eingehen. Dass ein analoges Verhalten im Falle der Zn(II)-DTPA vermisst wird, kann als Hinweis für einen anderen, eventuell mit der hohen Konzentration der injizierten Lösungen zusammenhängenden Mortalitätsmechanismus höherer Zn(II)-DTPA-Dosen aufgefasst werden; dafür sprächen auch die im Vergleich zu Ca-DTPA grösseren Regressionskoeffizienten (Figur 1).

Was das Verhältnis der toxischen Dosen beider Chelate betrifft, so sind, wie die Figur 2 zeigt, zwei grundsätzlich verschiedene Vergleichsmöglichkeiten gegeben: (a) Bei einer vorgegebenen Zahl von Chelatapplikationen muss zur Erreichung des gleichen toxischen Effekts die Einzeldosis der Zn(II)-DTPA 2,5mal grösser als bei Ca-DTPA sein. (b) Bei gleicher Höhe der Einzeldosen dagegen muss die Zahl der Zn(II)-DTPA-Applikationen um rund das 30fache erhöht werden. Die in (a) und (b) angegebenen Quotienten müssen insofern als Mindestwerte angesehen werden, als nach dem oben Gesagten die Möglichkeit besteht, dass die Toxizität der Zn(II)-DTPA in dem untersuchten Dosisbereich z. T. unspezifischer Art ist.

Die Erhöhung des therapeutischen Index ist somit nur im Falle (b) ausreichend gross, um die Verwendung der Zn(II)-DTPA in Erwägung ziehen zu können. Anders ausgedrückt, wird das Zn(II)-Chelat nur dann Vorteile bieten, wenn eine über längere Zeit fortgesetzte Chelatmedikation indiziert ist.

Summary. The lethal effect of daily intraperitoneal administrations of Ca-DTPA and Zn(II)-DTPA was studied in mice. Due to recovery processes, single chelate doses are non-additive and the cumulative LD_{50} increases with decreasing daily dosage. At a given number of administrations, Zn(II)-DTPA is 2.5 times less toxic than Ca-DTPA. However, if the daily dosage is kept constant and the number of administrations variable, Zn(II)-DTPA is 30 times less toxic.

A. Catsch und E. von Wedelstaedt

Institut für Strahlenbiologie, Kernforschungszentrum Karlsruhe (Deutschland), 4. Dezember 1964.

Gastric Excretion of C^{14}-Nicotine[1]

In an earlier investigation[2] we observed, using an autoradiographic method[3], that C^{14}-nicotine was accumulated in the gastric mucosa and also excreted into the stomach content. Several authors[4–7] have shown that nicotine interferes with the vascularity and motility of the stomach. The role of nicotine as a factor in causing peptic ulcers has been discussed by various writers[8]. This paper is an extension of our earlier studies presenting a quantitative analysis of the gastric excretion of C^{14}-nicotine and its metabolites.

The (−)-nicotine-methyl-C^{14} used in this investigation was synthetized by methylating (−)-nornicotine using formaldehyde-C^{14} in the presence of formic acid[2,9]. The C^{14}-nicotine thus obtained was radiochemically pure and had a specific activity of 66 μC/mg.

Mice, rats, and cats were used as experimental animals. A series of mice were injected with C^{14}-nicotine intravenously and sacrificed at 5, 15 and 30 min, and 1 and 4 h after the injection. The dose employed was 2.5 μg/g of body-weight, corresponding to 0.16 μC/g. Whole body autoradiography was performed according to the Ullberg method[3]. Figure 1 is an autoradiogram showing the

[1] This investigation was supported by grants from Svenska Tobaks AB and the American Medical Association.

[2] E. Hansson and C. G. Schmiterlöw, J. Pharmacol. *137*, 91 (1962).

[3] S. Ullberg, Acta radiol., Suppl. 118 (1954).

[4] M. I. Smith, Am. J. Physiol. *46*, 232 (1918).

[5] K. Westphal and H. Weselmann, Dtsch. Med. Wschr. *65*, 1229 (1939).

[6] H. Weselmann, Z. klin. Med. *136*, 683 (1939).

[7] J. R. Vane, Brit. J. Pharmac. *12*, 344 (1957).

[8] P. S. Larson, H. B. Haag, and H. Silvette, in *Tobacco* (The Williams & Wilkin Co., Baltimore 1961), p. 637.

[9] H. McKennis jr., E. Wada, E. R. Bowman, and L. B. Turnbull, Nature, Lond. *190*, 910 (1961).

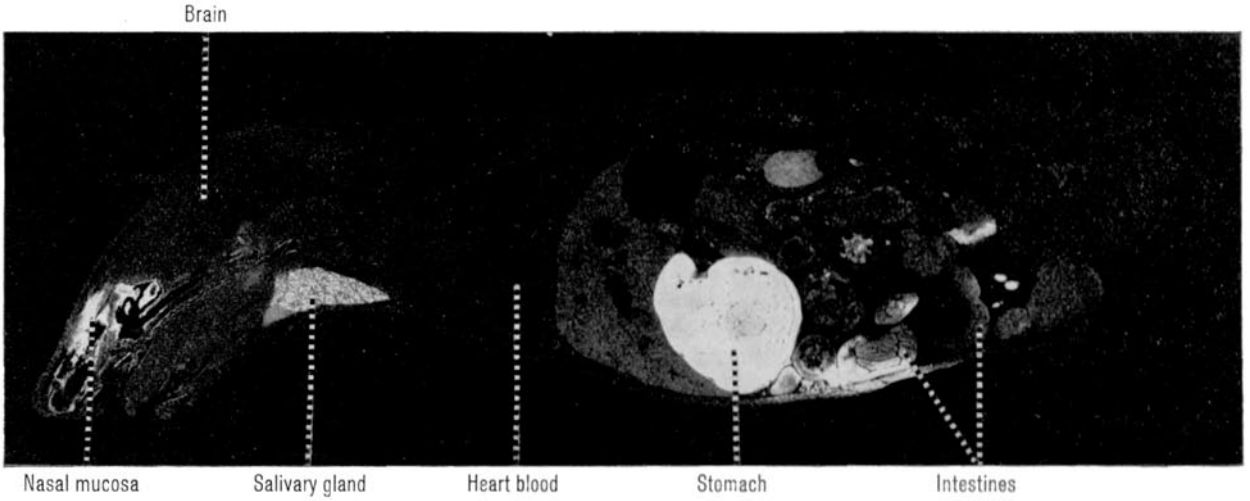

Fig. 1. Autoradiogram showing the distribution of radioactivity (light areas) in a mouse 1 h after intravenous injection of C^{14}-nicotine. Sagittal section through the entire body of the animal. Note the accumulation of radioactivity in the stomach.

distribution of C^{14} present as nicotine and/or its metabolites 1 h after injection. From this autoradiogram it is obvious that nicotine and/or its metabolites are concentrated in the stomach. Figure 2 shows an enlargement from a similar autoradiogram, obtained from a mouse sacrificed 15 min after the injection of C^{14}-nicotine. After this rather short time interval the amount of radioactivity is obviously higher in the gastric mucosa than in the stomach content, whereas after 1 h (Figure 1) a substantial amount of radioactivity has been excreted into the stomach.

In some cases we made autoradiograms of the excised mouse stomach. The stomach was cut open, wiped free of content, flattened out and put on X-ray film. Figure 3 shows both a photo of the cut-open stomach and the corresponding autoradiogram. It should be noted that radioactivity occurs only in the glandular part of the stomach.

In our experiments on rats and cats similar results were obtained.

The amount of radioactivity excreted in the stomach of mice and rats was determined in a series of experiments. In some cases only the stomach content was examined, but in other cases both stomach content and stomach wall were subjected to radioactivity measurements. Radioactivity was determined by means of a Packard Tri-Carb liquid scintillation counter. The results of these determinations showed rather wide variations, the values obtained varying from 1.8 to 10% of the amount of radioactivity initially administered to the animals as C^{14}-nicotine. In order to find an explanation for this variation we performed some experiments in rats, perfusing the stomach in situ with solutions of different pH levels.

Male albino rats weighing between 300–325 g were used in these experiments. They were starved overnight and then anaesthetized with pentobarbital. A polythene tube (PE 50) was inserted into the oesophagus and tied just above the stomach. The abdomen was opened and a glass cannula inserted into the stomach through a small incision in the duodenum. The abdomen was then closed with only the glass cannula protruding. The stomach was perfused continuously with a phosphate buffer and the perfusate sampled during periods of 30 min. The pH of the buffer varied in different experiments from pH 1 to pH 9. The perfusion rate was kept fairly constant at 0.5 ml/min. C^{14}-nicotine in a dose of 0.1 μg/g of bodyweight, corresponding to 0.007 μC/g, was administered into the femoral vein. The amount of radioactivity in the perfusion samples was then determined in the Packard Tri-Carb liquid scintillation counter.

The Table gives the values obtained from these experiments. The amount of radioactivity in the perfusates is expressed as % of the injected radioactivity present in nicotine. It is obvious that the highest excretion occurs at pH 1 (mean value 4.4%) and that the excretion diminishes as the pH is increased – at pH 9 the amount of radioactivity is down to 0.6%. In these experiments it was also shown that the peak in excretion is reached after approximately 1 h.

The question naturally arises: how much of the excreted radioactivity is present as nicotine and how much as metabolites? In order to answer this question samples of the perfusate were subjected to thin layer chromatography with subsequent radioassay as described in one of our earlier papers[10]. It was found that after 30 min 65% of the radioactivity was still present as nicotine but after 1 h only 25%. The remainder of the radioactivity was present in the one and only metabolite which could be detected in these experiments, viz. cotinine.

Our studies indicate an evident excretion of nicotine via the stomach. This process seems to be in the nature of a passive secretion, dependent on the pH level of the stomach content. It has been stated[11] that the passage of drugs from plasma (pH 7.4) to gastric juice (pH 1) depends on their pKa values. The basic drug nicotine seems

Excretion of radioactivity into rat stomach at different pH after intravenous injection of C^{14}-nicotine. Mean values from 3 different experiments in each group

pH	% of radioactivity excreted
1	4.4
3	2.5
7	0.9
9	0.6

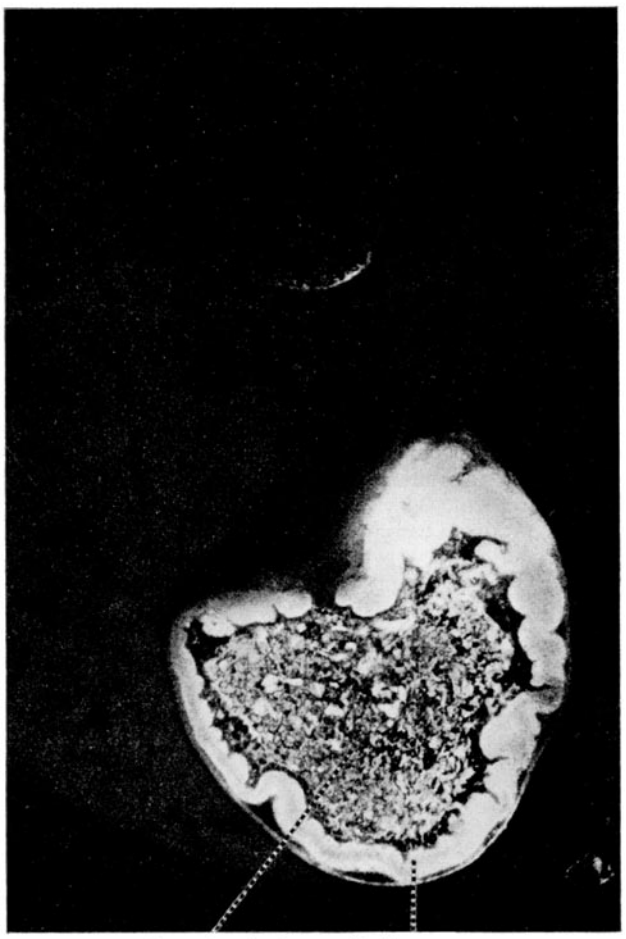

Fig. 2. Enlargement from a whole-body autoradiogram. Distribution of radioactivity in the mouse stomach mucosa and content 15 min after the intravenous injection of C^{14}-nicotine.

[10] E. HANSSON, P. C. HOFFMANN, and C. G. SCHMITERLÖW, Acta physiol. scand. *61*, 380 (1964).

[11] L. S. SCHANKER, Pharmacol. Rev. *14*, 501 (1962).

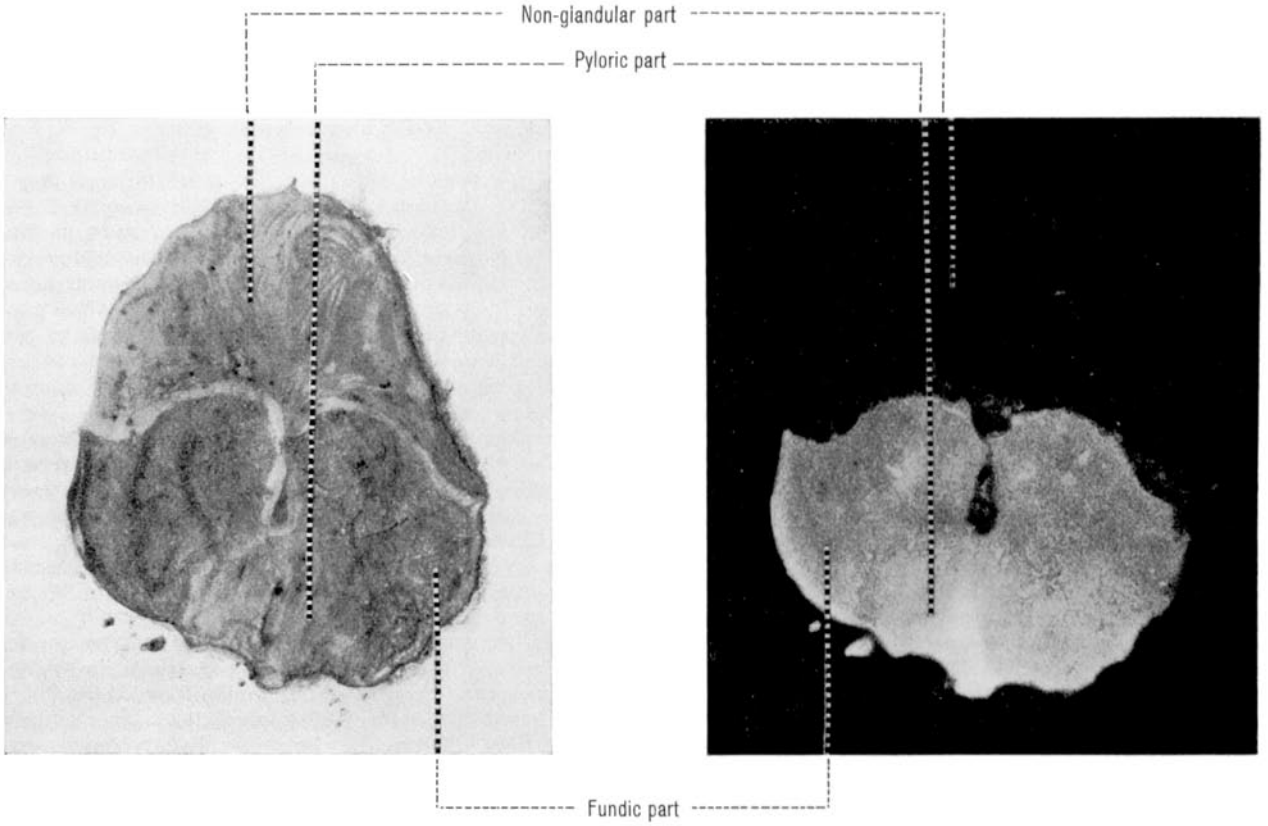

Fig. 3. To the left: a photo of the cut-open and flattened mouse stomach. To the right: the corresponding autoradiogram of the stomach. Note presence of radioactivity in the glandular part of the stomach. C^{14}-nicotine was injected intravenously 1 h before the stomach was excised from the animal.

to fit into the suggested pattern – the non-ionized nicotine passes into the acid stomach content and becomes ionized.

Naturally, no conclusions can be drawn from our present results as to the part played by nicotine in causing peptic ulcers. The mere fact, however, that nicotine is excreted via the gastric mucosa into the stomach content in substantial amounts, fulfils at least a prerequisite for the action of nicotine on the stomach wall.

Zusammenfassung. Der Übertritt von C^{14}-Nicotin aus der Magenschleimhaut von Mäusen, Ratten und Katzen in den Mageninhalt wurde autoradiographisch und mit Radioaktivitätsmessungen untersucht. Perfusionsversuche des Rattenmagens in situ ergaben eine pH-abhängige Nikotinausscheidung. Chromatographisch wurde die ausgeschiedene Radioaktivität teils im Nikotin selber, teils im Abbauprodukt Cotinin nachgewiesen.

G. Andersson, E. Hansson, and C. G. Schmiterlöw

Department of Pharmacology, Kungl. Veterinärhögskolan, Stockholm (Sweden), December 11, 1964.

Comparaison de l'effet de congélations et de lyophilisations sur la viabilité ultérieure d'embryons d'*Evonymus vulgaris* Miller (*E. europaeus* L.p.p)

Dans une précédente note[1], nous avons montré que le pourcentage d'embryons d'*Evonymus vulgaris* capables de germer après avoir subi une lyophilisation semble, au moins dans certaines limites, lié à leur teneur en eau.

Nous avons poursuivi nos recherches dans ce domaine, et, au cours du présent travail, nous avons essayé de déterminer, chez des embryons dont la teneur en eau a été amenée à des valeurs allant de 5,3 à 50%, la responsabilité relative de chacune des deux étapes de la lyophilisation (congélation et cryodessiccation) dans le taux de mortalité de ces embryons.

Pour ces recherches, nous avons utilisé des embryons d'*Evonymus vulgaris* après qu'ils aient été isolés de graines mûres et sèches; nous avons fait varier la teneur en eau

[1] C. Bulard et J. Monin, C. r. Acad. Sci. *258*, 1613 (1964).