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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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

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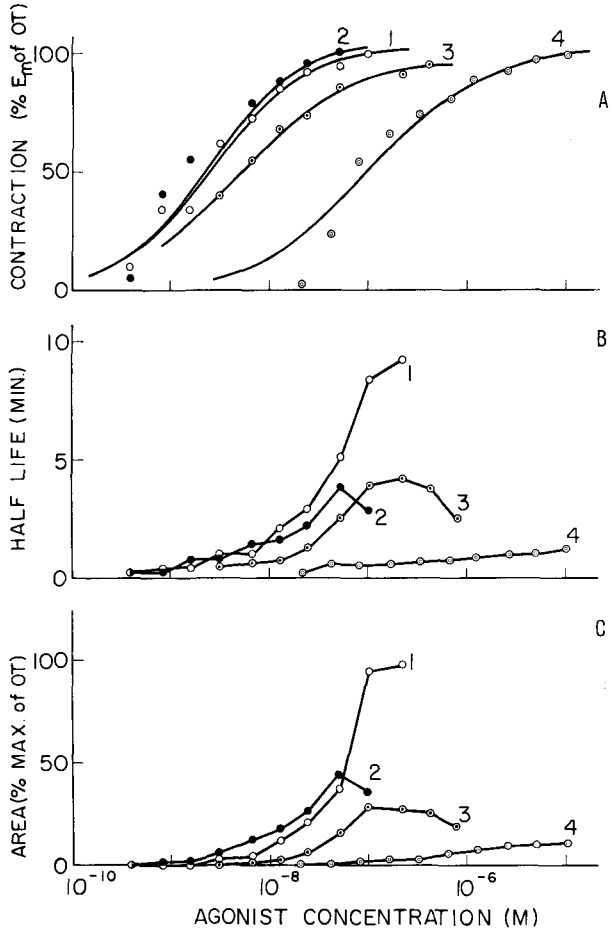
Table I. Parameters of dose-response curves for uterotonic response to neurohypophyseal hormones and analogs[a]

Peptide	pD_2	E_m	v
Oxytocin	8.61 ± 0.23	100.0 ± 2.6[b]	0.96 ± 0.14
Deamino-oxytocin	8.87 ± 0.07	103.9 ± 3.8	0.98 ± 0.37
[Asu[1,6]]oxytocin	8.30 ± 0.45	99.7 ± 9.6	0.76 ± 0.18
Lysine vasopressin	6.99 ± 0.75	102.6 ± 2.4	0.75 ± 0.11

[a] Values fitted according to WEIKER et al.[16] ± 95% confidence interval (see ref.[17]). [b] Reference value: E_m's in percent of oxytocin.



Dose-response curves on the isolated rat uterus of neurohypophyseal hormones and analogs: 1, oxytocin (○–○); 2, deamino-oxytocin (●–●); 3, [Asu[1,6]]oxytocin (○–○) and 4, lysine vasopressin (⊙–⊙). Panel A: log dose vs. maximum intensity of the first uterine contraction; lines are drawn according to theoretical calculated curves based on the dose-contraction data; symbols represent data points themselves. Panel B: log dose vs. half-time ($t_{0.5}$) of the initial contraction. Panel C: log dose vs. integral of the initial contraction curve to $t_{0.5}$, measured as area under the curve. For details of assay, measurement of response, and calculations, see text.

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coefficient', Table I). The differences in E_m and v values for various compounds are not statistically significant. The relative potencies calculated from the pD_2 values ($pD_2 = -\log c_{0.5}$)[13] of the present study for oxytocin and deamino-oxytocin are identical to those obtained by cumulative application of these two peptides[9,14], while the pD_2 values for AsuOT and LVP differed significantly when calculated from dose-response curves obtained by the two methods[15]. In contrast to the dose-intensity curves (Figure A), the log dose-half-life curves for the various peptides display significant differences in slope (Figure B).

To combine the intensity and time vectors, we plotted dose-area curves, using the planimetrically determined integrals (A) of the area under the response curve ($E(t)$). As may be seen in the Figure C, the log dose-dependence of A is similar to that of the corresponding $t_{0.5}$ curve. To determine the accuracy of the 3 parameters, intensity of contraction E, values A and $t_{0.5}$ were converted to a fraction of their maximal values and a two-way analysis of variance for each parameter and each substance was carried out (variations between uterine horns and dose levels were considered; residual variances, i.e., 'internal' errors of estimation, were compared by the F-test). In the case of oxytocin and DOT, as expected, the residual variance of $t_{0.5}$ is significantly greater than those of E and A (probability level $p > 97.5\%$). For AsuOT, the residual variance increases in the series $t_{0.5}$, E, A; all three variances are significantly different ($p > 97.5\%$). As for LVP, the residual variances display only insignificant differences. From the viewpoint of accuracy, the parameter A seems to be slightly better than the others, but obviously there is only little difference between the three.

The parameter A was found to be more sensitive than E at high hormone concentrations; in the dose range studied (i.e., 20–40 pmoles oxytocin/ml), the intensity of the first contraction was much less affected than parameter A which increased with increased dose (Table II). Analysis of variance indicated that the linear trend for parameter A was significant at the 1% level.

Discussion. The data in the Figure indicate that the relationship between the time and intensity parameters of the in vitro uterotonic response is different for different

Table II. Comparison of contractions determined by measuring the area under the contraction curve or by measuring the intensity of the first contraction of the isolated rat uterus stimulated by high doses of oxytocin[a]

Uterus	Series of injection	Parameter A[b] 20 pmole oxytocin/ml	30	40	Parameter E 20 pmole oxytocin/ml	30	40
Right	1	21	29	42	37	38	39
	2	22	28	34	36	39	39
	3	24	27	33	35	37	38
	4	20	25	29	30	37	38
Left	1	24	32	42	36	39	40
	2	23	29	37	38	40	41
	3	25	30	36	35	39	39
	4	19	30	31	26	40	40

[a] Area under the 1st contraction curve; E = maximal intensity of first contraction. [b] The slope coefficient a from equation $y = ax + b$ for parameter A is 6.62.

neurohypophyseal hormones and analogs. It appears that substances which affect the uterine motility not only change the intensity of the rhythmic contraction, but can also modify the time course of the individual contraction.

Three alternative explanations of these findings come to mind: 1. the differences in the time course of contractions are determined by the complicated nature of tonic/phasic relationships and by different receptor reserve for different substances; 2. separate receptors for different substances occur in myometrium; the very short duration of the LVP-induced contraction is particularly striking in this context; 3. the myometrium contains two populations of receptors, one for contraction and one for relaxation; different substances may have different affinities for each of the receptor populations.

The finding that the area under the contraction curve is a sensitive measure of the response of the uterus to high concentrations of hormone allowed us to use this parameter for rapidly measuring residual hormonal activities in a large number of samples after incubations of neurohypophyseal hormones with degradative enzymes, without prior dilution of samples[4]. The method was found to be valid for deamino-oxytocin and bradykinin as well as for oxytocin.

In summary, the time-response patterns of the isolated rat uterus to neurohypophyseal peptides was studied. At high doses of peptide a concentration-related effect upon the duration of the first contraction was found and the area under this contraction is useful for the bioassay of oxytocin and certain other peptides.

Zusammenfassung. Halbwertzeit und Zeit-Effekt-Integral für die erste Oxytocin-Kontraktion der isolierten Gebärmutter der Ratte wurden untersucht. Eine Verlängerung der Kontraktion wurde bei erhöhter Dosis beobachtet. Entsprechende unterschiedliche Abhängigkeiten für verschiedene uterotonisch wirkende Peptide sind deutlich. Zur Auswertung des Bioassays von Oxytoxin und gewisser Analogen wird die Verwendung des Integrals vorgeschlagen.

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The Methylation of Mercuric Chloride by Human Intestinal Bacteria

The ability of microorganisms in sediments of lakes and rivers to transform inorganic mercury to the far more toxic methylmercury and dimethylmercury is a well documented phenomenon[1,2]. There is now evidence that ingested mercuric chloride ($HgCl_2$) may be methylated, in vivo, in the rat intestine[3] and, in vitro, by human faeces[4]. However, one cannot infer from these experiments that the microbial flora of the intestine is responsible for the methylation reaction, since the gut contents contain several sources of metabolic activity other than bacteria[5]. We would like to present data on the ability of pure cultures of bacteria and yeasts, isolated from human faeces, to convert $HgCl_2$ to methylmercury.

Strains of *Escherichia coli*, streptococci, staphylococci, bacteroides and bifidobacteria were inoculated into a medium containing 0.1 *M* potassium phosphate buffer, pH 7.0, Bacto-peptone, Bacto-tryptone, yeast extract and D-glucose, each at 0.5% (w/v). Strains of lactobacilli were cultured in Oxoid MRS broth and yeasts in Oxoid Sabouraud medium. The cultures were incubated for 44 h at 37 °C under aerobic conditions, except for lactobacilli (under carbon dioxide) and bacteroides and bifidobacteria (under oxygen-free nitrogen) and in the presence of $^{203}HgCl_2$ (Radiochemical Centre, Amersham) at a final concentration of 5 µg/ml. Before use the solution of $^{203}HgCl_2$ was shaken with redistilled benzene to remove any organic impurities.

At the end of the incubation, methylmercury was extracted from the incubation mixture by the method described by WESTÖÖ[6]: the mixtures (2 ml) were acidified with 0.4 ml concentrated HCl and extracted with 2.4 ml redistilled benzene. A portion of the benzene extract was then chromatographed on silica gel G using chloroform-*n*-hexane (90:10, v/v) as developer[7]. The Rf values of methylmercuric chloride and $HgCl_2$ under these conditions were 0.85 and 0.2 respectively. The position of methylmercuric chloride was visualized by spraying with 0.04% (w/v) dithizone in chloroform and the zone scraped into a scintillation vial and counted for radioactivity. The identity of methylmercuric chloride was further confirmed by chromatography on silica gel G with *n*-hexane-acetone (70:30, v/v) as solvent[8].

Methylmercury was produced by a major proportion of strains of staphylococci, streptococci, yeasts and *E. coli*, but by only a small percentage of obligately anaerobic bacteria (bacteroides and bifidobacteria) and lacto-

Methylmercury synthesis by intestinal bacteria

Microorganism	No. tested	No. positive	Range (ng CH_3HgCl formed/ml/44 h)
Streptococci	6	4	2.1–5.8
Staphylococci	10	6	0.5–5.0
E. coli	5	3	0.9–3.0
Yeasts	9	4	0.7–1.7
Lactobacilli	9	1	0.5
Bacteroides / Bifidobacteria	10	3	0.4–0.6

The strains were incubated with $^{203}HgCl_2$ (5 µg/ml) for 44 h at 37 °C and any methylmercury formed was extracted into benzene and subjected to thin-layer chromatography as described in the text.

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