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Uptake of Labelled N-Nitrosomethylurea in the Pancreatic Islets

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Summary. Labelled N-nitrosomethylurea was injected intravenously into mice (C57/Bl strain) and Chinese hamsters. In whole-body autoradiograms the isotope was found to accumulate heavily in the islet tissue of the Chinese hamsters but not in that of the mice. The selective uptake in the islet tissue of Chinese hamsters was most pronounced after one hour. A dose of 50 mg/kg body weight of non-labelled N-nitrosomethylurea caused hyperglycemia in the Chinese hamsters while no hyperglycemia occurred in the mice at a dose of 150 mg/kg body weight. The results indicated that the diabetogenic effect of N-nitrosomethylurea is related to the ability of this substance, or a metabolite of it, to accumulate in the pancreatic islets.

Key words: Chinese hamsters — Mice — Pancreatic islets — ^{3}H-N-nitrosomethylurea — Whole-body autoradiography.

Introduction

N-nitrosomethylurea belongs to the N-nitroso compounds, a group of substances which have powerful biological properties including carcinogenicity, mutagenicity and teratogenicity (Lancet II, 1973; Sebranek and Cassens, 1973). There are several reports that small amounts of N-nitroso compounds are present in the human environment or may be formed endogenously under conditions existing in the human stomach (Sander, 1971; Crosby *et al.*, 1972). The potentially harmful effects that may arise from human exposure to these substances have been considered (Sander, 1971; Crosby *et al.*, 1972; Wolff and Wasserman, 1972; Lancet II, 1973, Sebranek and Cassens, 1973).

N-nitrosomethylurea is also an aglucone of the well known diabetogenic substance streptozotocin (Rerup, 1970). Recent studies in Chinese hamsters have shown that N-nitrosomethylurea may cause islet tissue damage and hyperglycemia (Wilander and Gunnarsson, in press). In normal mice, on the other hand, β-cell destruction can only be obtained with very high doses of N-nitrosomethylurea which cause severe general toxic effects and death, making it impossible to study any development of the hyperglycemia (Gunnarsson *et al.*, 1974).

It was considered of interest to investigate whether or not labelled N-nitrosomethylurea is accumulated in the pancreatic islets. This has been performed in the present study by using Chinese hamsters and mice of the C57/Bl strain. Blood glucose determinations after the administration of non-labelled N-nitrosomethylurea were also done and used as an indicator of the diabetogenic effect of this compound.

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Material and Methods

Blood Glucose Determinations

Thirty-five non-diabetic Chinese hamsters and 20 mice of the C57/Bl strain weighing about 25 gm each were used. They were injected intraperitoneally with 50, 100 and 150 mg/kg body weight of N-nitrosomethylurea (Pfaltz and Bauer Flushing, New York, USA) or a corresponding volume of plain buffer solution. The N-nitrosomethylurea was dissolved in a citrate-phosphate buffer, (McIlvain, 1921) pH 4.0 (Swann, 1968) at a concentration of 2% (w/v) immediately before use.

Blood samples were obtained after 2 days by the orbital bleeding technique (Riley, 1960). The animals were anaesthetized with ether prior to the puncture, which was performed with heparinized microhematocrit tubes (Drummond Scientific Company, USA) in the left orbital venous plexus. The blood glucose concentration was determined by a glucoseoxidase method with the aid of a commercially available kit (AB Kabi, Stockholm, Sweden).

Autoradiography

Six adult non-diabetic Chinese hamsters and 6 mice of the C57/Bl strain were used. The Chinese hamsters and the mice weighed about 25 gm each. The animals were fed a conventional diet for laboratory rodents and were given tap water *ad libitum*.

Five Chinese hamsters and 5 mice were each injected intravenously with 0.5 mCi (corresponding to 40 mg/kg body weight) of ^{3}H-N-nitrosomethylurea (labelled at the methyl group, spec. act. 48 mCi/mM, New England Nuclear, Boston, USA), dissolved in 0.2 ml of the citrate phosphate buffer, pH 4.0. One mouse and one Chinese hamster were injected with a corresponding volume of plain buffer solution. The mice were injected in a tail vein while the Chinese hamsters were injected in the jugular vein after being slightly anaesthetized with ether. One Chinese hamster and one C57/Bl mouse were then killed 5 minutes, 30 minutes, 1 hour, 4 hours, and 24 hours after the injection by being anaesthetized with ether and frozen in hexane cooled with solid CO_2 to $-78°$C. At the one-hour survival time the control animals were also killed. After freezing, the animals were embedded in a mixture of carboxymethyl cellulose and water. Autoradiography was then performed as decribed by Ullberg (1954, 1958). Thus sagittal sections (20 μ thick) of the whole animals were cut on tape (Minnesota Mining and Manufacturing Co., USA; tape No. 800) at $-15°$C in a microtome. The sections were then freeze-dried at $-15°$C and autoradiograms were obtained by attaching the sections to photographic plates (G5, Ilford). After an exposure time of about 1 month the photographic plates were developed in Kodak D19 and fixed in Gevaert G 305A. The sections were stained with hematoxylin-eosin for identification of the structures in the autoradiograms.

Results

Blood Glucose

The results of the blood glucose analyses are given in Table 1. As seen, there is a significant difference in the blood glucose concentration ($p < 0.001$) between the Chinese hamsters injected with N-nitrosomethylurea (50 and 150 mg/kg) and the control animals injected with the buffer solution alone. In the mice no blood glucose elevation was noted after any of the injected doses of N-nitrosomethylurea. Thus, in mice injected with 50, 100 and 150 mg/kg body weight of N-nitrosomethylurea the blood glucose concentrations did not differ from those of the animals injected with buffer solution alone ($p > 0.1$).

Autoradiography

Chinese Hamsters. In the whole-body autoradiograms radioactivity was seen to be accumulated in the pancreatic islets 30 minutes and 1 hour after the injection of ^{3}H-N-nitrosomethylurea (Figs. 1–2). The uptake was most pronounced after

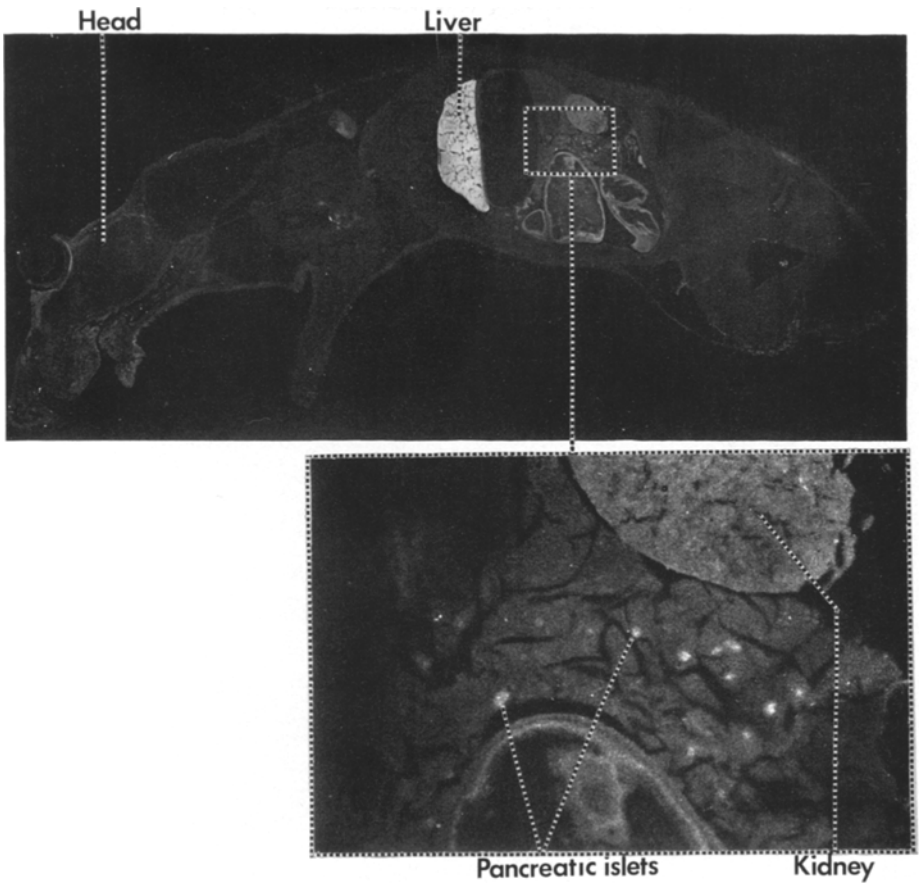

Fig. 1. Whole-body autoradiogram of a Chinese hamster 1 hour after an i.v. injection of ^{3}H-N-nitrosomethylurea showing an enlargement of the indicated pancreas. The pancreatic islets and the liver contain the largest amount of radioactivity (white areas) in the body

Table 1. Effect of intraperitoneally injected N-nitrosomethylurea on the blood glucose concentration in mice and Chinese hamsters after 2 days

Treatment	Blood glucose (mg/100 ml) M ± SEMs	
	Mice	Chinese hamsters
Buffer solution	132 ± 5 $n = 5$	96 ± 3 $n = 16$
N-nitrosomethylurea 50 mg/kg	138 ± 7* $n = 5$	159 ± 11** $n = 14$
N-nitrosomethylurea 100 mg/kg	142 ± 6* $n = 5$	—
N-nitrosomethylurea 150 mg/kg	126 ± 20* $n = 5$	305 ± 75** $n = 5$

*$p > 0.1$ in comparison with buffer injected mice.
**$p < 0.001$ in comparison with buffer injected Chinese hamsters.

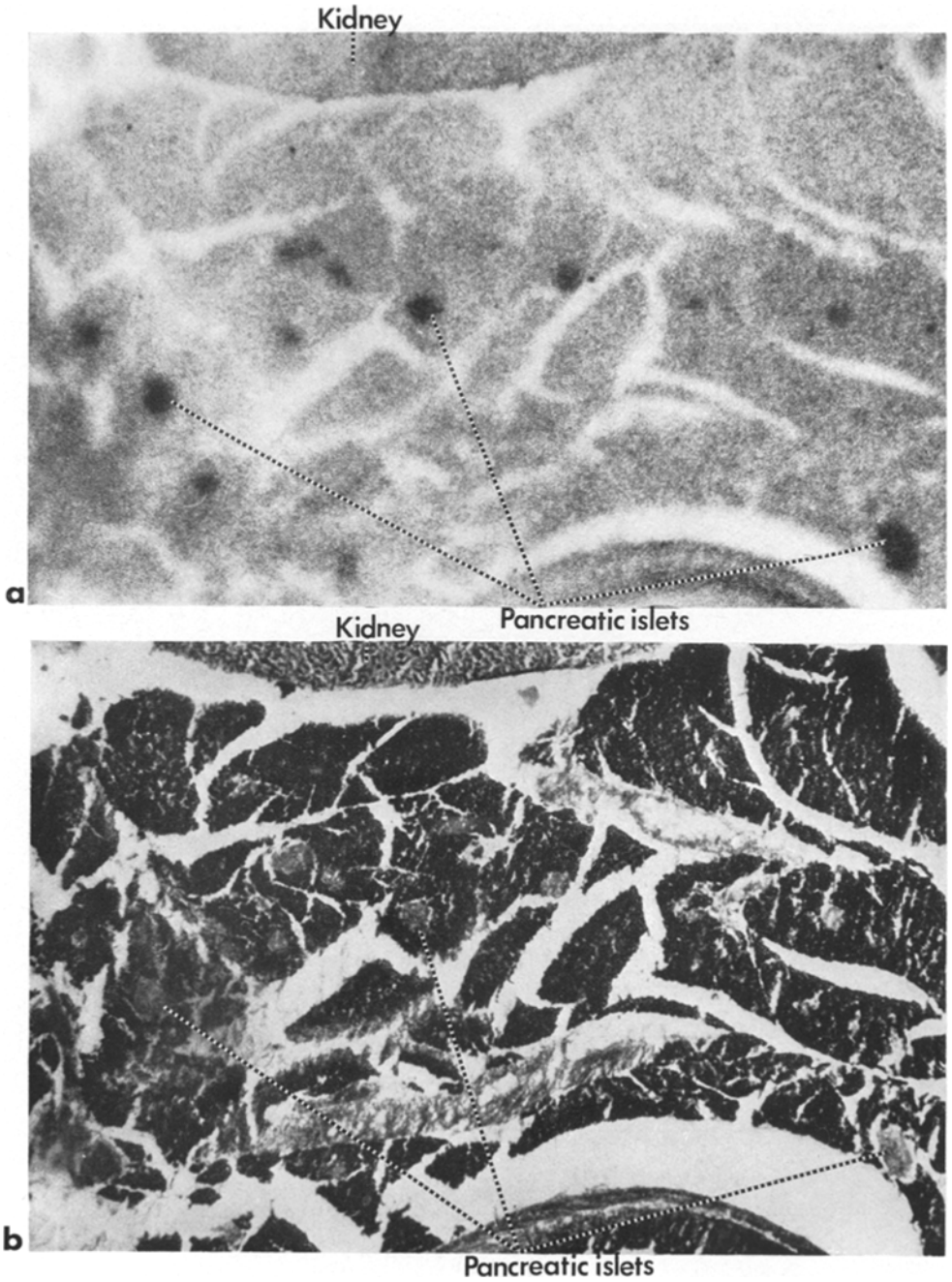

Fig. 2a and b. Detail of a whole-body autoradiogram of a Chinese hamster 1 hour after an i.v. injection of ^{3}H-N-nitrosomethylurea. (a) Autoradiogram, (b) hematoxylin-eosin stained section. High radioactivity is present in the pancreatic islets

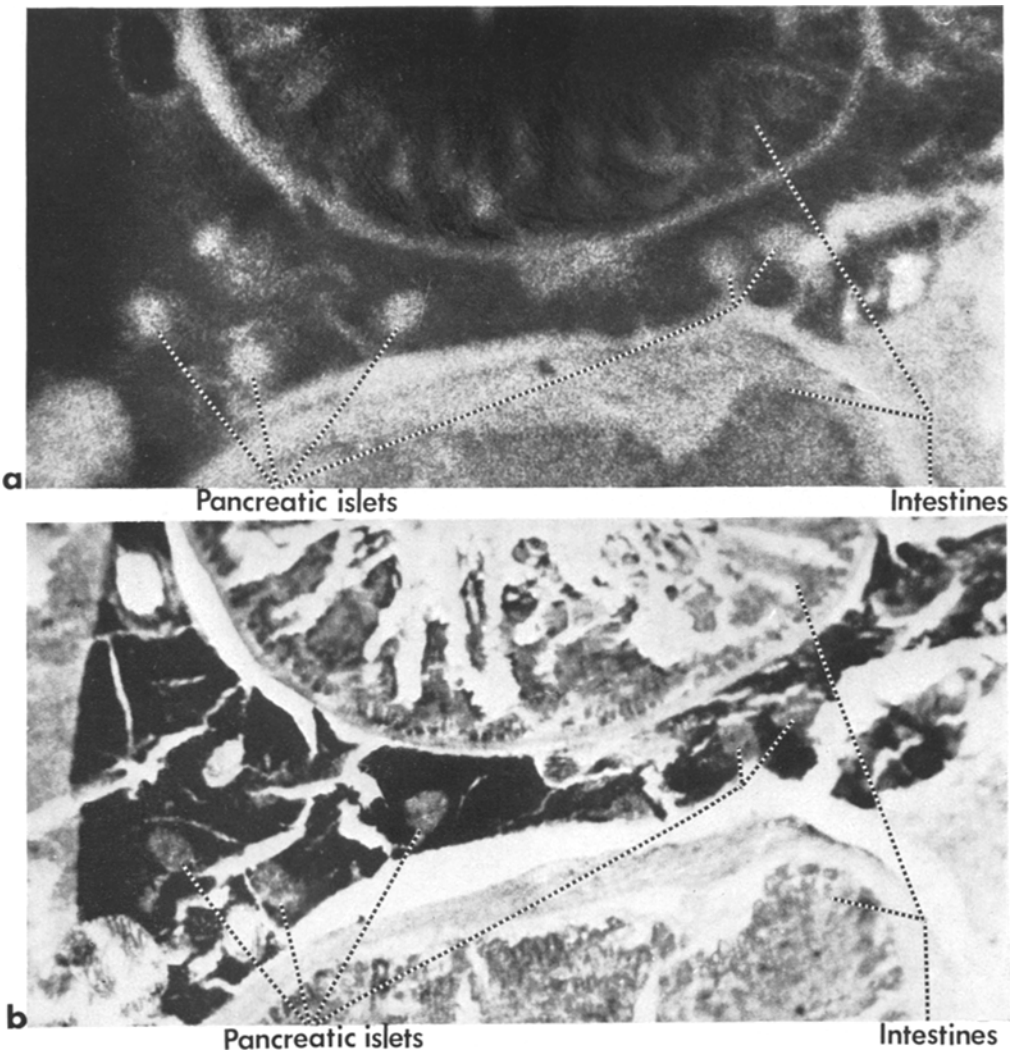

Fig. 3a and b. Detail of a whole-body autoradiogram of a mouse 1 hour after an i.v. injection of ^{3}H-N-nitrosomethylurea. (a) Autoradiogram, (b) hematoxylin-eosin stained section. The radioactivity in the islets is low. (The autoradiogram is overexposed to emphasize the lack of radioactivity in the islet tissue)

1 hour, at which survival time the radioactivity in the pancreatic islets, together with the liver, was the highest in the body. At later survival times the radioactivity in the pancreatic islets decreased and did not exceed that in the exocrine pancreas.

Mice. Throughout the entire experimental period the accumulation of radioactivity in the islet tissue of mice was low. Thus, the uptake of isotope in the islets was lower than that in the exocrine pancreas or in the blood (Fig. 3). Blackening of the autoradiograms from the control animals did not occur.

Discussion

In whole-body autoradiograms a high radioactivity was observed in the islet tissue of Chinese hamsters but not in that of mice after injection of labelled N-nitrosomethylurea. Why this species difference occurred is obscure. It may reflect some difference between the mice and the Chinese hamsters in regard to their transport systems or the intracellular metabolism of the islet cells. N-nitrosomethylurea rapidly degrades in serum (Swann, 1968). Since the highest radioactivity in the pancreatic islets of Chinese hamsters was found one hour after the injection of the labelled substance, it cannot be ruled out that the accumulated isotope was a metabolite of N-nitrosomethylurea which is formed in susceptible species. N-nitrosomethylurea is an alkylating agent, causing preferentially 7-alkylation of guanine in DNA and RNA (Pegg, 1973). However, if this reaction has any significance for the diabetogenic effect of N-nitrosomethylurea is not known at present. After one hour the radioactivity in the islet tissue of the Chinese hamsters decreased. This may be explained by islet cell destruction as it has been shown that approximately the same dose as was used in this investigation causes a total destruction of the islet cells after about 6 hours (Wilander and Gunnarsson, in press).

A dose of 50 mg/kg body weight of non-labelled N-nitrosomethylurea caused significant hyperglycemia in the Chinese hamsters while no hyperglycemia occurred at a dose of 150 mg/kg body weight in the mice. It thus seems that the diabetogenic properties of N-nitrosomethylurea is related to the ability of the substance or metabolite of it to be selectively accumulated in the pancreatic islets.

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