NTPP did not affect the lipaemic level (B); hydrocortisone caused a pronounced hyperlipaemia (C) which was decreased by the simultaneous administration of NTPP (D). Figure 2 reveals that hydrocortisone caused an elevation of both glycogen fractions in the liver (C), but this increase was partially inhibited by the simultaneous administration of NTPP (D). NTPP alone did not affect the glycogen level (B).

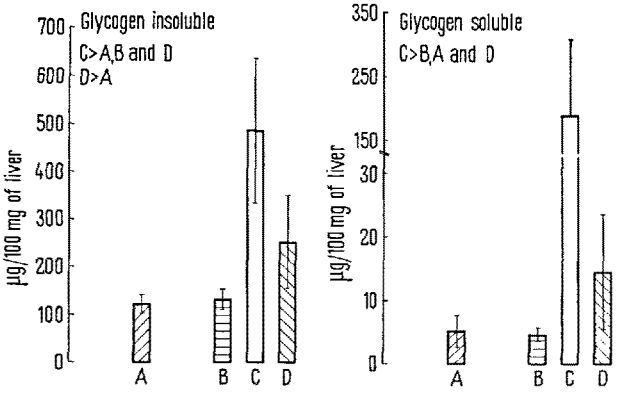

Fig. 2. The level of soluble and insoluble glycogen in liver after 10 days' treatment with NTPP and hydrocortisone. A, control; B, NTPP, C, hydrocortisone; D, NTPP + hydrocortisone. The differences are significant at $p = 0.05$.

Since the increase of both the lipid level in blood and the glycogen content in liver is a concomitant phenomenon of the proteino-catabolic action of glucocorticoids, the decrease both of lipaemia and of glycogen content caused by NTPP could be taken for an anticatabolic effect. This fact can serve as an explanation of why NTPP alone does not exert a substantial influence on lipaemia and glycogen levels in liver.

Zusammenfassung. Der durch Hydrokortison (zehntätige Zufuhr) erhöhte Spiegel der gesamten Lipämie im Serum und der löslichen und unlöslichen Glykogenfraktion in der Leber der Ratte wurde mittels gleichzeitiger 19-Nortestosteron-phenylpropionatzugabe erniedrigt, während 19-Nortestosteron-phenylpropionat allein die verfolgten Parameter nicht beeinflusste.

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Research Institute for Natural Drugs, Prague (Czechoslovakia), January 25, 1965.

Demethylation in vivo of Natulan, a Tumour-Inhibiting Methylhydrazine Derivative

The metabolism of the tumour-inhibiting methylhydrazine derivative RO 4-6467[1] (trade name: Natulan) has been investigated by OLIVERIO and KELLY[2] and by RAAFLAUB and SCHWARTZ[3]. These authors have found a very rapid dehydrogenation of the drug in the plasma and the appearance in the urine of N-isopropylterephthalamic acid. Furthermore it may be of interest that, in vitro, autoxidation of benzylhydrazine leads to the formation of monomethylhydrazine (BERNEISet al.[4]). N-demethylation of Natulan has been postulated by WEITZEL et al.[5,6]. These authors were able to detect formaldehyde after oxidation of the drug in vitro. The metabolism of iproniazid[7] is an example of dealkylation of an alkylhydrazine derivative.

Removal of the 1-methyl group and formation of N-isopropylterephthalamic acid suggests that the Natulan molecule is ultimately cleaved at both sides of the hydrazine group. However, the possibility of the splitting of the N–N bond, according to the classical example of prontosil[8], should also be considered.

This paper reports on the in vivo demethylation of 1-$^{14}CH_3$-Natulan in normal mice and rats, and in rats after pretreatment with phenobarbital. In addition, results obtained by perfusion of isolated rat livers are reported.

Methods. Male Swiss Albino mice weighing 20 g and male rats of 140–200 g (strain 'Füllinsdorf', Hoffmann-La Roche & Co.) were used for the in vivo experiments. Rats from the same source but weighing 250–280 g were used in the perfusion experiments.

All in vivo experiments were initiated by intraperitoneal administration of a single dose of 1-$^{14}CH_3$-Natulan. The metabolic cage and CO_2-trapping devices have been described recently[9]. The perfusion apparatus was a further development of the one described by MILLER et al.[10]. Radioassay was conducted in a TRICARB-liquid-scintillation spectrometer (Packard, Mod. 314 EX).

Results (see Tables). The total expired $^{14}CO_2$ was 19–21% after 24 h in mice and 19% after 12 h (extrapolated: 21% after 24 h) in rats in relation to the original radioactivity administered.

Three perfusion experiments with a perfusate containing 1-$^{14}CH_3$-Natulan (1 mM/l) yielded the following rates of $^{14}CO_2$-production at the steady state: 1.20, 1.25, and 1.43 μM/h/100 g body weight (mean value: 1.29). This corresponds to 75–80% of the mean $^{14}CO_2$ expired following administration in vivo (see Tables 2 and 3). The $^{14}CO_2$ values obtained by the perfusion technique decreased to 2% of the liver perfusion values when the circulating perfusate by-passed the liver.

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Table I. $^{14}CO_2$-expiration in mice

Animal number	1	2	3	4	5	Mean values
Body weight (g)	18.5	19	21	21	22	± S.D.
Natulan dose mg/kg	540	220	200	260	110	
$^{14}CO_2$ rate of expiration at the steady state (μM/h/100 g body weight)	3.5	3.6	2.5	3.3	2.5	3.1 ± 0.3
$^{14}CO_2$ total expiration after 7 h; % of original administered activity	10.5	15.4	9.1	12.1	12.5	11.9 ± 5.6

Table II. $^{14}CO_2$-expiration in rats

Animal number	1	2	3	4	Mean values
Body weight (g)	140	140	145	145	± S.D.
Natulan dose mg/kg	210	210	206	200	
$^{14}CO_2$ rate of expiration at the steady state (μM/h/100 g body weight)	1.75	1.80	1.80	1.45	1.7 ± 0.3
$^{14}CO_2$ total expiration after 7 h; % of original administered activity	12.9	13.0	10.0	9.3	11.3 ± 3.7

Table III. Induction experiments with phenobarbital in rats. Administration of Natulan (200 mg/kg i.p.) (a) before and (b) after induction of drug enzymes by phenobarbital. Microsomal enzymes were induced by injecting daily single doses of 100 mg/kg phenobarbital (Luminal Bayer) for five days. The second dose of Natulan was administered 48 h after the last phenobarbital injection

Animal number	1	2	3	4	Mean values Nos. 1–3
$^{14}CO_2$ expiration at the steady state:					
(a) before induction	1.50	1.66	1.60	1.05	1.59
(b) after induction (μM/h/100 g body weight)	3.80	4.20	3.50	2.67	3.83
Ratio of induction (b/a):	2.5	2.6	2.2	2.5	2.4

Discussion. Administration of 1-$^{14}CH_3$-Natulan to mice and rats is followed by expiration of $^{14}CO_2$. In rats the steady state lasts 3–5 h. Total amounts of $^{14}CO_2$ and of N-isopropylterephthalamic acid in 24 h are of the same order of magnitude[2,3].

The question arises as to where demethylation occurs in the metabolism of Natulan. A direct demethylation of Natulan is unlikely since the drug is rapidly and completely converted into the azo compound[3], which still carries the methyl group. Thus the substrate of demethylation may be the azo or hydrazone derivative or ultimately the hypothetical[3] metabolite monomethylhydrazine (MMH). Several findings, however, suggest that the demethylation of MMH is unlikely: (1) Equimolar doses of ^{14}C-MMH undergo considerably slower demethylation than Natulan in rats[11]. Whether the relatively polar MMH reaches the microsomal demethylases at the same rate when administered exogenously, is still questionable. (2) The steady state of $^{14}CO_2$ expiration occurs only 10 min after administration of Natulan. In that time the azo compound must have accumulated in large amounts[3], while the formation of MMH requires further metabolic steps. (3) MMH was not detected by RAAFLAUB and SCHWARTZ[3], although it would be expected to accumulate because of its low demethylation rate.

Demethylation of Natulan seems to occur primarily in the liver as shown by the comparison of the demethylation rates in vivo and those obtained by perfusion of the isolated liver. The considerable increase in the rate of demethylation in vivo by pretreatment with phenobarbital suggests that microsomal enzymes have been induced. This microsomal enzyme system thus seems to be responsible for the demethylation of Natulan. Experiments in vitro[12] confirm this result. In rats the rates of

[11] M. BAGGIOLINI, M. H. BICKEL, and F. S. MESSIHA, unpublished results.

[12] M. BAGGIOLINI, M. H. BICKEL, and F. S. MESSIHA, in preparation.

microsomal N-demethylation of Natulan and other drugs like methixen[13] and aminopyrine[14] are of a similar order of magnitude (1.7, 0.3, and 3.0 μM/h/100 g body weight respectively[15]).

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[15] Acknowledgment: The authors thank Messrs. F. Hoffmann-La Roche & Co. for a generous gift of $^{14}CH_3$-Natulan which was synthesized by D. J. WÜRSCH.

Zusammenfassung. Die intraperitoneale Verabreichung von 1-$^{14}CH_3$-Natulan bei der Maus und der Ratte hat eine Ausscheidung von $^{14}CO_2$ in der Atemluft zur Folge. Die Demethylierungsrate in vivo beträgt bei der Maus 3,1, bei der Ratte 1,7 μM/h/100 g Körpergewicht. Sie wird bei der Ratte durch Behandlung mit Phenobarbital mehr als verdoppelt. Über das Substrat der N-Demethylierung lässt sich keine sichere Aussage machen.

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Medizinisch-Chemisches Institut der Universität Bern (Switzerland), March 2, 1965.

β-Glucuronidase Activity in the Liver of Infant Mammals

The metabolism of glucuronides has received much attention because of its relationship to neonatal jaundice (Symposium 1959[1], Symposium 1963[2], MARSH and LEVVY[3], CONCHIE and LEVVY[4]). The development of conjugation ability has been studied to a large extent, but surprisingly enough the development of β-glucuronidase activity in the liver has been followed in mice only. It has been reported (KARANURAITNAM et al.[5]) that activity is much higher in new-born and infant mammals than in adult animals. It has been shown in previous work (HERINGOVÁ et al.[6]) that in the rat ileum there is a rise in activity after birth and a fall after weaning. This is in contrast to the finding of KARANURAITNAM et al.[5] for mice liver β-glucuronidase. Hence it was decided to study the development of β-glucuronidase activity in the liver of infant mammals of different species, i.e. those born in a mature and those born in an immature state.

Activity was determined according to TALALAY et al.[7] in the liver of rats, mice, rabbits and guinea-pigs. Phenolphthalinglucuronide served as the substrate, the pH being adjusted to 4.5 in acetate buffer. Activity is expressed in μg released phenolphthaline/mg wet weight/60 min at 38°C.

The Figure shows that activity in new-born *rats* is significantly lower than in adult animals. There is a rise after birth, and a fall to adult values at the time of weaning. In *guinea-pigs* activity is highest at birth and then gradually decreases up to day 24. In *rabbits* values increase up to day 3 and then remain unchanged. In *mice*

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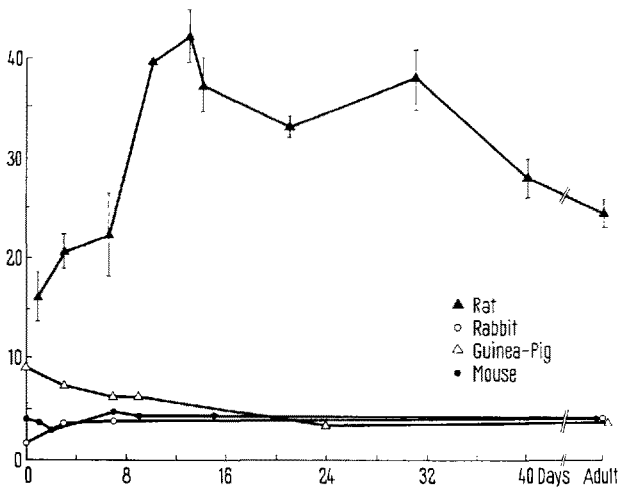

β-glucuronidase activity in the livers of different species. Abscissa: age in days after birth. Adult animals 3–4 months old. Male and female animals in the same proportion. Ordinate: β-glucuronidase activity expressed in μg phenolphthalin liberated/mg wet weight/60 min. The final concentration of phenolphthalinglucuronide in the assay according to TALALAY et al.[7] is 0.001 M. Each circle is the average of 10 determinations. Vertical lines: S.E., if larger than circle. Statistical significance: *Rats.* 1-day-old against 10, 13, 14, 20, 32, and 40-day-old and adult for $p < 0.001$. 3-day-old against 10, 13, 14, 22, and 32-day-old for $p < 0.001$, against 40-day-old for $p < 0.02$. 6-day-old against 10, 13, 14, 22, and 32-day-old for $p < 0.001$. 10-day-old against 22 and 32-day-old and adults for $p < 0.001$. 13-day-old against 22-day-old for $p < 0.02$, against 40-day-old for $p < 0.01$ and adults for $p < 0.001$. 14-day-old against 40-day-old and adults for $p < 0.001$. 22-day-old against 40-day-old and adults for $p < 0.01$. 32-day-old against 40-day-old for $p < 0.02$ and adults for $p < 0.001$. *Mice.* 0-day-old against 2-day-old for $p < 0.02$, against 7-day-old for $p < 0.05$. 1-day-old against 7-day-old for $p < 0.05$. 2-day-old against 7 and 9-day-old for $p < 0.001$, against adults for $p < 0.01$, 7-day-old against adults for $p < 0.02$. *Rabbits.* 0-day-old against 3 and 7-day-old and adults for $p < 0.001$. *Guinea-pigs.* 0-day-old against 3-day-old for $p < 0.02$, against 7-day-old for $p < 0.01$, against 9-day-old for $p < 0.02$, against 24-day-old and adults for $p < 0.001$. 7-day-old against 24-day-old for $p < 0.001$, against adults for $p < 0.01$. 9-day-old against 24-day-old for $p < 0.002$, against adults for $p < 0.01$, 24-day-old against adults for $p < 0.05$.