remained constant during 3 recrystallizations. In another experiment, MNG was isolated as pure crystals from the cummulative urine of rats receiving non-radioactive MNNG in DMSO at a dose of 100 mg/kg at 4-days intervals for one month (total dosage: 1100 mg) and identified by admixture with authentic sample. The third metabolite (III) has not yet been identified. It does not appear to be the general glucuronide because there was no change in chromatographic behavior of the radioactive spot on the chromatogram, even after the action of β-glucuronidase at 37 °C for 24 h in acetate buffer (pH 5.0).

In determining the metabolic sequence of MNNG, it is plausible to presume at least 2 possible pathways for the formation of NG in rats.

The first would be the N-demethylation of MNG and the second alternative is the nucleophilic attack of amino group on guanidine carbon, probably after elimination of hypothetic compound CH_3–N = N–OH.

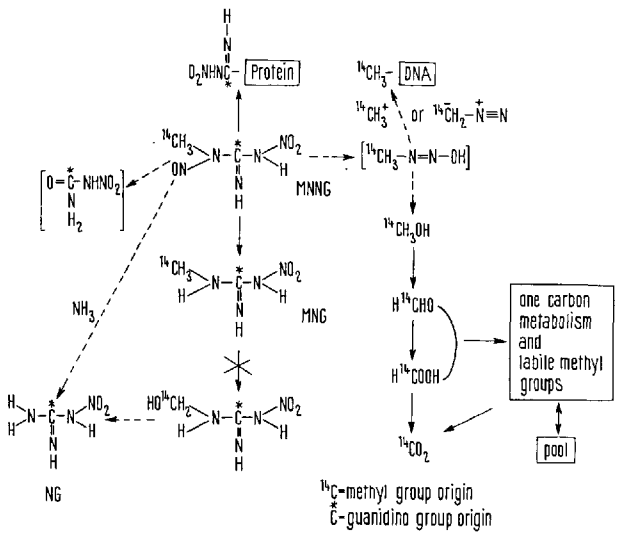
The first possibility was rejected due to the following in vivo and in vitro data. After an oral dosage of MNG (4.6 μCi/kg), methyl-labelled MNG was excreted unchanged in the rat and no $^{14}CO_2$ was detected in the expired air from rats, as shown in Table I. When methyl-labelled MNG was incubated in vitro with the rat liver homogenate fortified with co-factors at 37 °C for 30 min in air, and with constant shaking to determine the N-demethylation of MNG by the method of NASH[19], a measurable amount of formaldehyde labelled with ^{14}C was not obtained from the incubation mixture of MNG. Thus this fact is in favor of the hypothesis that MNG is very stable both in vivo and in vitro, and NG is not likely to be formed as a result of N-demethylation of MNG. Therefore, the alternate pathway in which MNNG reacts with amino group to give NG in the body would seem to predominate and can account for the formation of NG. Furthermore, our suggested metabolic route was supported by the fact that MNNG reacted with ammonia to give NG in vitro[20].

The authors wish to propose that the metabolic pathways of MNNG in rats are as shown in the Figure.

The distribution of ^{14}C in the organ at 48 h after a single oral dose of MNNG is shown in Table II. The organ-affinity was compared for level of ^{14}C in terms of relative specific activity (RSA). Although significant difference of the affinity among these organs was not observed, the values of RSA can be divided into 3 groups. The first group of relatively high RSA was found in forestomach, stomach contents, liver, intestines and kidneys. The second group indicating medium RSA-values included spleen, glandular stomach, oesophagus, heart and lungs with RSA value in the spleen close to the first group. Third group showed low RSA values and included brain, testicle, adrenal glands, prostate and muscles.

From the above data, one cannot say distinctly that there is stomach-specific affinity, since the comparison of the RSA values in our experiments did not clearly indicate sharply high values in the stomach. However, stomach-affinity must be examined at different time intervals before an exact conclusion could be attained.

The present results differ somewhat from those of KAWACHI et al.[14] in the experimental conditions, the oxidative ratio of guanidino- and methyl-carbons of MNNG and one of the metabolites, although the isolation of MNG is a common finding to us and the other group[14,21].



Presumed metabolic pathways of MNNG in rats.

Zusammenfassung. Versuche mit ^{14}C-markiertem Carcinogen MNNG ergaben an Ratten nach einmaliger Verabreichung der Substanz eine vorwiegend über die Niere erfolgende Elimination. Die Metabolite von MNNG in Verknüpfung mit verschiedenen Stoffwechselstufen und ihre Verteilung im Gewebe wurden näher verfolgt.

A. TANAKA and T. SANO[22]

Department of Radiochemistry,
National Institute of Hygienic Sciences,
Kamiyoga, Setagaya, Tokyo 158 (Japan), 5 April 1971.

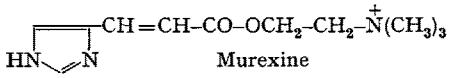
[19] T. NASH, Biochem. J. *55*, 416 (1953).
[20] M. NAKADATE, S. KAMIYA and I. SUZUKI, unpublished results.
[21] Acknowledgment. The authors wish to thank Drs. M. ISHIDATE and G. URAKUBO for their interest and support.
[22] Present address: Showa College of Pharmaceutical Sciences, Tsurumaki, Setagaya, Tokyo (Japan).

Occurrence of Dihydromurexine (Imidazolepropionylcholine) in the Hypobranchial Gland of *Thais (purpura) haemastoma*[1]

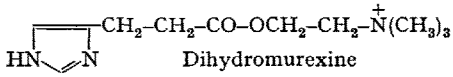
The hypobranchial gland of the Mediterranean snail *Murex trunculus* contains large amounts of urocanylcholine or murexine[2]. The same choline ester occurs also in *Murex brandaris, Tritonalia erinacea, Murex fulvescens, Thais lapillus, Urosalpinx cinerea, Concholepas concholepas*[3–5] and a number of other molluscs belonging to the families of Muricidae and Thaisidae[6]. The hypobranchial gland of *Thais floridana, Thais chocholata* and other related species contains, in its turn, senecioylcholine or β,β-dimethylacryloylcholine[7,8], and that of

Buccinum undatum, a member of the same order but not of the same family, and not a dye-secreting snail, contains acryloylcholine[9]. Different choline esters may be present in the same hypobranchial body. That of *Concholepas concholepas*, for example, contains both murexine and senecioylcholine[5].

This communication describes the occurrence in the hypobranchial body *Thais (purpura) haemastoma*, collected on the coasts of Latium (Italy) of dihydromurexine or imidazolepropionylcholine, a choline ester hitherto unknown in nature.



Murexine



Dihydromurexine

In a first experiment the whole hypobranchial body was removed from the living animal after cautious rupture of the shell and immediately extracted with 6 parts (w/v) of methanol. After 3 days the supernatant liquid was decanted and the tissue re-extracted with 5 parts of 80% methanol. The extracts were mixed and filtered. In another experiment the yellowish strip in the middle of the hypobranchial body was cut away from the remaining hypobranchial tissue and the 2 parts were extracted separately with methanol. The yellowish strip constituted 15–20%, in weight, of the total hypobranchial body. Part of the extracts was used as such, part was submitted to chromatography on an alkaline alumina column which was eluted with descending concentrations of ethanol[5].

Crude extracts and eluates from the alumina column were submitted to paperchromatography, thin-layer chromatography, high voltage electrophoresis and bioassay, using the isolated frog rectus abdominis muscle. Chromatograms and pherograms were developed with the Pauly reagent, the NNCD reagent (2-chloro-4-nitrobenzenediazoniumnaphthalene-2-sulphonate), the dichloroquinone chlorimide reagent, and finally the Dragendorff reagent[5].

Besides more or less conspicuous amounts of purple prepigments, and of senecioylcholine and free choline, large amounts of imidazolepropionic acid and of its methyl ester were found, together with a new imidazole choline ester which proved to be dihydromurexine.

Synthetic choline chloride, murexine chloride hydrochloride, senecioylcholine jodide, dihydromurexine dipicrate, urocanic acid, imidazolepropionic acid and the ethyl and methyl esters of imidazolepropionic acid were available for comparison.

Following chromatography on alumina column, the choline ester supposed to be dihydromurexine appeared in the 90% ethanol eluate. Its identification as dihydromurexine was based on the following data: a) Both the unknown imidazole choline ester and synthetic dihydromurexine showed the same colour shades with the Pauly reagent (pink red) and the Dragendorff reagent (orange red). b) On high voltage paper electrophoresis the unknown derivative showed the same mobility towards the cathode as synthetic dihydromurexine: $E_{1.8} = 1.1–1.2$ choline; $E_{5.8} = 1.3$ choline. c) On paper chromatography the Rf values of the unknown imidazole derivative and those of synthetic dihydromurexine were exactly the same in 3 solvent systems: Rf 0.1–0.12 in *n*-butanol-acetic acid-water (4:1:5), Rf 0.27–0.32 in 1-pentanol-pyridine-water (2:2:1) and finally Rf 0.91–0.93 in 20% KCl.

Similar results were obtained in thin-layer chromatography: no mobility with the chloroform-pentane mixture (1:1) and very poor mobility (Rf 0.01–0.02) with the butanol-acetic acid-water (4:1:5) mixture. Alkaline solvent systems could not be used owing to the lability of dihydromurexine. d) Upon hydrolysis with hydrochloric acid (*2N* HCl, 2 at 100 °C) amounts of the unknown derivative and of synthetic dihydromurexine showing the same biological activity on the frog rectus muscle or a Pauly reaction of the same intensity yielded equal amounts of choline and imidazolepropionic acid.

Dihydromurexine was totally localized in the yellowish strip occupying the middle of the hypobranchial gland. In fact, methanol extracts of this strip contained, when examined immediately after their preparation, as much as 15,000–17,000 µg dihydromurexine per g fresh tissue, as compared to the 150–200 µg/g occurring in the remaining hypobranchial tissue.

Large losses of the base occurred not only during chromatography on alumina column, but even during storage in the refrigerator of crude methanol extracts or ethanol eluates. This was due to the intensity of transesterification and hydrolysis processes.

On the frog rectus abdominis muscle dihydromurexine appeared to be 12–16 times as potent as murexine, on a weight basis, 10–12 times as potent as senecioylcholine and finally 6–9 times as potent as acetylcholine. All compounds were considered as free bases.

Dihydromurexine has been found in the hypobranchial body of several other molluscan species of the Thaisidae and Muricidae families.

A more thorough description of this choline ester, its precursors and metabolites will be presented in a paper in extenso, in which also the problem will be discussed of whether the different compounds found in methanol or acetone extracts of the hypobranchial body pre-exist in the living tissue or are artifacts due to the extraction procedure and/or to the successive manipulation of the extracts.

Riassunto. Gli estratti metanolici di organo ipobranchiale di *Thais (purpura) haemastoma* contengono cospicui quantitativi di un nuovo estere della colina, la diidromurexina o imidazolpropionilcolina. La base è localizzata esclusivamente nella striscia mediana giallastra dell'organo ipobranchiale, ove può raggiungere concentrazioni dell'ordine di 15–17 mg per g di tessuto fresco.

M. Roseghini

Istituto di Farmacologia medica I, Università di Roma, 13 March 1971.

[1] Supported by grants from the Consiglio Nazionale delle Ricerche, Roma.

[2] V. Erspamer and O. Benati, Biochem. Z. *324*, 66 (1953).
[3] V. Erspamer and A. Glaesser, Br. J. Pharmac. *12*, 176 (1957).
[4] V. P. Whittaker and I. A. Michaelson, Biol. Bull., Woods Hole *107*, 304 (1954).
[5] M. Roseghini, V. Erspamer, L. Ramorino and J. E. Gutierrez, Eur. J. Biochem. *12*, 468 (1970).
[6] M. Roseghini, to be published (1971).
[7] V. P. Whittaker, Biochem. J. *66*, 35 P (1957).
[8] M. J. Keyl, I. A. Michaelson and V. P. Whittaker, J. Physiol., Lond. *39*, 434 (1957).
[9] V. P. Whittaker, Biochem. Pharmac. *1*, 342 (1959).