International Journal of Pharmaceutics, 45 (1988) 101–110
Elsevier

IJP 01520

Nickel II elimination and autoradiographic distribution in normal and nickel-sensitized guinea pigs

J.C. Chevin [1], P. Maincent [1], J.M. Mougeolle [2], M. Jacque [1] and J.A. Martin [1]

[1] *Faculté des Sciences Pharmaceutiques et Biologiques, Université de Nancy I, Nancy (France) and* [2] *Hôpital Fournier, Service de Dermatologie-Centre Hospitalier Universitaire — Nancy I, Nancy (France)*

(Received 13 November 1987)
(Modified version received 28 December 1987)
(Accepted 5 January 1988)

Key words: Nickel; Nickel sensitization; Nickel absorption; Nickel distribution; Nickel elimination; Autoradiography; Guinea pig

Summary

This study investigates the differences in the elimination of ^{63}Ni (as $NiCl_2$) in normal and nickel-sensitized guinea pigs. The urinary elimination, 72 h after an intracutaneous injection, was significantly diminished in the sensitized animal (55%) compared to the animals used for control (63%). After an oral gavage, the fecal elimination was only significantly slowed down in the Ni-sensitized animals. After an intracutaneous injection, significantly more nickel was retained in the dermis of sensitized guinea pigs than in normal ones. The 24 h autoradiography study after $^{63}NiCl_2$ intracutaneous injection shows a general nickel distribution with a very high activity in the kidney and cartilages. No qualitative difference can be seen between sections of untreated and Ni-sensitized guinea pigs. 24 h after $^{63}NiCl_2$ oral gavage, in both Ni-sensitized and control guinea pig, a high radioactivity is present in the gastrointestinal tract, and in the periarteriolar sheaths of the splenic white pulp.

Introduction

Nickel is an essential nutrient (Nielsen and Ollerich, 1974; Diekert et al., 1980) but it can also induce cancer and allergy in man (Brown and Sunderman, 1980). Ni and Ni compounds have a strong sensitizing effect on the skin. Ni has been claimed to be the most common sensitizer (Flyvholm et al., 1984). It is an important problem for the general population since dermatitis may be due to a direct contact with Ni-containing objects, such as coins, watches, kitchen appliances, jewelry etc. and hand eczema occurs with a frequency of 20–60% in nickel-sensitive patients (Menne et al., 1982).

In a healthy man, serum nickel concentrations range from 1.6 to 7 μg/liter and urinary concentrations from 2 to 5 μg/liter, i.e., 2.6–9 μg/day for normal diuresis (Norseth and Piscator, 1979; Menne et al., 1978; Christensen et al., 1979; Cronin et al., 1980; Zober et al., 1984). An oral intake of 25 mg nickel sulfate brings the level of nickel serum up to 23.6 μg/liter within 2 h; 14.3 μg will pass into the urine within 7 h (Christensen et al., 1979), 35–50 μg on the first day, 35–45 μg on the second and 20–25 μg on the third day (Menne et al., 1978).

In nickel-sensitive subjects manifesting der-

Correspondence: J.C. Chevin, Faculté des Sciences Pharmaceutiques et Biologiques, BP 403, 54000 Nancy, France.

matitis, the Ni elimination and the Ni serum levels are either similar (Spruit et al., 1978; Gawkrodger et al., 1986) or inferior (Kaaber et al., 1979) to those of a healthy subject. The amounts of urinary excretion show that Ni-sensitive subjects tend to eliminate a smaller amount of nickel when daily absorbed than do healthy subjects (Christensen and Lagesson, 1981). Ingestion of nickel by patients with hand eczema and nickel allergy causes a flare-up of their eczema (Christensen et al., 1985); Flyvholm et al., 1984) and affects the Ni elimination. The oral intake of nickel sulfate exacerbates the allergic reactions; the serum concentration increases up to 23–89 μg/liter and the urinary excretion up to 95–206 μg/day (Beurey et al., 1980, Cronin et al., 1980). Nevertheless the absorption and the metabolism of nickel vary considerably per individual (Gawkrodger et al., 1986). The difference in the absorption rates is not the only factor responsible for clinical symptoms since the immunological state of the patient also interferes.

As regards rodents, nickel distribution and elimination have been studied in normal (healthy) animals, using ^{63}Ni-labelled nickel salts for liquid scintillation counting (Wase et al., 1954; Smith and Hackley, 1968; Sarkar, 1980). Whole-body autoradiography in mice given $^{63}NiCl_2$ (i.v. injection), shows a localization of nickel in the kidneys, the parenchyma of the lungs, the cartilages, the connective tissues and in addition, at long survival intervals, in the brain and spinal cord (Oskarsson and Tjalve, 1979).

Considering such disparate results, it was thought of interest to apply the experiments to guinea-pigs and to study the effects of nickel on healthy or sensitized animals, since the guinea-pigs proved to be fairly close to man in studies of sensitization (Magnusson and Kligman, 1969). Indeed, it seems that neither data nor autoradiography allowing a comparative study of the Ni distribution and elimination in healthy and sensitized animals have been published so far. This is why the following study was undertaken in order to investigate the fixation and the rate of nickel elimination both in healthy and Ni-sensitized guinea-pigs after parenteral and oral administrations.

Materials and Methods

Animals

Hartley female albino guinea pigs were housed in groups of 5 in wooden cages, in a room kept at a constant temperature of 20 °C and 60% relative humidity. This room was illuminated by daylight. Standard pellets (UAR 106 Villemoisson/Orge France) in galvanized iron containers and tap water in glass distributors were provided ad libitum.

Sensitization

Three month-old guinea pigs were sensitized according to the Maurer et al. (1979) method by repeated intracutaneous (i.c.) injections of nickel sulfate dissolved in Freund's complete adjuvant. Reactions to i.c. and epidermal tests were evaluated in erythemic intensity by means of the Draize et al. (1944) scale. This scale grades from 0 (no reaction) to 4 (a very intense redness). The measurement of the difference in thickness between the skin of the inflamed sector and that immediately adjacent was carried out with a "Kroplin Metallex" skin fold gauge with flat discs (10 mm in diameter). The sensitized animals used for the experiments showed responses from 1 to 3 to the i.c. and epidermal challenge tests.

Kinetics of elimination and tissue distribution

Intracutaneous (i.c.) administration. 0.14 ml/kg of body weight (b.wt.) of a solution (A) of $NiCl_2 \cdot 6H_2O$ 1 g/liter, NaCl 9 g/liter labelled with $^{63}NiCl_2$ (Amersham International PLC) were injected to 9 3-month-old Ni-sensitized guinea pigs (average weight 690 g) and 4 control guinea pigs of the same age (average weight 720 g). (Dose administered: 35.7 μg Ni/kg b.wt.; specific activity: 45.1 Bq per ng of total nickel).

Oral (p.o.) administration. 2.9 ml/kg b.w. of solution A labelled with $^{63}NiCl_2$ were administered by oral gavage to 6 Ni-sensitized and 4 control guinea pigs (average weight 710 and 850 g respectively; age: 3 months; dose administered: 714 μg Ni/kg b.w.; spec. act.: 2,22 Bq per ng of total nickel).

Sampling. The administrations were made at 08.00 h and the animals were placed straight away

in metabolism cages. Urine and feces were collected after 2, 6, 11, 24, 34, 48 and 72 h. Blood samples were taken by scarification of the marginal ear vein after 0.5, 1.5, 6, 11, 24, 34 and 48 h.

For the study of dermal retention, the i.c. injections were carried out on the depilated backs of the normal and sensitized animals using the same dose (35,7 μg Ni/kg). Sections of tissues (2 × 2 cm) around the injection point were removed (epidermis and dermis) after ether anesthesia at intervals of 1, 2, 6 and 24 h.

Analytical procedures. ^{63}Ni was counted by liquid scintillation (SN 3000 counter, IN Intertechnique, Plaisir, France). The whole blood samples and the urines were counted directly in a proportion of 15–50 μl per test. The feces were liquified by KOH (5 M) then 25 μl were taken for counting. The dermal sections were digested by a 3% papain solution, then by KOH (5M). The samplings for the counting varied from 25 to 100 μl according to the Ni tissue concentrations. Ni was dosed by flameless atom absorption spectrometry (Varian AA6-CRA 90) directly on drinking water and after sulfonitric mineralisation in standard pellets.

Whole-body autoradiography

I.c. injection. Two 12-month-old guinea pigs sensitized for 9 months and one 3-month-old control guinea pig were injected i.c. on the left side of the spinal column with 0.1 ml of solution A labelled with $^{63}NiCl_2$ (spec. act.: 148 Bq/ng Ni; dose administered: 35.7 μg Ni/kg b.w.).

Oral administration. One 12-month-old guinea pig sensitized for 9 months and two 12-month-old control guinea pigs were given $^{63}NiCl_2$ dissolved in 2 ml of solution A (spec. act.: 6.66 Bq/μg Ni; dose administered: 714 μg Ni/kg b.wt.). The Ni-sensitized animals were challenged 15 days before autoradiography study and exhibited a strong reaction (3) on the Draize scale to the challenge dose.

Autoradiography. The whole-body autoradiographic procedures were performed both on the Ni-sensitized animals (i.c. and p.o. administrations) and the i.c. control guinea pig according to Ullberg (1954).

The animals were killed 24 h after the administration of ^{63}Ni by deep anaesthesia with ether. They were frozen by immersion in isopentane cooled with liquid nitrogen and then embedded in a carboxymethyl cellulose gel. The frozen guinea pigs were sectioned sagittally with cryomat (LKB Cryomicrotome PMU 450). The freeze-dried sections (20 μm thick) were exposed at low temperature (−20 °C) against Kodak Industrex A X-ray films for 20 days. The relative evaluation of radioactivity on various tissues was performed on a same film by means of a densitometer (Densitocolor Volomat, Paris).

A series of dry sections of each guinea pig was treated twice for 2 min with a 5% (w/v) trichloracetic acid (TCA) solution and briefly (1 min) rinsed with distilled water. The sections were air-dried and then exposed against X-ray films.

The two 12-month-old control guinea pigs which had been given the Ni p.o. were killed in the same way, 24 h after the Ni administration. The kidneys, spleen and intestine were immediately removed and then frozen as previously described. The procedure for organ sections and the exposure on X-ray film were similar to the method described above.

Analysis of data. The results were analyzed statistically by the Student's *t*-test. Differences with $P < 0.05$ were considered significant.

Results

Elimination kinetics

The Ni blood concentration (concn) versus time profiles after i.c. and p.o. administrations are presented in Fig. 1 (values are expressed in ng Ni/g of blood). Cumulated urinary and fecal elimination amounts (Figs. 2–5) are given as a percentage of the initial dose administered and are summarized in Table 1.

The plateau of the fecal cumulated elimination curves show that the excretion (Figs. 3 and 5) is nearly over 48 h after i.c. and p.o. administrations.

The Ni urinary elimination is not completed after 72 h (Figs. 2 and 4). The total elimination (urine + feces) (Table 1) is important during the

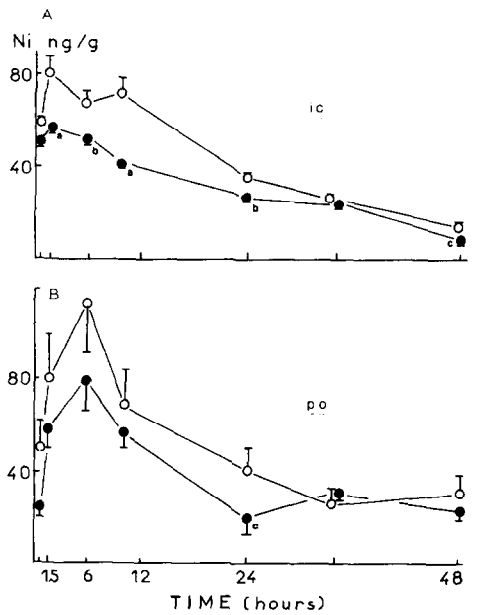

Fig. 1. Concentration of nickel (mean ± S.E.M.) in the blood of Ni-sensitized and control guinea pigs having received 35.7 μg Ni/kg i.c. (A) and 714 μg/Ni p.o. (B). ●, sensitized; ○, controls. Significance from controls: a, $P < 0.001$; b, $P < 0.02$; c, $P < 0.05$.

first 24 h for the i.c. injection and during the first 48 h for p.o. administration; then the elimination process goes on much more slowly.

It is possible to calculate the slopes of the urinary elimination curves between sampling times (Figs. 2, 4). The greatest slope is found in the interval 2–6 h on Fig. 2 and corresponds to the Ni

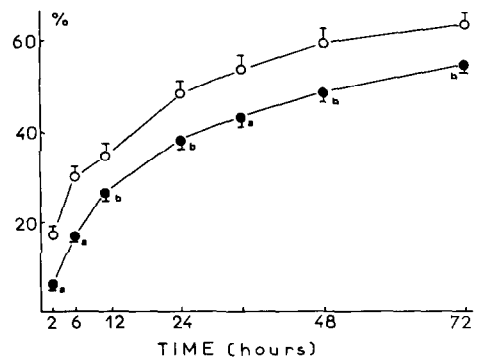

Fig. 2. Cumulated percentages (mean ± S.E.M.) of nickel urinary elimination after an i.c. injection to Ni-sensitized and control guinea pigs (quantities administered and symbols as in Fig. 1).

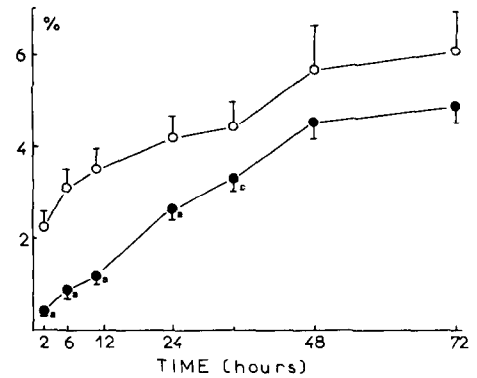

Fig. 3. Cumulated percentages (mean ± S.E.M.) of nickel fecal elimination after an i.c. injection to Ni-sensitized and control guinea pigs (quantities administered and symbols as in Fig. 1).

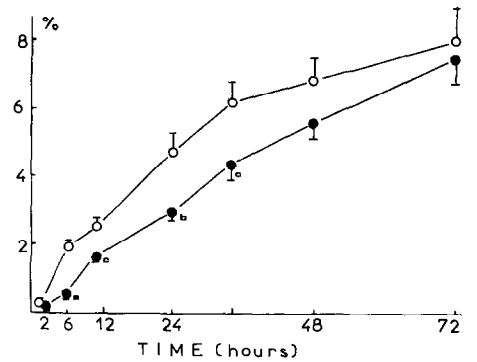

Fig. 4. Cumulated percentages (mean ± S.E.M.) of nickel urinary elimination after oral gavage to Ni-sensitized and control guinea pigs (quantities administered and symbols as in Fig. 1).

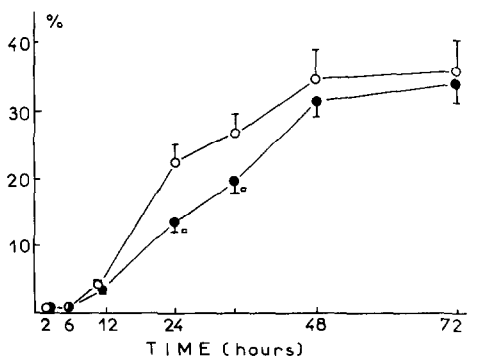

Fig. 5. Cumulated percentages (mean ± S.E.M.) of nickel fecal elimination after oral gavage to Ni-sensitized and control guinea pigs (quantities administered and symbols as in Fig. 1).

TABLE 1

Nickel in urine and feces

	I.c. administration		P.o. administration	
	Control	Sensitized	Control	Sensitized
	(4)	(9)	(4)	(6)
24 h	52.9 ± 2.5	40.9 ± 2 *	26.9 ± 2.3	16.2 ± 1.3 *
48 h	65.2 ± 2.4	53.3 ± 1.9 *	41.8 ± 3.9	36.8 ± 1.8
72 h	69.5 ± 2.1	59.5 ± 2.1 *	43.8 ± 3.8	41.5 ± 2.3
96 h	72 ± 2.4	n.d.	45.1 ± 4	n.d.
120 h	73.3 ± 2.2	n.d.	45.8 ± 3.7	n.d.

Cumulated percentages (urine − feces) (means ± S.E.M.) of elimination of the initial dose after intracutaneous and oral administrations of ^{63}Ni in control and Ni-sensitized guinea-pigs (i.c. injection: 35.7 μg Ni/kg, oral gavage: 714 μg Ni/kg b.w.) Numbers of animals in parentheses, n.d., not determined.
* Statistically different from controls ($P < 0.01$).

blood concn peak (Fig. 1) after i.c. injection for both the normal and the sensitized animals. In the case of the oral gavage, the Ni blood concn reached its peak at 6 h which corresponds to the greatest slope (interval 6–11 h) for the urinary elimination curve (Fig. 4) for both the normal and the sensitized animals.

The areas under the blood concn time curves from time 0–24 h (AUC_{0-24}) and from 0–48 h (AUC_{0-48}) were calculated by means of the linear trapezoidal rule. The relative Ni bioavailability for the two periods of time were calculated according to the following equation:

$$\text{Relative Bioavailability} = \frac{AUC_{po}}{AUC_{ic}} \times \frac{Dose_{ic}}{Dose_{po}}$$

After the oral gavage, the relative bioavailability of nickel (cf Table 2) is very low (about 6%), both in sensitized and control animals, showing the poor gastrointestinal absorption of Ni. Fig. 6 presents, as a function of time, the quantities of residual nickel in the dermis at the injection point in sensitized and untreated animals. The amounts are calculated in percentage of the initial quantity injected.

Autoradiography studies

Distribution picture after ic injection of $^{63}NiCl_2$. The distribution picture of the control animal, 24 h after the administration (Fig. 7), is characterized by a general nickel impregnation. A black area reveals radioactivity (RA) on the original autoradiography.

TABLE 2

Bioavailability parameters after i.c. and p.o. administrations of nickel in control and Ni-sensitized animals

Relative bioavailability (%) (reference i.c. route)	Sensitized animals	Control animals
0 → 24 h	6	5.6
0 → 48 h	6.3	5.9

I.c. dose 35.7 μg Ni/kg, oral dose 714 μg Ni/kg.

After measuring of optical densities on the film, the levels of RA in the various organs make it possible to notice a maximum activity in the kidney, the injection point, the cartilages.

The lung, blood, feces in descending colon, the connective tissues and the outer dermal layer had the second highest activity. RA was lower in the remaining tissues: heart, liver, spleen, pancreas, pituitary, content of stomach and cecum. There was no noticeable RA in the brain and spinal cord. The RA in the kidney is localized in the cortex and corticomedullary zone, especially in distal and proximal convoluted tubules. The cartilages can be easily seen at various locations such as conjugation cartilages of growing bones, hyaline costal and tracheal cartilages, elastic cartilages of the larynx and the ear.

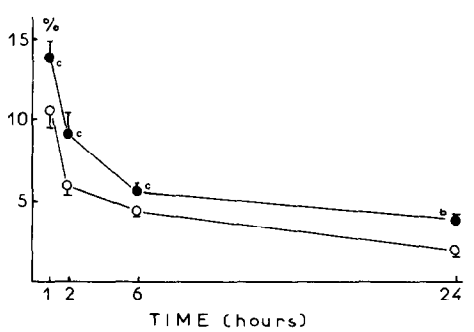

Fig. 6. Residual nickel in the dermis at the i.c. injection point in Ni-sensitized (●) and control (○) 3-month-old guinea pigs (mean ± S.E.M. of 5 tests). Values are expressed as percentages of the initial dose injected (quantities administered and symbols as in Fig. 1).

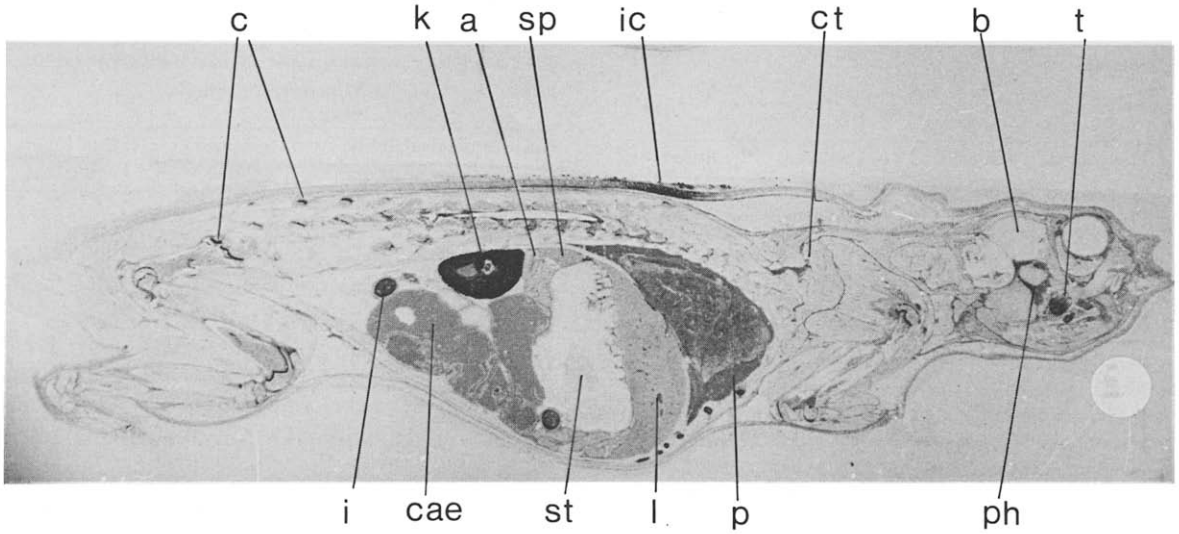

Fig. 7. Whole-body autoradiography of a control Hartley female guinea pig, 24 h after an i.c. injection of $^{63}NiCl_2$ (35.7 μg Ni/kg). a, adrenal; b, brain; c, cartilages; cae, cecum; ct, connective tissue; h, heart; i, intestine; ic, injection point; k, kidney, l, liver; p, lung; ph, pharynx; sp, spleen; st, stomach; t, tooth.

The medullary zone of the adrenal shows a higher RA than the cortical zone.

The RA of the pituitary is similar to that of the liver and spleen. However, activity appears to be greater in the antehypophysis than in the post-hypophysis.

The sections of the sensitized guinea pig (Fig. 8) exhibited the same distribution picture as the control despite the different ages of the animals.

When tape sections of the guinea pig were incubated in TCA solution, almost all RA was lost. Indeed the autoradiography reveals that only the kidneys had a very low intensity.

Distribution picture after oral administration of

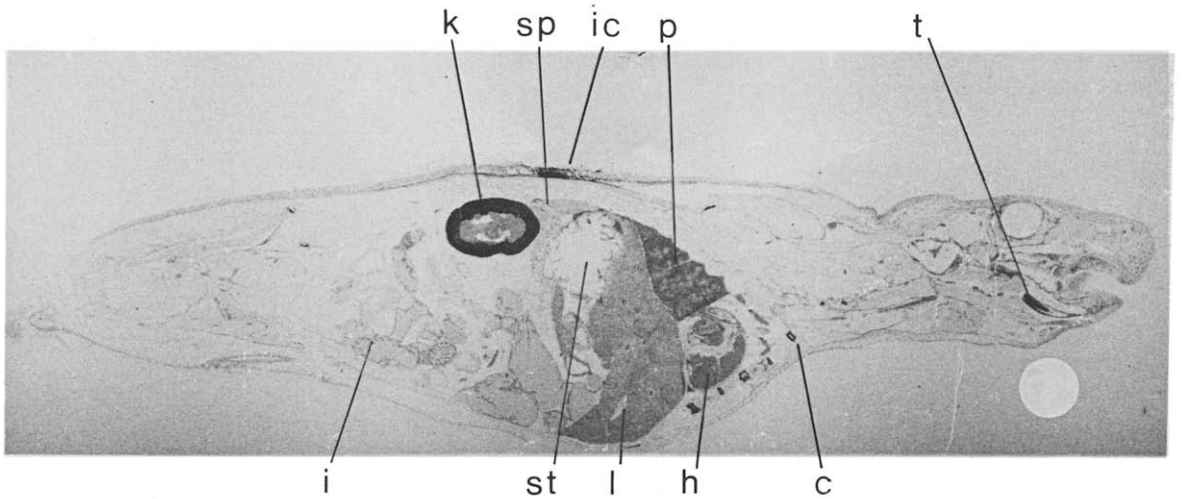

Fig. 8. Whole-body autoradiography of a nickel-sensitized Hartley female guinea pig, 24 h after an i.c. injection of $^{63}NiCl_2$ (35.7 μg Ni/kg) (same symbols as in Fig. 7).

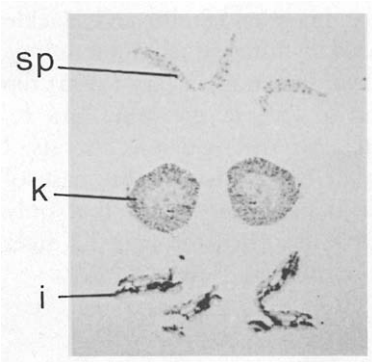

Fig. 9. Autoradiography of various organs sections (spleen, kidney, intestine) of a control Hartley female guinea pig after oral administration of $^{63}NiCl_2$ (714 μg Ni/kg) (same symbols as in Fig. 7).

$^{63}NiCl_2$. In Ni-sensitized guinea pig, at 24 h survival interval, high RA is present in the gastrointestinal tract especially in the content of the cecum and intestine. A great activity is also observed in the periarteriolar sheaths of the splenic white pulp.

The kidney shows low activity consistent with the small quantity of nickel absorbed from the gastrointestinal tract. The liver and the lung are barely perceptible on the autoradiography.

The sections of the tissues removed from control animals display activity fairly close to those of sensitized animals (Fig. 9). Nevertheless, by comparison with RA in each animal, a stronger RA is to be noticed on the splenic white pulp of sensitized animal compared to the control one.

Discussion

Elimination

Guinea pigs were chosen as experimental animals since Magnusson and Kligman (1969) consider that these laboratory animals exhibit epidermal reactions fairly close to those of a human being. If we are to believe Maurer et al. (1979), in the case of weak allergens as Ni, his sensitization method is more efficient with regard to the number of sensitized animals than the classical method of Magnusson and Kligman (1969).

The i.c. dose of 35.7 g Ni/kg of our challenge is not irritant and induced an allergic reaction in a Ni-sensitized guinea-pig (Buehler, 1965; Stevens, 1967; Magnusson and Kligman, 1969; Maurer et al., 1979). The p.o. dose was increased in order to obtain blood levels close to those found after i.c. injection.

It must be kept in mind that the standard pellets contain 0.6 μg Ni/g and that drinking water contains 3 μg Ni/liter (Beurey et al., 1980). These values correspond with those established by Smith and Hackley (1968) as well as Norseth and Piscator (1979).

Considering that a guinea pig drinks an average of 10 ml of water per day and eats 10–30 g of pellets per day, it can be inferred that the daily Ni ingestion varies from 6 to 18 μg. Thus the doses of Ni injected by i.c. are equivalent to those daily ingested by the animals. Yet these doses appear to be 20–400 times inferior to the doses that are liable to affect (when administered by parenteral route in rats) as many parameters as the level of the seric glucose (Clary, 1975; Horak and Sunderman, 1975), lipids (Hasan and Fatehyab, 1981), P 450 cytochrome (Sasame and Boyd, 1978; Yawets et al., 1984), prolactine (Clemons and Garcia, 1981), as well as the renal reabsorption rate (Linder and Foulkes, 1985) or the level of renal metallothionein in the case of mice (Maitani and Suzuki, 1983) which could all interfere with the animal metabolism.

Kinetics of elimination

After i.c. injection, the blood concn and the urinary elimination appeared to be significantly diminished in sensitized animals: the fecal elimination amount was significantly diminished during the first 36 h only.

After the p.o. gavage, the Ni elimination is weaker and slower than after an i.c. injection (cf Table 1). It should be remembered that the guinea pig is subject to nocturnal coecotrophy (Dorst, 1973) which may entail important variations in the nickel excretion, particularly after an oral gavage. In Ni-sensitized animals, the urinary and fecal eliminations are significantly slowed down during the first 36 h. After 48 h, the amounts excreted are similar in both sensitized and control animals;

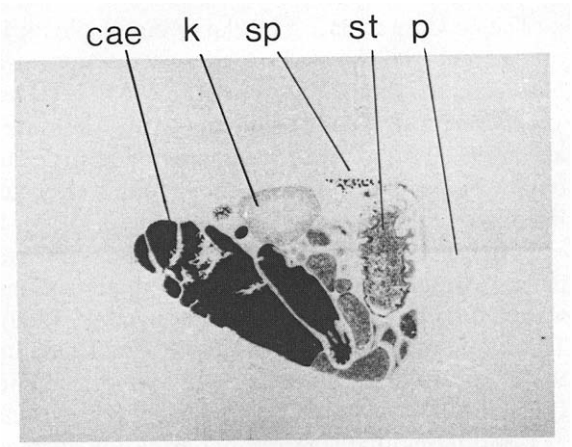

Fig. 10. Whole-body autoradiography of a nickel-sensitized Hartley female guinea pig 24 h after oral administration of $^{63}NiCl_2$ (714 μg Ni/kg) (same symbols as in Fig. 7).

there is scarcely any ^{63}Ni left in the feces but the process of urinary excretion goes on slowly.

Considering that the i.c. dose is nearly totally absorbed after 24 h (4% remaining in the dermis (cf Fig. 6), the bioavailability calculation reflects the very poor absorption of nickel by the oral route. Such figures are confirmed by the urinary excretion of nickel (see Fig. 4); the total urinary elimination after 48 h is about 5.5 and 6.8% of the initial dose for the sensitized and the control animals respectively.

After 24 h, the feces Ni elimination which corresponds to the amount of non-absorbed nickel is about 16.2 and 26.9% of the administered dose for the sensitized and the control animals respectively. Therefore it seems that nickel, in addition to its being poorly absorbed is somehow retained along the gastrointestinal tract by various components such as, possibly, mucine or gastrointestinal contents (cf. Fig. 10).

After 48 h, the relative bioavailability is around 6% but it is to be noted that the Ni absorption goes on, as seen by the continuous process of urinary elimination in the period 48–72 h (Fig. 4). Thus, after 5 days, as much as 55% of the administered dose remains (cf Table 1) in the animals.

Such data of urinary and fecal elimination after i.c. injections in healthy guinea pigs are consistent with the results found in rats; that is 60% eliminated in the urine and 6% in the feces 72 h after an i.v. injection (Smith and Hackley, 1968). Having used a different administration schedule (intratracheal injection), Clary (1975) reported an elimination of 75% in the urine and 15% in the feces 72 h after the injections in rats. Our data also seem to be relevant in the case of healthy mice since it has been shown that only a small percentage is absorbed through the digestive barriers (Oskarsson and Tjalve, 1979).

Dermal location

The Ni elimination curves in blood and urine (Figs. 1, 2, 4), both in sensitized and normal guinea pig, display identical profiles which means that the sensitization does not alter the elimination process.

After the i.c. injection, the Ni blood levels are always inferior in the sensitized animals compared to the normal ones. This fact is supported by the Ni dermic concn (Fig. 6) which is always significantly higher in sensitized animals than in normal ones.

Thus, the decreasing Ni elimination could suggest that the sensitized animals retain the nickel longer in their skins; the formation of erythema and edema associated with a delayed hypersensitivity reaction entails an increase in the number of lymphocytes (Brulos, 1978) which could lead to the obstruction of the vessels surrounding the local injection point, due to the formation of "leucocyte thrombus" (Uhr, 1966).

Autoradiography study

The guinea pigs were killed 24 h after the administration of nickel. This survival interval is necessary to induce a delayed-contact hypersensitivity reaction to the i.c. challenge dose (Stevens, 1967). As we have shown, however, the Ni elimination was already disturbed during this survival interval. Because the Ni excretion had caused the autoradiography prints to be slightly blurred, the specific activity of Ni was increased so as to improve the quality of the prints but the overall amount of administered Ni was unchanged. Similarly, the oral dose was increased to compensate for the poor Ni absorption by the gastrointestinal tract.

After an i.c. injection, the presence of ^{63}Ni in the cartilages is due to the inorganic and organic binding properties of chondroitin sulfate (Waser, 1977; Oskarsson and Tjalve, 1979; Larsson et al., 1981).

The distribution picture of $^{63}NiCl_2$ in the control animal parallels the results found by Oskarsson and Tjalve (1979) in mice after i.v. injections. The TCA treatment of sections were undertaken in order to obtain information on the protein-bound RA (Blomquist, 1972). In fact, the loss of RA after TCA incubation was attributed to the acid strength and the concn used which are powerful enough to desorb nickel-II from its cationic binding locations and valid conclusions cannot possibly be drawn regarding the fixation of nickel on the mucopolysaccharids and the proteinic structures. There was no appreciable difference in autoradiography distribution of ^{63}Ni between normal and Ni-sensitized guinea pigs, except for a slight difference in the RA intensity. It is particularly interesting to notice that no difference between normal and Ni-sensitized animals could be observed in spleen and thymus RA, organs that are usually involved in sensitization processes.

After an oral gavage, together with a considerable RA in the gastrointestinal tract, an important activity was observed in the splenic white pulp of the sensitized animal (cf. Fig. 10). The white pulp is rich in T cells (long-lived circulating lymphocytes) which mediate cellular immune reactions. Yet the very presence of the sheaths in the sections of the control animals although they have a lesser activity (same activity as in the kidney) seems to prove that the sensitization is not responsible for the phenomenon. Oskarsson and Tjalve (1979) have not reported such a Ni-splenic location after an oral gavage of $^{63}NiCl_2$ in mice at 7, 24 h and 5 days survival intervals. The periarteriolar sheaths of the splenic white pulp are not perceptible after i.c. injection. The thymus, another central limphoïd structure, does not appear on the autoradiography since lymphocytes recirculate to an extremely limited degree through the thymus. A very small activity can be seen in the lungs and an even smaller one in the liver. Oskarsson and Tjalve (1979) reported that the RA in these organs increased at 5 days survival interval in mice, which can be accounted for by the great amount of remaining nickel after 5 days.

To conclude, the Ni-sensitive state is responsible for a dermal retention leading to lower blood concns and lower urinary eliminations. We have shown by autoradiography that the qualitative Ni distribution after i.c. injection is not altered by the Ni-sensitive state of the animals. The behavior of nickel in healthy and sensitized animals presents the same pattern, with relation to the nickel urinary elimination reported by Christensen and Lagesson (1981) in Ni-sensitized men. The fact that the Ni-sensitized animal model responds differently to this metal will now allow us to study the influence of various chemical substances, such as chelating agents on the Ni retention, a research study which might be applied to man.

Acknowledgments

The authors would like to thank Dr. Chatelet (Laboratoire Roussel, UCLAF, France) for his expert assistance in the sectioning, autoradiographic exposures and identification of the anatomical structures. They are also grateful to Miss A. Berthin for helping in the preparation of the manuscript.

References

Beurcy, J., Mougeolle, J.M., Weber, M. and Martin, J.A., Allergie cutanée au nickel. Problème d'actualité. *Rev. Fr. Gastroenterol.*, 158 (1980) 39–49.

Blomquist, L., Mode of accumulation of iodophenylalanines in the exocrine pancreas and certain tumors. *Acta Radiol.*, Suppl. 322 (1972) 1–140.

Brown, S.S. and Sunderman Jr., F.W., *Nickel Toxicology*, Academic, London 1980.

Brulos, M.F., Résultats des mesures du potentiel sensibilisant de produits cosmétiques parfumés et non parfumés par une méthode expérimentale adaptée. *Rev. Inst. Pasteur Lyon*, 11 (1978) 153–169.

Buehler, E.V., Delayed contact hypersensitivity in the guinea-pig. *Arch. Dermatol.*, 91 (1965) 171–175.

Christensen, O.B., Beckstead, J.H., Daniels, T.E. and Maibach, H.I., Pathogenesis of orally induced flare-up reactions at old patch sites in nickel allergy. *Acta Derm. Venereol.*, 65 (1985) 298–304.

Christensen, O.B. and Lagesson, V., Nickel concentrations of blood and urine after oral administration. *Ann. Clin. Lab. Sci.,* 11 (1981) 119–125.

Christensen, O.B., Moller, M., Andrasko, L. and Lagesson, V., Nickel concentration of blood, urine and sweat after oral administration, *Contact Dermatitis,* 5 (1979) 312–316.

Clary, J.J., Nickel chloride-induced metabolic changes in the rat and guinea-pig. *Toxicol. Appl. Pharmacol.,* 31 (1975) 55–65.

Clemons, G.K. and Garcia, J.F., Neuroendocrine effects of acute nickel chloride administration in rats. *Toxicol. Appl. Pharmacol.,* 61 (1981) 343–348.

Cronin, E., Dimichiel, A.D. and Brown, S.S., Oral challenge in nickel-sensitive women with hand eczema. In Brown, S.S. and Sunderman Jr, F.W. (Eds.) *Nickel Toxicology.* Academic, London, 1980, pp. 149–152.

Diekert, G., Weber, B. and Thauer, R.K., Nickel dependence of factor F430 content in *Methanolbacterium Thermoautotrophicum. Arch. Microbiol.,* 127 (1980) 273–278.

Dorst, J., Appareil digestif et annexe. In Grasse, P. (Ed.), *Traité de Zoologie.* Masson, Paris, 1973, pp. 250–382.

Draize, J.H., Woodard, G. and Calvery, M.O., Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.,* 82 (1944) 377.

Flyvholm, M.A., Dalsgaard Nielsen, G. and Andersen, A., Nickel content of food and estimation of dietary intake. *Z. Lebensm. Unters. Forsch.,* 179 (1984) 427–431.

Gawkrodger, D.J., Cook, S.W., Fell, G.S. and Hunter, J.A.A., Nickel dermatitis: the reaction to oral nickel challenge. *Br. J. Dermatol.,* 115 (1986) 33–38.

Hasan, M. and Fatehyab, A.S., Effects of thallium, nickel and cobalt administration on the lipid peroxydation in different regions of the rat brain. *Toxicol. Appl. Pharmacol.,* 57 (1981) 8–13.

Horak, E. and Sunderman Jr., F.W., Effects of Ni II, other divalent metal ions, and glucagon upon plasma glucose concentrations in normal, adrenalectomized and hypophysectomized rats. *Toxicol. Appl. Pharmacol.,* 32 (1975) 316–329.

Kaaber, K., Menne, T., Tjell, J.C. and Veien, N., Antabuse treatment of nickel dermatitis. Chelation — a new principle in the treatment of nickel dermatitis. *Contact dermatitis,* 5 (1979) 221–228.

Larsson, B., Nilsson, M. and Tjalve, H., The binding of inorganic and organic cations and H^+ to cartilage in vitro, *Biochem. Pharmacol.,* 30 (1981) 2963–2970.

Linder, G. and Foulkes, E.C., Kinetics of renal aspartate reabsorption and its inhibition by metals in the intact rat kidney, *Environ. Res.,* 36 (1985) 241–247.

Magnusson, B. and Kligman, A.M., The identification of contact allergens by animal assay. The guinea-pig maximization test. *J. Invest. Dermatol.,* 52 (1969) 268–276.

Maitani, T. and Suzuki, K., Dose-dependent induction of metallothionein in kidneys of mice injected with indium and nickel ions, *Chem. Pharm. Bull.,* 31 (1983) 979–984.

Maurer, T., Thomann, P., Weirich, E.G. and Hess, R., Predictive evaluation in animals of the contact allergenic potential of medically important substances. II. Comparison of different methods of cutaneous sensitization with "weak" allergens. *Contact Dermatitis,* 5 (1979) 1–10.

Menne, T., Borgan, O. and Green, A., Nickel allergy and hand dermatitis in a stratified sample of the Danish female population: an epidemiological study including a statistic appendix. *Acta Derm. Venereol.,* 62 (1982) 35–41.

Menne, T., Mikkelsen, M.I. and Solgaard, P., Nickel excretion in urine after oral administration. *Contact Dermatitis,* 4 (1978) 106–108.

Nielsen, F.H. and Ollerich, D.A., Nickel: a new essential trace element. *Fed. Proc.,* 33 (1974) 1767–1772.

Norseth, T. and Piscator, M., Nickel. In Friberg, L., Nordberg, G.F. and Vouk, V.B. (Eds.), *Handbook on the Toxicology of Metals.* Elsevier, New York, 1979, pp. 541–553.

Oskarsson, A. and Tjalve, M., An autoradiographic study on the distribution of $^{63}NiCl_2$ in mice. *Ann. Clin. Lab. Sci.,* 9 (1979) 47–59.

Sarkar, B., Nickel in blood and kidney. In Brown, S.S. and Sunderman, Jr. F.W. (Eds.), *Nickel Toxicology,* Academic, London 1980, pp. 81–84.

Sasame, H.A. and Boyd, M.R., Paradoxical effects of cobaltous chloride and salts of other divalent metals on tissue levels of reduced glutathione and microsomal mixed—function oxidase components. *J. Pharmacol. Exp. Ther.,* 205 (1978) 718–724.

Smith, J.C. and Hackley, B., Distribution and excretion of nickel-63 administered intravenously to rats. *J. Nutr.,* 95 (1968) 541–546.

Spruit, P., Bongaarts, P.J.M. and De Jongh, G.J., Dithiocarbamate therapy for nickel dermatitis. *Contact Dermatitis,* 4 (1978) 350–358.

Stevens, M.A., Use of the albino guinea-pig to detect the skin sensitizing ability of chemicals. *Br. J. Industr. Med.,* 24 (1967) 189–202.

Uhr, J.W., Delayed hypersensitivity. *Physiol. Rev.,* 46 (1966) 359–407.

Ullberg, S., Study on the distribution and fate of ^{35}S labelled benzylpenicillin in the body. *Acta Radiol.,* Suppl. 118 (1954) 1–110.

Wase, A.W., Goss, D.M. and Boyd, M.J., The metabolism of nickel. 1. Spatial and temporal distribution of Ni^{-63} in the mouse. *Arch. Biochem. Biophys.,* 51 (1954) 1–4.

Waser, P.G., Mucopolysaccharides as unspecific receptors for cationic drugs. *Acta Pharmacol. Toxicol.,* 41, Suppl. 1 (1977) 134–135.

Yawets, A., Alter, A. and Oron, U., Biochemical and histological anomalies in the rat hepatic tissue following administration of bichromate and nickel in ionized form. *Toxicology,* 33 (1984) 145–155.

Zober, A., Kick, K., Schaller, K.H., Schellmann, B. and Valentin, H., "Normal values" of chromium and nickel in human lung, kidney, blood and urine samples. *Zbl. Bakt. Hyg. I. Abt. Orig. B,* 179 (1984) 80–95.