Evaluation of tissue response to dental alloys by subcutaneous implantation

Christer Bessing and Thomas Kallus
Department of Dental Materials and Technology, University of Umeå, Umeå, Sweden, and NIOM, Scandinavian Institute of Dental Materials, Oslo, Norway

Bessing C, Kallus T. Evaluation of tissue response to dental alloys by subcutaneous implantation. Acta Odontol Scand 1987;45:247–255. Oslo. ISSN 0001–6357.

The tissue response of two low-gold alloys (Midas® and Rajah®), two silver–palladium alloys (Albacast® and Alba V), and one type III gold alloy (JSC®) was studied after subcutaneous implantation of cylindrical implants in 24 guinea pigs. Castings of each alloy were inserted into polyethylene tubes and implanted by means of a standardized technique. Each animal received five implants representing the five alloys. The implants were left in place for 30 and 90 days. Microscopic evaluation of the implant sites showed that after 30 days the tissue reactions were slight to moderate, with only small differences between the alloys. After 90 days the reactions were slight to extreme. One of the silver–palladium alloys (Alba V) demonstrated several severe and extreme reactions after 90 days. The other silver–palladium alloy (Albacast) demonstrated the least tissue response of the five alloys in the 90-day group.
□ *Adverse effects; alternative alloys; biocompatibility testing; toxicity*

Christer Bessing, Department of Dental Materials and Technology, University of Umeå, S-901 87 Umeå, Sweden

Because of the increasing price of gold during the past two decades, a large number of alternatives for fixed prosthetic reconstructions have been introduced in dentistry. The alternatives can be classified into three major groups: low-gold alloys, silver–palladium alloys, and base metal alloys (1). Apart from problems with regard to the technical handling, the reduction or elimination of gold may have an impact on the corrosion behavior and tarnish (2–9) and on the biocompatibility of the alloys (10–18). In Au–Ag–Cu alloys with a low content of Au and an inhomogeneous or heterogeneous structure a preferential corrosion has been shown to occur in vitro (2–7), in which the Ag- and Cu-rich phases are attacked. In Ag–Pd-based alloys Pd reduces the corrosion and tarnish of Ag in alloys with 20–40% Pd (8).

The biocompatibility of an alloy is influenced by the resistance to corrosion in a biological environment and by the type and quantity of cations that can be released (15–17). The penetration of metal ions from various types of alloys into the surrounding tissues has been demonstrated in humans (18–20). It has been suggested that the release of metal ions by means of corrosion processes in the oral cavity may have several clinical effects such as toxic reactions, tissue lesions, metallic taste, and risk for sensitization (10).

Wright et al. (21) demonstrated a direct correlation between the Cu content in Au–Ag–Cu alloys and their cytotoxic reactions in vitro. In a human cell culture study, Leirskar (17) found that Ag^+ is more toxic than Cu^{2+}. Elevated levels of Cu and Hg were found in the tissues in a study in which different dental alloys had been implanted subcutaneously in rats (22). In another implantation study on guinea pigs, a Cu- and Pd-rich alloy induced acute inflammation (15).

The great majority of alloys in dentistry have been marketed and come into clinical practice without prior documentation of their biological properties. It can be questioned whether such routines provide an acceptable safety to the public. Moreover, the FDI supports studies to evaluate the safety of dental materials before use in humans (23). The aim of the present study

was therefore to evaluate the biocompatibility of four different alternative alloys and a type III alloy, using a standardized subcutaneous implantation technique.

Materials and methods

Four commercially available alloys and one experimental alloy for fixed prosthetic reconstructions were tested. The metal content of the alloys was analyzed by X-ray fluorescence and atomic absorption spectrophotometry and carried out at Analytica AB, Sollentuna, Sweden (Table 1).

Preparation of test specimens

Twenty-four cylindrical castings (diameter, 1.6 mm; length, 9.5 mm) were produced from each of the five alloys. The 24 castings were cast in the same ring, using a vacuum-pressure casting machine (Combilabor CL-G 77, Heraeus Edelmetalle GmbH, Hanau, FRG), utilizing round wax shapes, gauge 14 (Kerr Manufacturing Co., Romulus, Mich., USA). Investing, burnout, and casting were carried out in accordance with the manufacturers' instructions. No subsequent heat treatments were performed.

After being cast, the gold-containing alloys Midas®, Rajah®, and JSC® were cleaned in an ultrasonic cleaner and then pickled (Jel-Pac, J. F. Jelenko & Co., Armonk, N.Y., USA). The silver–palladium alloys Albacast® and Alba V were carefully sandblasted. The cylinders were cut off from the sprues with a thin separating disc. Both ends of each casting were finished with rubber wheels and finally polished with Shure Shine (Aurora Dental Specialities Co., Hillside, Ill., USA) and a buffing compound (Häxan, Barnängen AB, Stockholm, Sweden) on a chamois buffing wheel. The castings were cleaned in an ultrasonic bath with soap water and air-dried. Each casting was inserted into a polyethylene tube (Intramedic 7445, Clay Adams, Parsippany, N.Y., USA) with an outer diameter of 2.08 mm and an inner diameter of 1.57 mm, corresponding to the 1.6 mm diameter of the castings. The tubes were cut flush with the ends of the castings. The test specimens thus obtained had a length of approximately 9.5 mm. They were disinfected in 70% ethanol and stored dry at room temperature for 4 days before implantation.

Animals

Twenty-four female albino guinea pigs (Dunkin-Hartley strain, HB Sahlins försöksdjursfarm, Malmö, Sweden), weighing 400–500 g, were used for the study. The animals were caged in groups of four. Commercial pellet diet (Avels-Tillväxt foder, Ewos AB, Södertälje, Sweden) containing 600 mg/kg vitamin C and ordinary tap water were supplied ad libitum. The animals were weighed on experimental days d_0, d_3, and d_7 and thereafter every 7th day. The weight development of the animals was compared with the normal weight curve supplied by the breeder. Twelve animals were assigned to

Table 1. Composition of the alloys determined by X-ray fluorescence analysis (Analytica AB, Sollentuna)

Alloy trade name	Composition, weight per cent								
	Au	Pt	Pd	Ag	Cu	Zn	In	Sn	Ir
JSC®*	76	3.0	<0.1	8.5	11.5	0.5	<0.1	<0.2	0.04
Rajah®†	60	<0.1	3.4	25	10.0	0.85	0.3	<0.5	≤0.1
Midas®†	47	<0.1	6.5	37	8.0	0.95	<0.1	<0.5	<0.1
Alba V‡	<0.5	<0.1	40	54	<0.01	0.04	5.0	<0.5	0.2
Albacast®†	<0.5	<0.1	26	68	0.02	2.3	3.0	<0.5	≤0.1

* J. Sjöding AB, Spånga, Sweden.
† J. F. Jelenko & Co., Armonk, N.Y., USA.
‡ Experimental alloy, Heraeus Edelmetalle GmbH, Hanau, FRG.

each of the two different implantation periods of 30 and 90 days.

Implantation

Before implantation the animals were anesthetized with fluanisonum (Hypnorm® vet., Leo AB, Helsingborg, Sweden), 0.1 ml/100 g body weight, given intramuscularly in the left hind leg. The test specimens were loaded into a cartridge in a predetermined order. The cartridge was fitted in an injection device that enabled a standardized subcutaneous placement of the test specimens through a trochar (24). After the skin had been disinfected with 70% ethanol, a 5-mm-long incision was made in the middle of a clipped and shaved region on the caudal half of the back. Each animal received five implants representing the five alloys. The implants were inserted through the single incision and placed clockwise in five different positions. The incision was closed with a skin clamp, which was removed after 1 week.

Histologic processing

On each of the experimental days d_{30} and d_{90}, 12 animals were killed with an overdose of pentobarbital sodium (Mebumal® vet., 60 g/ml, ACO Läkemedel AB, Solna, Sweden), 0.3 ml/100 g body weight intraperitoneally. The implantation sites were reshaved and excised. The skin and underlying connective tissue was fixed in 10% neutral buffered formalin for at least 48 h. The implants were left in situ during paraffin embedding. Cutting of each paraffin block was started with a worn-out microtome blade. The paraffin block was oriented so as to enable the blade to cut tangentially to the alloy cylinder, until the blade chipped off the polyethylene surrounding the alloy. The test specimen was then carefully removed by applying a needle at the middle of the specimen and thus rolling and lifting it up from the block. The paraffin blocks were melted and the specimens re-embedded, ensuring that the empty lumen left by the cylinder was filled with paraffin. Eight to ten longitudinal sections, 5 to 6 μm thick, from the central plane of the lumen left by the removed test specimen were placed on each of five slides. Every second slide was stained with hematoxylin and eosin, which left approximately 30 sections to represent each implant site at the histologic evaluation.

Selected slides were stained with routine Brown and Brenn staining to assess the possible presence of microorganisms.

Histologic evaluation

The tissue response at the tube ends, where the test alloy had been exposed to the connective tissue, was compared with the reaction along the sides of the polyethylene tube, which served as a reference site—that is, a site unaffected by the alloy. The inflammatory response was rated with regard to its degree of cellularity and vascularity and with regard to the extent of the lesion. On the basis of a previously published gross rating scale (25), the reactions were classified as no, slight, moderate, severe, and extreme. After repeated intra- and inter-individual calibrations with other experienced investigators, the microscopic examinations were performed by one investigator whose evaluation had been calibrated to established histologic criteria (T. Kallus, N. R. Gjerdet, S. Syrjänen, I. A. Mjör. Ranking and evaluation of histologic tissue responses. Unpublished observations). The most intense of the two tube-end reactions was chosen to represent the implant site at the gross rating.

The gross rating was supported by microscopic analysis at higher magnifications, following the Fédération Dentaire International's recommended standard practices for biologic evaluation of dental materials with regard to number, type, and location of the inflammatory cells (23).

Results

Animals and implantation

In the course of the experiment all animals remained in good condition and gained weight in a normal manner as compared with the weight curve supplied by the breeder. The implantation sites did not show any skin irritation, and no specimen was lost during the excision procedures. However, owing to a mishap during implantation, one animal

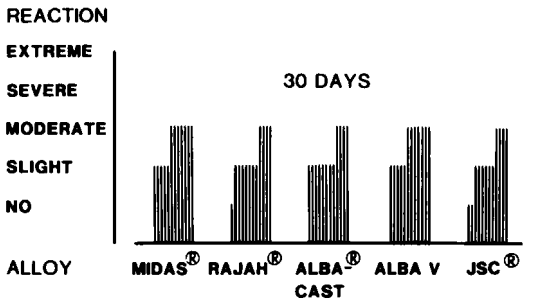

Fig. 1. Distribution of subcutaneous tissue response to crown and bridge alloys after 30 days' implantation period. Each bar represents the most intense tube-end reaction to one test specimen.

(no. 23) belonging to the d_{90} group did not receive the specimen from the alloy Midas.

Histologic evaluation

Gross rating. In Figs. 1 and 2 the ratings from the most intense tube ends are plotted, to enable a visual comparison between alloys. After 30 days, in increasing order of severity, the alloys were ranked: JSC, Rajah, Albacast, Midas, and Alba V (Fig. 1). There were only small differences between the tissue reactions to the alloys in the 30-day group, the reactions being slight to moderate. After 90 days, in increasing order of severity, the ranking was Albacast, Midas, JSC, Rajah and Alba V. Compared with the reactions after 30 days, a slight increase in tissue response was noted to JSC and Rajah. A definitely more pronounced increase in tissue response was observed to Alba V, which demonstrated several severe and extreme reactions at 90 days (Fig. 2). The tissue response to Midas and Albacast decreased slightly with time.

All ratings of the implant sites are presented in Tables 2 and 3. Generally, the difference in tube-end reaction from the two

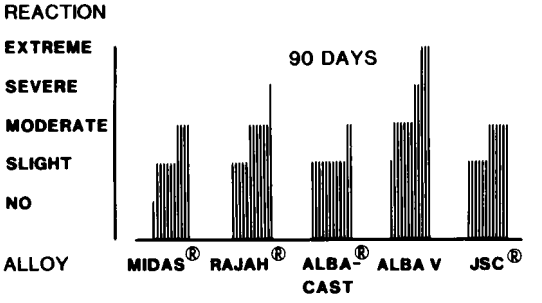

Fig. 2. Distribution of subcutaneous tissue response to crown and bridge alloys after 90 days' implantation period. Each bar represents the most intense tube-end reaction to one test specimen. One of the Midas values is missing owing to a mishap during implantation.

Table 2. Evaluation of tissue response after 30 days

Animal no.	Alloy									
	JSC®		Rajah®		Midas®		Alba V		Albacast®	
	−	+	−	+	−	+	−	+	−	+
1	0	2	0	1	**	2	1	2	1	1
2	0	1	0	2	1	2	1	1	2	2
3	0	1	1	1	0	2	1	2	0	1
4	0	1	0	1*	0	1*	2	2	0	1
5	1	1	1	1	0	2	0	1	1	1
6	0	2	1	1	1	2	1	2	2	2
7	1	2	0	0	1	2	1	2	0	2
8	1	1	1	2	1	2	**	1*	1	2
9	0	0	1	2	1	1	1	2	1	1
10	1	2	0	1	0	1	1	2	1	1
11	0	0	0	1	0	1	0	1	0	1
12	1	1	1	2	0	1	1	1	1	1

0 = no; 1 = slight; 2 = moderate; 3 = severe; and 4 = extreme reaction. + = most intense tube end, and − = less intense tube end.

* Intramuscularly.

** Not evaluated (torn at dissection).

Table 3. Evaluation of tissue response after 90 days

Animal no.	Alloy									
	JSC®		Rajah®		Midas®		Alba V		Albacast®	
	−	+	−	+	−	+	−	+	−	+
13	1	2	1	2*	1	2	1	2	0	1
14	1	2	1	2	1	1	1	3	1	1
15	0	1	0	2	0	0	1	4	0	1
16	0	1	1	1	0	1	**	4	0	1
17	**	1	1	2	1	2	0	2	0	1
18	0	1	1	3*	1	1	0	2	1	1
19	2	2	0	1	1	2	1	1	0	1
20	0	1	1	1	0	1	0	2	1	2
21	0	2	1	1	0	1	1	4	1	1
22	0	2	1	1*	0	1	1	2	1	2
23	1	2	1	2	***		2	3	0	1
24	**	1	2	2	1	2	1	2*	1	1

0 = no; 1 = slight; 2 = moderate; 3 = severe; and 4 = extreme reaction. + = most intense tube end, and − = less intense tube end.

* Intramuscularly.

** Only one end could be evaluated (torn at dissection).

*** Owing to a mishap during implantation, animal 23 did not receive the specimen from the alloy Midas.

ends of the same test specimen was not more than one level. However, in the Alba V implants a greater difference was sometimes seen after 90 days. The most intense tube-end reaction was then rated as extreme and the less intense tube-end reaction as slight (Table 3).

Microscopic evaluation. The histologic features were similar after the 30- and 90-day implantation periods. A fibrous capsule developed along the polyethylene tubes. The capsule showed increased cellular maturity, and its thickness diminished over time. In general, no cellular response was noted along the polyethylene tube. The degree of cellularity at the tube ends could easily be evaluated. No inflammatory reaction could be ascribed to the implantation technique per se.

The cellular analyses did not show any difference between tissue reactions to the five alloys after 30 days. Lymphocytes and fibroblasts dominated; that is, the inflammation was generally of the chronic type. However, occasional neutrophilic leukocytes could be seen in the close proximity of the alloy specimens exposed at the tube ends, indicating an acute inflammatory response in this location.

The tissue response after 90 days followed the above-described general histologic features, except for Alba V. Several Alba V specimens caused dense cellularity also at a distance from the tube end. The inflammatory lesions had a larger extension with increased vascularity and unspecific inflammatory cells. Foreign-body giant cells and macrophages with engulfed, granular material could be observed (Figs. 3 and 4).

Owing to the differences in tube-end reaction from the two ends of the same implant at 90 days, each Alba V implant of the 90-day group was selected to be stained with routine Brown and Brenn staining. No microorganisms could be detected at the microscopic evaluation.

Discussion

In previously published studies in which this implantation technique was used, 10–15% of the implanted specimens were lost because of technical and methodologic mishaps

(25, 26). Earlier methodologic failures have been ascribed to such loss of the polyethylene tubes in vivo, removal of tubes before sectioning, displacement of test material, and too short (14 days) implantation periods (25, 27, 28). After 14 days the operative trauma still 'overshadowed' possible effects from the implanted material. In the present study the standardized subcutaneous implantation technique now seems to have been developed to an adequate level of precision, considering that all 119 implanted test specimens became available for histologic evaluation. However, the higher frequency of lost specimens in the previous studies may to some extent be due to a higher initial tissue-irritating effect of the test materials.

Differences in response intensity between the two tube ends of the same implant have been reported previously and ascribed to scattered test material in the surrounding tissues (28). Implanted denture base polymers produced generally uniform reactions at the two tube ends (25). It is therefore not likely that the implantation procedure

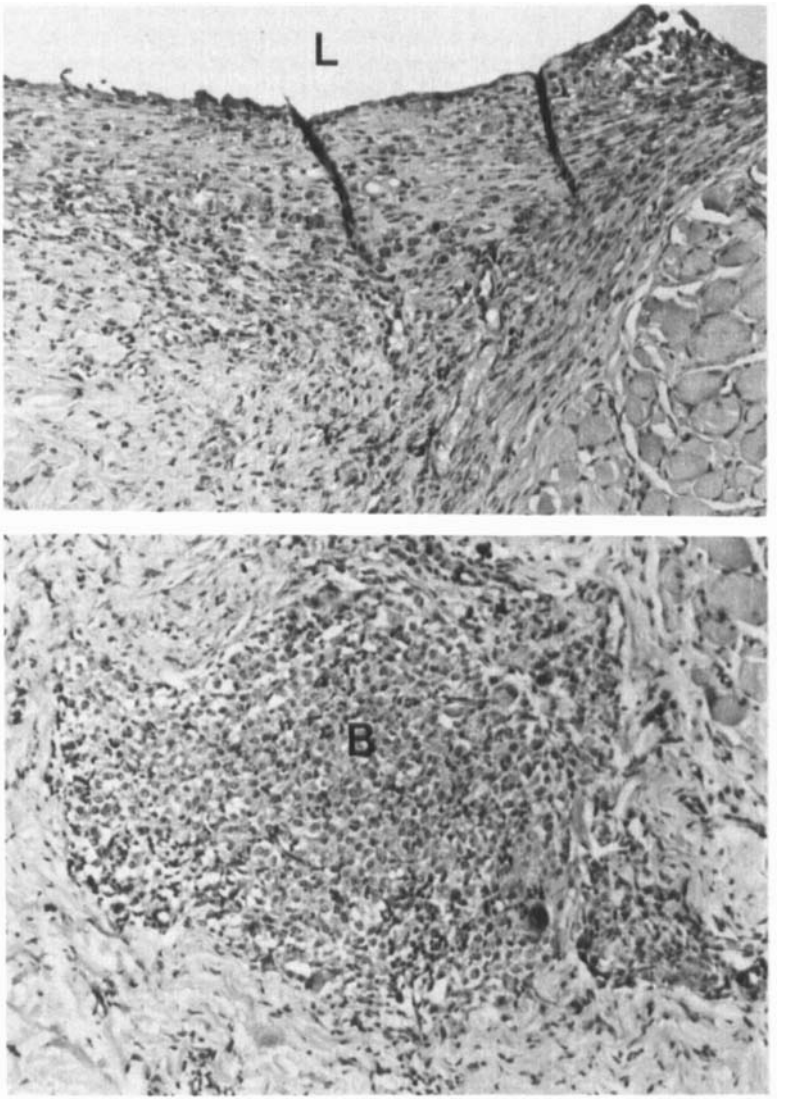

Fig. 3. Histologic tissue response to the alloy Alba V. Vascular and cellular reaction comprised fibroblasts, collagen, and unspecific inflammatory cells. A bolus-like formation (B) could be seen in several slides also at a distance from the tube end, demonstrating a large but localized extent of the inflammatory lesion. L = empty lumen left by the test specimen. (Hematoxylin and eosin; magnification, ×41.)

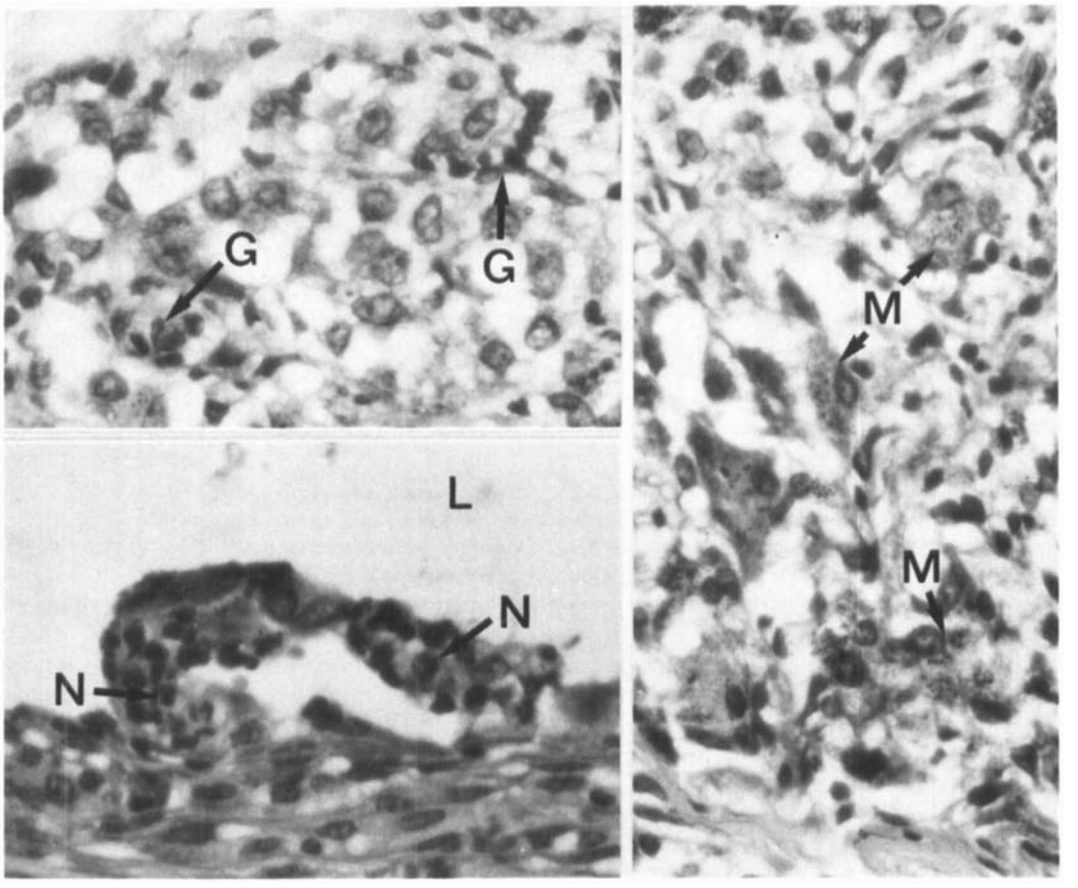

Fig. 4. The tissue response at a tube end when Alba V had been implanted. Note foreign-body giant cells (G), neutrophilic leukocytes (N), and macrophages (M) with engulfed, granular material. L = empty lumen left by the test specimen. (Hematoxylin and eosin; magnification, ×250.)

through a trochar would affect the histologic response to a significant extent. Furthermore, the absence of microorganisms at the tube ends also indicates that other explanations should be sought to elucidate the differences in tube-end reactions.

The differences in reaction at the tube end for Alba V may be explained by the fact that the alloy does not contain sufficient amounts of Zn to protect the other alloy elements, such as In, from oxidation during melting and casting of the alloy (Table 1). For example, oxides such as In_2O_3 and ZnO may be created during melting and casting. If these oxides, which corrode easily, are collected mainly at one end of the cylindrical casting, this could explain the enhanced tissue reaction seen at this end of the cylinder. A metallographic examination of the casting could confirm this assumption.

The release of metal ions by corrosion may contribute to the development of local toxic reactions from dental alloys (10–14, 17, 21). In an introductory study the metallographic characterization demonstrated the heterogeneous structure of the two silver–palladium alloys Albacast and Alba V (29). Furthermore, in a subsequent corrosion investigation of the same alloys it was shown that the corrosion resistance of the alternative alloys in the as-cast condition generally was inferior to that of the type III alloy tested (30). Results from ESCA analyses showed that a release of mainly In but also Ag from

the two silver–palladium alloys was evident, whereas Cu and Ag were released from the low-gold alloys Midas and Rajah and Cu from the type III alloy JSC.

The similar tissue response of the alloys JSC, Rajah, and Midas after 30 and 90 days' implantation (Figs. 1 and 2) would presumably be explained by the release of Cu. The slightly increased tissue response with time that occurred with the alloys JSC and Rajah may be due to a continuous formation and accumulation of corrosion products in the connective tissues of the implant sites.

All alloys studied contained various amounts of Ag. The most Ag-rich alloy, Albacast, showed mainly slight reactions after 90 days. It may be concluded that Ag^+, although cytotoxic in tissue cultures (17), could probably not be considered a harmful substance in at least four of the five alloys when evaluated in vivo in the present test system.

The almost total absence of Cu in the two Ag–Pd alloys means that there must be some other element(s) responsible for the tissue reactions. Pd did not induce any enhanced subcutaneous tissue response in a study by Niemi et al. (15). Both Ag–Pd alloys in the present study contain In. According to Browning (31), In is fairly non-toxic when administered orally but highly toxic by subcutaneous and intravenous injection. However, there are no published reports concerning the local effects of In in animals (32). Nevertheless, the In content of Alba V is approximately 67% higher than that of Albacast and may have contributed to the difference in tissue response.

The fact that the tissue response to Albacast decreased with time may be explained by a dissolution of Ag, followed by a surface enrichment of Pd (30, 33). Released Ag in the surrounding subcutaneous tissues may be dissolved in the tissue fluids and transported away from the implantation sites. As the concentration of Ag in the surface of the alloy implant decreases, the relative concentration of Pd increases. The surface becomes ennobled, and the tissue response decreases with time. A corresponding explanation, however, could not be used for Alba V. There may exist a corrosion mechanism that enables the alloy Alba V to release more In than is the case with Albacast. Differences in the solubility of the dissimilar elements released in tissue fluids are thought to play an important role, as is the rate of diffusion of elements and ions. Further investigations utilizing, for example, EDAX equipment are needed for the correlation between alloy elements and tissue reaction. Such studies are in progress.

In the present study Albacast demonstrated the least tissue response, whereas the other Ag–Pd alloy, Alba V, caused the most intense tissue response of the five alloys in the 90-day group. Alba V showed extreme tissue reactions from the subcutaneous tissues in 3 cases and severe reactions in 2 cases out of 12. The copper-containing alloys Midas, JSC, and Rajah were ranked between the two Ag–Pd alloys.

Alba V is an experimental alloy, not available on the market. All the other alloys studied have been used in dentistry for several years without any reports of adverse effects. The results of the subcutaneous screening test in this study seem to indicate a fairly good agreement with clinical experience. Previous to clinical testing in humans Alba V ought to be evaluated by means of a usage test in animals. Accordingly, the alloy Alba V has been excluded from a subsequent clinical investigation of alternative alloys for fixed prosthetic reconstructions.

One of the main concerns in biological testing of dental materials is the lack of scientific data on the correlation between biological and clinical tests; for example, a lack of correlation between implantation studies and pulp studies has been demonstrated (34). This lack of correlation between screening tests—that is, cell culture tests and implantation tests—and usage tests implies that true usage tests in humans is the most desirable (35). Therefore, testing of dental biomaterials should include a final clinical evaluation, until the clinical relevance of any screening test has been established.

Acknowledgements.—Ms Åsne Vinje, Faculty of Dentistry, University of Oslo, is gratefully acknowledged for her skillful processing of the histologic specimens. Financial support has been given by the Swedish Medi-

cal Research Council, grant B85-24X-06875-02A, and the Swedish Dental Trade Association.

References

1. Sarret DC, Richeson JS. Low-gold alloys for use in operative dentistry. Oper Dent 1982;7:63–74.
2. Wright DC, German RM, Gallant RF. Copper and silver corrosion activity in crown and bridge alloys. J Dent Res 1981;60:809–14.
3. Tucillo JJ, Nielsen JP. Observations of onset of sulfid tarnish on gold-base alloys. J Prosthet Dent 1971;25:629–37.
4. Herø H. Tarnishing and structures of some annealed dental low-gold alloys. J Dent Res 1984;63:926–31.
5. Burse AB, Swartz ML, Phillips RW, Dykema RW. Comparison of the in vitro and in vivo tarnish of three gold alloys. J Biomed Mater Res 1972;6:267–77.
6. Lang BR, Bernier SH, Giday Z, Asgar K. Tarnish and corrosion of noble metal alloys. J Prosthet Dent 1982;48:245–52.
7. German RM. Wright DC, Gallant RF. In vitro tarnish measurements on fixed prosthodontic alloys. J Prosthet Dent 1982;47:399–406.
8. Vaidyanathan TK, Prasad A. In vitro corrosion and tarnish analysis of the Ag–Pd binary system. J Dent Res 1981;60:707–15.
9. German RM. The role of microstructure in the tarnish of low-gold alloys. Metallography 1981; 14:253–66.
10. Smith DC. Tissue reaction to noble and base metal alloys. In: Smith DC, Williams DF, eds. Biocompatibility of dental materials. Vol. IV. Florida: CRC Press, 1982;51–77.
11. National Institute of Dental Research. Workshop: biocompatibility of metals in dentistry. J Am Dent Assoc 1984;109:469–71.
12. Glantz PO. Intraoral behaviour and biocompatibility of gold versus non precious alloys. J Biol Buccale 1984;12:3–16.
13. Mjör IA, Hensten-Pettersen A. The biological compatibility of alternative alloys. Int Dent J 1983; 33:35–40.
14. Picard B. Biocompatibilité des métaux dans le milieu buccal. Actual Odontostomatol (Paris) 1984;145:147–58.
15. Niemi L, Syrjänen S, Hensten-Pettersen A. The biocompatibility of a dental Ag–Pd–Cu–Au-based casting alloy and its structural components. J Biomed Mater Res 1985;19:535–48.
16. Niemi L, Hensten-Pettersen A. In vitro cytotoxicity of Ag–Pd–Cu-based casting alloys. J Biomed Mater Res 1985;19:549–61.
17. Leirskar J. On the mechanism of cytotoxicity of silver and copper amalgams in a cell culture system. Scand J Dent Res 1974;82:74–81.
18. Bergenholtz A, Hedegård B, Söremark R. Studies of the transport of metal ions from gold inlays into environmental tissues. Acta Odontol Scand 1965;23:135–46.
19. Fredén H, Helldén L, Milleding P. Mercury content in gingival tissues adjacent to amalgam fillings. Odont Rev 1974;25:207–10.
20. Söremark R, Wing K, Olsson K, Goldin J. Penetration of metallic ions from restorations into teeth. J Prosthet Dent 1968;20:531–40.
21. Wright DC, Gallant RF, Spangberg L. Correlation of corrosion behaviour and cytotoxicity in Au–Cu–Ag ternary alloys. J Biomed Mater Res 1982; 16:509–17.
22. Yli-Urpo A, Parvinen T. Metal degradation and tissue accumulation following subcutaneous implantation of combinations of materials. Proc Finn Dent Soc 1980;76:124–8.
23. Fédération Dentaire Internationale. Recommended standard practises for biological evaluation of dental materials. Int Dent J 1980;30:140–188.
24. Kallus T, Eklund G. Instrumentation for preparation and placement of subcutaneous implants. J Biomed Mater Res 1983;17:735–40.
25. Kallus T. Evaluation of the toxicity of denture base polymers after subcutaneous implantation in guinea pigs. J Prosthet Dent 1984;52:126–34.
26. Kallus T, Hensten-Pettersen A, Mjör IA. Tissue response to allergenic leachables from dental materials. Biomed Mater Res 1983;17:741–55.
27. Marion L, Haugen E, Mjör IA. Methodological assessments of subcutaneous implantation techniques. Biomed Mater Res 1980;14:343–57.
28. Olsson B, Sliwkowski A, Langeland K. Subcutaneous implantation for the biological evaluation of endodontic materials. J Endod 1981;7:355–69.
29. Bessing C, Bergman M. Metallographic characterization of four alternative alloys intended for fixed prostheses. Acta Odontol Scand 1986;44:101–12.
30. Bessing C, Bergman M, Thorén A. Potentiodynamic polarization analysis of low-gold and silver-palladium alloys and one high-gold alloy in three different media. Dental Mater (in press).
31. Browning E. Toxicity of industrial metals. London: Butterworths, 1969;164–8.
32. Fowler BA. Indium. In: Friberg L, Nordberg GF, Vouk VB, eds. Handbook on the toxicology of metals. Amsterdam: Elsevier/North-Holland Biomedical Press, 1979;429–34.
33. Gniewek J, Pezy J, Bater BG, Bockris JO'M. The effect of noble metal additions upon the corrosion of copper: an Auger-spectroscopic study. J Electrochem Soc 1978;125:17–23.
34. Mjör IA, Hensten-Pettersen A, Skogedal O. Biologic evaluation of filling materials. A comparison of results using cell culture techniques, implantation tests and pulp studies. Int Dent J 1977;27:124–9.
35. Langeland K. Correlation of screening tests to usage tests. J Endod 1978;4:300–3.

Received for publication 1 September 1986