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Thesis/Dissertation

Biodegradation of Orthodontic Applicances and Their Effects
on the Blood Level of Nickel and Chromium

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BIODEGRADATION OF ORTHODONTIC APPLIANCES AND THEIR EFFECTS
ON THE BLOOD LEVEL OF NICKEL AND CHROMIUM

by

Robert Dawson Barrett

A thesis submitted in partial fulfillment
of the requirements for the Master of
Science degree in Orthodontics
in the Graduate College of
The University of Iowa

May 1990

Thesis supervisor: Professor Samir E. Bishara

Graduate College
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Iowa City, Iowa

CERTIFICATE OF APPROVAL

MASTER'S THESIS

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INTRODUCTION

Austenitic stainless steels containing approximately 18 percent chromium and 8 percent nickel for orthodontic bands, brackets and wires is universally used in orthodontic practices. With the introduction of nickel-titanium alloys as orthodontic archwires in the 1970's an additional source of patient exposure to metal corrosion products has been introduced. Since the oral environment is particularly ideal for the biodegradation of metals due to its ionic, thermal, microbiologic and enzymatic properties some level of patient exposure to the corrosion products of these alloys is assured. JS

The primary metals of concern in orthodontic appliances are nickel and chromium. The potential health effects from exposure to nickel and chromium and their compounds has been scrutinized for over one hundred years. It has been well established that these metals possess the propensity to produce hypersensitivity, dermatitis and asthma. In addition, a significant carcinogenic and mutagenic potential have been demonstrated for compounds of both of these metals.

Whether orthodontists should be concerned about the

use of titanium alloys has not been fully determined since the health effects of exposure to titanium and titanium compounds is not fully known. What has been reported is mostly favorable for the safety and biocompatibility of pure titanium and titanium alloys used as implant materials in humans and animals. The research on titanium compounds is quite limited due to the relatively recent introduction of these materials for use in human and animal biological systems.

In order to assess the potential for short or long-term undesired health effects attributable to orthodontic appliances a determination must be made to quantify three factors: The rate orthodontic appliances release potentially harmful metal compounds in the oral environment, the degree humans absorb these metal compounds and finally the length these compounds are retained in the body.

PURPOSE

The purpose of this study is two-fold. First, to determine in vitro the corrosion rate of a typical banded and bonded fixed orthodontic appliance system including both stainless steel and nickel-titanium archwires in an environment representative of the human oral cavity. The corrosion products under investigation are to include compounds composed of nickel and chromium.

Secondly, to determine if an increase in the blood levels of nickel or chromium occurs in patients undergoing routine orthodontic therapy. If such an increase does occur, then how long does it persist.

Another objective of this study will be to determine if the blood levels of nickel and chromium in patients being treated through the use of nickel-titanium archwires differs appreciably from treatment involving standard stainless steel archwires.

LITERATURE REVIEW

Overview-Nickel

Nickel is widely distributed in nature and ranks twenty-fourth among elements in order of abundance on the earth. Nickel and its alloys are important because of their properties of hardness, toughness and resistance to oxidation and corrosion. The major uses of nickel include the production of stainless steels and other nickel alloys used for electroplating, battery manufacturing, in catalysts, coins and inorganic pigments.¹

Overview-Chromium

Chromium is also a predominant element in nature and ranks twentieth in order of abundance. Its primary uses include: metal alloys such as stainless steel, electroplating as a protective coating on other metals, in catalysts, in pigments, as a tanning agent for leather, textile printing, rubber goods and on magnetic recording tapes.^{1,2,3}

Overview-Titanium

Titanium ranks as the eighth commonest element and is widely distributed in the earth's crust. Titanium is

extensively used as a white pigment in paint, plastics and paper, in food as a coloring agent, in cosmetics and pharmaceuticals, in catalysts and in surgical implants.⁴

Biological Importance of Nickel

Nickel is an essential nutrient for several species of laboratory animals.⁵ In humans, a physiological role has not been proven, however, a role in pigmentation is thought to exist.⁶ A nickel deficiency has not been demonstrated in humans and the possible nickel requirement is probably very low.⁵ In very high doses, inorganic nickel may cause acute toxicity, and severe effects have been observed following respiratory exposure to particular nickel compounds.⁵ The major emphasis today is placed on allergenic and carcinogenic effects since it is extremely unlikely that anyone could encounter a toxic exposure to nickel in normal daily life.

Biological Importance of Chromium

Chromium in its trivalent form is an essential metal in man and in animals and plays an important role in insulin metabolism as the glucose tolerance factor.^{7,8} Adverse effects in mammals due to chromium deficiency have been demonstrated.⁹ The kidney is regarded as the critical organ in chromium toxicity with acute tubular necrosis and renal failure having been described following heavy exposure to water soluble chromium.⁹ High concentrations

of chromium are toxic causing precipitation of proteins and interfering with essential enzyme systems.⁸

Biological Importance of Titanium

No essential biological importance has been found for titanium.⁴ It is poorly absorbed from the alimentary tract and many titanium compounds have been found to be biologically inert.⁴ Toxic effects in humans caused by titanium compounds have not been reported in the literature.

Human Exposure to Nickel

Environmental exposure to nickel comes from many sources including the diet, atmosphere, drinking water, clothing fasteners, and jewelry which man is in contact with on a daily basis.

Diet

In the diet, the highest naturally occurring nickel levels, that is, above 1 mg/kg (1×10^{-3} gram/1000 gram) fresh weight, have been found in the following vegetables: peas, beans, lentils, lettuce, spinach, cabbage, and mushrooms.⁵ Seafood is often high in nickel with oysters, other shellfish and salmon approaching or exceeding a nickel level of 1 mg/kg.⁵ High nickel levels have also been found in chocolate, nuts, certain spices, baking powder, and flour-bread food items.⁵ The increased levels found in flour and baking powder are thought to originate from contamination during the milling process which takes place in vessels manufactured with nickel containing

alloys. Some prepared foods have been found to contain unusually high levels of nickel attributed to contamination by the processing procedures. Little or no nickel is typically found in most edible animal products such as meat, milk and eggs.⁶

The average dietary intake of nickel is approximately 200-300 μg [1×10^{-6} gram] per day, however this figure may vary significantly.^{5,6} Three to four times this amount will be ingested by a vegetarian, particularly when chocolate or nuts are included in the diet.⁵

Even though significant amounts of nickel are ingested from the diet there appears to be a mechanism in mammals limiting intestinal absorption similar to those for iron, manganese, copper and probably other essential metals. Large doses are required to overcome this mechanism.⁶ Fractional absorption from the gastrointestinal tract to blood is thought to be about 5%, of which 70% is rapidly excreted and 30% is transferred to the body tissues and retained an average of 200 days.¹⁰

The primary route of eliminating nickel is through the urine. Some is also excreted in sweat and only a minor amount is excreted through the fecal route.¹¹

Atmosphere

The major emissions of nickel to air arise from fuel oil consumption, industrial refining applications, waste incineration and from wind-blown dusts and volcanos.^{5,10} Suburban and rural areas usually exhibit air nickel levels from a few to several ng/m^3 [1×10^{-9} gram/cubic meter], but average levels in cities in excess of $100 \text{ ng}/\text{m}^3$ have been

documented.⁵ Indoor nickel levels are expected to be lower than outdoor concentrations. A general average is thought to be about 20 ng/m³ in urban areas and 10 ng/m³ in rural areas.⁵ Additional pulmonary intake may occur through smoking. One cigarette contains about 2 µg of nickel¹² of which about 10% is released into the mainstream smoke. Cigarette smoking may contribute much more nickel than living in an urban environment.⁵

Drinking Water

Nickel is often found in river water in amounts ranging from 1 to 30 µg per liter.⁶ Drinking water usually contains additional quantities of nickel due to leaching from nickel containing or nickel plated pipes. A maximum of 957 µg/liter has been demonstrated in "first draw" drinking water in the USA (the initial water released from the faucet after sitting in a building's water pipes over night).⁵ Tests conducted in the USA have revealed that 97% of the 2053 drinking water samples tested had nickel concentrations below 20 µg/liter and 80% of the samples had less than 10 µg/liter.¹³

Clothing Fasteners and Jewelry

Percutaneous uptake of nickel takes place and appears to occur mainly through sweat ducts and hair follicles.¹⁴ Common items from which nickel exposure occurs in humans include: jeans buttons, earrings, zippers, rings, watch bands, bracelets, necklaces, eyeglass frames and coins. In these sources of exposure sweat plays an important role in leaching the nickel from the metal object.

Iatrogenic Exposures

Iatrogenic exposures to nickel containing alloys can come from joint prostheses, plates and screws for fractured bones, surgical clips and steel sutures, pacemaker leads, prosthetic heart valves, intravenous fluids and dental alloys. From an in vitro study designed to analyze the leaching of nickel from nickel containing dental alloys into autoclaved human saliva a release of approximately 4.2 $\mu\text{g}/\text{cm}^2/\text{day}$ was reported by Moffa.¹⁵ Other research has also shown the release of nickel from base metal alloys of the type used in fabricating dental crowns, bridges and partial denture frameworks.¹⁶ In vitro release of nickel has also been demonstrated with orthodontic bands and brackets (40 $\mu\text{g}/\text{day}$ for a full mouth appliance)¹⁷, heat-treated orthodontic archwires (0.26 $\mu\text{g}/\text{cm}^2/\text{day}$ for stainless steel and 0.11 $\mu\text{g}/\text{cm}^2/\text{day}$ for chromium-cobalt archwires when heat-treated to 600° C)¹⁸, and silver soldered orthodontic wires (0.023 $\mu\text{g}/\text{cm}/\text{day}$ for stainless steel and 0.005 $\mu\text{g}/\text{cm}/\text{day}$ for chromium-cobalt wire pairs soldered together)¹⁹.

Human Exposure to Chromium

Chromium, like nickel, is also an ubiquitous component of the environment. Sources for man include; the diet, atmosphere, drinking water, and several iatrogenic sources.

Diet

Though all plant and animal tissues appear to contain chromium²⁰, the chromium content of most foods is considered to be extremely low.³ The distribution of

chromium in foods is similar to that of nickel. Ranges in food groups have been reported as: vegetables = 20-50 $\mu\text{g}/\text{kg}$, fruits = 20 $\mu\text{g}/\text{kg}$, and grains and cereals = 40 $\mu\text{g}/\text{kg}$.³ The mean daily intake of chromium from food has been estimated to be 280 μg .³

The capacity of humans to absorb chromium is greatly influenced by the oxidation state of the chromium ion. Compounds with chromium in the III+ oxidation state are poorly absorbed and appear mainly in the feces in insoluble, complex form.²⁰ Hexavalent chromium (Cr VI+) is the primary form in which this metal is absorbed by man, however, when hexavalent chromium is introduced orally and subsequently swallowed it tends to be reduced to the trivalent form by saliva and especially by gastric juice.²¹ Therefore, this mechanism provides an important protective barrier against toxicity to chromium through oral intake. Only about 2% of ingested hexavalent chromium compounds are absorbed from the gastrointestinal tract.³ Once hexavalent chromium is absorbed into the blood stream it is selectively accumulated in erythrocytes which reduce it to the trivalent form.²¹ In tissue or organ systems chromium is not known to concentrate selectively. Excretion of absorbed chromium occurs mainly via the urine.³

To summarize, for chromium to be absorbed by man it must be in the hexavalent oxidation state. The majority of this form is reduced to the trivalent form prior to absorption taking place. What hexavalent chromium is absorbed becomes stored in the erythrocytes where it is reduced to the trivalent form. Circulating chromium is in the trivalent oxidation state.²²

Atmosphere

The concentration of chromium in the air has been reported to be in the range of 0.002-0.02 $\mu\text{g}/\text{m}^3$.³ Approximately 200 urban locations were evaluated in 1960-1969 and had annual mean concentrations of 0.01-0.03 $\mu\text{g}/\text{m}^3$. In non-urban areas, the level of chromium was less than 0.01 $\mu\text{g}/\text{m}^3$.³ Common sources of chromium in the air are: the burning of coal, manufacturing facilities for metallurgical chromium and chromium chemicals, cement producing plants, automobile catalytic emission control systems, and the wearing of chromium containing asbestos brake linings for motor vehicles. The chromium content of cigarette tobacco in the United States has been reported as 0.24-6.3 mg/kg.³

The National Institute for Occupational Safety and Health estimated in 1975 that there are 175,000 persons in the U.S. who are potentially exposed occupationally to hexavalent chromium and listed 104 occupations in which such exposure could occur. The most significant of these industries include: chromite ore processing including ferrochromium alloys and stainless steel production, chromium plating industries, welding, chromium pigment industries (paints and dyes), and leather tanning.³

Drinking Water

A survey of chromium content of 15 North American rivers showed levels of 0.7-84 $\mu\text{g}/\text{liter}$ ($\mu\text{g}/\text{l}$), with most in the range of 1-10 $\mu\text{g}/\text{l}$.³ Levels in public water supplies ranged from no detectable content to 36 $\mu\text{g}/\text{l}$ of chromium with the median equal to 0.43 $\mu\text{g}/\text{l}$.

Iatrogenic Exposures

Iatrogenic exposures to chromium primarily come from the use of stainless steel medical and dental appliances or prostheses. Stainless steel is commonly used for joint prostheses, plates and screws for fractured bones, surgical clips and steel sutures. In addition, orthodontic appliances are predominantly manufactured from stainless steel and partial denture frameworks are constructed using alloys containing chromium.

In vitro release of chromium has been demonstrated from orthodontic bands and brackets (36 $\mu\text{g}/\text{day}$ for a full mouth appliance)¹⁷ and silver soldered orthodontic wires (0.011 $\mu\text{g}/\text{cm}/\text{day}$ for stainless steel and 0.0004 $\mu\text{g}/\text{cm}/\text{day}$ for chromium-cobalt wire pairs soldered together)¹⁹.

Human Exposure to Titanium

Like the two previously discussed metals, titanium enters the body through a number of routes. It is present in the diet and in drinking water, breathed in from the air, and is used in medicine and dentistry as either a pure metal or in alloys with other metals.

Diet

Though titanium is extremely abundant in the earth's crust and in the soil it is poorly absorbed and retained by plants and animals so that the levels in the tissues are generally much lower than those in the environment to which the organisms are exposed. No evidence that titanium performs any vital function in either plants or animals nor that it is necessary for growth has yet been produced.

The most significant concentrations of titanium found in food sources occur in vegetables and cereals. One report²⁰ examined a variety of plants for titanium and found levels ranging from 100-5000 $\mu\text{g}/\text{kg}$ with a high proportion of the values lying close to 1000 $\mu\text{g}/\text{kg}$. For the cereal grains the titanium present was concentrated in the outer, branny layers. The daily intake from dietary sources has been estimated at between 300 and 2000 μg of the element.⁴ Titanium is poorly absorbed from the gastrointestinal system.

Atmosphere

The literature reveals very few references regarding the measurement of titanium levels in the environment. The highest peak levels of titanium in the air in an occupational environment have been 50 mg/m^3 .⁴ Titanium has been found to accumulate in the lung with age but not in the liver or kidney.²⁰ The primary source for this accumulation is thought to be from the inhalation of titanium containing atmospheric dust.

Drinking Water

Titanium is quite often found in drinking water with concentrations ranging from 0.5 to 15 $\mu\text{g}/\text{l}$.⁴

Iatrogenic Exposures

Titanium comprises a significant portion of the metals used for medical and dental implant materials. It has been shown that titanium is released in quantifiable amounts from porous titanium implant materials.²³ It was found that larger amounts of titanium were present in the blood

and urine of animals with titanium fiber implant specimens than without.

In contrast to both nickel and chromium, titanium is a very acidic ion and as such it does not bind effectively to biological ligands (organic molecules which bind metal ions). This inhibits the ability of titanium to diffuse through the body when used as an implant material. Thus, as a rule, titanium tends to accumulate in the tissues surrounding its placement in surprisingly large quantities.

Systemic Effects of Nickel

As more information is gained concerning the potential health risks of biomedical metals and alloys there has been an increase in the level of concern over their use. There are several reasons for this concern:

1. The possibility of direct toxic effects.
2. The possibility for sensitization of the patient which may have serious consequences for those individuals with a cardiac pacemaker or a hip prosthesis.
3. The potential to produce allergic reactions which may lead to rejection of an implanted material.
4. The possibility of exacerbating episodes of allergic dermatitis when hypersensitive individuals are exposed to additional sources of these metals.
5. The known high incidence of allergy to some metals, particularly to nickel.
6. The possibility that an increased cancer risk is produced by the use of certain metals.

Nickel Allergy

An allergic response is defined as a pathologic process induced by immune (antigen/antibody) responses.²⁴ For all practical purposes it is synonymous with hypersensitivity which is more generally defined as an exaggerated response to a foreign agent.

Metal sensitivity usually develops to metal salts as haptens (partial antigens which must be linked to proteins in order to induce antibody production) and it is the binding of the metal ions to the body proteins (usually skin) that causes the sensitivity to the metal ion.²⁵ Nickel is a very common sensitizer and produces more instances of allergic contact dermatitis than all other metals combined.²⁴ The first cases of nickel allergy caused by articles in everyday use were reported in the early 1930's.²⁶ The incidence of nickel allergy has increased dramatically in this century.⁵ At present, the incidence of nickel allergy in the general population has been reported to be from 7.2 to 31.9% in females and between 1 and 20.7% in males.^{25,27,28,29,30,31} The majority of the literature (with the exception of Blanco-Dalmau, et. al.²⁸) supports an incidence for nickel allergy very close to 10% for females and 1% for males.

Most causes for nickel allergy have been attributed to dermatological exposure to nickel alloys. Common sources of exposure include: hairpins, earrings, eyeglass frames, necklaces, zippers, wire bra supports, buttons on jeans, coins, pens, watch bands, bracelets and rings. The ten-fold higher hypersensitivity rate for women has been explained by the fact that they typically come in contact

with these articles much more frequently than do men. The primary sites and sources for sensitization has shifted during the 1900's from the suspenders used to hold up women's stockings to pierced earrings and metal buttons in blue jeans.^{27,30,31} Women with pierced ears were found to be six times more likely to have a positive patch test to nickel as compared to women without pierced ears.¹⁵ For men the incidence for a positive patch test was 33 times higher in the sample with pierced ears as compared to those without. The nickel exposure in these individuals comes from inexpensive "gold post" earrings which contain nickel plating under the thin gold plating. This gold plating is quickly worn or chipped off exposing the underlying nickel surface. Due to the increased uses of nickel alloys in jewelry and as clothing fasteners the incidence of nickel allergy in females has been shown to be steadily increasing from the period of 1948 to 1978.³⁰ The mean age of onset for allergic contact dermatitis has been reported in one study to be 23.1 years.²⁶ In another study involving female twins the heritability of nickel allergy was calculated to be approximately 60%.²⁷

In males, the most common sensitizers outside of occupations that involve nickel compounds seem to be wristwatches and the metal buckles found on many watchbands.²⁸ If the incidence of males wearing pierced earrings continues to increase a significant jump in the nickel allergy rate for males will be likely.

The literature contains numerous reports of nickel containing dental prostheses being responsible for episodes of expressed nickel allergy.^{32,33,34,35,36} Alloys

consisting of chromium, nickel and cobalt have been found to be the most frequent dental material causing allergy.³⁴ Allergic reactions due to the nickel content of stainless steel wire used by oral surgeons to fix jaw fractures has also been reported.^{37,38} Other references have described allergic reactions due to nickel alloys in orthodontic appliances.^{39,40,41}

Though it has been demonstrated that nickel-containing dental alloys may precipitate manifestations of an already present nickel allergy, it has not been decided whether long-term intraoral exposure to such alloys can result in an induced nickel sensitivity. It is possible that through intraoral exposure to nickel-containing alloys a subclinical hypersensitivity may progress to a clinical allergy state. At present, there is no direct evidence that the intraoral use of nickel-containing alloys will result in an induced sensitivity.¹⁵

Clinical symptoms of nickel allergy require a higher level of exposure when the allergen is applied to the oral mucosa as compared to an epidermal exposure. One study found that 5 to 12 times higher concentrations of the allergen have to be applied on the oral mucosa as compared to the skin in order to elicit microscopic reactions on the mucosa.³² This explains why not all individuals with nickel hypersensitivity exhibit symptoms when nickel containing dental alloys are used in their oral cavities. Moffa, et al.,⁴² found that 30% of known nickel sensitive individuals had an allergic reaction to an intraoral exposure of a nickel-chromium dental alloy. Eighty percent of these same individuals reacted to a skin application of

this same dental alloy.

Another reason for the lower incidence of reactions to nickel containing dental alloys is the frequent alloying with chromium which lends passivity to the alloy thus greatly reducing the amount of free nickel ions in the oral environment.⁴³ In comparison to the 30% reaction rate to an intraoral exposure to nickel-chromium alloys in nickel sensitive individuals, van Loon, et al.⁴⁴ found a 100% reaction rate for five subjects when a pure nickel plate (3x5 mm) was kept in contact with oral mucosa. This further supports the contention that nickel, when alloyed with chromium, has less potential for leaching than when it is in a pure state.

The dental profession is interested in determining if patients are becoming sensitized to the metal alloys which are being used in dental prostheses. In a study published by Moffa, et al.⁴⁵, they found no correlation between the incidence of nickel or chromium allergies and the presence of dental prostheses containing these metals. This suggests that patients are unlikely to become sensitized to nickel in a dental prostheses.

Contrary to most studies, one report has suggested that the incidence of nickel and chromium hypersensitivity among individuals who in the past underwent orthodontic treatment, is smaller than in other individuals.⁴⁶ Results of their animal study found that it was easier to subsequently sensitize animals which were not previously exposed to the intraoral administration of nickel and chromium metal than to sensitize animals which were previously exposed. They suggested that non-presensitized

individuals may become tolerant to metal sensitizers as a result of presenting metals through the oral cavity.

Research has shown that allergens released in the mouth may result in, maintain or worsen allergic reactions in other parts of the body without producing any local reactions in the oral mucosa.^{5,32} It has been hypothesized that if the oral mucosa is sensitized, the skin is also sensitized. However, if the skin is sensitized, the oral mucosa may not be.⁴⁷

Symptoms of allergic reactions to dental alloys have included severely inflamed hyperplastic gingival tissue surrounding crowns fabricated with a nickel containing alloy.³⁵ Two other cases reported a significant loss of alveolar bone within 18 months of crown placement.³⁶ When stainless steel bone fixation wires were used in nickel sensitive patients, two reports of allergic reactions included edema of the throat, palate and gums³⁷ and osteomyelitis³⁸.

In 1974, the Swedish National Board of Health and Welfare issued a statement containing a warning against the use of dental alloys containing more than 1% by weight of nickel.

Reactions to nickel in orthodontic appliances have also been reported. Reports in three articles on four cases of allergic reactions to either headgear facebows or neck straps in teenage girls have been presented.^{39,40,48} Symptoms included inflammation of the commissures of the mouth which later progressed to include the lips, cheeks, and eyelids; edema of the lips and cheeks followed by eyelid edema and cheilitis; and local skin inflammation

with blistering and ulceration. A rather severe allergic response has been reported from nickel-titanium orthodontic archwires.⁴¹ Within a few days the patient experienced a burning sensation in the oral mucosa which led to a loss of seven pounds in body weight due to the pain and difficulty in eating. After a month, large erythematous macular lesions were seen through out the mouth. The buccal mucosa, dorsal tongue, and palatal mucosa were extensively involved and to a lesser degree, lesions were present on the labial mucosa of both lips. No response was exhibited by this patient to the stainless steel orthodontic brackets and bands.

In a study conducted on nickel-sensitized animals (rabbits) no reactions were provoked from intradermal exposure to as-received stainless steel or chromium-cobalt orthodontic wires.⁴⁹ When soldered specimens of these wires were implanted, moderate to extreme local reactions occurred. The soldered chromium-cobalt wire elicited the most severe reactions.

Even though frank symptoms of nickel allergy to stainless steel orthodontic bands or brackets have not been reported in the literature it has been suggested that the metals in these appliances may well contribute to the gingivitis in some patients who observe rigorous oral hygiene procedures and who have well-fitting appliances.⁴⁷

The potential for allergic reactions to the somewhat new nickel-titanium alloy archwires may be expected to be of more significance as compared to stainless steel alloys for two reasons. First, the nickel-titanium archwires possess a much higher concentration of nickel (50-55% Ni)

when compared to stainless steel alloys (10-17% Ni). Secondly, chromium passivates the surface in stainless steel thus inhibiting corrosion and restricting leaching of nickel into the environment. Since chromium is not a constituent of nickel-titanium alloys they do not possess the passivating surface layer with which to inhibit leaching of the nickel in the oral environment. Titanium also has the ability to impart some degree of passivation to metal alloys. This may improve the corrosion resistance of nickel-titanium alloys.

Nickel Dermatitis

Dermatitis or eczema are the most common reasons why individuals with nickel allergy seek medical attention leading to an accurate diagnosis of their sensitivity. In many sensitive individuals dermatitis is the only symptom of their allergic condition. It is estimated that about 5-13% of all cases of eczema are caused by contact with nickel or nickel compounds.¹³ Hand eczema occurs with a frequency of 20-60% in nickel-sensitive patients.³⁰ Not all nickel-sensitive individuals however report a history of dermatitis. In one study, 38 percent of nickel sensitive patients had no history of nickel dermatitis when questioned before testing.⁵⁰

Nickel dermatitis may occur at the site of direct contact with nickel (primary) or in other distant areas which are involved when the dermatitis spreads (secondary). Furthermore, nickel dermatitis does not necessarily appear at all sites of contact with the metal.

The most common sites for dermatitis to occur in

nickel sensitive individuals is on either the palm or dorsum of the hand, the wrist, face, arm, neck or periorbital areas.⁵¹ In Denmark, 64% of the nickel-sensitive patients in one study had or had had eczema from contact with metallic buttons in blue jeans.⁵²

Many nickel-sensitive persons have reported that their dermatitis due to nickel was much worse in the summer.⁵³ It has been suggested that in many instances sweating and/or pressure appear to play a role in the production of nickel dermatitis.⁵³ The chloride radical in sweat is apparently an important factor in dissolving the metallic nickel, permitting the soluble nickel salts to act.

In a double-blind study involving the oral administration of nickel sulfate in subjects with contact allergy to nickel and had hand eczema, an aggravation of the eczema was found in nine of the twelve patients.⁵⁴ In seven of the patients this was accompanied by secondary eruptions including outbreaks of earlier, healed eczema. It was concluded that the ingestion of small amounts of nickel may be of greater importance in maintaining the hand eczema than external contacts with the metal.

Nickel Induced Asthma

Another adverse reaction to nickel compounds which has been reported is asthma. The majority of the precipitating causes for these reported cases is occupational exposure to inhaled nickel compounds, specifically, nickel sulfate.^{55,56,57} In one case,⁵⁷ the subject was also found to have asthma induced by inhalation of chromium sulfate.

Nickel induced asthma has also been reported from

implanted nickel containing alloys.⁵⁸ In this case the initiating agent of the subject's asthma was stainless steel surgical clips which remained in the abdomen from a previous surgical episode. When these surgical clips were removed the subject became symptom free and no longer required medication for her asthmatic condition.

Patch Testing for Nickel Allergy

The most frequently used method to test for an allergy is the epicutan test or Patch test which is effective in the case of contact allergens. When the test is positive the skin reactions range from erythema to blisters. In the case of nickel the standard patch test consists of applying a small amount of a 5% nickel sulfate in a petrolatum base or a 5% nickel sulfate solution in the center portion of a square Band-Aid of good quality.⁵⁹ The patch is applied to the medial aspect of the upper arm, which has been precleaned with an alcohol swab. This is left in place and undisturbed for 48 hours. A Band-Aid without any reagent on it is placed next to the other to serve as a control. After 48 hours the Band-Aids are removed and the skin is cleansed with alcohol or acetone to remove any adhesive residue. The tests are read 20 minutes later and the reaction is recorded according to the following scale:

No reaction	(0)
Erythema	(+)
Erythema and papules	(++)
Erythema, papules, and vesicles	(+++)
Marked edema with vesicles	(++++)

Cases in which nickel-sensitive patients have not

responded positively to standard patch testing have been reported. It was observed in one study that two patients responded positively only when a patch test containing 10% nickel sulfate was employed.⁵³

In a study evaluating 11 nickel alloys it was found that for all but one alloy a linear relationship existed between the amount of nickel released during a corrosion test in synthetic sweat and the severity of nickel-sensitive patients' response to patch testing.⁶⁰ Alloys with a nickel release exceeding $1 \mu\text{g}/\text{cm}^2/\text{week}$ gave a strong patch test reaction and those below $0.5 \mu\text{g}/\text{cm}^2/\text{week}$ a weak reaction. One alloy (Inconel 600), however, showed a strong reactivity in spite of a low nickel corrosion level. In this study, stainless steel had a low rate of corrosion and only 3% of the nickel-sensitive persons gave a positive patch test to it.

The Council on Dental Therapeutics of the American Dental Association has issued an opinion that patch tests should be performed by a professional trained in the administration and interpretation of these tests.⁶¹ Referral to allergists or dermatologists who specialize in this testing is strongly recommended. It is the opinion of the Council that patch tests for nickel are not to be used indiscriminately for all patients since these tests may induce sensitivity in individuals who were not sensitive to nickel-containing alloys prior to patch testing. They also recommend that nickel-containing alloys not be used in patients who are known to be hypersensitive to nickel.

Systemic Effects of Chromium

Like nickel, the predominant systemic effects in humans from exposure to chromium compounds is allergy, including dermatitis and asthma, and an increased carcinogenic risk.

Chromium Allergy

As a metal responsible for producing hypersensitivity in humans, chromium is second only to nickel in its incidence of atopic reactions.^{39,62} In one report the incidence of chromium sensitivity was found to be 10% in males and only 3% in females³⁹ while in the other study it was reported to be 12% in the 1,312 patients examined.

Chromium differs from nickel in that it is not antigenic in metal form, only as salts which result from the corrosion of chromium based alloys and usually only as hexavalent salts.^{63,64} While the concentration of chromium is somewhat higher (17-20%) than nickel (10-17%) in stainless steel alloys, typically there is less chromium released.⁶³ Chromium allergy related to contact in the mouth is rarely reported⁶⁵ and it is unlikely that patients become sensitized through exposure to chromium-containing dental alloys or appliances.⁶³

Since chromium is usually alloyed with nickel and sometimes with cobalt which is another significant metal allergen, it can be difficult to determine which metal is responsible for causing allergic reactions. Suspected sensitivity to chromium is best verified by patch testing to individual metals to rule out reactions to nickel, cobalt, and others. Again, indiscriminate patch testing is

contraindicated due to the possibility for inducing hypersensitivity from the patch testing procedure itself.⁶⁵

Chromium Dermatitis

In sensitized individuals chromium alloys can produce dermatitis in several forms. Chromium dermatitis may be manifested as eczematous eruptions, dry dermatitis, lichenified tissue, or ulceration.²⁴ The dermatitis produced in individuals sensitive to chromium may continue for a number of years.

Chromium Induced Asthma

Similar to nickel, chromium compounds have been shown to be able to induce asthmatic episodes in sensitized individuals.^{57,64} In both of these cases the initial exposure to the chromium came from occupational exposures to fumes, in one case for ten years⁶⁴ and in the other for only one week.⁵⁷

Systemic Effects of Titanium

Very few systemic effects due to titanium have been reported in the literature. In the relatively short period in which titanium has been used as a biomedical material it has not been associated with adverse effects typical of either nickel or chromium. The literature contains no reports of titanium allergy, dermatitis or asthma. Medicinal compounds of titanium such as titanium dioxide or occupational exposure to titanium dioxide dusts have no reported adverse effects.^{4,66} Additionally, titanium containing alloys used as surgical implants have not been associated with cancer or other adverse effects following

long term contact with tissues.⁴ Titanium compounds appear to be biologically inert.

Nickel Toxicity

Two types of toxicity are possible when dealing with exposure to metals in humans. Acute toxicity or poisoning occurs when massive quantities of the metal or metal compounds are ingested, inhaled or in some manner absorbed by an individual. For this to occur a major industrial accident or poisoning would be required and only individuals with occupations involving these compounds would be at risk for such an exposure. The second form of metal toxicity is chronic toxicity which is the potential for causing a toxic effect by long term exposure to a metal or metallic compound. There does exist the potential for chronic nickel toxicity to occur in the general population though reported cases are infrequent. Immunoglobulin A (IgA) nephropathy, a form of glomerulonephritis characterized by the glomerular deposition of immune complexes thought to contain IgA, has been reported as a consequence after placement of nickel alloy base dental crowns.⁶⁷ Symptoms developed several years after the first crown was placed and shortly after the second crown was inserted. The nephropathy worsened with the placement of the third crown. Once the three crowns were removed the condition dramatically resolved, with kidney function normalizing after 14 months.

Nickel Carcinogenicity

A carcinogen may be defined as an agent whose

administration leads to a statistically significant increase of malignant neoplasms as compared with that in appropriate untreated control animals.⁶⁸ This differs from mutagenicity which is the ability of an agent to produce a statistically significant increase in the incidence of qualitative or quantitative change in the genetic material.⁶⁸ There does, however, appear to be a strong correlation between mutagenicity and carcinogenicity.

The literature contains numerous references to the carcinogenicity of nickel and nickel compounds to both animals and humans.^{8,11,13,15,50,69,70,71,72,73,74,75,76,77} One ranking placed nickel and chromium as the most carcinogenic of the metallic elements.⁷⁰ Most of the reported incidences of nickel carcinogenicity are from occupational exposures to inhaled nickel compounds. The tumor sites have primarily been the lung and the nasal mucosa, and less frequently, the larynx and stomach.^{8,11,13,72,73} Certain nickel compounds have been labelled as extremely potent carcinogens after inhalation, but the cancer risk is felt to be limited to conditions of occupational exposure.¹¹

The carcinogenic risk of a nickel compound does not depend on the mode of administration but is inversely related to its solubility in aqueous media: dust of metallic nickel, nickel subsulfide (Ni_3S_2), nickel oxide (NiO), nickel carbonyl ($\text{Ni}[\text{CO}]_4$) and nickelocene (dicyclopentadienylnickel), all of which are almost insoluble in water at 37°C , are carcinogenic, whereas, soluble nickel salts such as nickel chloride (NiCl_2), nickel sulfate (NiSO_4) or nickel ammonium sulfate

(Ni[NH₄]₂[SO₄]₂) seem to be without carcinogenic properties.¹¹ It has been established that nickel sulfide (NiS), nickel oxide, and metallic nickel dust are the most carcinogenic agents of all of the nickel compounds encountered.^{13,15} The active agent in carcinogenesis is believed to be the metal ion.⁷⁵

The latency period from the time of exposure to nickel compounds to the development of cancer has been reported to vary from 12 to 25 years⁷⁶. Others reported averages of 22 years⁷⁰; 24 years with a range of 10 to 40 years¹¹; and 25 years with a range of 5 to 40 years⁷⁷.

In two studies involving stainless steel manufacturing, no increases in either lung cancer nor sinus cancer were found in the individuals employed in these plants.¹ In one animal study, however, stainless steel foils were found to induce sarcoma formation when imbedded subcutaneously.⁷⁸

Studies involving the oral administration of nickel compounds have failed to show any increase in cancer incidence.^{15,50,71} There is no experimental evidence that nickel compounds are carcinogenic when administered by oral or cutaneous routes.^{15,50}

In animal cells, soluble and insoluble nickel compounds have been shown to cause a number of genetic effects, including chromosome aberrations, gene mutations, and morphological transformations.^{68,69,70,72,73} Nickel compounds affect the genetic material by inducing mutation through direct chemical reaction with the DNA or through interference with DNA replication, DNA repair, and chromosome folding and distribution.⁶⁸ Compared with many

mutagenic organic compounds, the efficacy of the nickel compounds is low.⁶⁸

Chromium Toxicity

Chromium toxicity has only been reported from occupational exposures with absorption taking place through the respiratory tract.⁹ The kidney is the primary organ affected by high levels of chromium exposure though gastric and enteric lesions have also been reported.⁹ Acute tubular necrosis and renal failure have been described following heavy exposure to hexavalent chromium.⁹ When chromate workers were studied some evidence of kidney dysfunction was found.⁹ The damage was found to be reversible and the workers became resistant to the effects of more severe or prolonged exposures.

The method of action in the toxicity of hexavalent chromium is a function of its strong oxidizing power which, once inside the cell, becomes rapidly reduced to trivalent chromium. It is this reduced form of chromium which forms stable coordination complexes with several different ligands thereby interfering with their physiological functions.⁷⁹ Toxic effects due to chromium include precipitating proteins and interfering with essential enzyme systems.⁸

Chromium Carcinogenicity

Similar to the nickel compounds, it has been established that several chromium compounds also are known carcinogens. Numerous articles have been published documenting the carcinogenicity of various chromium

compounds in either animals or humans.^{8,21,65,73,74,75,77,79,80,81,82}

Only occupational exposures to chromium compounds have a documented increase in carcinogenic risk. Other forms of exposure may also contain some risk, however, no investigations have yet revealed an increase in the cancer rate from any non-industrial sources of chromium. Carcinogenesis related to dental and medical applications has not been reported.⁶⁵

The primary target organ for chromium induced carcinoma is the lung.^{65,73,80,81,82} Other reported tumor locations include the nasal cavities, larynx and the gastrointestinal tract.⁸¹ Nearly all of the human exposure from industrial sources has been from the inhalation of chromium containing vapors. For this reason, and also due to the fact that many chromium compounds are poorly absorbed by the body, the respiratory tract is the site of the majority of occupational chromium exposures.

Primarily it has been the hexavalent chromium compounds which have been found to be carcinogenic.^{74,75,77,81,82} Trivalent chromium compounds have not been found to possess a significant carcinogenic risk owing largely to their inability to be absorbed across cell membranes. A mechanism for chromium carcinogenesis has been hypothesized⁷⁴: Hexavalent chromium enters cells through the sulphate transport system and is reduced to trivalent chromium by cell organelles within the cell. The trivalent form is the one that interacts with genetic material or genetic processes and causes mutation which can later develop into cancer.

Important to the understanding of chromium carcinogenesis is the dose-response relationship for this metal. A linear relationship predicts some, however slight, carcinogenic risk with exposure to even the smallest amount of the carcinogen. The risk increases in a linear fashion with increases in the level of exposure. A threshold response relationship differs in that a certain threshold must be reached before a risk of carcinogenesis occurs. There is sound scientific evidence that chromium carcinogenesis is regulated by threshold phenomena.²¹ In order to minimize the carcinogenic risk with a threshold response relationship it is important that only exposure levels above the threshold be prevented and not all exposure to the agent. This is important considering that chromium is an essential element in man and therefore must be present in low levels for human absorption.

The average latency period for chromium carcinogenesis has been reported to be approximately 20 years with a range of between 10 and 47 years.^{77,81}

Chromium has also been reported to be mutagenic in animals and humans.^{2,3,73,79,83} Again, only the hexavalent form of chromium has been shown to possess mutagenic potential.⁸³ After it crosses the cell membrane it is reduced to trivalent chromium which is the main final genotoxic agent. The primary effect of intracellular trivalent chromium is inhibition of DNA synthesis; RNA and protein synthesis are secondarily impeded.²

Titanium Toxicity

Adverse effects from titanium containing compounds

have not been reported.⁴ Titanium compounds appear to be biologically inert in humans.

Titanium Carcinogenicity

In a few animal studies, certain titanium compounds were found to induce local sarcomas at the site of injection.^{75,84} Other studies have shown no increase in tumor frequency or other adverse effects compared with control animals.^{4,73} At present, the data are insufficient for an evaluation of the carcinogenic activity of titanium. There is no evidence to suggest that titanium compounds have acted as human carcinogens.⁴

Mutagenic activity has only minimally been investigated with one report showing no effects⁷³ and another only minimal effects on chromosomes.⁴ Cell transformation has not been observed.

Determination of Nickel, Chromium and Titanium Concentrations in Blood

Methods used to determine blood levels of nickel, chromium, or titanium include atomic absorption spectrophotometry, emission spectrometry, spectrophotometry, and neutron activation analysis. The most commonly used technique of analysis for all three of these metals is atomic absorption spectrophotometry.^{7,20,22,85,86,87,88,89,90,91,92,93,94,95,96}

Several studies have determined typical levels of nickel in whole blood for normal individuals. Reported mean values are: 2.4 ± 0.5 ng/ml,⁸⁵ 4.8 ± 1.3 ng/ml,⁸⁷ 6.0 ± 1.0 ng/ml,⁸⁶ and 30 ± 19 ng/ml²⁰. The mean concentration

of blood nickel in men has not been found to differ significantly from that in women.⁸⁷

Normal whole blood levels for chromium have been reported as: 0.371 ng/ml,⁹⁴ and 1.4 ± 0 ng/ml²².

Titanium levels in whole blood for humans has not yet been reported. One study has reported on the titanium concentration in serum, urine and lungs for baboons when titanium fiber bone implants were inserted.⁹⁶ They reported increases in titanium concentrations for urine and lung tissue following implant placement but no significant increases in serum concentrations. It was not known why titanium levels increased in urine and lung tissue but not in blood serum.

In another experiment in which titanium implants were placed in sheep femurs, increases in titanium concentrations in both serum and urine appeared as soon as one week after implantation.⁹⁷

Corrosion of Biomedical Materials

In order for individuals to absorb trace metals from medical or dental materials corrosion of the appliances must first occur. Corrosion may be defined as the breakdown of a metal or its alloy due to electrochemical interaction with its environment.⁹⁸

In analyzing the corrosion of these materials several variables need to be considered. First, the composition of the alloy affects the degree to which it corrodes. Secondly, the chemical and thermal environment in which the material is located will determine to what degree corrosion occurs. Thirdly, the amount of surface area and the degree

of surface smoothness of the material will also influence how much corrosion takes place.

Most biomedical materials are alloys of several metals. Certain metals corrode significantly more than others and some impart a certain degree of resistance to corrosion when alloyed with other metals. In stainless steel alloys, iron is the metal with the greatest corrosion potential. The presence of carbon in stainless steel increases its corrosion whereas the presence of chromium, titanium or molybdenum in an alloy decreases corrosion through a process known as passivation. Passivation is the process of forming a protective oxide coat on the surface of the metal. This oxide coating effectively terminates any chemical reaction between the metal and its environment and thus protects the material from corrosion.

The chemical and thermal environment of an alloy plays an important role in the corrosion process. In biological systems the presence and activity of the chloride ion has been viewed as the major factor in determining corrosion potentials. Nickel release from dental alloys has been shown to be proportionate to the acidity of the oral environment.¹⁶ Recently, it has been shown that the presence of proteins can also influence both the rates and mechanisms of corrosion of surgical-grade stainless steel.⁹⁹ Both the loss of metal and the concentration of dissolved nickel were significantly greater when the saline solution contained serum proteins. It was concluded that serum proteins interfere with the initial passivation process of stainless steel. The oral fluids are known to contain both chloride ions and proteins.

Increases in temperature, in the range found in biological systems increases corrosion rates.¹⁰⁰ The oral environment undergoes shifts in temperature due to the temperatures of foods and beverages consumed by the individual. This thermal cycling will tend to increase corrosion rates for intraoral metal appliances.

Increasing the surface area of a metal object will lead to an increase in corrosion due to a greater exposure of metal atoms to biological fluids. Decreasing the smoothness of a metal or alloy will predispose it to uneven distribution of the passivating layer and encourage biocorrosion. Bending or scratching will also partially destroy the passivated oxide coat increasing the susceptibility to corrosion.⁹⁸ Once the oxide coat is damaged it is not easily reformed.

Corrosion of Implants

It has been fairly well accepted that all metallic implants corrode.^{8,101,102} Stainless steel and cobalt-chrome hip replacements have been found to corrode at a rate of between 0.15 and 0.3 $\mu\text{g}/\text{cm}^2/\text{day}$.¹⁰¹ When stainless steel implants were implanted in the back muscle of rabbits chromium and nickel concentrations increased markedly in the surrounding muscle of all animals.¹⁰³ In some of the animals the nickel content also rose in the lung, spleen and kidneys. When titanium implants were used the titanium content of the surrounding muscle rose significantly in all the animals and one of the four rabbits showed a marked tendency to retain titanium in the spleen and lung. Transport of the metals to the other organs most probably

occurred via the bloodstream.

Corrosion of Dental Alloys

It is well established that base metal alloys corrode in the oral environment thus liberating metals into the saliva which is subsequently ingested.^{8,29,63,70,100,104} Nickel leaches out of nickel-chromium alloys of the type used for crowns in significant amounts up to 6.2 mg/cm²/year when measured in an artificial saliva solution.¹⁰⁰ Stainless steel alloys corrode similarly in the mouth and give off nickel, but not in as great a quantity. In a study comparing corrosion rates of seven different non-precious dental alloys, stainless steel was found to have a rate which was only 0.34% of the corrosion rate for the three nickel-chromium alloys.¹⁰⁰

Corrosion of Orthodontic Appliances

The primary alloys of importance in orthodontic appliances are stainless steel, used for bands, brackets, archwires and retainer wires (70% iron, 18-20% chromium, 8-10% nickel, 1-4% other elements); cobalt-chromium alloys used for archwires and retainer wires (40% cobalt, 20% chromium, 15% nickel, 15% iron, 10% other elements); beta-titanium alloys used for archwires (78% titanium, 11.5% molybdenum, 6% zirconium, 4.5% tin); and nickel-titanium alloys also used for archwires (52-55% nickel, 44-45% titanium, 1-3% other elements).

In a 1983 study, the equivalent of a half arch orthodontic appliance made of stainless steel bands, brackets, and archwires were placed in a 0.05 percent

sodium chloride solution at 37° C. It was observed that approximately three times more nickel was solubilized than chromium.¹⁷ It was found that nickel was released as a soluble compound while chromium was released primarily in an insoluble form. After 12 days in the sodium chloride solution, the appliance released an average of 121 µg of soluble and 4 µg of insoluble nickel along with 40 µg of soluble and 72 µg of insoluble chromium.

In a 1987 study, stainless steel and chromium-cobalt archwires were placed in artificial saliva at 37° C. for one week. An average of 0.036 and 0.18 µg/cm² of nickel was released, respectively, from these two archwire alloys.¹⁸

Another study in 1986 found that commercial recycling of stainless steel brackets increased the corrosion rate in an artificial saliva system when compared to new brackets.¹⁰⁵

In an evaluation of the corrosion in an in vitro system for the four types of archwire alloys (stainless steel, chromium-cobalt, beta-titanium, and nickel-titanium) it was found that the nickel-titanium alloy displayed appreciable corrosion damage to its surface whereas the other three alloys exhibited very little damage.¹⁰⁶ They concluded that the passive film on the nickel-titanium alloy was ineffective in preventing the corrosion of the alloy surface and was not as stable as that on the other alloys.

In another study comparing nickel-titanium archwires to their stainless steel counterparts, no evidence of corrosion pits on either type of archwire was seen under a

scanning electron microscope.¹⁰⁷ This study made use of archwires which were retrieved after clinical service for periods ranging from one to eight months.

It has also been shown that heat-treating cobalt-chromium and stainless steel archwires can increase the release of metal from these wires by 15 to 60 times the amount of as-received wires.¹⁸ Silver soldering of the same two types of orthodontic archwires also decreases the corrosion resistance of these alloys. Furthermore, stainless steel wires liberated more nickel and chromium compared to the cobalt-chromium wires when soldered.¹⁹

At this time there have been no reports on the effects of orthodontic appliances on the blood levels of nickel, chromium and titanium. Since it is known that these appliances do corrode in an oral environment and that adverse health effects have been associated with some of these metals and metal compounds it is important to determine whether orthodontic patients accumulate measurable concentrations of these three metals during the course of their orthodontic therapy.

SUMMARY OF THE LITERATURE REVIEW

Biological Importance of Nickel, Chromium and Titanium

The use of nickel, chromium and titanium compounds for the construction of biomedical appliances is quite common. Chromium is known to be an essential element for humans and animals^{7,8} while nickel is essential for some animals⁵, a similar role in humans has not been proven.⁶ No essential biological importance for titanium has been found.⁴

Human Exposure Sources

Human exposure to nickel, chromium and titanium occurs via the diet, atmosphere, drinking water, clothing fasteners, jewelry, and iatrogenic uses of articles containing these metals.

Diet

The major dietary sources for these three metals are vegetables, grains and cereals.^{3,5,20} Significant concentrations of nickel have also been found in seafood, chocolate, nuts and certain spices.⁵ The average dietary intake for these three metals has been estimated to be: nickel = 200-300 $\mu\text{g}/\text{day}$ ^{5,6}, chromium = 280 $\mu\text{g}/\text{day}$ ³, titanium = 300-2000 $\mu\text{g}/\text{day}$ ⁴.

Mechanisms for limiting the intestinal absorption of nickel^{5,10}, chromium^{3,21}, and titanium^{4,23} have been identified. The oxidation state of chromium uniquely

affects its ability to be absorbed by man. Only the hexavalent form (Cr VI+) is readily absorbed.²¹ The primary route for elimination of absorbed nickel and chromium from the body is through the urine.

Atmosphere

Atmospheric exposure to nickel comes primarily from fuel oil consumption, industrial air emissions, waste incineration and from wind-blown dusts and volcanos.^{5,10} Average levels in urban areas are reported to be about 20 ng/m³ and 10 ng/m³ for rural areas.⁵ Cigarette smoke also contains nickel compounds and may contribute much more nickel than living in an urban environment.⁵

Atmospheric chromium sources are the burning of coal, chromium manufacturing facilities, cement producing plants and automobile emissions.³ Chromium compounds are also present in cigarette smoke.³ Urban locations typically have been reported to have atmospheric levels for chromium of between 10 and 30 ng/m³ while non-urban areas have levels less than 10 ng/m³.³

Titanium concentrations in the air are primarily from atmospheric dust.²⁰ Average environmental levels for titanium have not been reported.

Drinking Water

Nickel concentrations in drinking water generally measure below 20 µg/liter.¹³ Average chromium levels in drinking water have been reported as 0.43 µg/liter.³ Levels for titanium are reported to range from 0.5 to 15 µg/liter.⁴

Clothing Fasteners and Jewelry

Clothing fasteners (metal buttons, zippers, etc.) and jewelry articles are common sources of nickel exposure. Sweat plays an important role in leaching the nickel from such metal objects.¹⁴

Iatrogenic Exposures

Iatrogenic exposures to nickel, chromium and titanium can occur from joint prostheses, dental implants and orthodontic archwires.^{5,8,18,19,23,25,41,66,77,90,93,95-98,101,106,107} Exposure to nickel and chromium may occur through orthopedic plates and screws, surgical clips and steel sutures, pacemaker leads, prosthetic heart valves, dental alloys and orthodontic appliances.^{5,8,15-17,24,32-40,42-44,48,63,65,67,70,76,100,104,105} Nickel exposure may also occur from intravenous fluids.⁵

Nickel release from dental alloys have been reported as $4.2 \mu\text{g}/\text{cm}^2/\text{day}$.¹⁵ For full mouth orthodontic appliances a release rate of $40 \mu\text{g}/\text{day}$ of nickel and $36 \mu\text{g}/\text{day}$ of chromium has been reported.¹⁷ For heat-treated stainless steel orthodontic archwires the release rate for nickel was found to be $0.26 \mu\text{g}/\text{cm}^2/\text{day}$.¹⁸

Systemic Effects of Nickel, Chromium and Titanium

The predominant systemic effects in humans from exposure to nickel or chromium compounds is allergy, including dermatitis and asthma. Both toxic effects and an increased cancer risk have been reported from these metals, however, these are primarily found only from occupational

exposures. The literature contains no reports of titanium allergy, dermatitis or asthma. Additionally, titanium containing alloys used as surgical implants have not been associated with cancer or other adverse effects following long term contact with tissues.⁴

Allergy

Allergy is the most common adverse effect produced by both nickel and chromium.^{5,13,15,24-45,48,52-54,62-65} The literature supports an incidence for nickel allergy of 10% for females and 1% for males^{25,27,29-31} and an incidence for chromium allergy of 10% for males and 3% for females³⁹.

Most causes for nickel and chromium allergies have been attributed to dermatological exposures to these metals or to compounds containing these metals. Pierced earrings and metal buttons in blue jeans have been found to be responsible for a significant amount of the cases of nickel hypersensitivity in women.^{27,30,31} In males, the most common sources of sensitization to nickel are occupational exposures, wristwatches and the metal buckles found on many watchbands.²⁸

Several reports have documented that nickel containing dental prostheses can be responsible for episodes of expressed nickel allergy.³²⁻³⁶ Orthodontic appliances have also been found to produce hypersensitivity reactions.³⁹⁻⁴¹ At present, there is no direct evidence that the intraoral use of nickel-containing alloys will induce a hypersensitive state in a previously non-sensitized individual.¹⁵

Symptoms of allergic reactions to dental alloys have included severely inflamed hyperplastic gingival tissue surrounding crowns fabricated from a nickel containing alloy³⁵, alveolar bone loss from a similar crown³⁶ and edema of the throat, palate and gums³⁷. In addition, osteomyelitis was reported when stainless steel bone fixation wires were used in the jaws of nickel sensitive patients.³⁸

Nickel allergy reactions to orthodontic appliances have been reported following the use of headgear facebows and neck straps^{39,40,48} and also following the insertion of nickel-titanium orthodontic archwires⁴¹.

Dermatitis

Reports of dermatitis or eczema have been reported from exposure to both nickel and chromium compounds but not to titanium.^{13,24,25,30,34,50-54,63,65} In many sensitive individuals dermatitis is the only symptom of their allergic condition. Nickel dermatitis has been reported to occur in 62% of sensitive patients.⁵⁰ Dermatitis does not necessarily appear at all sites of contact with the metal. Oral exposures to metal allergens may prolong or worsen dermatitis reactions at other skin locations.

Asthma

The literature contains reports of asthmatic reactions to both nickel and chromium compounds.^{55-58,64} Most cases of metal induced asthma have been from occupational exposure to inhaled nickel or chromium compounds.

Patch Testing for Nickel and Chromium Allergy

The most frequently used method to identify an allergic individual is the epicutan test or Patch test. Allergies to both nickel and chromium may be tested for in this manner. False negative reactions have been reported in testing for nickel allergies in known sensitive individuals.⁵³ Such testing should not be used indiscriminately since these tests may induce sensitivity in individuals who prior to testing were not sensitive.

Nickel and Chromium Toxicity

Acute toxic reactions have only been reported following occupational exposures to high concentrations of nickel or chromium dusts or fumes. Chronic or long-term toxicity has been reported in at least one case from dental crowns fabricated from a nickel containing alloy.⁶⁷

Nickel and Chromium Carcinogenicity

The fact that nickel, chromium and their compounds present a known cancer risk in certain forms has been well documented in the literature.^{8,11,13,15,21,50,65,69-77,79-82} One ranking placed nickel and chromium as the most carcinogenic of the metallic elements.⁷⁰ Nearly all of the reported cases of nickel and chromium induced carcinoma have occurred from occupational exposures to inhaled metal compounds. The primary tumor locations are the lung and nasal mucosa.^{8,11,13,65,72,73,80-82} Not all nickel and chromium compounds have carcinogenic potential. For nickel compounds, risk is inversely related to its solubility in

an aqueous media.¹¹ For chromium compounds, carcinogenic risk has only been identified with compounds in which the chromium is in a hexavalent oxidation state.^{74,75,77,81,82} There is no experimental evidence that nickel or chromium compounds are carcinogenic when administered by oral or cutaneous routes.^{15,50,65}

The average latency period from the time of exposure to these metal compounds to the development of cancer has been reported to be between 20 and 25 years.^{11,70,76,77,81}

At present, the data are insufficient for an evaluation of the carcinogenic activity of titanium. There is no evidence to suggest that titanium compounds have acted as human carcinogens.⁴

Determination of Nickel, Chromium and Titanium Concentrations in Blood

Normal whole blood concentrations for nickel have been reported to be between 2.4 ± 0.5 ng/ml and 30 ± 19 ng/ml.^{20,85-87} For chromium the average values have been reported as 0.371 ng/ml⁹⁴ and 1.4 ± 0 ng/ml²². Titanium levels in whole blood for humans has not yet been reported though increases in concentrations have been found in animals after titanium implant placement.⁹⁷

Corrosion of Biomedical Materials

In respect to dental and medical appliances, corrosion must first occur in order for individuals to absorb these trace metal compounds.

Several factors become involved when determining the corrosion rate of dental and medical appliances such as the composition of the material, the chemical and thermal

environment of the material, the surface area and the degree of surface smoothness.

Corrosion of Orthodontic Appliances

In vitro studies on the corrosion of stainless steel orthodontic bands, brackets, and archwires have documented that both nickel and chromium are liberated as corrosion products in an artificial saliva medium.¹⁷ Commercial recycling of orthodontic brackets, heat-treating or silver soldering of archwires all increased the corrosion rate of these appliances.^{18,19,105}

Because it has been shown that the oral environment is corrosive to orthodontic appliances and because adverse health consequences can be produced by some of the corrosion products of these appliances it is important to determine whether the use of orthodontic appliances significantly affects the level of nickel, chromium and titanium in the blood of individuals treated with these appliances.

SPECIFIC OBJECTIVES

The first objective of this study is to compare in vitro the corrosion rate of a standard orthodontic appliance consisting of bands, brackets and stainless steel or nickel-titanium archwires. The corrosion products to be analyzed will include nickel and chromium. Evaluation will be conducted while immersed for 4 weeks in a prepared artificial saliva medium at physiologic temperature (37°C). Previous studies have not investigated the effects on corrosion of nickel-titanium alloys when used in conjunction with stainless steel appliances and many studies have not used either an artificial saliva solution or a solution containing any protein component. This study will seek to determine if any increase in corrosion may occur from possible galvanic action when nickel-titanium archwires are used with stainless steel orthodontic appliances. An artificial saliva solution will be employed which will include a protein component to most closely simulate the corrosive environment of the oral cavity.

The second objective of this study is to determine whether orthodontic patients accumulate measurable concentrations of these two metals in their blood during their initial course of orthodontic therapy. Blood samples will be collected at three different time periods: Prior to the placement of any orthodontic appliances; two months after the placement of the appliances while nickel-titanium

archwires are still being utilized; four to five months after the placement of the appliances when stainless steel archwires are being utilized. An analysis of blood samples in individuals undergoing orthodontic therapy has not previously been reported.

METHODOLOGY

In Vitro Study

Ten sets of bands and brackets simulating an average orthodontic appliance used for the maxillary arch with a full complement of teeth were utilized. Each maxillary set comprised the following: two second molar bands with buccal tubes and welded lingual buttons, two first molar and second premolar bands with buccal twin brackets and welded lingual buttons, two each of first premolar, canine, lateral and central incisor brackets used for direct bonding to enamel. Average size second premolar, first and second molar bands were selected. All ten sets of appliances were identical in the size and type of bands and bonds utilized. The material from which the bands were constructed was American Iron and Steel Institute (A.I.S.I.) type 305 stainless steel with type 316 for the brackets and tubes.* Bondable brackets were made of type 303 and 304 stainless steel.† Chemical composition of these alloys and several other A.I.S.I. stainless steels can be found in Table 1. All bands and brackets were used in the as-received condition.

*Ormco Corporation (personal communication).
Glendora, California. 1989.

†GAC International Inc. (personal communication).
Central Islip, New York. 1989.

Table 1. Composition of Selected Orthodontic Appliances as Specified by American Iron and Steel Institute (A.I.S.I.) Type of Stainless Steel*

AISI Type	Orthodontic Appliance	Cr %	Ni %	C %	Mn %	Si %	P %	S %	Mo %	Cu %	N %	Cb, Ta %	Fe %
303	Unitek brackets ¹⁰⁸ GAC Brackets ¹⁰⁹	17-19	8-10	0.15	2.0	1.0	0.20	0.15	0.60	-	-	-	66.9-70.9
304	Unitek mesh bases ¹⁰⁸ GAC mesh bases ¹⁰⁹ Ormco ligature wire ¹¹⁰	18-20	8-10	0.08	2.0	1.0	0.045	0.03	-	-	0.10	-	66.35-70.85
305	Unitek bands ¹⁰⁸ Ormco bands ¹¹⁰ "A" Co bands ¹¹¹	17-19	10.5-11	0.12	2.0	1.0	0.045	0.03	-	-	-	-	Balance
310	"A" Co regular brackets ¹¹¹	24-26	19-22	0.25	2.0	1.5	0.045	0.03	-	-	-	-	Balance
316	Ormco cast brackets ¹¹⁰	16-18	10-4	0.08	2.0	1.0	0.045	0.03	2-3	-	0.10	-	Balance
316L	American regular brackets ¹¹² Ormco mesh bases ¹¹⁰	16-18	10-14	0.03	2.0	0.75	0.045	0.03	2-3	-	0.10	-	Balance
17-4	American mini brackets ¹¹² "A" Co mini brackets ¹¹¹	15.5-17.5	3-5	0.07	1.0	1.0	0.040	0.30	-	3-5	-	0.15-0.45	Balance

Cr = Chromium Ni = Nickel C = Carbon Mn = Manganese Si = Silicon
P = Phosphorus S = Sulfur Mo = Molybdenum Cu = Copper N = Nitrogen
Cb = Columbium Ta = Tantalum Fe = Iron

* The American Society for Metals: 1985 Materials and Processing Databook. Metall Progress 128:42-49, 1985.

No attempt was made to cover or exclude the inner surface of the bands or the bonding bases of the bonds from possible corrosion as was previously suggested by Park and Shearer¹⁷. The present approach was considered to eliminate any other potential sources of nickel, chromium or titanium which would be introduced into the experimental situation. In a clinical situation the inner surface of the bands would be coated with a cementing medium and the bond bases would be covered with a composite bonding material. Therefore, it could be assumed that the amount of metal available in the solution is approximately twice as much as would be released if the inner surfaces of the bands and brackets were covered. This could also be considered approximately equal to the exposed surface area of two entire arches of appliances after cementation and bonding to the teeth.

Five sets of the whole arch appliances were ligated to rectangular stainless steel archwires and the other five sets were ligated to rectangular nickel-titanium (Nitinol) archwires. Both types of archwires were 12.5 cm in length and had a cross-sectional dimension of 0.017 by 0.025 inches. The length for the archwires was determined from an ideal typodont set-up with appliances in place from the distal of the right second molar tube to the distal of the left second molar tube.

The stainless steel archwire was idealized with cuspid offset and molar bayonet bends and then heat-treated in an electrical furnace at a temperature of 425° C for 5 minutes. This temperature setting is the mid-point of the heat treatment range of 370° to 480° C as recommended by

Phillips.¹¹³ The five minute time period for heat treatment was selected to ensure that the archwires were fully brought up to the heat treatment temperature. This time period for heat treatment also fell into the time range reported by Phillips.¹¹³

The nickel-titanium archwires were used in an as-received condition after being cut to the specified length. The incisor, canine and first premolar brackets and the second premolar and first molar bands were ligated to the archwires with standard 0.01 inch stainless steel ligature wire (A.I.S.I. type 304*).

A small bend was placed at each end of all archwires to keep the second molar bands which had buccal tubes from sliding off of the wire. After construction of the simulated full arch orthodontic appliances they were cleaned in acetone and dried.

Experimental Conditions

Nickel and chromium release was tested by placing each of the ten appliances in separate polyethylene screw-top bottles containing 100 ml. of artificial saliva. The simulated saliva medium consisted of 0.4g NaCl, 1.21g KCl, 0.78g $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.005g $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, 1g Urea [$\text{CO}(\text{NH}_2)_2$] and 1000 ml distilled and deionized water. The artificial saliva formula was a modification of that used by Gjerdet and Hero,¹⁸ the difference being that their formula included 0.795g of $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ and only 0.4g of KCl. When this formula was employed, interference occurred with the

*Ormco Corporation (personal communication).
Glendora, California. 1989.

atomization of chromium in the atomic absorption spectrophotometer. This interference completely prevented the measurement of chromium in the original artificial saliva formula. The interference was later found to be caused by the presence of the calcium ions. In order to replace the chloride ion when the calcium chloride was deleted, an equimolar amount (0.81g) of potassium chloride was added to the 0.4g of KCl already in the formula. Potassium chloride was chosen over sodium chloride since it more closely matches the corrosive properties of calcium chloride than does sodium chloride. After thoroughly mixing of the saliva medium its pH was adjusted to 6.75 ± 0.15 with 10 N NaOH. The pH value coincides with that reported for human saliva.¹¹⁴

At first, protein was added to this formula due to the work of Brown and Merritt⁹⁹ which showed an increase in corrosion when a saline solution contained serum proteins. Albumin was selected as the protein component due to its presence in saliva¹¹⁴ and its ready availability. The albumin, which was derived from eggs, was found to be unsatisfactory due to the high endogenous concentration of nickel. No protein component was found suitable for inclusion into the artificial saliva formula.

The sample bottles were placed in an Environmental Incubator Shaker Model G-24* (see Figure 1) and agitated slowly at 37° C for 4 weeks. On days 1, 7, 14, 21 and 28 the entire 100 ml. of artificial saliva solution was

*New Brunswick Scientific Co. New Brunswick, New Jersey.

removed and replaced with a fresh solution except on day 28 when the experiment was ended so the solution was not replaced. This was done in order to avoid saturating the artificial saliva medium with corrosion products.

Nickel and chromium analyses were performed on the samples removed on each of these days for each of the ten experimental groups. This resulted in a total of 50 samples, 25 for the stainless steel archwires and 25 for the nickel-titanium archwires.

Titanium release from the nickel-titanium archwires was not able to be measured since no procedure for its analysis has been developed for the equipment which was available for this project.

Four control solutions were utilized and consisted of a 100 ml. artificial saliva sample placed in the same type polyethylene bottle but without orthodontic appliances. The control samples were kept in the environmental incubator shaker along with the experimental samples. Two of the control samples were analyzed after one week while the other two control samples were analyzed at the end of the four week experimental period.

The analysis of the artificial saliva samples was performed according to the Applications Manual for the Scintrex AAZ-2 Zeeman Modulated Absorption Spectrophotometer.¹¹⁵ Two specimens from each sample were analyzed for each metal and the mean value was taken as representative of the true concentration for that sample. If the two readings for any sample differed by more than 10 percent from the mean value then additional specimens were analyzed until three values were recorded which fell within

a ten percent variation from the mean value.

Measurement Technique

The In-vitro analyses were performed by flameless atomic absorption spectrophotometry* (see Figures 2 and 3). Atomic absorption is a technique based on the unique spectrum of each element. For every element analyzed, characteristic wavelengths are generated in a discharge lamp (hollow cathode lamp) and in turn are absorbed by a cloud or vapor of that element. The amount of absorption is proportional to the concentration of the element which is vaporized into the light beam.¹¹⁶

The samples to be analyzed were injected into the atomic absorption spectrophotometer with a 5-40 μL micropipette[§] (see Figures 4 and 5). A 10 μL sample size was utilized as specified by the procedures manual.

Commercially available nickel and chromium standard stock solutions were used to prepare working standards of 5, 10, 20 and 40 ng/ml with distilled and deionized water. Calibration plots were generated at the start of every run utilizing freshly prepared working standards. All glassware was first cleaned with a 1:1:1 solution of sulfuric acid, nitric acid and water and then stored containing 0.6 Normal nitric acid. All water used in this study was deionized by a 5 stage Milli-Q plus water

*Scintrex Model AAZ-2 Zeeman Modulated Atomic Absorption Spectrophotometer. Scintrex Limited. Concord, Ontario, Canada.

§Finnpipette Digital 5-40 μL Micropipette. Labsystems. Helsinki, Finland.

purification system.* This was conducted to remove any potential metal contamination from the glassware. Prior to use, all glassware was rinsed at least three times with deionized water, inverted and allowed to dry.

In Vivo Study

This portion of the study involved 31 subjects who were about to start comprehensive orthodontic therapy at The University of Iowa College of Dentistry. Selection criteria included the following: subjects were at least 11 years of age, their treatment plan involved orthodontic therapy that required the use of complete banded and bonded edgewise stainless steel appliances (patients being treated with any ceramic brackets were excluded). All subjects volunteered for this project. Of approximately 55 individuals asked to participate, 31 accepted.

The age of the subjects who participated in the study ranged between 12 and 38 years. An explanation of the research project was given in verbal and written form, a consent form was completed, demographic information was obtained, and an allergy questionnaire was completed to identify any existing allergies in general and any possible metal allergies in particular. This was accomplished for all participants of the study. The patient's parent completed the forms when the patient was under 18 years of age and patient's over 18 years completed their own forms. A sample of the allergy questionnaire is included in Appendix A.

*Millipore Corporation. Bedford, Massachusetts.

A baseline blood sample was taken through standard venipuncture techniques in acid washed trace element study Vacutainer blood collection tubes containing 143 USP units of Sodium Heparin as an anticoagulant.* The baseline samples were obtained prior to the fitting or cementation of any bands or bonds. Another blood sample was taken in the same manner after orthodontic appliances had been in place for approximately two months. During this time period nickel-titanium archwires were primarily used. A third blood sample was collected after appliances had been in place between 4 and 5 months. At this point, stainless steel archwires were predominantly being utilized.

The three blood samples were frozen and when all three samples had been collected they were shipped to a commercial medical laboratory for analysis by atomic absorption spectrophotometry.† The three blood samples for each patient were analyzed in succession on the same day to eliminate equipment variance which may have occurred if blood samples were analyzed on separate days.

Titanium levels in blood were not a part of this study for two reasons: First, the analysis of blood samples for trace levels of titanium is extremely expensive, and secondly, no known adverse health effects are attributed to increased levels of titanium exposure in humans. Therefore, we chose to limit our analysis of subjects blood samples to nickel and chromium which were felt to be the

*Becton-Dickinson Type 6527. Rutherford, New Jersey.

†Smith, Kline and Beecham Medical Laboratories. Van Nuys, California.

most important metals to which patients would be exposed to while undergoing orthodontic therapy.

A total of ninety blood samples from 17 female and 13 male subjects were sent frozen to the commercial medical laboratory for analysis. We were unable to obtain a third blood sample from one of the original thirty-one subjects participating in this study. Therefore, the results from this subject are not included in our findings.

Statistical Analysis

In-Vitro Study

A general linear models procedure including Duncan's multiple range test was used to determine if differences existed between the two independent variables: archwire type (stainless steel versus nickel titanium) and time (days 1, 7, 14, 21 and 28), and the dependent variables: metal concentration (nickel and chromium). Probabilities and Duncan's tests were performed on all variables. The Duncan's test is a procedure which simultaneously compares means of multiple groups in order to delineate differences between the groups while classifying similar groups together. An alpha of 0.05 was chosen.

Figure 1. Model G-24 Environmental Incubator Shaker.



Figure 2. Scintrex Model AAZ-2 Zeeman Modulated Atomic
Absorption Spectrophotometer. Front view.

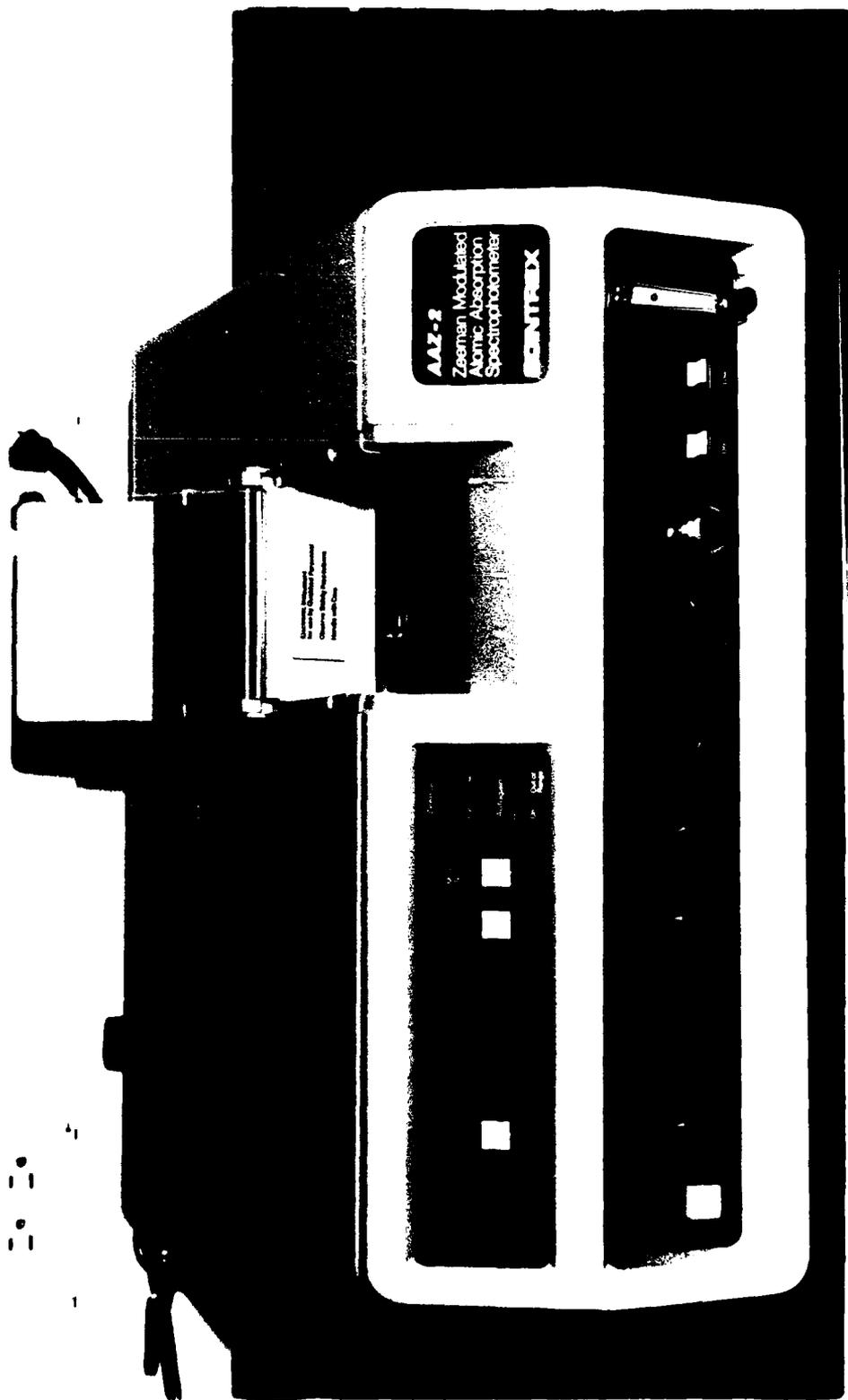


Figure 3. Scintrex Model AAZ-2 Zeeman Modulated Atomic Absorption Spectrophotometer. Top view with cover raised.

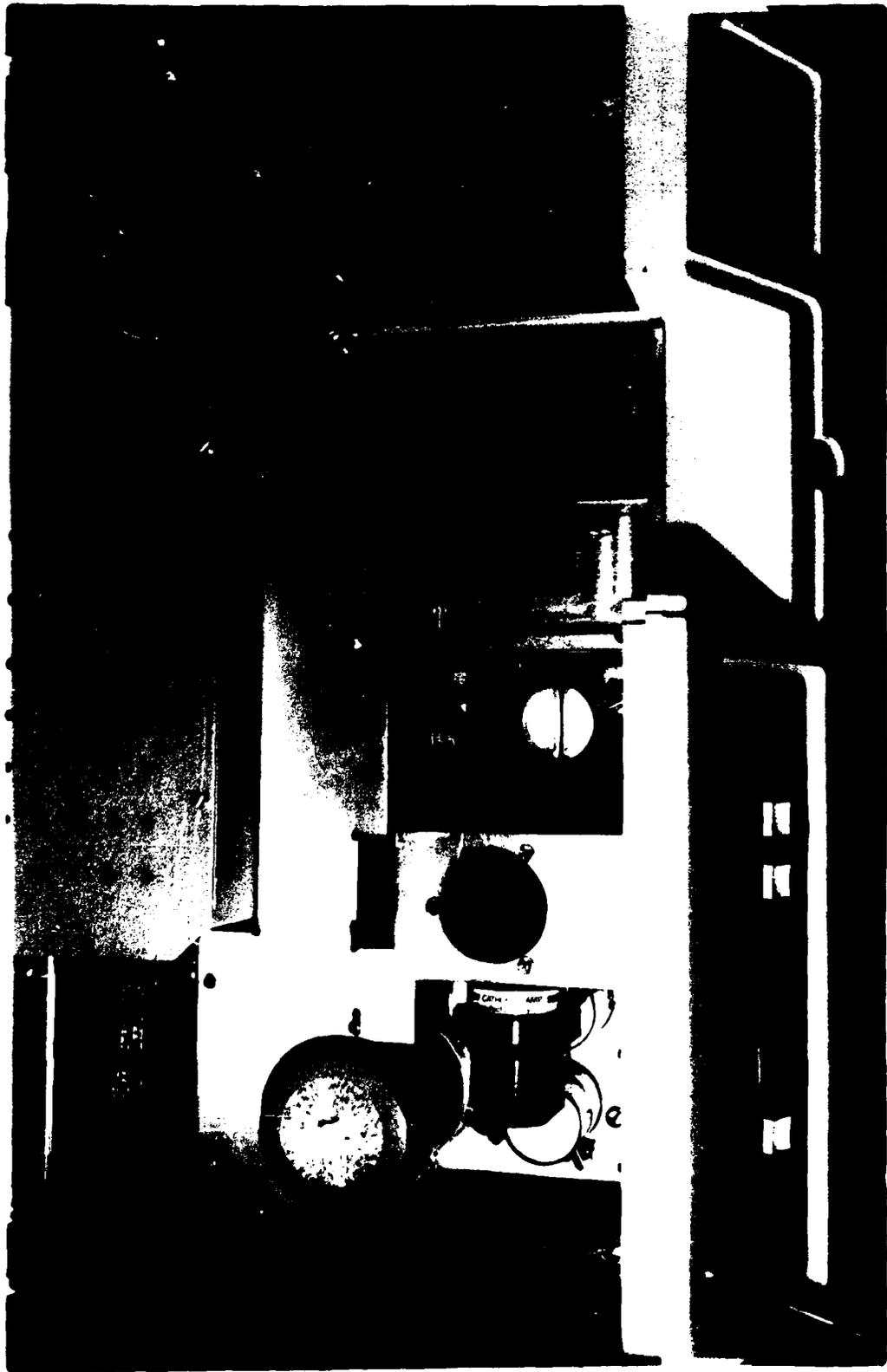


Figure 4. Finnpiquette 5-40 μ L micropipette.

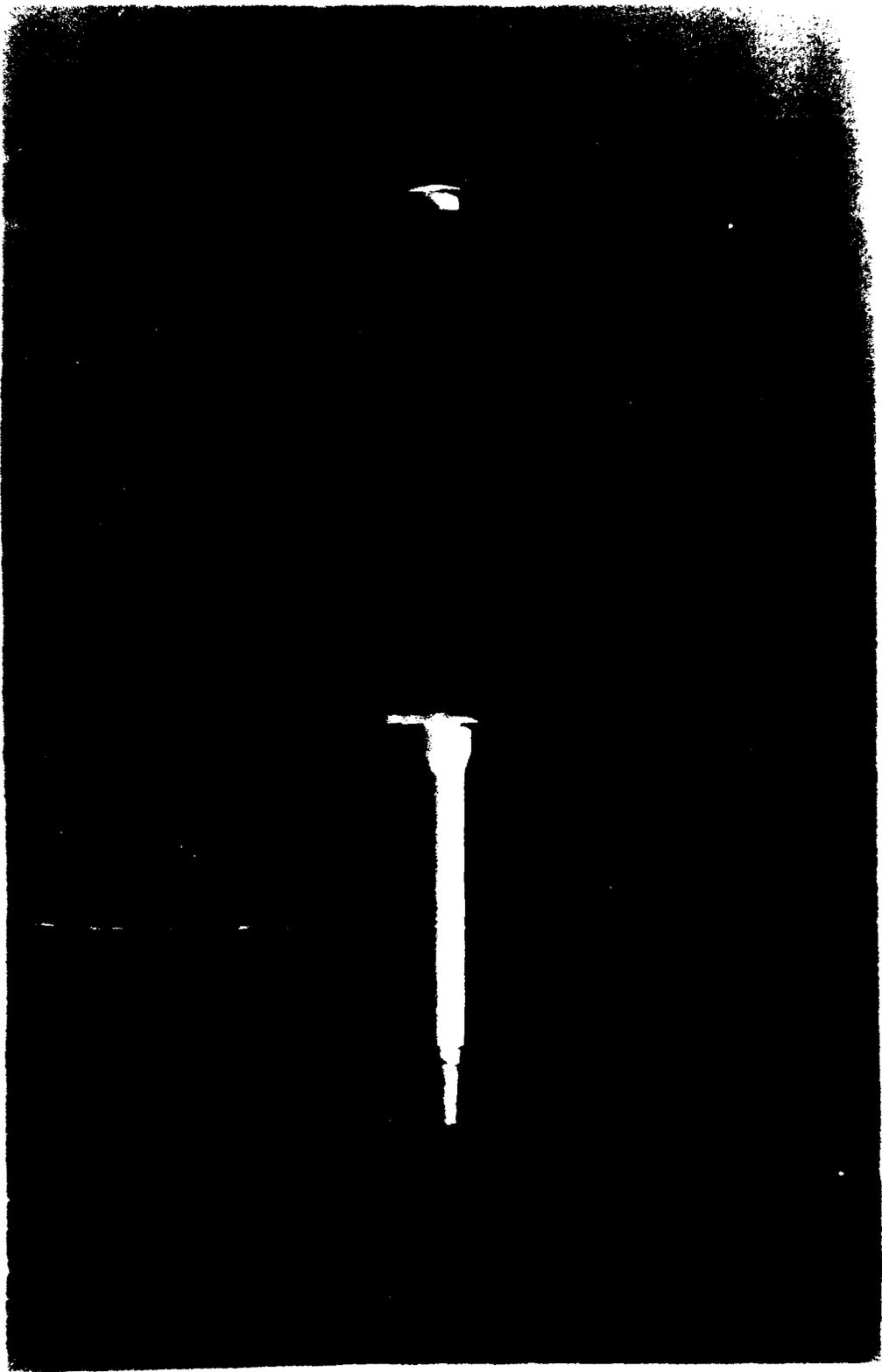


Figure 5. Injection technique for a 10 μ L sample utilizing the micropipette.



FINDINGS

In Vitro Study

During the course of this study no rust-colored precipitates were noticed in any of the sample containers. Localized areas of rust-colored corrosion were apparent on a few of the brackets and bands. The corrosion occurred primarily at either the bracket-mesh base or bracket-band interface. This visible corrosion was evident on less than 10 percent of total number of brackets and bands utilized in this study.

The independent variables considered in this portion of the project were archwire type (stainless steel versus nickel titanium) and time (days 1, 7, 14, 21 and 28). The dependent variables consisted of the concentration of nickel and chromium as measured in the artificial saliva solutions.

The nickel concentration results for the five stainless steel and five nickel titanium archwire appliances are presented in Appendix B, Table 5. The results for chromium concentrations for the same appliances are presented in Appendix B, Table 6. The statistical analyses of these results are presented in Table 2 for

nickel and in Table 3 for chromium. The changes in the mean nickel concentration over time are charted in Figure 6 and the same relationship for chromium are presented in Figure 7.

Reliability of the Measurement

Additional sample measurements were performed when the first two readings for any particular sample failed to fall within a range that was ten percent of their mean value. Of the fifty-four samples analyzed for nickel only one required additional sample measurements. The reliability of the measurement, therefore, for nickel was 98 percent.

For chromium, seven of the fifty-four samples analyzed required additional sample measurements. For the chromium analyses the reliability of the measurement was 87 percent.

Nickel

A peak occurred in the nickel concentration of the artificial saliva on day 7. The release of this metal steadily decreased during the subsequent three week period. The overall pattern of nickel release was similar for appliances fabricated from either stainless steel or nickel-titanium archwires (Figure 6).

The corrosion of the appliances fabricated with either stainless steel or nickel-titanium archwires was analyzed with respect to nickel concentration. The analysis of variance indicated a statistically significant difference

in nickel concentrations released in the solution with time ($p = 0.0001$).

When the magnitude of the corrosion of the appliances attached to stainless steel archwires was compared to those attached to nickel titanium, no significant differences were found ($p = 0.5792$).

When both variables of time and archwire type were combined a significant difference was found indicating that the corrosion rate of these appliances for nickel differs significantly over the time periods tested when the appliances with stainless steel archwires are compared to those with nickel titanium archwires ($p = 0.0232$). Duncan's Multiple Range Test indicated that a significant difference was present at the 14 day period . In other words, nickel release was significantly greater from the stainless steel archwires as compared to the nickel titanium archwires at day 14 ($p = 0.0264$).

Chromium

The release of chromium into the artificial saliva solution showed the greatest increase through day 14. The release rate leveled off between day 14 and 21 and then increased moderately at day 28. The overall pattern of chromium release was similar for appliances fabricated from either stainless steel or nickel-titanium archwires (Figure 7).

When chromium levels released from the appliances constructed from stainless steel archwires were compared to those of nickel titanium archwires no significant differences were present ($p = 0.5456$).

As with nickel, the number of days which had elapsed since the initiation of the experiment did make a significant difference in the corrosion level of all appliance types ($p = 0.0065$).

On the other hand, no significant differences were found in the chromium levels over time when the appliances fabricated with stainless steel archwires were compared to those made with nickel titanium archwires ($p = 0.6411$). This was contrary to the results obtained from nickel.

The Interrelationship of Nickel and Chromium Levels

The nickel concentration in the artificial saliva was at a significantly higher level than that of chromium at each time period ($p = 0.0001$). The lowest difference was recorded on day 28 where the nickel concentration averaged 5.5 times the chromium concentration. The greatest difference occurred on day 7 where the nickel levels were 236 times those of chromium. Over the five time intervals tested, the nickel concentrations averaged 37.3 times higher than the chromium concentrations.

Table 2. In Vitro Statistical Results: Nickel

	Archwire Type	NICKEL (ppb)				Significance [§]
		Mean	Standard Deviation	Std. Error of the Mean	Range	
Day 1	SS	2865	1299	581.1	1560-4410	n.s.
	NiTi	4290	1423	636.4	2620-5760	
Day 7	SS	7518	1473	658.9	5805-9060	n.s.
	NiTi	8408	1274	569.7	7680-10,680	
Day 14	SS	5220	1773	793.1	2895-7860	sig.*
	NiTi	2763	973.0	435.2	1725-3880	
Day 21	SS	1928	1317	589.0	790-3850	n.s.
	NiTi	1575	1614	721.9	204-4026	
Day 28	SS	1262	1217	544.5	220-3318	n.s.
	NiTi	702	625.7	279.8	193-1788	

[§] Significance determined by Duncan's Multiple Range Test.
Alpha = 0.05 n.s. = not significant sig. = significant

* p = 0.0264

Table 3. In Vitro Statistical Results: Chromium

	Archwire Type	CHROMIUM (ppb)				Significance [§]
		Mean	Standard Deviation	Std. Error of the Mean	Range	
Day 1	SS	21.2	8.52	3.81	12.8-34.3	n.s.
	NiTi	16.4	7.30	3.26	8.0-27.8	
Day 7	SS	23.7	12.6	5.65	12.1-43.2	n.s.
	NiTi	43.6	24.7	11.1	14.1-79.4	
Day 14	SS	125.5	159.8	71.5	20.5-405.0	n.s.
	NiTi	154.1	71.7	32.1	26.0-191.0	
Day 21	SS	132.4	80.9	36.2	60.5-264.0	n.s.
	NiTi	102.3	48.9	21.9	16.3-137.0	
Day 28	SS	233.1	250.4	112.0	68.5-672.0	n.s.
	NiTi	126.9	111.3	49.7	13.1-252.0	

[§] Significance determined by Duncan's Multiple Range Test.
 Alpha = 0.05 n.s. = not significant sig. = significant

Figure 6. Nickel Concentration in Artificial Saliva for
Different Archwire Types Versus Time.

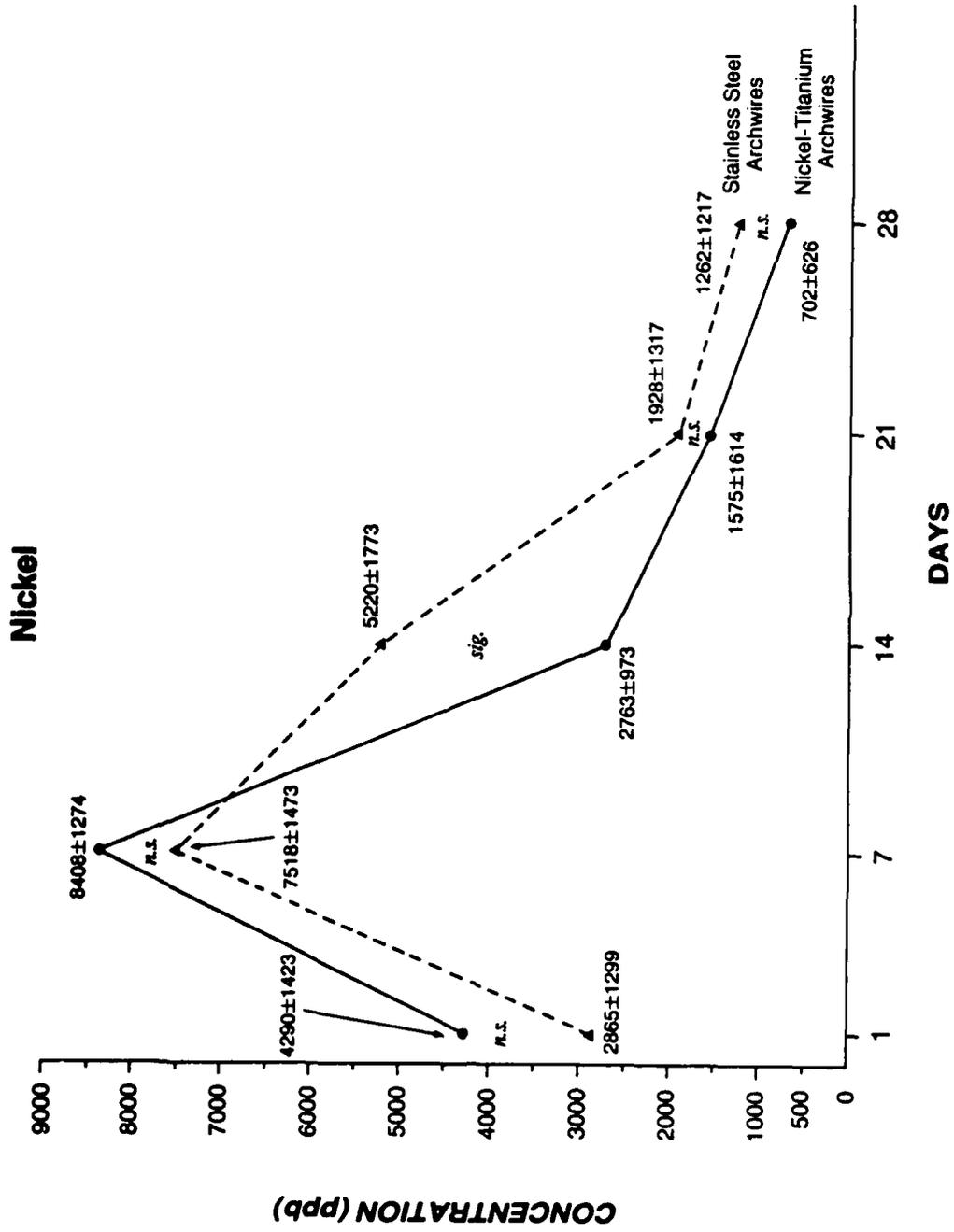
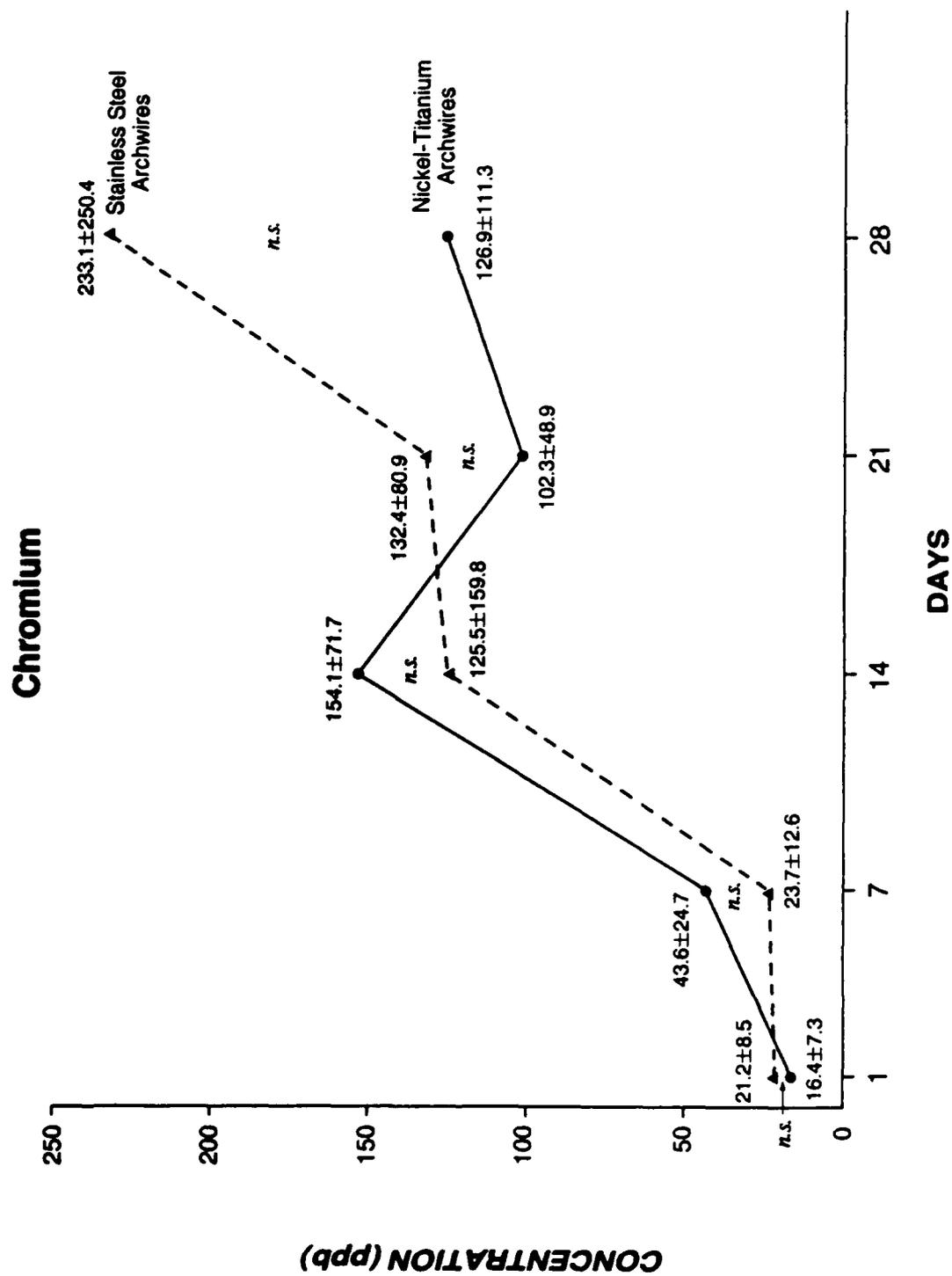


Figure 7. Chromium Concentration in Artificial Saliva for
Different Archwire Types Versus Time.



In Vivo Study

Subjects

An analysis of the allergy questionnaires completed by the 31 subjects revealed a positive suspected metal allergy in five subjects or 16.1 percent. All of the positive responses were by females and all symptoms were in response to ear rings. The percentage of females reporting metal allergy symptoms was 28.8% (5 of 18 subjects). The symptoms reported were: red, itchy or flaky skin at the location of the ear rings. No symptoms of any suspected metal allergies were reported by the males.

Blood Levels

The results for the blood concentrations of nickel in patients undergoing orthodontic treatment are presented in Table 4. Results for blood levels of chromium could not be determined with the equipment presently available at the commercial laboratory. When the analysis was attempted it was found that the proteins present in whole blood completely interfered with the determination of the chromium levels. The chemical treatment of the blood samples used for nickel analysis were different and the interference encountered in the analysis for chromium was not encountered in the analysis for nickel. Due to these difficulties the results and discussion of the in vivo portion of this project will be limited to only the

analysis of nickel levels.

Nickel

No demonstrable increase in the blood level of nickel was found during the four to five month course of orthodontic treatment. For the 93 blood samples analyzed, 77 (82.8%) were below the detection limit of 0.4 ppb. Ten were either 0.4 or 0.5 ppb and only six had higher values (range = 0.8 to 1.3 ppb). The occurrence of levels at or above the detection limit were equally distributed in each of the three sampling time periods. No pattern of increase in nickel blood levels occurred over these three time periods.

For the five subjects which acknowledged a sensitivity to metal ear rings only three (20 percent) of their fifteen blood samples were at or above the detection limit for nickel and all of these occurred at the 2 month time period. The three measureable levels were 0.4, 0.5 and 0.8 ppb.

Since only 17.2 percent of blood samples were at or above the detection limit no statistical analysis of this data was deemed appropriate. Any form of statistical treatment for this data would be meaningless since the majority of these values were not significantly different from zero.

Table 4. In-Vivo Results: Nickel

Patient Number	Time 0 ^a	Time 1 ^b	Time 2 ^c
Females			
1	0.4	<0.4	0.4
2	0.4	<0.4	<0.4
3	<0.4	0.8	0.4
4	<0.4	<0.4	<0.4
5	<0.4	<0.4	<0.4
6	<0.4	0.4	<0.4
7	<0.4	<0.4	<0.4
8	<0.4	<0.4	1.3
9	<0.4	<0.4	<0.4
10	0.5	<0.4	<0.4
11	<0.4	<0.4	<0.4
12	<0.4	<0.4	<0.4
13	<0.4	0.5	<0.4
14	<0.4	<0.4	<0.4
15	<0.4	0.8	<0.4
16	<0.4	<0.4	0.5
17	0.9	<0.4	<0.4
18	<0.4	<0.4	<0.4

Table 4. (continued)

Patient Number	Time 0 ^a	Time 1 ^b	Time 2 ^c
males			
19	<0.4	<0.4	<0.4
20	<0.4	<0.4	<0.4
21	0.8	<0.4	<0.4
22	<0.4	1.0	<0.4
23	0.4	<0.4	<0.4
24	<0.4	<0.4	<0.4
25	<0.4	<0.4	<0.4
26	<0.4	<0.4	0.4
27	<0.4	<0.4	<0.4
28	<0.4	<0.4	<0.4
29	<0.4	<0.4	<0.4
30	<0.4	<0.4	<0.4
31	<0.4	<0.4	<0.4

Units = Parts per Billion (ppb) in Blood

Level of Detection = 0.4 ppb

^aTime 0 = Prior to orthodontic appliance placement.

^bTime 1 = Approximately 2 months following orthodontic appliance placement.

^cTime 2 = Approximately 4 to 5 months following orthodontic appliance placement.

DISCUSSION

In Vitro Study

Though no rust-colored precipitates were visible in the sample bottles as was previously observed in the Park and Shearer¹⁷ study, corrosion was seen at localized areas adjacent to the spot welds of the brackets-mesh bases and the brackets to the bands. No attempt was made in the present study to remove and examine these attached corrosion products. Park and Shearer¹⁷ found that the precipitated corrosion products contained much higher amounts of chromium than nickel while the saline solutions contained more nickel than chromium. They also found that nickel was released primarily as a soluble compound, while chromium was released primarily as an insoluble form. Since no rust-colored precipitates were evident in the sample bottles no analysis for insoluble forms of nickel and chromium were conducted. However, it is possible that some insoluble precipitates containing nickel and chromium were formed but were undetectable by visualization. Therefore, all the results of the in vitro study are representative of the solubilized forms of nickel and chromium.

Nickel

When the concentrations of nickel were compared to the various time intervals, a significant difference was observed ($p = 0.0001$). A maximum level of corrosion was found at day 7 and all subsequent concentration levels demonstrated a progressive decline. These results are consistent with the those of previous studies. Park and Shearer¹⁷ found that the corrosion of their orthodontic appliances reached a plateau after six days and did not increase appreciably thereafter. Menne, et al.⁶⁰ found that nickel release was at its maximum after one week when compared to either weeks three and six. They used an artificial sweat medium that consisted of 0.5% sodium chloride, 0.1% lactic acid and 0.1% urea. Marek and Treharne¹¹⁷ analyzed the corrosion of stainless steel shavings in Ringer's solution for a period of sixty days. They found a maximum nickel release through day five, subsequently the release rate slowed and reached a constant value for the remainder of the test period.

Two explanations are possible for this behavior: First, the nickel present on the surface of the stainless steel may quickly corrode during the first seven days of the experiment then the rate of release drops off as surface nickel is depleted. Secondly, corrosion products may have formed on the surface of the appliances after seven days thus somewhat blocking additional corrosion of

the nickel. When the results of the chromium levels are considered, as will be discussed in the next section, it appears that the first hypothesis seems to be more likely.

Comparison of nickel release from the appliances with stainless steel archwires to those with nickel titanium archwires revealed no significant difference in the nickel levels in the artificial saliva medium for all time periods except day 14 ($p = 0.0264$). This is most likely a random occurrence since at all other time intervals there was no significant difference present.

The basic pattern of nickel release over time is quite similar for the two archwire types (Figure 6). When both time and archwire type were related to nickel corrosion levels the relationship was found to be significant at the $p = 0.0232$. Here again, the only significant difference was at the 14 day time period. Random occurrence is also believed to be responsible for this result.

The total release of nickel during the four week period of this study averaged $13.05 \mu\text{g}/\text{day}$. Even if this figure is doubled to simulate the equivalent release from a fully banded and bonded maxillary and mandibular appliances, the release rate of $26.1 \mu\text{g}/\text{day}$ is approximately one-tenth the previously reported average daily dietary intake of $200\text{-}300 \mu\text{g}/\text{day}$ ^{5,6}. These levels are even higher than the anticipated levels since in this study both the inside of the bands as well as the bracket

bases were exposed to the artificial saliva medium. In practice, these surfaces would be covered by cement or composite bonding adhesive and would not be as available or susceptible to corrosion.

Chromium

As with the nickel levels, a significant relationship was found when the chromium levels in the artificial saliva medium were compared at the various time intervals ($p = 0.0065$). The level of chromium released was found to increase up to day 14, at which point it levels off (Figure 7). The numerical disparity in chromium release between the two archwire types at day 28 was not found to be significant.

Contrary to the nickel results, the level of chromium corrosion did not decrease after day 7. Therefore, it is unlikely that a buildup of corrosion products occurred at this time since such an occurrence would have made the concentrations of both metals decrease with time after day 7. It is much more likely that the nickel concentration on the surface of the appliances is being depleted much faster than is the chromium concentration.

The total release of chromium during the four week period of this project averaged $0.35 \mu\text{g}/\text{day}$. Doubling this figure to simulate orthodontic appliances having been placed on both dental arches would give a release rate of

0.7 $\mu\text{g}/\text{day}$. This is approximately 0.25 percent of the reported average daily dietary intake of 280 $\mu\text{g}/\text{day}$ ³ for chromium. As discussed earlier this figure is also an over exaggeration of the chromium release rate from a fully banded and bonded orthodontic appliance.

The Interrelationship of Nickel and Chromium Levels

Throughout the course of this study the concentrations of nickel in the artificial saliva were always much higher than those recorded for chromium. Previous reports¹⁷ indicate that slightly more nickel than chromium is released when stainless steel corrodes, however, the magnitude of the difference has been much smaller than the results of this project. The reason for the decreased release rate for chromium when compared to nickel has been attributed to the formation of a protective layer of chromium oxides which inhibits the further release of chromium.^{18,60} On the basis of the results of Park and Shearer¹⁷ who found that nickel corrosion products are much more soluble in a saline solution than those of chromium it seems probable that the large differences found in the present study are due primarily to the solubility characteristics of these two metals and not to a 37 times higher release rate for nickel.

In Vivo Study

Nickel

No discernable trend towards a systemic increase in the blood levels of nickel were found in patients fitted with full orthodontic appliances. Only 17.2 percent of the samples contained a nickel level that was at or above the detection limit of 0.4 ppb. The occurrence of detectable levels of nickel in the blood was distributed randomly over the three time periods of this study and never exceeded 1.3 ppb. No correlation between orthodontic therapy and an increase in nickel blood levels was found in these results.

Several possibilities exist for the infrequent and random distribution of these slightly higher values. Contamination from the stainless steel venipuncture needle could have caused these higher readings. This could occur if a small piece of stainless steel, from the needle, was carried into the venipuncture tube during drawing of the blood sample. Another possibility is that these higher blood levels may correspond with the consumption of foods containing a high trace level of nickel which produced a transitory increase in the subjects' blood nickel levels at the time these blood samples were obtained. It is felt that these small and infrequent increases are due to some random variation in the patients' habits and not the result of nickel absorption from their orthodontic appliances.

Regardless, all the blood levels of nickel found in these subjects were below the mean levels previously reported in the literature (2.4 ± 0.5 ppb⁸⁵, 4.8 ± 1.3 ppb⁸⁷, 6.0 ± 1.0 ppb⁸⁶, and 30 ± 19 ppb²⁰). Thus, none of the subjects displayed a blood level which was greater than normal on any of the sampling periods. There was no clinically or statistically significant increase in the nickel blood levels detected during the first 4 to 5 months of orthodontic therapy in the subjects of this study.

The five subjects who were identified through the metal allergy questionnaire as being sensitive to metal ear rings did not evidence nickel blood levels that were any higher than non-sensitive individuals. This study detected no differences in the blood levels for nickel in patients with a suspected sensitivity to nickel and/or chromium when compared to individuals without such sensitivity.

Chromium

The frozen whole blood samples could not be analyzed for chromium due to the high protein content of whole blood which interfered with its determination. The analysis of chromium would have required the use of blood serum samples instead of whole blood samples. The original choice to use whole blood samples was based on the information obtained from the literature which indicates that chromium is selectively bound to the red blood cells. Once the whole

blood samples were frozen it became impossible to extract the serum component. The use of blood serum samples would have provided a partial indication for changes in the chromium blood levels during orthodontic therapy.

CONCLUSIONS

In Vitro Study

The following conclusions can be drawn from this study:

1. Orthodontic appliances release measurable amounts of nickel and chromium when placed in an artificial saliva medium.

2. The nickel release from orthodontic appliances kept in an artificial saliva solution reaches a maximum after approximately 1 week. Thereafter, the release diminishes with time during the 3 additional weeks of this study.

3. The chromium release from orthodontic appliances kept in an artificial saliva solution increases during the first 2 weeks, at which time the release rate levels off and does not increase greatly during the subsequent 2 week period.

4. The release of nickel and chromium from stainless steel and nickel-titanium archwires is not significantly different for the two archwire types.

5. Over the 4 week duration of this study the concentration of soluble nickel was 37 times higher than

the concentration of soluble chromium.

6. When compared to reported average daily dietary intake for nickel, the estimated release rate from full mouth orthodontic appliances approaches 10% of this value.

7. When compared to reported average daily dietary intake for chromium, the estimated release rate from full mouth orthodontic appliances approaches 0.25% of this value.

In Vivo Study

1. No appreciable increase in nickel blood levels occur in patients with fully banded and bonded orthodontic appliances during the first 4 to 5 months of orthodontic therapy.

2. Orthodontic therapy utilizing appliances made of nickel containing alloys does not contribute, in a measurable way, to the patient's blood level for nickel.

Total Study

1. The results obtained from both parts of this study indicate that orthodontic bands, bonds, archwires and ligature wires currently used for orthodontic treatment do not significantly increase the levels of patient exposure to nickel or chromium.

Suggestions for Further Study

1. It would have been interesting to conduct the in

vitro portion of this study for a longer duration, possibly eight or ten weeks to see if a steady rate of corrosion would have occurred.

2. It also would have been beneficial to dry some of the artificial saliva samples entirely and then to dissolve the resulting residue in nitric acid to see if some of the chromium was present in an insoluble form during the initial analysis. Possibly more chromium could have been recovered with this procedure.

3. The protein source initially included in this portion of the study had to be excluded since it contained high concentrations of nickel. Other protein sources could be evaluated for their appropriateness for this particular project and included in the artificial saliva formula. This would have provided a medium which more closely resembles natural saliva.

4. Due to the difficulties in analyzing whole blood samples for trace metals it would be preferable to analyze blood serum instead. The original choice of whole blood was based on the information obtained from the literature that indicates that chromium is selectively bound by the red blood cells. On the other hand, presently available laboratory techniques cannot analyze whole blood samples for chromium, hence it would have been less complicated if blood serum was collected. Serum would have provided a partial indicator for changes in the chromium blood levels

during orthodontic therapy.

Since no increase in nickel blood levels were found within the 5 month period of this study, there was no need to obtain additional blood samples at later time intervals.

5. Further research is required in order to determine if the low levels of release of nickel and chromium from orthodontic appliances are of clinical significance in sensitizing patients or eliciting a contact hypersensitivity reaction in patients who have a prior history of contact hypersensitivity to nickel and/or chromium.

SUMMARY

The purpose of the first part of this study was to determine the in vitro corrosion rate in terms of nickel and chromium release for a typical banded and bonded fixed orthodontic appliance system including stainless steel or nickel-titanium archwires in an environment representative of the human oral cavity. The purpose of the second part of this study was to determine if an increase in the blood levels of nickel or chromium occurs in patients undergoing routine orthodontic therapy.

The in vitro findings indicated that orthodontic appliances corrode when placed in an artificial saliva solution, releasing measurable quantities of both nickel and chromium. The rate of metal release was not found to be constant over time. The nickel release reached its maximum level on day 7 and then decreased. The chromium release rate rose rapidly through day 14 and then levelled off for the remaining two weeks of the study.

The rate of release for nickel was found to be 37 times greater than that for chromium over the course of this study. Chromium's lower solubility in a saline solution may be responsible for the difference.

The release of nickel from orthodontic appliances utilizing nickel-titanium archwires was not found to differ significantly from levels released by appliances fabricated with stainless steel archwires.

The estimated in vitro release rate for both nickel (26.1 $\mu\text{g/day}$) and chromium (0.7 $\mu\text{g/day}$) from orthodontic appliances should not be considered as a major source of exposure when compared to normal dietary intake levels for nickel (200-300 $\mu\text{g/day}$ ^{5,6}) and chromium (280 $\mu\text{g/day}$ ³). The potential for patient sensitization to these metals from orthodontic appliances remains unknown however.

The results of the in vivo study did not reveal any detectable increase in blood levels of nickel in patients undergoing orthodontic therapy.

The results obtained from this study indicate that orthodontic bands, bonds, archwires and ligature wires currently used for orthodontic treatment do not significantly increase the levels of patient exposure to nickel and chromium.

Orthodontists should be aware of the potential for sensitization in patients treated with modern orthodontic appliances. Additionally, those in the orthodontic profession should consider the possibility of provoking hypersensitivity reactions when treating patients who are already sensitive to objects containing nickel or chromium. The unexplained occurrence of gingival or mucosal

inflammation, erythema, or the report of a burning sensation in the patient's oral tissues should be investigated as a potential allergic response to their orthodontic appliances. The health history completed on each patient should seek to identify individuals who have experienced previous atopic reactions to these metals.

APPENDIX A
METAL ALLERGY QUESTIONNAIRE

Patient Name: _____ Sex _____ Birth Date _____

1.) Do you have any allergies? Yes _____ No _____

If yes, to what materials? _____

If yes, what type of reaction did you have? _____

2.) Can you tolerate costume jewelry, metal watch bands, ear rings or other metal objects in close contact with your skin?

Yes _____ No _____.

3.) Have you ever noticed redness, itching, or a rash from:

a. costume jewelry Yes _____ No _____

b. ear rings Yes _____ No _____

c. metal materials in any clothing Yes _____ No _____

d. other metal objects Yes _____ No _____

4.) If you have had any reactions or allergies to any metal objects:

a. How long ago did it occur? _____

b. How long did the reaction last? _____

c. Did you receive any treatment? _____

If so, what type of treatment? _____

Who provided the treatment? _____

APPENDIX B
RAW DATA FOR IN VITRO STUDY

Table 5. In-Vitro Results: Nickel

Day 1:				
<u>Bottle No.</u>	<u>Sample #1</u>	<u>Sample #2</u>		<u>Mean</u>
SS 1	2090	2300		2195
SS 2	3960	4860		4410
SS 3	1540	1580		1560
SS 4	2020	2080		2050
SS 5	3940	4280		4110
NiTi 1	5540	5580		5560
NiTi 2	2900	3220		3060
NiTi 3	4700	4200		4450
NiTi 4	2880	2360		2620
NiTi 5	5800	5720		5760
Day 7:				
<u>Bottle No.</u>	<u>Sample #1</u>	<u>Sample #2</u>	<u>Sample #3</u>	<u>Mean</u>
SS 1	8010	9360	-	8685
SS 2	9570	8220	9390	9060
SS 3	6300	6000	-	6150
SS 4	5730	5880	-	5805
SS 5	7680	8100	-	7890
NiTi 1	7920	7920	-	7920
NiTi 2	7520	8320	-	7920
NiTi 3	7640	8040	-	7840
NiTi 4	7040	8320	-	7680
NiTi 5	10,520	10,840	-	10,680
Blank 1	0	0	-	0
Blank 2	0	0	-	0

Table 5. (Continued)

Day 14:

<u>Bottle No.</u>	<u>Sample #1</u>	<u>Sample #2</u>	<u>Mean</u>
SS 1	2970	2820	2895
SS 2	4500	5130	4815
SS 3	5730	5070	5400
SS 4	7980	7740	7860
SS 5	5130	5130	5130
NiTi 1	2480	2480	2480
NiTi 2	1800	2280	2040
NiTi 3	3900	3480	3690
NiTi 4	1770	1680	1725
NiTi 5	3900	3860	3880

Day 21:

<u>Bottle No.</u>	<u>Sample #1</u>	<u>Sample #2</u>	<u>Mean</u>
SS 1	880	700	790
SS 2	1092	1080	1086
SS 3	1124	1216	1170
SS 4	3860	3840	3850
SS 5	2696	2792	2744
NiTi 1	216	192	204
NiTi 2	2454	2316	2385
NiTi 3	790	900	845
NiTi 4	420	410	415
NiTi 5	4164	3888	4026

Table 5. (Continued)

Day 28:

<u>Bottle No.</u>	<u>Sample #1</u>	<u>Sample #2</u>	<u>Mean</u>
SS 1	248	192	220
SS 2	796	712	754
SS 3	696	644	670
SS 4	3180	3456	3318
SS 5	1280	1420	1350
NiTi 1	540	590	565
NiTi 2	404	404	404
NiTi 3	592	528	560
NiTi 4	188	198	193
NiTi 5	1824	1752	1788
Blank 3	0	0	0
Blank 4	0	0	0

Units = Parts per Billion (ppb)

SS = Stainless Steel archwire

NiTi = Nickel-Titanium archwire

Table 6. In-Vitro Results: Chromium

Day 1:				
<u>Bottle No.</u>	<u>Sample #1</u>	<u>Sample #2</u>	<u>Sample #3</u>	<u>Mean</u>
SS 1	21.9	20.5	19.3	20.6
SS 2	14.5	14.9	-	14.7
SS 3	13.7	11.9	-	12.8
SS 4	33.7	34.9	-	34.3
SS 5	23.1	23.9	-	23.5
NiTi 1	16.9	18.7	-	17.8
NiTi 2	14.9	13.9	14.9	14.6
NiTi 3	25.0	30.6	-	27.8
NiTi 4	14.9	12.4	-	13.7
NiTi 5	8.5	7.1	8.5	8.0
Day 7:				
<u>Bottle No.</u>	<u>Sample #1</u>	<u>Sample #2</u>	<u>Sample #3</u>	<u>Mean</u>
SS 1	43.6	42.8	-	43.2
SS 2	21.4	24.8	-	23.1
SS 3	12.0	14.2	-	13.1
SS 4	12.2	12.0	-	12.1
SS 5	26.4	27.4	-	26.9
NiTi 1	81.8	77.0	-	79.4
NiTi 2	41.4	50.2	45.2	45.6
NiTi 3	46.2	55.6	-	50.9
NiTi 4	12.8	15.4	-	14.1
NiTi 5	29.4	25.4	29.4	28.1
Blank 1	0	0	-	0
Blank 2	0	0	-	0

Table 6. (Continued)

Day 14:

<u>Bottle No.</u>	<u>Sample #1</u>	<u>Sample #2</u>	<u>Sample #3</u>	<u>Mean</u>
SS 1	92.4	95.2	-	93.8
SS 2	91.0	77.0	-	84.0
SS 3	411.0	399.0	-	405.0
SS 4	21.0	20.0	-	20.5
SS 5	25.6	22.4	-	24.0
NiTi 1	180.0	210.0	177.0	189.0
NiTi 2	189.0	180.0	-	184.5
NiTi 3	198.0	184.0	-	191.0
NiTi 4	23.8	28.2	-	26.0
NiTi 5	178.0	182.0	-	180.0

Day 21:

<u>Bottle No.</u>	<u>Sample #1</u>	<u>Sample #2</u>	<u>Mean</u>
SS 1	256.0	272.0	264.0
SS 2	162.0	145.0	153.5
SS 3	83.0	97.0	90.0
SS 4	56.0	65.0	60.5
SS 5	98.4	90.0	94.2
NiTi 1	126.0	127.0	126.5
NiTi 2	121.0	102.0	111.5
NiTi 3	120.0	120.0	120.0
NiTi 4	17.4	15.2	16.3
NiTi 5	140.0	134.0	137.0

Table 6. (Continued)

Day 28:

<u>Bottle No.</u>	<u>Sample #1</u>	<u>Sample #2</u>	<u>Sample #3</u>	<u>Mean</u>
SS 1	115.0	115.0	-	115.0
SS 2	225.0	184.0	-	204.5
SS 3	68.0	69.0	-	68.5
SS 4	686.0	658.0	-	672.0
SS 5	107.8	103.6	-	105.7
NiTi 1	85.4	74.9	-	80.2
NiTi 2	49.0	50.4	48.3	49.2
NiTi 3	248.5	231.0	-	239.8
NiTi 4	13.0	13.2	-	13.1
NiTi 5	243.0	261.0	-	252.0
Blank 3	0	0	-	0
Blank 4	0	0	-	0

Units = Parts per Billion (ppb)

SS = Stainless Steel archwire

NiTi = Nickel-Titanium archwire

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