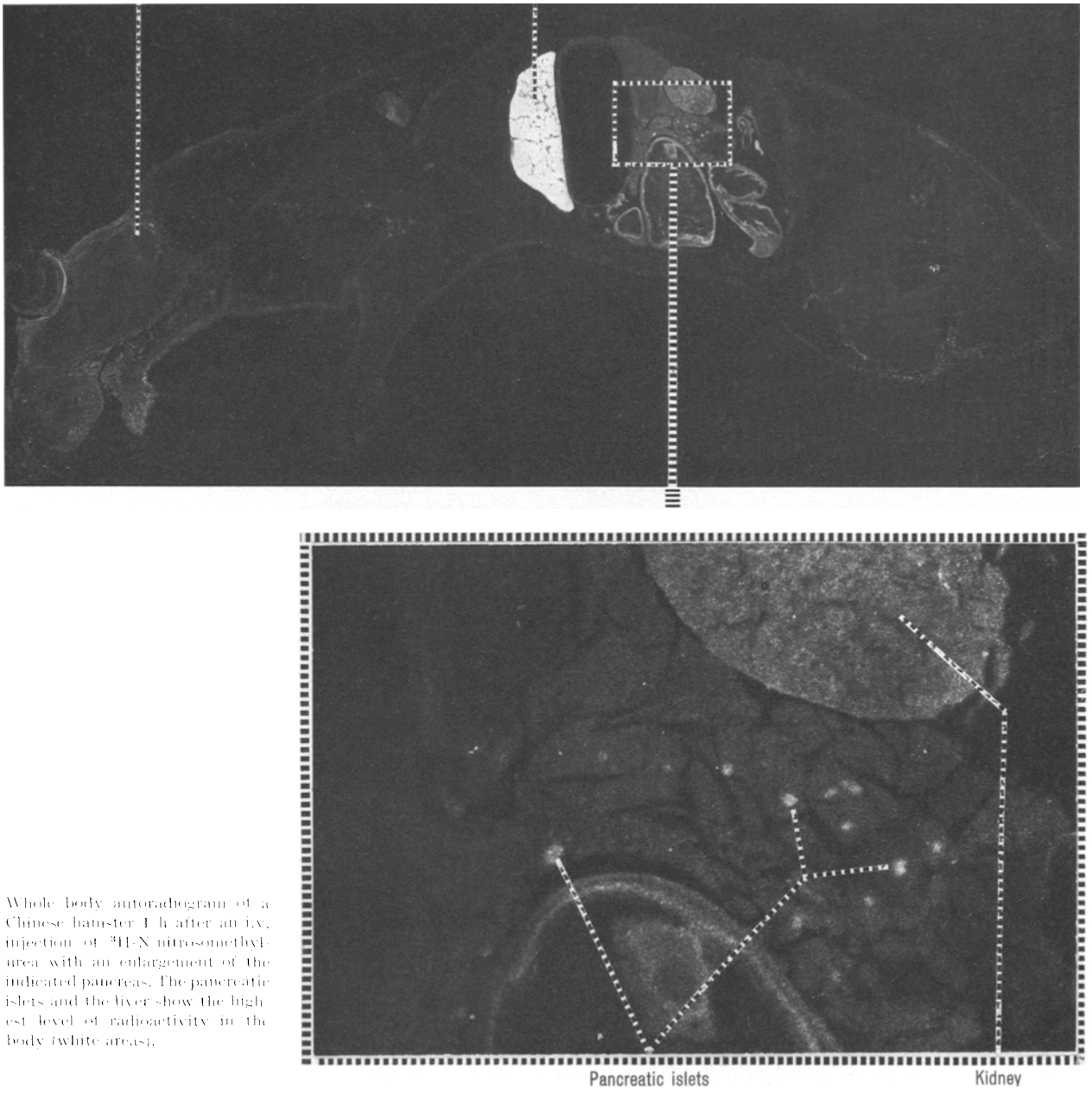
Specific Uptake of Labelled N-Nitrosomethylurea in the Pancreatic Islets of Chinese Hamsters

The environmental nitrosamines are considered to be potentially harmful to humans since many substances in this group have powerful biological properties under experimental conditions[1]. In addition to carcinogenic, teratogenic and mutagenic effects, a diabetogenic action of one of these substances, namely N-nitrosomethylurea (NMU) has recently been emphasized[2]. NMU is the aglucone of the well-known diabetogenic substance, streptozotocin (STZ). In mice the glucose carrier of STZ has been claimed to facilitate uptake of its cytotoxic group NMU into the islets[3] and the attempts to explain the mode of action of NMU are based on its non-selective distribution in the pancreatic islets. We have investigated the distribution of labelled NMU by whole-body autoradiography in mice and Chinese hamsters and have found an accumulation in the islet tissue of the latter species.

Non-diabetic adult Chinese hamsters and C57-B1 mice were injected i.v. with ^{3}H-N-nitrosomethylurea (^{3}H-methyl-labelled, spec. act. 48 mCi/mM: New England Nuclear, Boston Mass.).The injected dose was 40 mg/kg body weight (0.5 mCi/animal). The animals were killed at various time intervals up to 24 h after the injections. Autoradiograms of whole-body sagittal sections were made as described earlier[4]. The time of exposure was about 1 month.

In Chinese hamsters injected with labelled NMU, a high radioactivity occurred in the pancreatic islets. The selective uptake was observed after 30 min. At the 1 h survival interval, the radioactivity in the pancreatic islets and in the liver was the highest in the body (Figure).

[1] P. N. Magee, Fd Cosmet. Toxic. *9*, 207 (1971).
[2] C. Berne, R. Gunnarsson, C. Hellerström and E. Wilander, Lancet *1*, 173 (1974).
[3] T. Anderson, P. Schein, M. McMenamin and D. J. Conney, Clin. Invest. *54*, 672 (1974).
[4] S. Ullberg, Acta radiol. suppl., *1954*, 118.



Whole body autoradiogram of a Chinese hamster 1 h after an i.v. injection of ^{3}H-N nitrosomethylurea with an enlargement of the indicated pancreas. The pancreatic islets and the liver show the highest level of radioactivity in the body (white areas).

At later survival intervals, the radioactivity in the pancreatic islets decreased and did not exceed that in the exocrine pancreas or in the blood. Contrary to the results in the Chinese hamsters, the radioactivity in the pancreatic islets of the mice was low and did not exceed the level of the blood at any survival interval. NMU degrades rapidly in serum[5] and it is possible that the accumulated isotope represents a metabolite of NMU in the Chinese hamsters.

In mice, pancreatic islet β-cell destruction has been obtained only with high doses of NMU (230 mg/kg) which caused severe general toxic effects and death, making it impossible to study any potential development of hyperglycemia[6]. In Chinese hamsters, on the other hand, doses of 50 mg/kg of NMU resulted in overt diabetes[7]. It thus appears that the diabetogenic property of NMU is related to the ability of the substance or a metabolite of it to be selectively accumulated in the pancreatic islets. The mechanism of accumulation needs further investigation. The pronounced differences in the uptake of labelled NMU in the islets of Chinese hamsters and mice makes it difficult to anticipate the distribution of NMU in the islet tissue of other species (including man). Further studies in this respect are necessary to evaluate the possible role of the environmental N-nitroso-compounds in the etiology of diabetes mellitus[8].

Summary. Upon the administration of ^{3}H-N-nitroso-methylurea, a selective accumulation of radioactivity was observed in the pancreatic islets of the Chinese hamster, but not of the mouse.

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[5] P. SWANN, Biochem. J. *110*, 49 (1968).
[6] R. GUNNARSSON, C. BERNE and C. HELLERSTRÖM, Biochem. J. *140*, 487 (1974).
[7] E. WILANDER and R. GUNNARSSON, Acta path. microbiol. scand., in press.
[8] Supported by the Swedish Environmental Protection Board (grant no. 7-23/75).

Time-Response Patterns of Isolated Rat Uterus to Neurohypophyseal Peptides[1]

It has been suggested that oxytocin may have a chronotropic effect on the uterus, i.e., that it would change the resting frequency of contraction-relaxation cycles[2,3]. In our experiments, however, these changes were either absent or irregular and we found instead a concentration-related effect of hormone upon the duration of the first contraction[4]. The possibility that this phenomenon might be helpful in allowing the extension of the dose range in hormone assays, prompted some basic investigation of these time effects.

Materials and methods. Oxytocin (OT)[5], lysine vasopressin (LVP)[6], crystalline [1-β-mercaptopropionic acid] oxytocin (deamino-oxytocin, DOT)[7] and crystalline [1, 6-aminosuberic acid]oxytocin (AsuOT)[8] were used.

Uteri from adult virgin Sprague-Dawley albino rats (170–250 g) in proestrus or estrus were mounted for bioassay[9] by isometric contraction[10] using Mg^{++}-free van Dyke-Hastings solution[11]. Dose-response curves were obtained by increasing peptide concentrations in a geometric series until the tissue contracted maximally and then further until either a decline in the maximal response was seen or the time required for the response to return to the 50% level was greater than 10 min. Each contraction was allowed to pass its maximum and then to decline to at least the 50% level prior to washout of peptide. Only one analog was tested on each pair of uterine horns from the same animal, and in each instance oxytocin was also tested alternately on the same tissue.

The uterotonic response to each concentration of agonist was described in terms of maximal intensity reached and in terms of half-life ($t_{0.5}$) of the response; $t_{0.5}$ was measured from time of administration to the time in which the response to a given dose diminished to half of its maximal intensity. In addition, the integrals (A) for each response, corresponding to the area under the response curve from time zero to time $t_{0.5}$ ($A = \int_0^{t_{0.5}} E(t)_x \, dt$) were measured planimetrically and plotted vs. dose of agonist. Since the dose-area curve appeared linear at agonist concentrations where the dose-intensity curve had reached a plateau, it seemed plausible that high concentrations of oxytocin could be assayed by measuring area under the curve of the initial contraction instead of maximal intensity of this contraction. To test linearity at such high concentrations, 4 series of injections of oxytocin (one series consists of 20, 30 and 40 pmoles hormone injected in random order) were carried out on paired uterine horns. The areas under initial contraction curves were measured and the results statistically examined for linearity.

Results. Uterotonic responses to OT, DOT, AsuOT and LVP differed from each other quantitatively as well as qualitatively. Their roughly parallel log dose-intensity curves, obtained by stepwise increase of peptide concentration with repeated washout between individual challenges, is shown in the Figure. The measured values can be fitted by a modified logistic function[12] $E = E_m c^v / c_{0.5}^v + c^v)$, where E is a contraction in response to a hormone concentration c, E_m maximal attainable contraction, $c_{0.5}$ concentration causing a half-maximal response (0.5 E_m), and v an exponential constant ('Hill

[1] Supported by USPHS Grant No. AM-18399 and Swiss National Science Foundation Grant Nos. 3.424.70 and 3.2080.73
[2] H. JUNG in *Oxytocin* (Eds. R. CALDEYRO-BARCIA and H. HELLER; Pergamon Press, Oxford 1961), p. 87.
[3] J. J. POSEIRO and L. NORIEGA-GUERRA, in *Oxytocin* (Eds. R. CALDEYRO-BARCIA and H. HELLER; Pergamon Press, Oxford 1961), p. 158.
[4] M. KOIDA, J. D. GLASS, I. L. SCHWARTZ and R. WALTER, Endocrinology *88*, 633 (1971).
[5] M. MANNING, J. Am. chem. Soc. *90*, 1348 (1968).
[6] J. MEIENHOFER and Y. SANO, J. Am. chem. Soc. *90*, 2996 (1968).
[7] H. TAKASHIMA, V. DU VIGNEAUD and R. B. MERRIFIELD, J. Am. chem. Soc. *90*, 1323 (1968).
[8] T. YAMANAKA, S. HASE, S. SAKAKIBARA, I. L. SCHWARTZ, B. M. DUBOIS and R. WALTER, Molec. Pharmac. *6*, 474 (1970).
[9] R. WALTER, B. M. DUBOIS and I. L. SCHWARTZ, Endocrinology *83*, 979 (1968).
[10] P. HOLTON, Br. J. Pharmac. *3*, 328 (1948).
[11] R. A. MUNSICK, Endocrinology *66*, 451 (1960).
[12] R. B. PARKER and D. R. WAUD, J. Pharm. exp. Therap. *177*, 1 (1971).