoassay (Hales & Randle, 1963) by using crystalline insulin from this strain of mouse (kindly prepared by Novo A/S, Copenhagen, Denmark) as standard. Separate experiments showed that nicotinamide did not interfere with the immunoassay procedure.

#### Determination of islet NAD<sup>+</sup> and ATP content

To study the effects of streptozotocin and *N*-nitrosomethylurea on islet NAD<sup>+</sup> and ATP concentrations, obese-hyperglycaemic mice were anaesthetized with ether 2 h after injection, and the pancreas

was excised and immediately frozen in isopentane chilled to -160°C with liquid N<sub>2</sub>. In two groups of animals 500 mg of nicotinamide/kg was injected intravenously 10 min after streptozotocin or *N*-nitrosomethylurea. NAD<sup>+</sup> and ATP were extracted from islets which were micro-dissected from freeze-dried cryostat sections of pancreas. After the samples had been weighed on a quartz-fibre balance (Lowry, 1953) NAD<sup>+</sup> was extracted and NADH simultaneously destroyed in 0.05 M-HCl. A total of 1.9 µl of extract was added to the same volume of a reaction mixture composed of 0.1 M-sodium pyrophosphate, pH 9.5, 1.2 M-ethanol, 70 mM-semicarbazide and 80 munits of alcohol dehydrogenase (EC 1.1.1.1). After 20 min at +24°C the samples were diluted to 15 µl and the NADH formed in the reaction was measured with a photokinetic technique by using bacterial luciferase (Brolin *et al.*, 1971, 1972). ATP was extracted in 9.8 µl of 0.02 M-NaOH for 15 min at +60°C and measured photokinetically by using firefly lantern extract (Wettermark *et al.*, 1970).

## Results

### Islet morphology and blood glucose concentrations

Plate 1 shows that *N*-nitrosomethylurea (78 mg/kg) was cytotoxic in obese-hyperglycaemic mice, but the lesions were in no case as extensive as those after an equimolar dose of streptozotocin (200 mg/kg) (Plate 1b). Table 1 shows that streptozotocin increased the initial hyperglycaemia in the obese-hyperglycaemic mice. However, notwithstanding its B-cytotoxic effect, *N*-nitrosomethylurea did not further increase the blood sugar concentrations. In fact, the blood glucose decreased after *N*-nitrosomethylurea and this presumably can be attributed to a general toxic effect of this drug, which causes a decreased food intake. The latter rapidly decreases the blood sugar concentrations in these mice (Mayer *et al.*, 1953; Christophe *et al.*, 1959).

Table 1. Effects of streptozotocin and *N*-nitrosomethylurea on blood sugar concentrations in obese-hyperglycaemic mice

Blood sugar was determined before and at 2 and 7 days after an intravenous injection of streptozotocin (200 mg/kg), *N*-nitrosomethylurea (78 mg/kg) or 0.154 M-saline alone. Each group consisted of four mice. The values are given as means ± S.E.M.

	Day ...	Blood sugar (mg/100 ml)		
		0	2	7
Controls		200 ± 47	221 ± 27	148 ± 22
Streptozotocin		271 ± 51	466 ± 23	346 ± 127
<i>N</i> -Nitrosomethylurea		137 ± 36	65 ± 6	43 ± 19

In the lean mice neither hyperglycaemia nor B-cell destruction was induced by a dose of 78 mg of *N*-nitrosomethylurea/kg. On a molar basis this is equivalent to 200 mg of streptozotocin/kg, which produced

both a considerable hyperglycaemia (Fig. 1) and marked degeneration of the B cells, which was similar to that seen in the obese animals. Higher doses of *N*-nitrosomethylurea (156 and 234 mg/kg) resulted in a high mortality within a few days and development of a diabetic syndrome was therefore difficult to follow. However, 24 h after treatment with the highest dose, i.e. 234 mg/kg, there were clear signs of islet-cell degeneration.

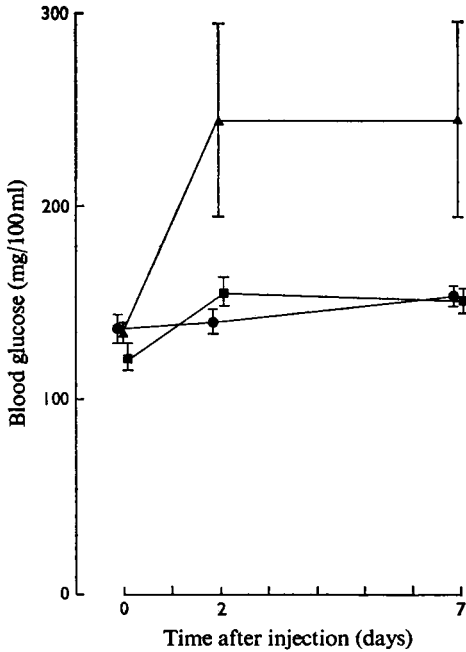


Fig. 1. Effects of streptozotocin and *N*-nitrosomethylurea on blood glucose concentrations in normal lean mice

Blood glucose concentrations during the first week after injection of streptozotocin (200 mg/kg) ( $\Delta$ ) or an equimolar dose of *N*-nitrosomethylurea ( $\blacksquare$ ) are shown. Controls received a corresponding amount of saline ( $\bullet$ ). Only streptozotocin induced a permanent hyperglycaemic state.

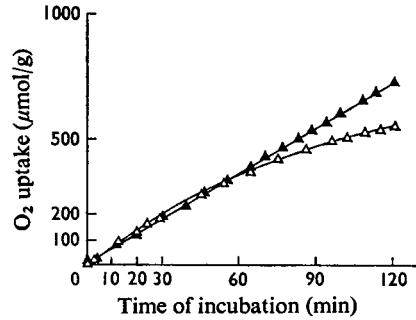


Fig. 2. Effect of streptozotocin on islet-cell respiration

The oxygen uptake of islets isolated from obese-hyperglycaemic mice killed 10 min after injection of streptozotocin (200 mg/kg) was measured in Cartesian diver microrespirometers. One to three islets were incubated in a diver in 1.0  $\mu$ l of Krebs-Ringer phosphate buffer with 16.7 mM-glucose and the gas phase was ambient air. The graphs represent the respiratory rate in two single divers. After streptozotocin treatment ( $\Delta$ ) the rate decreased during the second hour of incubation. When the incubation medium was supplemented with 10 mM-nicotinamide the oxygen consumption of islets from streptozotocin-injected animals was normal and linear throughout the incubation period ( $\Delta$ ). *N*-Nitrosomethylurea exerted identical effects on islet respiration (cf. Table 2).

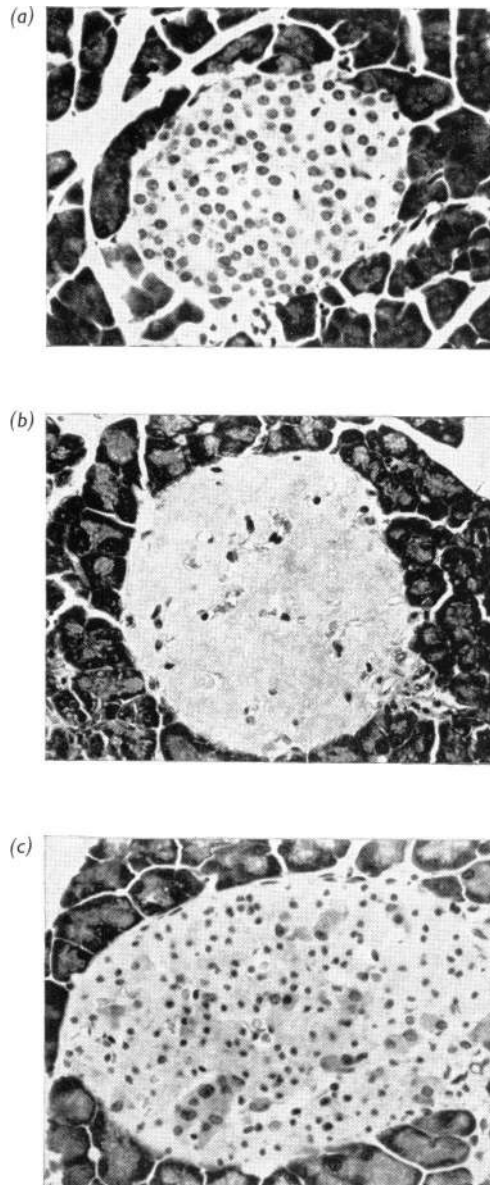
Table 2. Effects of streptozotocin and *N*-nitrosomethylurea on islet oxygen uptake

Obese-hyperglycaemic mice were killed 10 min after injection of streptozotocin (200 mg/kg) or *N*-nitrosomethylurea (78 mg/kg). Islets were isolated by micro-dissection and respiration was followed for 2 h in Cartesian divers and the oxygen uptake was calculated separately for the first and second hours. The medium was Krebs-Ringer phosphate buffer with 16.7 mM-glucose and the gas phase was ambient air. Each experimental group consisted of two obese-hyperglycaemic mice. The values are expressed as  $\mu$ mol/h per g dry wt. and are means  $\pm$  S.E.M. for the numbers of observations given.

	No. of observations	Nicotinamide in medium (10 mM)	Oxygen uptake ( $\mu$ mol/h per g dry wt.)		Differences between 1st and 2nd h
			1st h	2nd h	
Controls	5	—	374 $\pm$ 25	351 $\pm$ 31	23 $\pm$ 10
Streptozotocin	7	—	347 $\pm$ 23	215 $\pm$ 17	132 $\pm$ 12*
Streptozotocin	8	+	359 $\pm$ 21	339 $\pm$ 21	20 $\pm$ 4
<i>N</i> -Nitrosomethylurea	8	—	381 $\pm$ 16	293 $\pm$ 21	88 $\pm$ 15†
<i>N</i> -Nitrosomethylurea	7	+	371 $\pm$ 24	339 $\pm$ 27	32 $\pm$ 12

\*  $P < 0.005$ .

†  $P < 0.05$ .



EXPLANATION OF PLATE I

*Light-microscopy of mouse pancreatic islets*

The effects of streptozotocin and *N*-nitrosomethylurea on pancreatic-islet morphology were studied in obese-hyperglycaemic mice killed 24 h after intravenous injection. In contrast with the control animals treated with saline (*a*), the islets after streptozotocin (200 mg/kg) (*b*) or after an equimolar dose of *N*-nitrosomethylurea (78 mg/kg) (*c*) displayed marked signs of cell death with nuclear pyknosis and cell disintegration. Chrome-haematoxylin staining was used. Magnification  $\times 240$ .

Table 3. *Effects of streptozotocin and N-nitrosomethylurea on islet glucose oxidation*

Obese-hyperglycaemic mice were killed 10 min after injection of streptozotocin (200 mg/kg) or *N*-nitrosomethylurea (78 mg/kg) and islets were isolated after collagenase digestion of the pancreas. Details are given in the text. Each group consisted of at least three animals. Results are given as means  $\pm$  s.e.m., with the number of observations in parentheses.

Concn. of glucose ...	Glucose oxidation ( $\mu$ mol/h per g dry wt.)	
	3.3 mM	16.7 mM
Controls	7.3 $\pm$ 1.0 (25)	29.0 $\pm$ 3.8 (24)*
Streptozotocin	2.9 $\pm$ 0.2 (19)†	7.1 $\pm$ 0.7 (24)*†
<i>N</i> -Nitrosomethylurea	2.2 $\pm$ 0.3 (19)†	4.9 $\pm$ 0.6 (20)*†

\*  $P < 0.001$  in comparison with 3.3 mM-glucose.

†  $P < 0.001$  in comparison with controls.

#### Oxygen consumption and glucose oxidation

The respiration of islets from the control animals in the presence of 16.7 mM-glucose proceeded at an approximately linear rate during 2 h incubation. By contrast islets from the streptozotocin- and *N*-nitrosomethylurea-treated animals respired at a constant rate during the first hour of incubation, after which there was a marked tendency to a decreased oxygen uptake (Fig. 2). A quantitative evaluation of these changes is given in Table 2. The streptozotocin-treated animals exhibited the greatest decreases, the respiratory rate falling to about 40% of the initial value. Addition of 10 mM-nicotinamide to the medium during the isolation and incubation procedures prevented the streptozotocin- and *N*-nitrosomethylurea-induced respiratory inhibition (Table 2). The effects of streptozotocin and *N*-nitrosomethylurea on the islet glucose oxidation are given in Table 3; both substances strongly inhibited, to about the

Table 4. *Effects of streptozotocin and N-nitrosomethylurea on insulin release*

Each experimental group consisted of three to five obese-hyperglycaemic mice. After a 20 min preincubation period at 3.3 mM-glucose groups of three to five islets were incubated for 30 min at 3.3 or 16.7 mM-glucose. Results are given as means  $\pm$  s.e.m., with the numbers of observations in parentheses.

Concn. of glucose ...	Nicotinamide in medium (10 mM)	Insulin released (ng/30 min per $\mu$ g dry wt.)	
		3.3 mM	16.7 mM
Controls	—	2.5 $\pm$ 0.3 (21)	5.6 $\pm$ 0.7 (22)*
Controls	+	1.5 $\pm$ 0.1 (24)	7.2 $\pm$ 0.7 (23)*
Streptozotocin	—	2.0 $\pm$ 0.3 (33)	2.4 $\pm$ 0.3 (34)
Streptozotocin	+	0.8 $\pm$ 0.1 (23)	4.6 $\pm$ 0.4 (24)*
<i>N</i> -Nitrosomethylurea	—	4.1 $\pm$ 0.5 (28)	5.0 $\pm$ 0.7 (28)
<i>N</i> -Nitrosomethylurea	+	1.6 $\pm$ 0.2 (22)	7.3 $\pm$ 0.8 (24)*

\*  $P < 0.001$  in comparison with insulin release at 3.3 mM-glucose.

Table 5. *Influence of streptozotocin and N-nitrosomethylurea on the islet ATP and NAD<sup>+</sup> concentrations*

The pancreases from obese-hyperglycaemic mice were excised under ether anaesthesia 2 h after injection of streptozotocin (200 mg/kg) or *N*-nitrosomethylurea (78 mg/kg) and rapidly frozen in isopentane ( $-160^{\circ}\text{C}$ ). Nicotinamide (500 mg/kg) was injected 10 min after streptozotocin or *N*-nitrosomethylurea. ATP and NAD<sup>+</sup> were measured photokinetically in freeze-dried sections of pancreatic islets. Four islet sections were analysed from each animal and each experimental group consisted of four animals. Results are given as means  $\pm$  s.e.m.

	NAD <sup>+</sup> ( $\mu$ mol/g dry wt.)	ATP ( $\mu$ mol/g dry wt.)
Controls	0.97 $\pm$ 0.14	10.2 $\pm$ 1.0
Streptozotocin	0.08 $\pm$ 0.03*	3.4 $\pm$ 0.7*
Streptozotocin + nicotinamide	0.86 $\pm$ 0.12	11.4 $\pm$ 0.5
<i>N</i> -Nitrosomethylurea	0.15 $\pm$ 0.03*	8.7 $\pm$ 1.0
<i>N</i> -Nitrosomethylurea + nicotinamide	1.02 $\pm$ 0.10	10.4 $\pm$ 0.2

\*  $P < 0.005$  in comparison with controls.

same extent, the oxidation of glucose at low and high glucose concentrations.

#### *Glucose-induced insulin secretion*

Streptozotocin completely abolished the stimulatory effect of 16.7mM-glucose on the insulin release *in vitro* (Table 4). A similar block of the glucose effect was observed with *N*-nitrosomethylurea, but there may also have been an increase in the insulin secretion at the low external glucose concentration. Addition of 10mM-nicotinamide to the incubation medium restored the deficient secretory response to glucose after both streptozotocin and *N*-nitrosomethylurea.

#### *Islet NAD<sup>+</sup> and ATP contents*

As shown in Table 5 streptozotocin markedly decreased the NAD<sup>+</sup> concentration in the islet cells. In equimolar amounts *N*-nitrosomethylurea also decreased the NAD<sup>+</sup> to about the same extent, whereas injection of nicotinamide 10min after either streptozotocin or *N*-nitrosomethylurea, completely prevented the decrease in NAD<sup>+</sup>. Other experiments (not shown) indicated that the decrease in NAD<sup>+</sup> after streptozotocin and *N*-nitrosomethylurea was not due to a shift to the reduced state of the nucleotide.

Streptozotocin caused a significant fall also in the ATP concentrations in the islet cells, and this was prevented with nicotinamide. In contrast, it was doubtful whether the ATP concentration was altered by *N*-nitrosomethylurea.

#### **Discussion**

It is well known that both streptozotocin and *N*-nitrosomethylurea are extremely labile in solution at physiological pH and body temperature and their half-life in blood is of the order of 5–10min (Schein & Loftus, 1968). Nevertheless, in the present study we exposed the islets to the drugs *in vivo* rather than *in vitro*, since the morphological lesions in the B cells *in vivo* are well documented. In addition, non-specific effects such as might occur *in vitro*, for example by prolonged exposure of the islets to high concentrations of streptozotocin or *N*-nitrosomethylurea or their degradation products, were minimized. It should be noted, however, that the total time from the injection of the drugs into the animal to the start of the islet incubations *in vitro* was about 1h. Since permanent damage to the B cells by streptozotocin can be prevented by nicotinamide for up to 2h after administration of streptozotocin (Stauffacher *et al.*, 1970) it is possible that the present data reflect only the initial toxic phase of the drug action.

The data presented here confirm previous reports of a highly toxic action of streptozotocin on the B

cells of mice. The additional finding that *N*-nitrosomethylurea exhibits a similar though lesser toxicity is of considerable interest, since *N*-nitrosomethylurea has previously been regarded as non-diabetogenic. Although in the present study *N*-nitrosomethylurea did not produce a manifest hyperglycaemia this was presumably due either to its marked side effects or, perhaps, to the survival of a sufficient number of B cells to maintain the blood sugar concentration within a normal range. Nevertheless, there was extensive morphological damage to the B cells in both normal and obese-hyperglycaemic mice. The reason for the higher susceptibility of the obese-hyperglycaemic mice remains, however, a matter of speculation, particularly when it is borne in mind that streptozotocin is as potent in obese-hyperglycaemic mice as in their lean littermates. Although streptozotocin and *N*-nitrosomethylurea seem to differ in potency with respect to their B-cytotoxic action, the acute metabolic effects on the B cells appeared remarkably similar. Thus both drugs decreased the glucose-stimulated oxygen uptake, glucose oxidation by the islet cells and insulin secretion in response to glucose. The inhibitory actions on both the respiration and the insulin release were readily reversed by nicotinamide *in vitro*. Since the glucose-stimulated oxygen uptake reflects the rate of glucose oxidation (Hellerström & Gunnarsson, 1970) the present results indicate that nicotinamide reversed also the oxidation of this substrate. The present observations conform to a report by Dean & Matthews (1972), who observed an inhibiting effect of both streptozotocin and *N*-nitrosomethylurea on the generation of action potentials in mouse B cells. Wyse & Dulin (1971) also reported a marked decrease in glucose oxidation by islets removed from rats 15min after they had been treated with streptozotocin. Although these data indicate that streptozotocin has a marked short-term action on islet metabolism it remains to be clarified to what extent this is causally related to its destructive effects on the B cells. The similarity between the acute metabolic actions of streptozotocin and *N*-nitrosomethylurea, and alloxan, another B cytotoxin, supports such a relationship (Gunnarsson & Hellerström, 1973). However, since streptozotocin produced more extensive islet-cell degeneration than did *N*-nitrosomethylurea, certain additional properties of the streptozotocin molecule must enable it to exert a greater effect on the B cell. Such an effect might be, for example, a stronger binding to the B cell membrane by means of the glucose residue of the streptozotocin molecule, or a facilitated transport into the cell. In fact, 2-deoxyglucose, which may interfere with membrane transport sites for glucose (Kipnis & Cori, 1959), has been shown to protect the B cells from the diabetogenic effect of streptozotocin (Dulin & Wyse, 1969b). On the other hand preloading with high doses of glucose did not inhibit the streptozotocin effect either in rats

(Dulin & Wyse, 1969b; Zawalich & Beidler, 1973) or mice (R. Gunnarsson, unpublished work), suggesting an alternative mechanism for protection by 2-deoxyglucose.

Previous studies have shown that streptozotocin causes a rapid and marked depletion of islet NAD<sup>+</sup> and this action has been regarded as the primary molecular mechanism behind the destruction of the B cells. Since NAD<sup>+</sup> concentrations are decreased by streptozotocin also in the liver, but without permanent damage to the liver cells, it has been postulated that B cells are particularly sensitive to decreased concentrations of NAD<sup>+</sup>. The observation that nicotinamide, which is a precursor of NAD, has a protective action both with regard to the induction of diabetes (Schein *et al.*, 1967) and the depletion of islet NAD<sup>+</sup> (Ho & Hashim, 1972) in the streptozotocin-treated animal supports this theory. The present data confirm both the decrease of islet NAD<sup>+</sup> by streptozotocin and the prevention of this by nicotinamide. The virtually identical effects on islet NAD<sup>+</sup> produced by the B-cytotoxic N-nitrosomethylurea moiety and streptozotocin fit in with the view that the NAD<sup>+</sup> depletion alone is sufficient to explain the B-cell degeneration. The observation that islet ATP concentrations were significantly decreased by streptozotocin but not by N-nitrosomethylurea probably reflects the more extensive injuries seen after streptozotocin rather than any major differences between the molecular actions of streptozotocin and N-nitrosomethylurea on the B cell. In all, the present results strongly suggest that N-nitrosomethylurea is the B-cytotoxic part of the streptozotocin molecule, though the presence of a glucose residue in this molecule may potentiate the toxic action. The depression of the oxidative metabolism of the islets and the lack of insulin response to a glucose challenge after administration of either drug suggests that both these cellular activities depend on the maintenance of normal NAD<sup>+</sup> values. These observations conform with a report by Deery & Taylor (1973) who demonstrated that an inhibitor of NAD synthesis, azaserine, blocked the insulin release caused by both sugars and amino acids. In addition, the present observations support the view that NAD<sup>+</sup> depletion is incompatible with survival of the B cell. It remains to be clarified whether a similar mechanism is of significance for the degeneration of the B cell observed in other types of spontaneous or induced diabetes. It also remains to be settled whether other nitrosamines share the diabetogenic property of N-nitrosomethylurea. Since it is known that nitrosamines are present in human food (Crosby *et al.*, 1972) the possible role of these compounds in diabetes aetiology deserves consideration (Berne *et al.*, 1974).

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