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COMPARISON BETWEEN BIOLOGICAL ASSAYS AND THE FERROXYL TEST FOR STUDYING CORROSION OF EXPERIMENTAL METAL IMPLANTS

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Since the use of metal (plates, nails, screws and wires) in surgery attempts have been made to devise physical methods and chemical tests comparable to biological assays, which are very time-consuming. Long-term biological tests are, of course, decisive in the evaluation of the merits and limitations of a given sort of metal. But in an *in vitro* experiment various details can be studied under controlled conditions, e. g. corrosion tendency of various metallic implants. It is also comparatively easy to make large series of experiments.

The simplest types of stainless steels are Cr-Fe alloys, characterized by a chromium content of at least 12 %. When exposed to the air or acid solutions these steels are "passive", *i. e.* the surface is covered with a thin oxide coating invisible to the naked eye,[1] which increases the resistance of the alloy to corrosion. To be properly effective, however, the oxide film must form on a metallically clean surface, for which reason any scales on the surface due to manufacturing process must be removed by chemical or mechanical means.

Stresses in the surface of the steel caused by vigorous mechanical working also reduce the chemical resistance.

Observations with electron microscopy have made it probable that the passive film consists in part of bristle-like crystals of Cr_2O_3. A condition necessary for making the steel surface entirely passive is that the spaces between these crystals be filled with absorbed oxygen.

These simple chromium steels, formerly widely used for surgical purposes, are far surpassed by the austenitic chromium-nickel steels which are still less corrosive, particularly if the latter are alloyed with molybdenum.

From a medical point of view the following types of corrosion should be considered:

1. Pitting.
2. Crevice or gap corrosion.
3. Genuine galvanic corrosion ("Bimetallic element").
4. Stress corrosion.

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[1] The thickness of the passive film is 300—400 Å according to MAHLA and NIELSEN 1948.

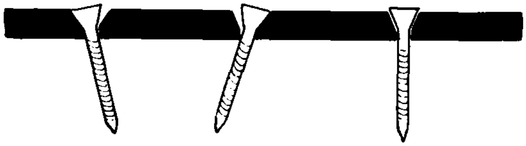

Fig. 1. Minute gaps are often formed between screw head and plate or flange.

No conditions exist in biologic tissues for the other types of corrosion which will, consequently, not be dwelt on here. (BERG 1958.)

1. Pitting is a corrosion attack, which is limited to local spots, and which occurs above all, in the presence of chloride solution. The attack is supposed to take place in two steps. In the first step weak points in the passive film are penetrated by chloride ions. The active points formed in this way are then deepened electrochemically, during which process the active point is the anode which delivers Fe-ions to the solution, while the passive surface serves as the cathode. Since the cathode surface is much larger than the anode surface, there will be a high current density at the point of attack, which results in a deep, local attack of corrosion.

Under the conditions existing in the human body — about 37.5° C and a Cl-ion content corresponding to about 0.9 % NaCl — austenitic steel without molybdenum will be rather soon attacked by pitting. However, if the steel is alloyed with about 2.7 % molybdenum (the nickel content is raised at the same time in order to maintain the austenitic structure) the resistance to corrosion will be higher. A steel of this kind and consisting of approximately 18 % Cr, 11 % Ni and 2.7 % Mo, is Firth Vickers grade FMB, AISI 316 and Avesta Jernverk's grade 832 SK.

2. Crevice corrosion might occur in narrow spaces between steel surfaces or between the surfaces of steel and other materials. In these gaps the steel undergoes corrosion to a greater extent than elsewhere. It is supposed that the oxygen content in such spaces is below the value required for maintaining the passive film. (The electrolyte concentration of the solution might also be raised owing to stagnation in such spaces, a fact that causes increased corrosion.)

Crevice corrosion must be considered in the field of surgery. The use of plates in connection with screws and nails almost always involves the formation of minute gaps, such as between a screw head and a flange or a plate. It is difficult to achieve complete contact over the whole circumference (fig. 1).

3. Genuine galvanic corrosion occurs when two different metals or alloys immersed in an electrolyte are brought into contact with each other. The electromotive force generated, and thereby the rate of corrosion, varies

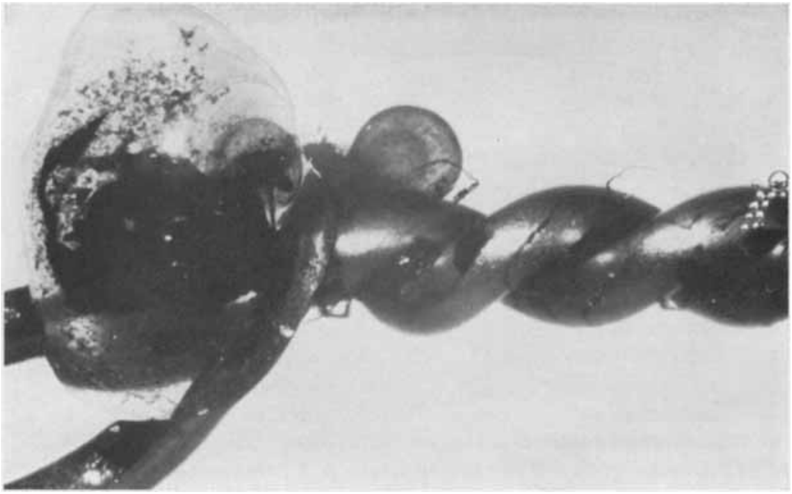

Fig. 2. Cold worked 18—8-steel in the form of cerclage. (B according to table 2.) Positive ferroxyl test. This picture was taken after 8 hours exposure to the ferroxyl gel. The colour of the bubbles is intense blue (Turnbull blue.) Magn. 10.

with the relative positions of the metals or alloys in the series of electrochemical potentials. The less precious component, *i. e.* the one which has a higher position in the series, will be the anode of the galvanic element and is, consequently, attacked by corrosion.

Table 1 presents a galvanic series where the metals and alloys have been put together in certain groups. Although the boundary-lines between them are rather diffuse, it could be established that, in general, metals and alloys within one and the same group can be brought into contact with each other without any great risk, provided the electrolyte present is not highly dissociated.

In this connection it should, however, be pointed out that the passive alloys 38—44 are not passive in all solutions. In a physiological NaCl-solution as well as in the body fluids these alloys are quickly activated, for which reason groups 38—44 must be transferred to groups 8—13, and, consequently, cannot be connected with vitallium, for example. The longer the distance between the metals or the alloys in the series, the greater is the risk of galvanic corrosion. The "passive surface" is a secondary protection against this primary risk.

A factor to be considered is also the size of the surface of the two electrodes in a supposed galvanic element. If the surface of the anode is

TABLE 1.

Galvanic series for different metals and alloys.

1. Magnesium alloys
2. Zinc
3. Galvanized steel and iron

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4. Aluminium, pure, and Al without copper
5. Aluminium of compound type with anodic coating
6. Cadmium alloys
7. Aluminium vith copper

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8. Carbon steel
9. Steel with an addition of copper
10. Cast iron
11. Chromium steel 4—6 % Cr, active
12. *Chromium steel 12—30 % Cr, active*
13. Ni alloyed cast iron

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14. *18—8 stainless steel, active*
15. *18—8 stainless steel with 3 % Mo, active*
16. Tinman's solder (lead + tin)
17. Lead
18. Tin
19. Muntz metal
20. Manganese bronze
21. Tolrin bronze
22. Marine bronze
23. Phosphor bronze

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24. Nickel, active
25. Inconel (80 % Ni, 5 % Fe, 15 % Cr), active
26. Titanium, active
27. 80—20 Ni-Cr alloy
28. Brass
29. Admiralty brass
30. Aluminium brass
31. Tombac
32. Nickel silver
33. Copper
34. Copper-nickel alloy
35. Siliceous bronze
36. Monel metal

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37. Silver solder
38. Nickel, passive
39. Inconel, passive
40. 80—20 Ni-Cr alloy, passive
41. *Chromium steel (12—18 % Cr), passive*
42. *18—8 stainless steel, passive*
43. *Chromium steel (23—30 % Cr), passive*
44. *18—8 stainless steel with 3 % Mo, passive*
45. *Vitallium*

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46. Graphite
47. Amalgam
48. Quicksilver
49. Gold
50. Titanium, passive
51. Platinum

very small as compared with that of the cathode, the current density of the former, and thus also the rate of corrosion will be high. If the surface is very large, the anode will be slowly corroded. The cathode is always protected from corrosion.

4. Stress corrosion. An example of this is the influence of cold-working. On cerclage or other types of cold-working local "depassivation" of the surface of the material can occur, *i.e.* the protective passive layer will

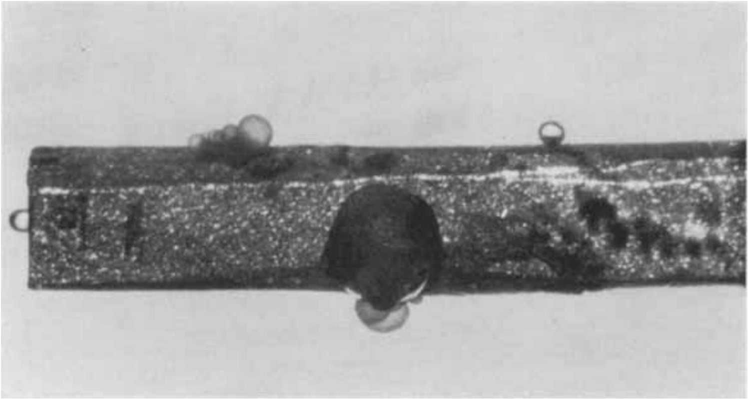


Fig. 3. Soft vitallium. (E according to table 2.) Positive ferroxyle test. This picture was taken after 8 hours exposure to the ferroxyl gel. The colour of the bubbles is dark brown-grey. Magn. 10.

be broken mechanically, and a local element can appear between the surrounding passive surface and the active surface. Therefore, the use of this process increases the risk of corrosion.

Cobalt alloys of the Stellite-type

The cobalt alloys of most interest from a surgical point of view are the stellites (Co, Cr), vitallium (Co, Cr, Mo) and neutrilium (Co, Cr, Ni, W).

The present investigation is concerned only with vitallium. In these experiments also so-called soft vitallium Co, Cr, Ni, Mo was studied. It has been used in dentistry, but never in osteosynthetic materials.

Vitallium must be cast and cannot be worked. It is rather brittle, and according to BECHTOL *et al.*, it will not tolerate cold treatment like stainless steel. It does not corrode so readily as stainless steel. The good anticorrosion properties of vitallium are probably ascribable to the formation of chromate on the surface. BECHTOL *et al.* further suggest that vitallium is sensitive to cathodic depolarisation. High local oxygen content can be dangerous. When coupled in galvanic series with stainless steels vitallium will obviously be the cathode and be protected, while the steel will be the anode and site of corrosion.

PETERSEN & EMNÉUS (1960) have focused attention on these problems and have shown that the so-called ferroxyl test is

Fig. 4. Soft and hard vitallium spot welded together. (D+E according to table 2.) Positive ferroxyl test. This picture was taken after 24 hours exposure to the ferroxyl gel. The colour of the bubbles is dark brown with a violet tint. (This may represent galvanic or crevice corrosion.) Magn. 10.

useful in the investigation of the corrosion of alloys containing iron and also cobalt and nickel.

The method, which is simple, is outlined below.

To a 0.9 % solution of NaCl is added potassium ferricyanide and gelatin. With ferricyanide ionised iron forms Turnbull blue; with nickel, a yellow brown precipitate and with Co a black brown violet precipitation.

The composition of the indicator gel, which is heated to 40° C, is as follows:

NaCl 0.9 % in distilled water	1000 g
Gelatin	50 g (5 %)
Potassium ferricyanide	20 g (2 %)

A number of alloys and elements of surgical interest were tested by EMNÉUS & STENRAM (1960) on chickens. The pieces of stainless steel and cobalt alloys, which were implanted in

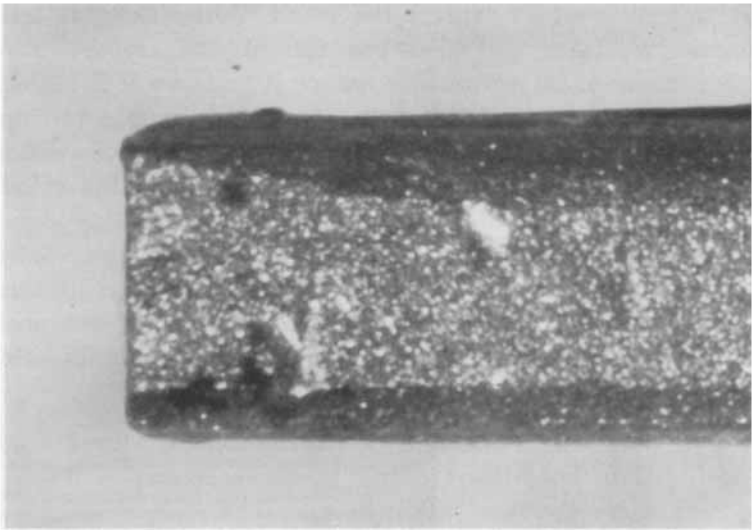


Fig. 5. Soft vitallium. (E according to table 2.) Negative ferroxyl test. The colour of the small spots is dark or blue. Magn. 20.

the animals, were tested with ferroxyl gel to ascertain any correlation between the results of the corrosion test and of the biologic test.

The metal implants removed from the animals at autopsy were taken out *en bloc* with the surrounding tissue and fixed in the conventional way. When the specimen was afterwards embedded the metal implants were removed. They were thoroughly cleaned in soda solution and then in ether.

They were then placed separately in ferroxyl gel and studied for the first few hours, also photographically. The metal implants were covered with ferroxyl gel in transparent plastic bags so that they could be inspected from all angles. Broadly speaking, colour indication, a sign of anodic corrosion, could be observed already after a few minutes or within 1 hour. This applies to the stainless steels. As pointed out by PETERSEN & EMNÉUS, demonstration of cobalt from the cobalt alloys requires a longer time.

It was difficult to express the result of the ferroxyl tests numerically. Once corrosion has started, the reaction proceeds because of its provocative nature (PETERSEN & EMNÉUS): therefore if the test is positive, there will be a strong development of colour within about a day. The tendency to corrosion can be fairly well judged from the rate at which the colour develops.

In the present investigation the results are therefore graded according to the time necessary for the colour (*i. e.* at least one anodic point) to develop. Sometimes the result was positive already within a minute or so, in others not until after one hour or more. Three grades were distinguished:

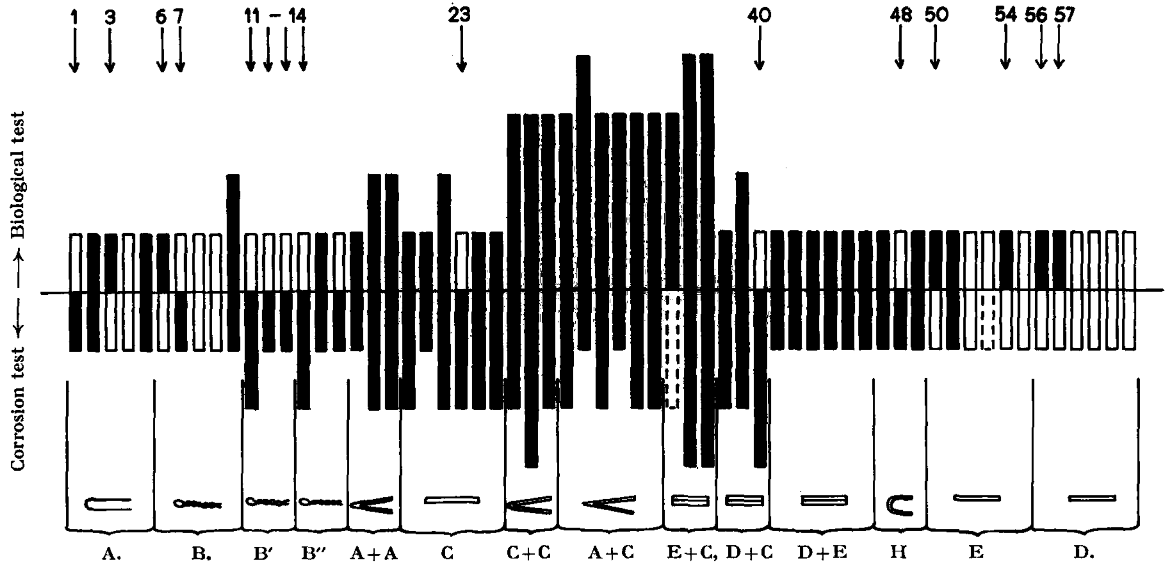
+ only slight reaction during first hour, strong reaction first after 1 day
++ clear reaction within 1 hour
+++ clear reaction within some minutes, strong reaction after 1 hour.

In table 2 only the amount of pigment in the tissue around the implants has been taken from the investigation of EMNÉUS & STENRAM. Attempts to grade the reaction according to fibrosis or amount of lymphocytes with the corrosion test would be inappropriate. In this connection it should be pointed out that the corrosion test is only a supplementary test and cannot replace the biologic test.

Turnbull blue positive pigment can, however, be regarded as a measure of corrosion of iron-containing alloys since the animals were allowed to survive for 3 months. Ionised iron from decomposed blood pigments can be neglected, since the traumata used in the present investigation were small and the animals were not killed until 3 months after insertion of the implants.

Turnbull negative pigment can *probably* be regarded as a manifestation of corrosion of non-ferrous alloys, since none was found around the iron-containing alloys or in the surroundings of the platinum implants. The products of corrosion around the cobalt alloys in the ferroxyl test were analysed

TABLE 2.



A. Sandviken 2R2. 18 % Cr and 9 % Ni, the rest Fe.
B. Sandviken 2R2, coldworked, "Cerclage", four twists.
C. Avesta 249 (Chromium steel, 17.5 % Cr).
D. Hard vitallium (65 % Co, 30 % Cr, 5 % Mo). Dental AB — Austenal.
E. Soft vitallium (52 % Co, 19 % Cr, 28 % Ni, 0.5 % Mo). Dental AB — Austenal.
H. Contracid (61 % Ni, 15 % Cr, 19.5 % Fe, 2.5 % Mo, 2 % Mn). Haereus, Hanau.

B′ After coldworking the steel was pickled.
B″ After coldworking the steel was annealed and pickled.
A+A or C+C means that the two pieces were spot welded together. The spot welding process took only a fraction of a second. This was the only simple way to unite the pieces. It appears that this welding process *per se* will not influence the corrosion resistance. After *spot welding* the *implants* of steel were pickled and those of vitallium and contracid sand blasted.
Column with *interrupted* outline indicates specimen lost.

X-ray-spectrographically by Bæcklund of Avesta Jernverk's roentgen spectrographic laboratory and were found to consist of Co-, Cr- and Ni-salts.

The metal pigment in the granulation tissue around a metal implant must be regarded as a sort of summation effect from corrosion that has proceeded during the most of the experimental period. Corrosion can, for example, have occurred in the main in the beginning of the experimental period and then ceased, and part of the metal pigment can have been reabsorbed by the time the animal was killed. On the other hand, corrosion might have started late and be most active at the very time the experiment was terminated.

A corrosion test of the ferroxyl type is a short-term test, which means that the iron or cobolt ions are ionised with a certain intensity (fairly rapidly). It should therefore be recollected what was said about gradation, *i. e.* the results should be graded according to the time necessary for the colour to develop.

The metal implants had been in the animals for 3 months, during which they were exposed to NaCl, which is a very strong corrosive. In those cases where both the biologic test and the *in vitro* corrosion test were negative there was strong reason to suppose that the metal or alloy is resistant to corrosion. If the result of the biologic test was uncertain but the *in vitro* test positive, one might assume that the environments in the animal were less corrosive than in the ferroxyl test. How a positive biologic test in association with a negative *in vitro* test should be interpreted is discussed (see Discussion). It should be stressed that the biologic environment is not constant but continuously changing.

The investigation of Emnéus & Stenram also included testing of titanium and platinum. However, there is no simple corrosiontest available for titanium.

Inspection of table 2 will show a certain general agreement between the results of the biologic test and of the corrosion test. In 43 of the 61 tests there was complete agreement.

In 41 the biologic test was positive.

In 44 the ferroxyl test was positive.

In 6 the biologic test was positive and the ferroxyl test negative.

In 10 the ferroxyl test was positive and the biologic test negative.

DISCUSSION

Validity of test

In the discussion of discrepances between the two tests the provocative properties of the ferroxyl test should be borne in mind.

If corrosion has begun at a given site, it will tend to be encapsulated by formation of a ferricyanide membrane with an isolated corrosion with a progressive tendency as a result. A specific ionic environment, which promotes corrosion, is maintained and increases.

This applies to implants 1, 7, 11, 12, 13, 14, 16, 23, 40, in table 2. Concerning implant 48 one can only say that contracid is an alloy whose behaviour in the biologic test and in the ferroxyl test is not known sufficiently to warrant commentation.

In addition it should be remembered that the biologic test is based on histologic sections taken from two levels only (see EMNÉUS & STENRAM). Consequently pigment may have been present but not at the very levels studied.

In those 10 cases where the biologic test was negative and the ferroxyl test positive 7 consisted of 18—8-steel, 1 of chromium steel, 1 of contracid, and 1 of hard vitallium and chromium steel in contact.

Concerning the so-called deposition

When working 18—8-steel, particles of metal from the tools may be transferred to it (BOWDEN, WILLIAMSON and LAING 1955). If only the metal transferred goes into solution but does not start any corrosion, it will probably be dissolved within 3 months and then the ferroxyl test will be negative.

In addition, it must be remembered that ionisation of iron from haemoglobin can occur. It appears, however, less probable that any such iron can persist after 3 months (EMNÉUS & STENRAM).

This applies in particular to implant (Nos. 3 and 6 in table 2).

It appears likely that the ferroxyl test used on cobalt alloys is provocative but experience with these metals is still limited. Judging from Nos. 50, 54, 56 and 57 it would not be so.

It should, however, be observed that as far as the two cases (50, 54) are concerned, in the ferroxyl test of soft vitallium a faint colour is sometimes seen under the binocular microscope which might very well be due to nickel or cobalt. Fig. 5.

Since experience with this alloy is limited, it was never interpreted as positive test. Only when the colour was seen with the naked eye was the test said to be positive. Fig. 3.

One cannot expect Co- and Ni-ferricyanide to be as good an indicator as Fe-ferricyanid (Turnbull-blue). Furthermore it is clear that if the passive film of an alloy of stellite-type is damaged, the dissolution of Co will take place much slower than the dissolution of Fe in activated Cr-Ni-steel.

CONCLUSIONS

The ferroxyl test can rapidly demonstrate the presence of ionisation of iron and cobolt alloys. When corrosion is considerable, there is almost complete agreement between the results of the biologic test and the ferroxyl test.

When corrosion is slight, the ferroxyl test appears to be more sensitive, at least regarding stainless steel. This provocative tendency has not been shown to hold for cobalt alloys.

Since most of the alloys used in surgery contain iron (stainless steel) or cobalt (vitallium), the ferroxyl test is useful for studying details of the tendency to corrosion.

X-ray-spectrographic analysis of the corrosion products from cobalt alloys in the ferroxyl test showed that they contain a fair amount of cobalt, which stains brown-black-violet.

EMNÉUS & STENRAM assumed that the so-called Turnbull negative pigment found in the tissues around soft vitallium implants consists of cobalt. The results obtained in the present investigation confirmed this assumption.

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