Pure & Appl. Chem., Vol. 51, pp. 1375-1389. 0033-4545/79/0601-1375 $02.00/0
Pergamon Press Ltd. 1979. Printed in Great Britain.
© IUPAC

INTERNATIONAL UNION OF PURE
AND APPLIED CHEMISTRY

CLINICAL CHEMISTRY SECTION

COMMISSION ON TOXICOLOGY

RADIOACTIVE ^{63}Ni IN BIOLOGICAL RESEARCH

Prepared for publication by
K. S. KASPRZAK
F. W. SUNDERMAN, JR.
University of Connecticut,
Farmington, USA

PERGAMON PRESS
OXFORD · NEW YORK · PARIS · FRANKFURT

RADIOACTIVE ^{63}Ni IN BIOLOGICAL RESEARCH*

Kazimierz S. Kasprzak and F. William Sunderman, Jr.**
Radiochemical Laboratory, Institute of General Chemistry
Technical University of Poznań, 60-965 Poznań, Poland, and
Department of Laboratory Medicine, School of Medicine
University of Connecticut, Farmington, CT 06032, U.S.A.

Abstract - Applications of ^{63}Ni in biological research are reviewed, with emphasis upon recent investigations of nickel metabolism and toxicology in experimental animals. The radiochemistry of ^{63}Ni is summarized, including consideration of the preparation of certain ^{63}Ni compounds (*e.g.* $^{63}Ni(CO)_4$ and $^{63}Ni_3S_2$) that are of current interest in toxicology, teratology and cancer research. Practical guidance is given regarding the detection and quantitation of ^{63}Ni in biological materials by autoradiography and liquid scintillation spectrometry.

INTRODUCTION

Interest in nickel among biochemists, biologists and clinical scientists has recently increased as a consequence of several scientific developments. First, nickel has been shown to be an essential trace nutrient for mammals (1-3). Second, urease has been identified as the first known nickel metalloenzyme (4-6). Third, recent epidemiological studies have confirmed the long established observation that exposure of nickel refinery workers to certain nickel compounds is associated with increased risks of cancers of the lung and nasal cavities (7-9). Fourth, prenatal exposure of rats to nickel carbonyl has been found to induce ocular malformations in rats (10). Fifth, the relationship between dietary intake of nickel and exacerbations of nickel dermatitis has become a controversial topic among dermatologists (11,12). Sixth, the major role of the kidney in the excretion of nickel (13) and the polycythemia that occurs in rats after intrarenal injection of αNi_3S_2 (14,15) have created interest in the effects of nickel on the kidney. In order to investigate these various subjects, numerous investigators are employing nickel radioisotopes for radiotracer studies. This review of ^{63}Ni in biological research is intended to provide a succinct resumé of this topic, and a convenient introduction to the pertinent literature of the past two decades. For references to the earlier literature, readers are referred to a valuable monograph which was published by Kirby in 1961 on "The Radiochemistry of Nickel" (16). For discussions of the metabolism, toxicology and carcinogenicity of nickel compounds, readers are referred to recent review articles and monographs (17-21).

NICKEL RADIOCHEMISTRY

Certain characteristics of the seven unstable isotopes of nickel are listed in Table 1. Four of these isotopes (^{56}Ni, ^{65}Ni, ^{66}Ni, and ^{67}Ni) are impractical for radiotracer studies. Although ^{57}Ni has only been employed in a single clinical investigation (24), ^{57}Ni appears to be advantageous for use in nuclear medicine, since it has a relatively short half-life (36 h) and since its high-energy radiation can readily be measured in the presence of the low-energy radiation of its ^{57}Co daughter. ^{59}Ni might be convenient for certain biochemical applications, owing to its long half-life (8 x 10^4 years) and soft X-irradiation. However, production of ^{59}Ni is expensive, and only low specific activities can be obtained (up to 4.45 Ci/gram-atom). ^{63}Ni is the ideal nickel radioisotope for most investigations, because it has a long half-life (92 years) and low energy of beta emission (67 keV), and it is

---

* This review was written on behalf of the Subcommittee on Environmental and Occupational Toxicology of Nickel, Commission on Toxicology, Section on Clinical Chemistry, of the International Union of Pure and Applied Chemistry. This work was supported by research grants EV03140 from the U.S. Department of Energy and ES01337 from the U.S. National Institute of Environmental Health Sciences.

** To whom requests for reprints should be addressed at P.O. Box G, Farmington, Connecticut 06032, U.S.A.

TABLE 1. Radioactive isotopes of nickel (16,22,23)

Isotope	Half-life	Type of decay	Maximum energy of emitted radiation (keV)* beta		gamma	
^{56}Ni	6.4 d	EC**	-		1580	[*0.15*]
					810	[*0.8*]
					480	[*0.4*]
					170	[*1.0*]
^{57}Ni	36 h	β^+[50%] EC†	854	[*87%*]	1920	[*0.2*]
			720	[*11%*]	1758	[*0.1*]
			350	[*2%*]	1378	[*1.0*]
					108	[*0.2*]
^{59}Ni	$8x10^4$ y	EC	-		500	
					60	
					38	
					15	
^{63}Ni	92 y	β^-	67±0.5	[*100%*]	-	
^{65}Ni	2.5 h	β^-	2140	[*58%*]	1490	[*1.0*]
			1020	[*12%*]	1120	[*0.5*]
			650	[*30%*]	370	[*0.2*]
^{66}Ni	55 h	β^-‡	200	[*100%*]	-	
^{67}Ni	50 s	β^-§	4100	[*50%*]	1260	
			3200	[*20%*]	900	
			2300	[*30%*]	890	

* Energies of radiation from the main routes of decay. The numbers in brackets for gamma rays indicate the abundance of the particular quantum relative to the most abundant one [*1.0*].

** Electron capture, which leads to radioactive daughter - $^{56}Co/EC,\beta^+$, $T_{1/2}$ = 77.2 days.

† Electron capture to radioactive $^{57}Co/EC$, $T_{1/2}$ = 270 days.

‡ The decay leads to radioactive $^{66}Cu/\beta^-$, $T_{1/2}$ = 5.1 min.

§ The decay leads to radioactive $^{67}Cu/\beta^-$, $T_{1/2}$ = 59 hours.

available in high specific activity (up to 3.87 kCi/gram-atom). The soft beta emission of ^{63}Ni is readily counted by liquid scintillation spectrometry, and it can provide autoradiograms with exceptionally high resolution.

The ^{63}Ni nuclide originates from the stable ^{62}Ni isotope after nuclear reaction with a neutron [$^{62}Ni/n,\gamma/^{63}Ni$]. The nickel target is customarily enriched in ^{62}Ni, but it usually contains significant amounts of the other stable isotopes of nickel (^{58}Ni, ^{60}Ni, ^{61}Ni, and ^{64}Ni). Therefore, in addition to ^{63}Ni, neutron bombardment yields certain other radioisotopes of nickel, as well as radioisotopes of cobalt. The yield of ^{59}Ni is relatively low, and ^{65}Ni disappears by fast decay. Contamination by cobalt radioisotopes necessitates the use of ion-exchange procedures to separate Ni and Co. Typically, the radioactive metal target is dissolved in HCl (12 mole/liter) and passed through an anion-exchange column (Dowex 1). ^{63}Ni is present in the effluent, while Co isotopes remain adsorbed to the resin (25). Acidified aqueous solutions of $^{63}NiCl_2$ or $^{63}NiSO_4$ can be purchased from nuclear centers of countries throughout the world. Other radioactive compounds of ^{63}Ni can be prepared in the researcher's laboratory by reprocessing the ^{63}Ni salts or they can be obtained from certain companies that specialize in custom production of ^{63}Ni compounds (Isotope Products Laboratories, Burbank, California 91504, U.S.A.; New England Nuclear Corporation, Boston, Massachusetts 02118, U.S.A.).

Water-soluble ^{63}Ni compounds

Practical methods for preparation of water-soluble ^{63}Ni compounds are included in Kirby's monograph (16). In general, water-soluble nickel compounds can be labelled with ^{63}Ni by adding the proper amount of $^{63}NiCl_2$ to an aqueous solution of the compound. The $^{63}NiCl_2$ should have the highest possible specific activity in order to minimize contamination with chloride. If the presence of chloride is unacceptable, it can be removed from the product by ion-exchange chromatography (25). Alternatively, the ^{63}Ni can be processed through nickel carbonate by precipitation, washing, and dissolution in the desired acid. In the preparation of nickel salts, one must remember that nickel hydroxide starts to precipitate about pH 6.7. This can be prevented by addition of aqueous ammonia. A useful tabulation of nickel compounds of biological interest has been published in the IARC Monograph on Nickel and Nickel Compounds, including pertinent remarks about their chemical and physical properties and their methods of preparation (26).

Metallic ^{63}Ni powder

High purity preparations of ^{63}Ni powder can be prepared by thermal decomposition of $^{63}Ni(CO)_4$ above 453 K (16,26). Metallic ^{63}Ni powder may also be prepared by reduction of nickel oxalate or formate with carbon monoxide at 723 K (27), or by reduction of nickel oxide with hydrogen at 973-117 K (28). Finely divided ^{63}Ni may also be produced by reduction of nickel acetate with sodium borohydride at ambient temperature (29,30). Wienzierl and Webb (29) prepared ^{63}Ni by adding 1.6 g of $NaBH_4$ to 100 ml of a solution of $^{63}NiCl_2$ (10 mmole/liter, 145 mCi/mole) in acetic acid (10 mmole/liter). The resulting black precipitate of ^{63}Ni was filtered, washed with acetic acid (10 mmole/liter) and water, and finally dried *in vacuo*. Perlstein *et al* (30) used a 30% molar excess of $NaBH_4$ for reduction of nickel acetate. The precipitate of ^{63}Ni was washed with distilled water until the washings became neutral and then stored in water under an H_2 atmosphere. Nickel powders can be also labelled with ^{63}Ni by the isotope exchange method (31), but this method is not recommended since the product is strongly influenced by surface contamination with oxides and sulfides.

Radioactive nickel carbonyl

Nickel tetracarbonyl, $Ni(CO)_4$, is a volatile liquid (b.p. 316 K, m.p. 254 K), which is an intermediate in the Mond process for refining nickel matte. Nickel carbonyl is of great interest in biology and medicine, owing to its toxicity, carcinogenicity, and teratogenicity (10,19,21,26). ^{63}Ni-labelled nickel carbonyl is usually prepared by direct action of carbon monoxide on powdered metallic ^{63}Ni at 363-383 K and 0.5-1.0 atm of CO pressure (27). The reaction is performed in an all-glass system of tubes whereby CO is slowly passed over a layer of ^{63}Ni powder. The CO can be generated by dropping formic acid into concentrated H_2SO_4. The resulting $^{63}Ni(CO)_4$ is trapped in a receiving flask that is cooled with a mixture of dry ice (solid CO_2) and ethanol. The outflowing gas is bubbled through a Vigreaux column that contains ethanolic iodine or bromine solution, in order to destroy any escaping nickel carbonyl gas. This procedure is relatively time-consuming, and it may take several hours to obtain a few grams of $^{63}Ni(CO)_4$ with approximately 90% efficiency. The specific activity of $^{63}Ni(CO)_4$ that has been obtained by this procedure has been as high as 2.6 Ci/mole (32,33). $^{63}Ni(CO)_4$ can also be prepared at ambient temperature by passing CO through a suspension of ^{63}NiS in the presence of metallic Fe powder and sodium thiosulfate (34), or through a mixture of $^{63}Ni(II)$-dithiocarbamate and sodium sulfide in 62% ethanol (35). These processes are inefficient since they involve disproportionation reactions ($2\,^{63}Ni(II) \rightarrow \,^{63}Ni(CO)_4 + \,^{63}Ni(IV)$-complex). Theoretically, 50% of the labelled ^{63}Ni is lost in the Ni(IV)-complex. In practice, the yield of $^{63}Ni(CO)_4$ is approximately 30% (35).

Radioactive nickel monosulfides

Amorphous radioactive nickel monosulfide (^{63}NiS) is prepared by addition of sodium sulfide, ammonium sulfide, thioacetamide, or gaseous hydrogen sulfide to aqueous solutions of ^{63}Ni salts (16,36-38). The precipitated ^{63}NiS is washed, dried *in vacuo*, and stored under nitrogen. The ^{63}NiS that is prepared by such procedures is devoid of crystal structure, based upon X-ray diffractometry. It should be noted that in the presence of oxygen, Ni_3S_4 or NiS_2 may also be precipitated. If the reaction is conducted in slightly acidic medium in the absence of oxygen, slow crystallization of millerite (βNiS) may occur (39).

Crystalline radioactive nickel monosulfides ($\alpha^{63}NiS$ and $\beta^{63}NiS$) are prepared by direct reaction between ^{63}Ni powder and elemental sulfur (28,40-42). Stoichiometric amounts of both reagents are placed in a quartz tube, evacuated, and sealed. Heat is required to initiate the vigorous and potentially explosive reaction. When the synthetic monosulfide has been annealed for many hours in an inert atmosphere at 555 ± 25 K, the low-temperature phase ($\beta^{63}NiS$) is produced. When annealing is performed in an inert atmosphere at 823 ± 50 K, followed by rapid quenching, the high-temperature phase ($\alpha^{63}NiS$) is produced. The crystal structure of the low-temperature form (βNiS, millerite) is rhombohedral, whereas the crystal structure of the high-temperature form (αNiS) is hexagonal (43,44). The three forms of nickel monosulfide (amorphous NiS, αNiS and βNiS) are stable in an inert atmosphere at ambient temperature (20).

Radioactive nickel subsulfide

Nickel subsulfide (αNi_3S_2) is a naturally occurring mineral (heazelwoodite) and it is an intermediate product during sintering of nickel-copper sulfide ores. Nickel subsulfide is of great interest in cancer research, owing to its remarkable carcinogenic potency (20,26). Nickel subsulfide is a metallic-lustrous, yellow-bronze, brittle substance which melts incongruently at 1058-1067 K. At ambient temperatures, nickel subsulfide exists only in a rhombohedral crystal structure (αNi_3S_2) (41). αNi_3S_2 undergoes polymorphic transition at 826 K to tetragonal βNi_3S_2, which cannot be sustained by quenching (20,40,41,45). The various sulfides of nickel have characteristic X-ray diffraction patterns (Fig. 1), and powder X-ray diffractometry is requisite for their identification (20,41,46).

$\alpha^{63}Ni_3S_2$ is most satisfactorily prepared by direct reaction of ^{63}Ni powder with elemental sulfur (20). Both ingredients are mixed in stoichiometric amounts and placed in a quartz tube, which is then evacuated and sealed. The tube is heated in order to initiate the exothermic reaction, with appropriate precautions owing to the possibility of an explosion. The contents of the tube are annealed at 573-1073 K for at least 24 hours (41). The product is consistently $\alpha^{63}Ni_3S_2$. This procedure has been simplified by reacting ^{63}Ni powder with a 2- to 3-fold excess of sulfur in a porcelain crucible under an inert atmosphere or graphite layer (40,47). The excess sulfur vapor is protection against O_2 contamination, which would lead to formation of nickel monoxide. The crucible is kept in an annealing furnace for several days until the excess sulfur has evaporated. The sulfur pressure drops rapidly when the stoichiometric proportion of $\alpha^{63}Ni_3S_2$ is reached, and protracted annealing does not, in practice, influence the product (28,48). $\alpha^{63}Ni_3S_2$ can also be prepared by thermal decomposition of $\alpha^{63}NiS$ at 1093 ± 60 K (49-51), or by reduction of anhydrous $^{63}NiSO_4$ with H_2 at 573-623 K (20,52-54).

LIQUID SCINTILLATION COUNTING OF ^{63}Ni IN BIOLOGICAL MATERIALS

Liquid scintillation spectrometry is practically universally used for quantitation of ^{63}Ni in biological materials, since this technique is more sensitive, precise and convenient than alternative methods, such as the use of windowless gas counters, solid scintillators, and semiconductive devices. The optimal experimental conditions for measurement of ^{63}Ni by liquid scintillation counting have been evaluated by several investigators (55-59). Despite the apparent simplicity of liquid scintillation counting of ^{63}Ni, applications of this technique to biological materials are susceptible to artefactitious interferences. Therefore, each researcher should critically investigate the validity of the particular technique that he plans to employ for preparation of biological samples prior to liquid scintillation counting of ^{63}Ni. Owing to the nettlesome problem of losses of ^{63}Ni by adsorption on glassware, a non-radioactive nickel salt should routinely be added to the samples as a carrier. Liquid scintillation spectrometry of ^{63}Ni should be performed in plastic (polypropylene or polyethylene) counting vials rather than in soda glass vials, in order to avoid losses of ^{63}Ni by incorporation into the glass. The samples should be allowed to remain in the dark for 24 to 48 hours prior to liquid scintillation spectrometry in order to avoid errors that may be caused by chemiluminescence.

The radioactivity of ^{63}Ni in small samples (10 to 25 μl) of homogeneous, aqueous fluids such as urine or plasma, can be counted directly after addition of 15 ml of a suitable toluene-based liquid scintillation fluid. A typical scintillation fluid for ^{63}Ni-counting contains 2,5-diphenyloxazol ("PPO", 4 to 6 g) and 1,4-bis-[2-(5-phenylaxazolyl)]-benzene ("POPOP", 0.075 to 0.2 g) per liter of toluene (60). Another satisfactory scintillation fluid for ^{63}Ni-counting contains 2-(4'-t-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxdiazole ("Butyl-PBD", 7.5 to 8.0 g), alone or combined with 2-(4-biphenylyl)-6-phenylbenzoxazole ("PBBO", 0.5 g) per liter of toluene (61-65). The scintillation fluid should contain the minimum amount of methanol that is requisite to ensure miscibility of the toluene with the aqueous ^{63}Ni-containing sample. Otherwise, excessive quenching is obtained. For optimal counting efficiency, scintillation fluids that contain proprietary surfactants (*e.g.* "Bio-Solv", Beckman Instruments Inc., Fullerton, California 92634, U.S.A. or "Aquasol", New England Nuclear Corporation, Boston, Massachusetts 02118, U.S.A.) can be employed. Water-soluble ^{63}Ni compounds can be counted directly in dioxane-based or xylene-based scintillation fluids (47), but such fluids are more expensive and generally yield lower counting efficiencies than toluene-based fluids. Other methods employ suspensions of powdered anthracene (57,58) or scintillating cation-exchange resin in the form of small beads (66).

In order to measure ^{63}Ni in biological materials (*e.g.* whole blood, tissue homogenates, or large volumes (0.1 to 0.5 ml) of plasma) which contain significant amounts of protein, the samples can be treated with "solubilizing agents" prior to liquid scintillation counting (67). Such solubilizers belong to a class of quaternary ammonium bases with high molecular weight (general formula = $R_2R'_2NCl$ or $R_2R'_2NOH$), which are miscible with toluene or methanol. Examples of proprietary toluene-soluble quaternary ammonium bases include "Soluene-100" (Packard Instrument Co., Downers Grove, Illinois 60515, U.S.A.); "Protosol" (New England

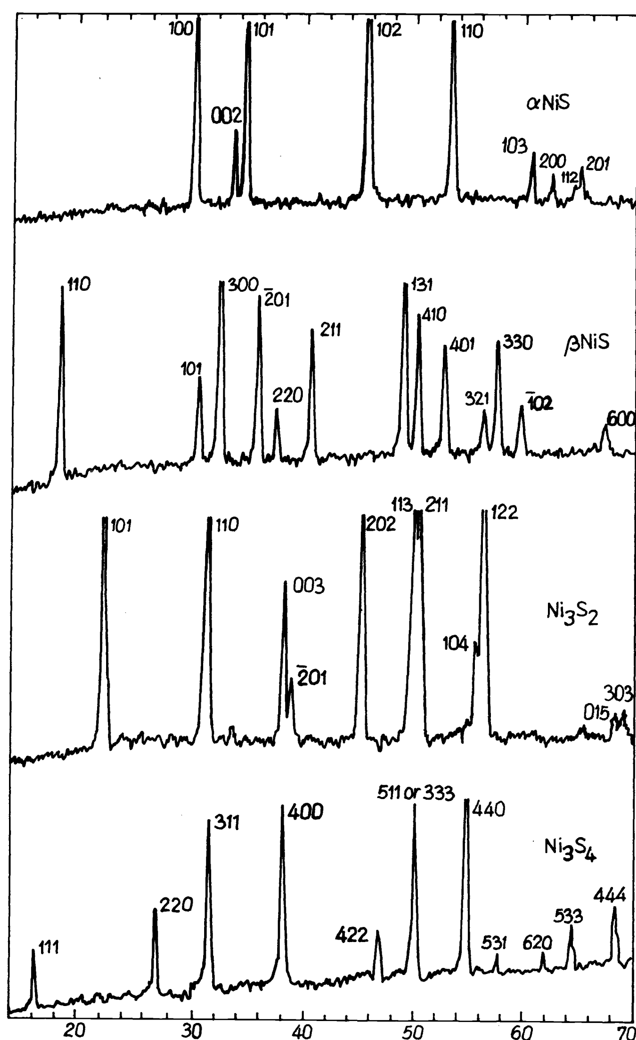

Fig. 1. X-ray diffraction patterns of nickel sulfide powders (αNiS, βNiS, αNi_3S_2 and Ni_3S_4), as described by Kullerud and Yund (41), Kasprzak (20) and Kasprzak and Sunderman (46). The abscissa is the angle of 2θ. The ordinate is X-ray intensity in a linear scale. Miller's indices of the reflecting crystal planes are indicated over the peaks, according to Kullerud and Yund (41).

Nuclear Corporation, Boston, Massachusetts 02118, U.S.A.), and "NCS Solubilizer" (Amersham-Searle Corp., Des Plains, Illinois 60016, U.S.A.). Examples of methanol-soluble quaternary ammonium bases include "Hyamine hydrochloride" (Rohm and Haas Co., Philadelphia, Pennsylvania 19105, U.S.A.) and Digestin (E. Merck Co., Darmstadt, Federal Republic of Germany). According to the alternative technique of Newman (65) and Stevens *et al* (68), digestion of protein-containing fluids and tissue homogenates can be performed with aqueous NaOH solution (1 mole/liter). The digest is then mixed with "Bio-Solv II" (Beckman Instruments Co., Fullerton, California 92634, U.S.A.), which is a high molecular weight detergent that renders the aqueous phase miscible with toluene-based scintillation fluid. The maximum weight of tissue which can be dissolved and counted in a standard scintillation vial is 0.2 g by the "Bio-Solv II" technique, with digestion in NaOH for 1 hour at 308-323 K (68). The corresponding maximum weight of tissue is 0.5 g by the "NCS-Solubilizer" technique, with digestion for 36 hours at 343-353 K (68). Particular attention should be directed to complete dissolution of small pieces of tissue in the solubilizer, since the counting efficiency is often different for ^{63}Ni in the solvent compared to that present within minute fragments of tissue. These differences in counting efficiency may be unsuspected, since they cannot be measured by either internal or external standardization procedures. Colored samples such as

blood or liver homogenate can be bleached with a few drops of saturated benzoyl peroxide in toluene (or, less satisfactorily, with 30% H_2O_2) prior to mixing with the liquid scintillation fluid (67).

Liquid scintillation counting of ^{63}Ni in large samples (1 to 7 g) of solid biological materials (*e.g.* tissues or feces) usually entails preliminary acid digestion, and extraction of a ^{63}Ni-chelate into an organic solvent. For example, Sunderman *et al* (51) digested muscle samples (5 to 7 g) in a mixture of nitric, sulfuric and perchloric acids. ^{63}Ni was chelated with ammonium pyrrolidine dithiocarbamate and extracted into 6 ml of methylisobutylketone (MIBK). The MIBK extract was evaporated overnight in a waterbath at 310 K under a stream of N_2. The residue was decolorized with 2 drops of 30% H_2O_2 and 2 drops of acetone, and evaporation was continued to dryness. Liquid scintillation spectrometry was performed in a toluene-based scintillation fluid. Quantitative extraction of ^{63}Ni from mineralized aqueous samples can also be achieved with various dioximes dissolved in toluene or chloroform (69, 70). Tissues may be destroyed by use of a muffle furnace instead of acid digestion, but the dry ashing technique is subject to poor recovery of ^{63}Ni if porcelain, vycor or quartz crucibles are used instead of platinum crucibles (70). Moreover, platinum crucibles may catalyze the pyrolytic destruction of carbonaceous material.

The efficiency of counting of ^{63}Ni in biological samples is influenced by (a) the color of the sample, (b) the content of quaternary ammonium base or NaOH, (c) the presence of quenching agents, (d) the concentrations of the phosphors, (e) the volume of the liquid scintillation fluid, and (f) the design and characteristics of the spectrometer. Nickel itself causes quenching, if the nickel concentration exceeds 120 mg/liter of scintillation fluid (71). The counting efficiency of measurements of ^{63}Ni in biological materials by liquid scintillation spectrometry is usually in the range from 40 to 60%. The counting efficiency of each scintillation vial can be monitored by the internal standard ("spiking") method. This technique involves the addition of a small volume (10 to 20 µl) of a ^{63}Ni-containing solution of known radioactivity to each previously counted scintillation vial. The vials are counted once again and the observed increments in radioactivity are divided by the known radioactivity that was added. Other methods of evaluating the counting efficiency are discussed in detail by Simon (60).

AUTORADIOGRAPHY OF ^{63}Ni

Several investigators (64,72-75) have employed autoradiography for qualitative identification of ^{63}Ni-binding to proteins, amino acids and other ligands in biological materials. The ^{63}Ni-complexes are separated by chromatography on a cellulose thin-layer plate or by electrophoresis on a cellulose acetate strip, and the plate or strip is immediately dried in a desiccator at ambient temperature or in an oven at 373 K. The dried plate or strip is taken to a darkroom and placed in direct apposition to the emulsion side of an X-ray film ("Gaestar" film, Ansco Co., Rochester, New York 14650, U.S.A. or "NS-54 X-ray film", Eastman Kodak Co., Rochester, New York 14650, U.S.A.). The film and dried plate or strip are wrapped in aluminium foil to provide a light-tight packet, and the packet is stored in a dry box at room temperature for 15 to 90 days, depending upon the ^{63}Ni radioactivity. The method can detect as little as 0.3 nCi of ^{63}Ni per sample. Generally, 10^6 to 10^8 particles/sample are necessary to obtain a distinct spot on X-ray emulsion (60). At the completion of the exposure period, the X-ray film is developed and fixed according to the manufacturer's directions, and the chromatographic plate or electrophoretic strip is stained for proteins, amino acids, or other constituents as desired. In autoradiography of ^{63}Ni-containing samples, it is not practical to place any isolating foil between the X-ray film and the chromatographic plate or electrophoretic strip. Therefore, it is always essential to test nonradioactive control samples that contain all of the ingredients of the test sample excepting ^{63}Ni, inasmuch as prolonged exposure and direct contact of reducing compounds with the X-ray emulsion may cause chemigraphic artifacts which mimic spots of radioactivity.

A few investigators (47,76-79) have successfully performed autoradiography of ^{63}Ni in histological sections. The critical step is the tissue fixation procedure. Best results are obtained by use of the cryostatic microtome. If fixatives such as methanol or phosphate-buffered or neutral formalin are employed, there is always a possibility that the fixative may dissolve or displace nickel from its original binding sites. The cryostat-prepared or fixed tissue section is covered with autoradiographic emulsion and placed in a desiccator over $CaCl_2$ at 277 K. After exposure in the dark for 1 week to 6 months, the emulsion layer is developed, and the underlying histologic section is stained with hematoxylin-eosin. The autoradiogram remains precisely superimposed upon the histologic section, so that the location of the silver grains in the emulsion indicates the histological localization of ^{63}Ni.

To date, the most elegant examples of ^{63}Ni-autoradiography of biological samples have been the whole-body autoradiograms of mice that have been performed by Oskarsson and Tjälve (77, 78). These investigators have prepared coronal sections of entire mice by use of a sledge

cryomicrotome, and they have demonstrated the tissue localization of ^{63}Ni following administration of $^{63}NiCl_2$ and $^{63}Ni(CO)_4$ (77,78). Insofar as the authors can ascertain from published reports, ^{63}Ni has not been employed for electron microscopic autoradiography, but there appears to be no practical or theoretical obstacle to the use of ^{63}Ni for such studies.

RADIATION CONTROL IN ^{63}Ni EXPERIMENTATION

External sources of ^{63}Ni are not hazardous for man, since the maximum range of the emitted beta particle is very short (6.6 mg/cm^2), and the radiation is completely absorbed by the walls of glass or plastic containers, as well as by protecting gloves and the epidermis. Following administration of ^{63}Ni to experimental animals, their carcasses, tissues, fluids and excreta should not be considered as hazardous sources of external radiation. Precautions must be taken to minimize internal exposures to ^{63}Ni by accidental ingestion or inhalation. Safety standards that pertain to internal exposures to beta-emitting radioisotopes have been formulated and are enforced in practically every nation of the world.

In order to check for contamination of working surfaces with ^{63}Ni, it is convenient to take smears with filter-paper strips. The filter-paper strips may be wetted with solutions of ^{63}Ni-complexing agents (*e.g.* EDTA, 0.1 mole/liter, or citric acid, 50 g/liter) or dilute nitric acid (0.2 mole/liter). The filter-paper strips are dried and placed directly into vials for liquid scintillation counting.

Decontamination of ^{63}Ni on working surfaces and glassware is accomplished by washing or soaking in an EDTA solution or in a commercial decontamination fluid ("Radiac Wash", Nuclear R & D Corp., Berkley, Michigan 48072, U.S.A.). If necessary, glassware may be soaked overnight in nitric acid (6 mole/liter) in order to remove adsorbed ^{63}Ni. Decontamination of skin can be accomplished by washing with soap and with "Radiac Wash" or citric acid solution (10 g/liter). Decontamination of ^{63}Ni from stainless steel or other nickel-containing alloys (*e.g.* in syringes, needles, infusion pumps) is difficult, since the ^{63}Ni may enter the alloy by isotope-exchange. In such cases, decontamination with acids and leaching agents may damage the surface of the metal part. Re-exchange in a nonradioactive nickel bath is therefore recommended. Decontamination procedures are discussed in detail by Zimon (80).

APPLICATIONS OF ^{63}Ni IN STUDIES OF NICKEL METABOLISM AND TOXICOLOGY

Studies of the metabolism and toxicology of ^{63}Ni in animals and plants are tabulated in Table 2, in order that researchers may sift the procedures that have been employed and identify experimental approaches that may be applicable to their own particular studies. A detailed review of nickel metabolism and toxicology is unnecessary in this paper, since these topics have been summarized in several monographs and articles (18,19,21,96,97). However, the following brief resumé of ^{63}Ni metabolism is included for the reader's orientation.

Following oral administration of $^{63}Ni(II)$ to animals, most of the ^{63}Ni remains unabsorbed and is excreted in the feces (85,95). A minor fraction of the ^{63}Ni is absorbed from the intestine, enters the plasma, and is excreted primarily via the urine (62,85,95). Following parenteral administration of $^{63}Ni(II)$ to animals, ^{63}Ni is rapidly accumulated in kidney, pituitary, lung, skin, adrenal and ovary or testis (63,77,79,81,82,87,88). During 3 to 5 days after parenteral administration of $^{63}Ni(II)$, the ^{63}Ni is excreted via the urine and to a minor degree via the bile (74,81,82,88). Transport of ^{63}Ni in blood is accomplished by serum albumin (61,74) and by several ultrafiltrable serum ligands, which appear to be amino acids or small polypeptides (64,74). A major fraction of serum nickel is bound to macroglobulin ("nickeloplasmin"), but nickeloplasmin does not appear to have an important role in the transport and excretion of nickel (62,64,75). Although several investigations have suggested the presence of intracellular ^{63}Ni-binding macromolecules, little is known regarding their identity or properties (29,32,86,90,92,94). The kinetics of $^{63}Ni(II)$ metabolism in rodents have been described by two-compartment models (84,88,89,93).

The metabolism of $^{63}Ni(CO)_4$ differs markedly from that of $^{63}Ni(II)$, since (a) nickel carbonyl is highly volatile and can be absorbed by the lungs and eliminated in the exhaled breath, (b) nickel carbonyl can enter the erythrocyte, where it is slowly decomposed to liberate carbon monoxide and Ni(II), and (c) nickel carbonyl is lipid-soluble and can readily cross the blood-brain barrier (32,33,78). Relatively insoluble nickel compounds such as $^{63}Ni_3S_2$ also differ from $^{63}Ni(II)$ in their metabolism. Following *im* injection of $^{63}Ni_3S_2$ in rats, ^{63}Ni is very slowly mobilized from the injection site (46,51). Studies of the *in vitro* reactions of $^{63}Ni_3S_2$ during incubation in rat serum and rat serum ultrafiltrate have identified mechanisms whereby this carcinogenic compound may be solubilized and excreted (46). A three-compartment model of ^{63}Ni-kinetics has been formulated based upon measurements of ^{63}Ni in tissues, plasma and excreta of rats following *im* injection of $^{63}Ni_3S_2$ (51).

TABLE 2. Studies of metabolism and toxicology of radiolabelled nickel compounds

Authors & Date	Nickel compounds	Species & routes*	Samples†	Methods	Observations
Wase *et al* (81) 1954	$^{63}NiCl_2$	Mouse (i.p.)	B,P,U,F,T	Acid digestion, electro-deposition, gas-flow detector	Rapid ^{63}Ni uptake by kidney and lung.
Norgaard (24) 1955	$^{57}NiSO_4$	Man (p.c.)	Skin-washings	Geiger-Müller counter	Dermal ^{57}Ni absorption equal in normal and Ni-sensitive subjects.
Smith & Hackley (82) 1968	$^{63}NiCl_2$	Rat (i.v.)	B,U,F,T	Acid digestion, scint. counting	Rapid ^{63}Ni uptake by kidney and excretion in urine.
Sunderman & Selin (32) 1968	$^{63}Ni(CO)_4$, $^{63}NiCl_2$	Rat (i.v., inh.)	Br,S,U,F,T	NCS solubilizer, scint. counting	$^{63}Ni(CO)_4$ excretion in breath; $^{63}Ni(II)$ excretion in urine and feces.
Kasprzak & Sunderman (33) 1969	$^{63}Ni(CO)_4$	Rat (i.v.)	Br,U,B,S	Scint. counting	$^{63}Ni(CO)_4$ oxidation in RBC, and release of $^{63}Ni(II)$ into serum.
Tiffin (73) 1971	$^{63}NiCl_2$	Tomato, cucumber, corn, carrot, peanut (n.s.)	Exudate, rootsap	Electrophoresis, autoradiography, planchet counting	^{63}Ni-binding to organic ligands.
Soestbergen & Sunderman (74) 1972	$^{63}NiCl_2$	Rabbit (i.v.)	S,Bi,U	Electrophoresis, dextran gel chromatog., autoradiography, scint. counting	^{63}Ni-binding to serum albumin and to ultrafiltrable ligands which are excreted in urine or bile.
Weinzierl & Webb (29) 1972	^{63}Ni dust	Rat (i.m.)	Muscle homogenate	Dextran gel chromatog., acid digestion, scint. counting	^{63}Ni-binding to ultrafiltrable ligands.
Webb & Weinzierl (83) 1972	$^{63}NiCl_2$ ^{63}Ni dust	Mouse fibroblasts *in vitro)*	Subcellular fractions	Acid digestion, scint. counting	Rapid ^{63}Ni uptake and localization in nuclei (>50% in nucleoli).
Nomoto *et al* (75) 1973	$^{63}NiCl_2$	Rabbit (i.v.)	S	Chromatography, electrophoresis, scint. counting	^{63}Ni-binding to serum nickeloplasmin, albumin and ultrafiltrable ligands.
Onkelinx *et al* (84) 1973	$^{63}NiCl_2$	Rat, rabbit (i.v.)	S,B,U,T	Scint. counting	^{63}Ni-kinetics described by 2-compartment model.

TABLE 2. (continued)

Authors & Date	Nickel compounds	Species & routes*	Samples†	Methods	Observations
Callan & Sunderman (61) 1973	$^{63}NiCl_2$	Rat, man, rabbit, pig, dog *(in vitro)*	Albumin	Scint. counting	^{63}Ni-affinity of dog and pig albumin is less than human, rat or rabbit albumin.
Ho & Furst (85) 1973	$^{63}NiCl_2$	Rat (oral, i.p.)	U,F	Scint. counting	3-6% of oral dose of ^{63}Ni was absorbed and excreted in urine.
Decsy & Sunderman (62) 1974	$^{63}NiCl_2$	Rabbit (i.v., oral)	S	Chromatography, electrophoresis, scint. counting	*In vivo* ^{63}Ni labelling in nickeloplasmin.
Parker & Sunderman (63) 1974	$^{63}NiCl_2$	Rabbit (i.v.)	T	NCS solubilizer, scint. counting	Highest ^{63}Ni concentrations in kidney, pituitary, skin & lung.
Kasprzak (47) 1974	$^{63}Ni_3S_2$	Rat (i.m.)	T	Autoradiography	^{63}Ni persisted for many months at the injection site.
Asato *et al* (64) 1975	$^{63}NiCl_2$	Rabbit (i.v.)	S	Chromatography, autoradiography, scint. counting	^{63}Ni-binding to serum ultrafiltrable ligands.
Hutchinson *et al* (76) 1975	$^{63}NiCl_2$	Human lumphocyte *(in vitro)*	C	Autoradiography	^{63}Ni-binding to lymphocyte cell surface.
Jacobsen & Jonsen (86) 1975	$^{63}NiCl_2$	Mouse bone *(in vitro)*	T	Acid digestion, scint. counting	Absorption of ^{63}Ni to macromolecules in bone cells.
Clary (87) 1975	$^{63}NiCl_2$	Guinea pig (s.c.)	T	Soluene solubilizer, scint. counting	Highest ^{63}Ni concentrations in kidney, pituitary and lung.
Sunderman *et al* (51) 1976	$^{63}Ni_3S_2$	Rat (i.m.)	T,U,F	Acid digestion, ^{63}Ni-APDC extract. Scint. counting	$^{63}Ni_3S_2$ is slowly solubilized from site of i.m. injection.
Sunderman *et al* (88) 1976	$^{63}NiCl_2$	Rat (i.p., i.m.)	S,U,T	Acid digestion, ^{63}Ni-APDC extraction, scint. counting	Renal ^{63}Ni clearance increased by triethylenetetramine.
Chausmer (89) 1976	$^{63}NiCl_2$	Rat (i.v.)	T	Acid digestion, scint. counting	^{63}Ni uptake in kidney, lung, liver, spleen and bone.
Kasprzak & Sunderman (46) 1977	$^{63}Ni_3S_2$	Rat serum *(in vitro)*	S	Ultrafiltration, scint. counting	$^{63}Ni_3S_2$ slowly dissolves in rat serum.

TABLE 2. (continued)

Authors & Date	Nickel compounds	Species & routes*	Samples†	Methods	Observations
Jacobsen *et al* (90) 1977	$^{63}NiCl_2$	Human salivary gland *(in vitro)*	T	Electrophoresis, chromatography, scint. counting	^{63}Ni incorporation into macromolecules.
Oskarsson & Tjälve (77,78) 1977	$^{63}Ni(CO)_4$	Mouse (i.v., inh.)	T	Whole-body autoradiography	Uptake of $^{63}Ni(CO)_4$ in lung, brain and adrenals. Uptake of $^{63}Ni(II)$ in lung, kidney & connective tissue.
Sunderman *et al* (79) 1978	$^{63}NiCl_2$	Pregnant rat (i.m.)	A,T	Autoradiography, acid digestion, ^{63}Ni-APDC extraction, scint. counting	Transplacental transport of ^{63}Ni and fetal uptake; ^{63}Ni localization in maternal kidney, lung, adrenal, ovary and pituitary.
Cataldo *et al* (72,91) 1978	$^{63}NiCl_2$	Soybean (n.s.)	T	Autoradiography, acid digestion, scint. counting	^{63}Ni uptake into leaves, stems and seeds; ^{63}Ni binding to organic complexes.
Jacobsen *et al* (92) 1978	$^{63}NiCl_2$	Pregnant mouse (i.p.)	T	Alkaline digestion, scint. counting	^{63}Ni transport to progeny via placenta and lactation.
Chausmer *et al* (93) 1978	$^{63}NiCl_2$	Rat liver *(in vitro)*	T	Protosol solubilizer, scint. counting	^{63}Ni uptake into hepatocytes *in vitro* is not competitive with Ca(II).
Charles *et al* (94) 1978	$^{63}NiCl_2$	Rat (i.t.)	T	Acid digestion, scint. counting	^{63}Ni binding to high-affinity sites in lung.
Spears *et al* (95) 1978	$^{63}NiCl_2$	Lambs (oral)	U,F,T	Acid digestion, scint. counting	Oral ^{63}Ni is excreted primarily in feces, but a small fraction of the dose is found in urine.

* inh., inhalation; i.p., intraperitoneal; i.t., intratracheal; i.v., intravenous; n.s., nutrient solution; p.c., percutaneous.

† A, amniotic fluid; B, blood; Bi, bile; Br, breath; C, cells; F, feces; P, plasma; S, serum; T, tissues; U, urine.

APPLICATIONS OF ^{63}Ni IN ENVIRONMENTAL RESEARCH

Environmental contamination with ^{63}Ni results from neutron activation, which may occur during nuclear explosions. Beasley and Held (98) measured ^{63}Ni in biological samples and in water and soil samples from the region of the nuclear arms test in the Bikini atoll. They concluded that ^{63}Ni has a long effective life-time in the ocean, and proposed that ^{63}Ni measurements might be useful as a tracer of oceanic processes (98). Harvey and Sutton (56) described a technique for analysis of ^{63}Ni in liquid radioactive waste from nuclear power stations.

APPLICATIONS OF ^{63}Ni AS A BIOCHEMICAL REAGENT

Ho (99) proposed the use of ^{63}Ni(II) as a reagent for radiochemical assay of long-chain free fatty acids in serum. The method requires only 10 µl of serum, and is conveniently adapted for automated liquid scintillation spectrometry. Kalb and Levitski (100) and Huet and Bernhard (101) showed that ^{63}Ni can be used for radiolabelling of concanavalin A, a plant lectin that binds to cell membranes. ^{63}Ni-labelling enables quantitation of concanavalin A by liquid scintillation counting, and it may permit subcellular localization of the lectin by autoradiography.

REFERENCES

1. A. Schnegg and M. Kirchgessner, Z. Tierphysiol. Tierernahr. Futtermittelkd. 36, 63-74 (1975).
2. A. Schnegg and M. Kirchgessner, J. Vitamin Nutr. Res. 46, 96-99 (1976).
3. M. Anke, M. Partschefeld, M. Grün and B. Groppel, Tiere. Arch. Tierernahr. 28, 83-90 (1978).
4. N. E. Dixon, C. Gazzola, R. L. Blakeley and B. Zerner, J. Amer. Chem. Soc. 97, 4131-4133 (1975).
5. W. R. Gordon, S. S. Schwemmer and W. S. Hillman, Planta 140, 265-268 (1978).
6. J. C. Polacco, Plant Physiol. 59, 827-830 (1977).
7. R. Doll, J. D. Mathews and L. G. Morgan, Brit. J. Ind. Med. 34, 102-105 (1977).
8. L. Kreyberg, Brit. J. Ind. Med. 35, 109-116 (1978).
9. R. Doll, Brit. J. Ind. Med. 15, 217-223 (1958).
10. F. W. Sunderman, Jr., P. R. Allpass, J. M. Mitchell, R. C. Baselt and D. M. Albert, Science 203, 550-553 (1979).
11. O. B. Christensen and H. Möller, Contact Dermatitis 1, 136-141 (1975).
12. K. Kaaber, N. K. Veien and J. C. Tjell, Brit. J. Dermatol. 98, 197-201 (1978).
13. P. H. Gitlitz, F. W. Sunderman, Jr. and P. J. Goldblatt, Toxicol. Appl. Pharmacol. 34, 430-440 (1975).
14. G. Jasmin and J. L. Riopelle, Lab. Invest. 35, 71-78 (1976).
15. S. M. Hopfer, F. W. Sunderman, Jr., T. N. Fredrickson and E. E. Morse, Ann. Clin. Lab. Sci. 8, 396-402 (1978).
16. L. J. Kirby, The Radiochemistry of Nickel, National Academy of Sciences, U.S.A., Washington, D.C. (1961).
17. R. T. Barton, J. Otolaryngol. 6, 412-422 (1977).
18. National Institute of Occupational Safety and Health. Criteria for a Recommended Standard: Occupational Exposure to Inorganic Nickel. U.S. Government Printing Office, Washington, D.C. (1977).
19. K. S. Kasprzak, Metabolism of the Carcinogenic Nickel Compounds. Poznań Technical University Press, Poznań (1978).
20. K. S. Kasprzak, Ni_3S_2: Chemistry, Applications and Carcinogenicity. Poznań Technical University Press, Poznań (1978).
21. F. W. Sunderman, Jr. Ann. Clin. Lab. Sci. 7, 377-398 (1977).
22. B. S. Dzhelepov and L. K. Peker, Decay Schemes of Radioactive Nuclei. U.S.S.R. Acad. Sci. Press, Moscow (1958).
23. I. P. Selinov, Izotopy, Nauka Press, Moscow (1970).
24. O. Norgaard, Acta Derm.-Venereol. 35, 111-117 (1955).
25. J. Tölgyessy and S. Varga, Nuclear Analytical Chemistry. II. Radioactive Indicators in Chemical Analysis, University Park Press, London (1972).
26. International Agency for Research on Cancer, Evaluation of the Carcinogenic Risk of Chemicals to Man. Vol. 11, Nickel and Nickel Compounds, Lyon (1976).
27. K. Mayer and J. P. Wibaut, Rec. Trav. Chim. Pays-Bas. 56, 356-358 (1937).
28. T. Rosenkvist, J. Iron Steel Inst. 176, 35-57 (1954).
29. S. M. Weinzierl and M. Webb, Brit. J. Cancer 26, 279-291 (1972).
30. M. T. Perlstein, M. Z. Atassi and S. H. Cheng, Biochim. Biophys. Acta 236, 174-182 (1971).
31. A. N. Nesmeyanov, Radiokhimia, Khimia Press, Moscow (1972).

32. F. W. Sunderman, Jr. and C. E. Selin, Toxicol. Appl. Pharmacol. 12, 207-218 (1968).
33. K. S. Kasprzak and F. W. Sunderman, Jr., Toxicol. Appl. Pharmacol. 15, 292-303 (1969).
34. G. Chiusoli and G. Mondelli, Nickel Carbonyl, Belg. Pat. No. 619,286 (1962).
35. W. Hieber and R. Brück, Z. Anorg. Allgem. Chemie 269, 28-39 (1952).
36. H. Remy, Lehrbuch der Anorganischen Chemie, Geest und Portig K.G., Leipzig (1959).
37. D. H. Klein, D. G. Peters and E. H. Swift, Talanta 12, 357-362 (1965).
38. F. W. Sunderman, Jr. and R. M. Maenza, Res. Commun. Chem. Pathol. Pharmacol. 14, 319-330 (1976).
39. J. Jellinek, Transition Metal Sulfides. In: Inorganic Sulphur Chemistry, (G. Nickless, Ed.), Elsevier, New York (1968).
40. M. A. Sokolova, Dokl. Akad. Nauk. SSSR 106, 286-289 (1956).
41. G. Kullerud and R. A. Yund, J. Petrology 3, 126-175 (1962).
42. D. H. Antonsen and D. B. Springer, Nickel Compounds. In: Encyclopaedia of Chemical Technology, (R. E. Kirk and D. F. Othmer, Eds.), Vol. 13, J. Wiley & Sons, New York (1967).
43. J. D. Grice and R. B. Ferguson, Can. Mineralogist 12, 248-252 (1974).
44. V. Rajamani and C. T. Prewitt, Can. Mineralogist 12, 253-257 (1974).
45. A. Westgren, Z. Anorg. Allgem. Chemie 239,82-84 (1938).
46. K. S. Kasprzak and F. W. Sunderman, Jr., Res. Commun. Chem. Pathol. Pharmacol. 16, 95-108 (1977).
47. K. S. Kasprzak, Res. Commun. Chem. Pathol. Pharmacol. 8, 141-149 (1974).
48. D. Dingledy and W. M. Barnard, J. Chem. Educ. 44, 693-694 (1967).
49. J. E. Hiller and K. Probsthain, Geologie 5, 607-616 (1956).
50. J. S. Kirkaldy, G. M. Bolze, D. McCutcheon and D. J. Young, Metallurg. Trans. 4, 15-19 (1973).
51. F. W. Sunderman, Jr., K. S. Kasprzak, T. J. Lau, P. P. Minghetti, R. M. Maenza, N. Becker, C. Onkelinx and P. J. Goldblatt, Cancer Res. 36, 1790-1800 (1976).
52. G. Pannetier, J. Abegg and A. Chatalic, Compt. Rend. Acad. Sci. 251, 1784-1786 (1960).
53. G. Pannetier and J. Abegg, Bull. Soc. Chim. France 186-194 (1961).
54. G. Pannetier and J. Abegg, Acta Chim. Acad. Sci. Hung. 30, 127-146 (1962).
55. C. E. Gleit and J. Dumont, Intern. J. Appl. Radiat. Isot. 12, 66 (1961).
56. B. R. Harvey and G. A. Sutton, Intern. J. Appl. Radiat. Isot. 21, 519-523 (1970).
57. J. J. Law, J. W. Smith and M. W. Scott, Anal. Biochem. 50, 635-638 (1972).
58. J. J. Law, J. W. Smith and M. W. Scott, Experientia 29, 377-378 (1973).
59. I. S. Bhat, R. S. Iyer and S. Chandramouli, Anal. Chem. 48, 224 (1976).
60. H. Simon, Anwendung von Isotopen in der Organischen Chemie und Biochemie. Band II. Messung von Radioaktiven und Stabilen Isotopen. Springer Verlag, Berlin (1974).
61. W. M. Callan and F. W. Sunderman, Jr., Res. Commun. Chem. Pathol. Pharmacol. 5, 459-472 (1973).
62. M. I. Decsy and F. W. Sunderman, Jr., Bioinorg. Chem. 3, 95-105 (1974).
63. K. Parker and F. W. Sunderman, Jr., Res. Commun. Chem. Pathol. Pharmacol. 7, 755-762 (1974).
64. N. Asato, M. v. Soestbergen and F. W. Sunderman, Jr., Clin. Chem. 21, 521-527 (1975).
65. F. M. Newman, Technical Report No. 551, Beckman Instruments Inc., Fullerton, California (1973).
66. A. H. Heimbuch, H. Y. Gee, A. DeHaan and L. Leventhal, Int. Atomic Energy Agency Monograph S. M. 61/56, Vienna (1965).
67. D. L. Hansen and E. T. Bush, Anal. Biochem. 18, 320-332 (1967).
68. A. Stevens, E. Estrada, M. Pollay and R. Kaplan, Anal. Biochem. 37, 1-10 (1970).
69. K. S. Kasprzak, Z. Górski and T. Kopczyński, Ann. Clin. Lab. Sci. 8, 497 (1978).
70. D. Ader and M. Stoeppler, J. Anal. Toxicol. 1, 252-260 (1977).
71. J. P. Adloff and M. Bacher, Bull. Soc. Chim. France 1321-1323 (1961).
72. D. A. Cataldo, T. R. Garland, R. E. Wildung and H. Drucker, Plant Physiol. 62, 566-700 (1968).
73. L. O. Tiffin, Plant Physiol. 48, 273-277 (1971).
74. M. v. Soestbergen and F. W. Sunderman, Jr., Clin. Chem. 18, 1478-1484 (1972).
75. S. Nomoto, M. I. Decsy, J. R. Murphy and F. W. Sunderman, Jr., Biochem. Med. 8, 171-181 (1973).
76. F. Hutchinson, T. M. MacLeod and E. J. Raffle, Brit. J. Dermatol. 93, 557-561 (1975).
77. A. Oskarsson and H. Tjälve, Acta Pharmacol. Toxicol. 41(Suppl. 1), 158-159 (1977); Ann. Clin. Lab. Sci. 9, 47-59 (1979).
78. H. Tjälve and A. Oskarsson, Proc. Europ. Soc. Toxicol. 18, 211-214 (1977).
79. F. W. Sunderman, Jr., S. K. Shen, J. M. Mitchell, P. R. Allpass and I. Damjanov, Toxicol. Appl. Pharmacol. 43, 381-390 (1978).
80. A. D. Zimon, Desaktivatsiya, Atomizdat Press, Moscow (1975).
81. A. W. Wase, D. M. Gross and M. J. Boyd, Arch. Biochem. Biophys. 51, 1-4 (1954).
82. J. C. Smith and B. Hackley, J. Nutr. 95, 541-546 (1968).
83. M. Webb and S. M. Weinzierl, Brit. J. Cancer 26, 292-298 (1972).
84. C. Onkelinx, J. Becker and F. W. Sunderman, Jr., Res. Commun. Chem. Pathol. Pharmacol. 6, 663-676 (1973).

85. W. Ho and A. Furst, Proc. West. Pharmacol. Soc. 16, 245-248 (1973).
86. N. Jacobsen and J. Jonsen, Path. Europ. 10, 115-121 (1975).
87. J. J. Clary, Toxicol. Appl. Pharmacol. 31, 55-65 (1975).
88. F. W. Sunderman, Jr., K. Kasprzak, E. Horak, P. Gitlitz and C. Onkelinx, Toxicol. Appl. Pharmacol. 38, 177-188 (1976).
89. A. B. Chausmer, Nutr. Rep. Int. 14, 323-336 (1976).
90. N. Jacobsen, I. Brennhovd and J. Jonsen, J. Biol. Buccale 5, 169-175 (1977).
91. D. A. Cataldo, T. R. Garland and R. E. Wildung, Plant Physiol. 62, 563-565 (1978).
92. N. Jacobsen, I. Alfheim and J. Jonsen, Res. Commun. Chem. Pathol. Pharmacol. 20, 571-584 (1978).
93. A. B. Chausmer, C. H. Rogers and A. V. Colucci, Nutr. Rep. Int. 18, 249-258 (1978).
94. J. M. Charles, S. J. Williams and D. B. Menzel, Toxicol. Appl. Pharmacol. 45, 302 (1978).
95. J. W. Spears, E. E. Hatfield, R. M. Forbes and S. E. Koenig, J. Nutr. 108, 313-320 (1978).
96. F. W. Sunderman, Jr., F. Coulston, G. L. Eichorn, J. A. Fellows, E. Mastromatteo, H. T. Reno and M. H. Samitz, Nickel. National Acad. Sci., U.S.A., Washington, D.C. (1975).
97. F. W. Sunderman, Jr., Nickel. In: Disorders of Mineral Metabolism, (F. Bronner and J. W. Coburn, Eds.), Academic Press, New York (1979).
98. T. M. Beasley and E. E. Held, Science 164, 1161-1163 (1969).
99. R. J. Ho, Anal. Biochem. 36, 105-113 (1970).
100. A. J. Kalb and A. Levitzki, Biochem. J. 109, 669-672 (1968).
101. C. Huet and W. Bernhard, Intern. J. Cancer 13, 227 (1974).