these intervals activity was increased in only 50 per dent of the animals. Several factors could contribute to this, e.g. (a) transient release of CPK from damaged muscle which is rapidly cleared from the plasma, (b) variable time responses in different animals, (c) defective absorption of drug from the peritoneal cavity.

The temporal development of PCPK changes in this study are similar to those of the muscle pathology as muscle necrosis is first observed 2 days after treatment [8]. Although, histologically only a few fibres showed changes, the release of CPK is sufficient to measurably increase the level in the plasma. The mechanism of vincristine myopathy is at present unknown, but because of the short time interval involved, the results suggest a myogenic rather than a neurogenic lesion. Findings supporting this view are the unchanged levels of PCPK up to two weeks in rats after peroneal or sciatic nerve section [9], where muscle atrophy is a continuous process, and also following a subcutaneous injection of the neuromuscular blocking agents *d*-tubocurarine and succinylcholine [10].

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REFERENCES

1. J. Hildebrand and C. Coers, *Eur. J. Cancer* **1,** 51 (1965).
2. W. G. Bradley, *J. Neurol. Sci.* **10,** 133 (1970).
3. R. Yasin, B. P. Hughes and J. A. Parker, *Lab. Invest.* **29,** 207 (1973).
4. S. B. Rosalki, *J. Lab. clin. Med.* **69,** 696 (1967).
5. W. E. Worthy, P. Whitehead and D. M. Goldberg, *Enzym. biol. clin.* **11,** 193 (1970).
6. F. A. Craig, J. C. Smith and F. F. Foldes, *Clinica. chim. Acta* **15,** 107 (1967).
7. S. Segal, in *Non-Parametric Statistics: For the Behavioural Sciences*, p. 116. McGraw-Hill, New York (1956).
8. R. Yasin and B. P. Hughes, manuscript in preparation.
9. H. Y. Meltzer, *Exp. Neurol.* **40,** 547 (1973).
10. H. Y. Meltzer and P. Margulies, *Biochem. Pharmac.* **20,** 3501 (1971).

Biochemical Pharmacology, Vol. 24, pp. 746–747. Pergamon Press, 1975. Printed in Great Britain.

Diabetogenic activity of deoxy-2-[[(ethylnitrosoamino) carbonyl]amino]-D-glucopyranose

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The diabetogenic agent streptozotocin, is composed of the cytotoxic moiety 1-methyl-1-nitrosourea attached to the carbon-2 position of glucose. In addition to producing a permanent diabetic state in animals, mediated through the specific destruction of the pancreatic beta cell [1], this compound has demonstrable clinical activity against human islet cell carcinomas [2]. The diabetogenic activity of streptozotocin has been correlated with an inhibition of nicotinamide adenine dinucleotide (NAD) synthesis in the pancreatic islets of Langerhans [3, 4] with subsequent beta cell necrosis. While all compounds having an $R—\overset{\overset{NO}{|}}{N}—(CH_2)_{1-2}H$ end group have been demonstrated to depress hepatic NAD concentrations, only streptozotocin has been shown to be diabetogenic [5]. To explore further these structure–activity relationships and the importance of the glucose carrier for diabetogenicity, the pharmacologic properties of deoxy-2-[[(ethylnitrosoamino)carbonyl]amino]-D-glucopyranose (DENU; Upjohn U-30,964, NSC-174793) [6], a glucose-containing nitrosourea, identical in structure to streptozotocin except for the presence of an ethyl end group, were studied.

Male Swiss mice weighing 17–26 g and maintained on Purina laboratory chow pellets and water *ad lib.* were used for all studies. DENU was dissolved in 0·005 M citrate buffer, pH 4·5, immediately prior to use; each dose was administered intravenously at a volume of 0·1 ml/10 g of body weight. Animals were fasted for 18 hr prior to drug administration. Control animals received equal volumes of the citrate buffer diluent.

Five days after drug administration, mice were sacrificed and plasma glucose [7] and immunoreactive insulin concentrations [8] were determined on blood obtained by car-

Table 1. Mean plasma glucose and immunoreactive insulin concentration 5 days after treatment with intravenous DENU administered at doses of 500–2500 mg/kg in Swiss mice

Dose (mg/kg)	Plasma glucose* (mg/100 ml)	P†	Plasma insulin* (μU/ml)	P†
Control	128		86	
500	132	>0·1		
1000	181	<0·05		
1500	174	<0·05		
2500	276	<0·01	21	<0·01

* Mean value for five mice.
† Compared to control.

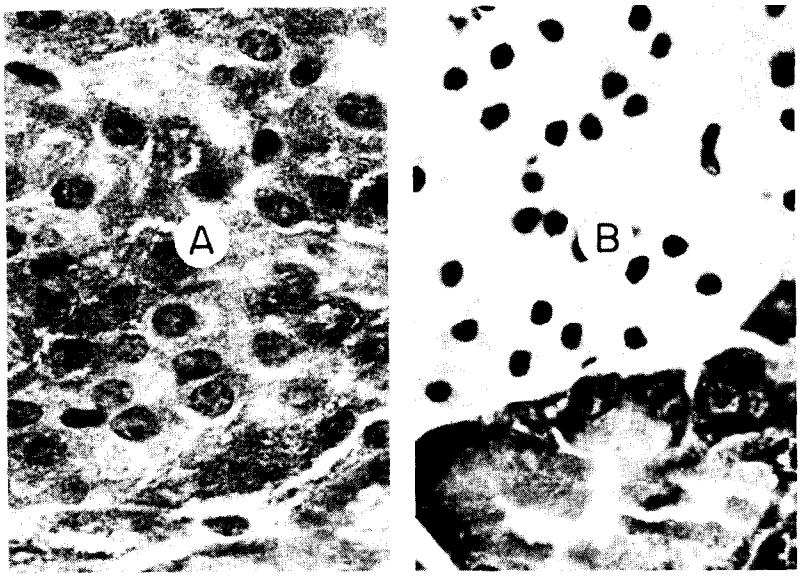

Fig. 1. Photomicrograph (360×) of pancreatic islets of Langerhans from mice receiving intravenous 0·005 M citrate buffer, pH 4·5 (A) or DENU, 2500 mg/kg (B). In contrast to the normal beta cells in the buffer control, B demonstrated loss of intracellular granules and cellular membranes, and pyknotic nuclei. Normal pancreatic exocrine cells are seen at the lower border of the DENU-treated islet (B).

diac puncture. The pancreas from each animal was placed in a 10% formalin solution, and 6 μm sections were stained with an aldehyde fuchsin trichrome technique [9].

Pancreatic islets were isolated 3 hr after treatment using techniques previously described [4]. The individual islets were lysed by the addition of 0·1 N HCl and five cycles of freeze thawing. The NAD content was determined using a radiometric cycling assay [4, 10].

Hepatic NAD content 3 hr post-treatment was determined spectrophotometrically using alcohol dehydrogenase (Boehringer). Protein determinations were performed by the standard method of Lowry *et al.* [11].

Using graded doses of DENU, significant hyperglycemia could be produced. Compared to control animals, a dose of 1000 mg/kg produced a moderate increase in plasma glucose, 181 mg/100 ml ($P < 0·05$); 2500 mg/kg produced definite hyperglycemia with a mean plasma glucose of 276 mg/100 ml ($P < 0·01$) (Table 1), which correlated with a reduced plasma insulin value of 21 μU/ml, compared to a control mean of 86 μU/ml ($P < 0·01$). At a dose of 2500 mg/kg, hepatic NAD content was depressed to 0·37 μmole/g of liver from a control mean of 0·62 μmole/g of liver 3 hr after treatment; similarly, pancreatic islet NAD was depressed to 0·112 pmole/islet from a control mean of 0·834 pmole/islet ($P < 0·01$). Histologic examination of pancreatic islets, from animals treated with DENU, 2500 mg/kg, 5 days previously demonstrated degranulation and necrosis of the beta cells (Fig. 1).

Previously reported structure–activity studies with nitrosamine and nitrosourea compounds have demonstrated the

$$\begin{array}{c} NO \\ | \end{array}$$

ability of the $-N-(CH_2)_{1-2}H$ end group to depress hepatic NAD concentrations. Those compounds which contained an ethyl end group required approximately a 10-fold increase in dosage to produce a decrease in hepatic NAD comparable to that obtained with a methyl end group [5]. Streptozotocin, a methyl nitrosourea attached to a glucose carrier, is the only representative of this class of agents that has been demonstrated to be diabetogenic. It has been shown that the glucose portion of the molecule facilitates the uptake of the cytotoxic group into the pancreatic islet [10].

DENU is identical in structure to streptozotocin except for the presence of an ethyl end group. A dose of 2500 mg/kg of DENU was required to produce a diabetic state comparable to that observed with streptozotocin at a dose of 200 mg/kg. This is consistent with our prior observations of the relative inefficiency of nitroso compounds containing an ethyl end group to depress NAD concentrations [5]. This study further supports the correlation of pancreatic islet NAD depression for the diabetogenicity of streptozotocin and establishes the ability of a class of glucose-containing nitrosourea compounds to produce beta cell destruction.

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REFERENCES

1. W. Stauffacher, I. Burr, A. Gutzeit, D. Beaven, J. Veleminsky and A. E. Renold, *Proc. Soc. exp. Biol. Med.* **133,** 194 (1970).
2. P. S. Schein, R. A. DeLellis, C. R. Kahn, P. Gorden and A. R. Kraft, *Ann. intern. Med.* **79,** 239 (1973).
3. C. Ho and S. A. Hashim, *Diabetes* **21,** 789 (1972).
4. P. S. Schein, D. A. Cooney, M. G. McMenamin and T. Anderson, *Biochem. Pharmac.* **22,** 2625 (1973).
5. P. S. Schein, *Cancer Res.* **29,** 1226 (1969).
6. B. K. Bhuyan, T. J. Fraser, H. H. Buskirk and G. L. Neil, *Cancer Rep.* **56,** part 1, 709 (1972).
7. G. R. Kingsley and G. Getchell, *Clin. Chem.* **6,** 466 (1960).
8. J. Roth, P. Gorden and I. Paspan, *Proc. natn. Acad. Sci. U.S.A.* **61,** 138 (1968).
9. J. C. Sieracki, J. E. Michael and D. A. Clark, *Stain Technol.* **35,** 67 (1960).
10. T. Anderson, P. S. Schein, M. McMenamin and D. Cooney, *J. clin. Invest.*, **54,** 672 (1974).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).