

AN X-RAY SPECTROGRAPHIC INVESTIGATION OF THE SOFT TISSUE AROUND TITANIUM AND COBALT ALLOY IMPLANTS

By

HANS EMNÉUS, UNNE STENRAM and JOHANNES BAECKLUND

Emnéus & Stenram (1960) studied the reaction of soft tissue around metal implants in chickens. Histologic sections often showed pigment around the implants. This pigment may have come from the implants. If so the pigment and the metal implants must contain the same element. In some cases the pigment was Turnbull positive, which is regarded as evidence of ionized iron. In the Turnbull reaction potassium ferricyanide reacts with ferro ions with precipitation of ferroferricyanide, so-called Turnbull blue. It should, however, be observed that the iron demonstrated need not emanate from the metallic implant but may be derived from the body e.g. decomposed haemoglobin. Turnbull negative pigment was found around titanium and cobalt alloy implants. It is difficult to ascertain the metal component, if any, of such pigment, though it is known that cobalt ions react with potassium ferricyanide to give a dark brown precipitate with a violet hue, while nickel ions give a yellow brown precipitate (Petersen & Emnéus, 1960). Chromium, molybden and titanium give no precipitate with ferricyanide. Further analysis of the Turnbull negative pigment was therefore considered desirable. Microradiography with the apparatus available failed. Roentgenspectrographic analysis of tissue sections and slices, however, proved successful. The positive and some representative negative results are reported below.

The literature on the solubility of vitallium and titanium in living tissue is scanty. Using spectrochemical analysis Gillieson (1955) showed the presence of cobalt and chromium in granulation tissue around some vitallium implants in human tissue. While the present paper was being

prepared for the press, Ferguson, Laing & Hodge (1960) published an investigation in which they demonstrated by spectrochemical methods titanium, zirconium, nickel, cobalt, chromium, molybden and iron in the tissue around metallic implants containing these elements. They gave no account of any histologic examinations.

MATERIAL AND METHODS

The x-ray spectrograph used in the experiments was of commercial type and has been described in detail by Parrish & Engström (1956) and Bunn & Parrish (1956).

The instrument consists of an x-ray source, which irradiates the object with white radiation, *i.e.* radiation distributed over a wide range of wave lengths, a spectrograph, which separates the rays retransmitted from the measuring object into components of different wave lengths and a registering apparatus, which measures the intensity of one component at a time from the spectrograph.

The white rays which impinge upon the object are largely absorbed by the latter and cause a certain amount of fluorescent radiation. This contains x-rays characteristic of the elements present in the sample, and the radiation is divided up into lines by the spectrograph. The wave-lengths of the lines are characteristic of the element which they belong to, and, since the wave-length can be determined by measurement of the angle in the spectrograph, different elements can be identified in this way. The intensity of a certain line is dependent on the content of the element in the object but is also influenced by the thickness of the specimen if it is thin, and by the presence of heavy elements in the sample. Those elements absorb part of the fluorescent radiation.

In the experiments the roentgen ray source consisted of a roentgen tube with gold anode fed from a well-stabilized high voltage generator with 54 kV and 30 mA direct current. The roentgen tube had been checked in previous experiments with analytical objects with high x-ray scattering coefficients (water, plastics) and the radiation had been examined for characteristic lines. Gold and copper lines were found in addition to the continuous white background. The copper line was, however, very weak.

The grating of the spectrograph consisted of a ground lithium fluoride crystal with $2d = 4.028$. A series of measurements were made with repeated adjustments of the spectrograph setting to pass the angle of the metal line sought, in order to register *background and line* with

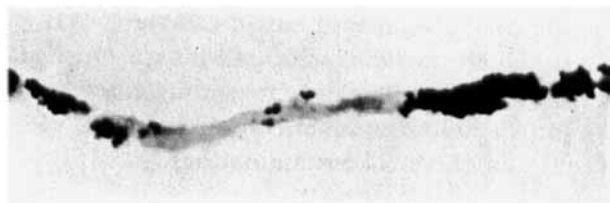


Fig. 1.

A piece of thin connective tissue near a buried titanium rod. Turnbull negative pigment in phagocytes. A non-stained section was analyzed with the x-ray spectrograph. See table 1 and diagram 1. Magn. $\times 760$.

good accuracy. The intensity was measured with a scintillation detector and electronic scaler. This detector absorbs roentgen rays in a sodium iodide crystal which on absorption of the rays, gives off a light flash. This flash is received by an extremely sensitive photocell, a photomultiplier, and is counted by the electronic counter as an impulse. If the roentgen radiation is intense many flashes per unit of time will be noted. Because the roentgen radiation has the character of quantum radiation the measuring process has a certain statistical uncertainty and, to decrease this, a large number of quanta must be recorded. If the radiation is weak, the measuring time must be long. In these experiments about 12,000 impulses were recorded, which corresponds to a standard deviation in the measurement of intensity of about 1 per cent. It is this statistical uncertainty and accordingly the intensities and measuring times that decide the maximum sensitivity of the spectrograph in an investigation of this type. The experiments were carried out in the following two ways.

1. $7\ \mu$ thick paraffin sections from the material accounted for previously (Emnéus & Stenram, 1960) were placed in the receptacle of the spectrograph on a thin film of supporting nylon. Some of these sections were adjacent to such sections containing a fairly large amount of pigment concentrated in a small area (Fig. 1).

2. Whole paraffin-embedded pieces of tissue were cleaned of paraffin by means of warm, distilled water and then placed in the receptacle in the same way.

RESULTS

Investigation on titanium.

Titanium could be demonstrated in $7\ \mu$ thick sections from the 2 experiments where considerable amount of black, Turnbull-negative pig-

ment had been observed histologically (Emnéus and Stenram, 1960, exp. no. 16) (see tables 1 and 2). When paraffin was removed and the rest of the tissue examined *en bloc*, titanium could not be demonstrated (table 3) in 16 other experiments (Emnéus and Stenram, 1960, exp. no. 16 and 17).

TABLE 1

Anode material: gold. Voltage 54 kV. Current 30 mA. Scintillation detector 1250 V. LiF-crystal. Spectrograph slit 14 mm. Grid no. 2. Division factor 4. Spectrograph evacuated. Damping of amplifier 34 dB. Sectioned tissue from animal 281. See diagr. 1.

2 θ	Imp./30 sec.	2 θ	Imp./30 sec.
85.00	2830	86.00	3080
20	2780	10	2980
40	2820	20	2920
60	2860	50	2830
80	2970		
90	3020		
95	3100		

Standard deviation ± 30 impulses/30 sec.

The maximum of the Ti-line is at 85.95° in our spectrograph and no disturbing lines appear in the neighbourhood. It is therefore concluded that titanium is present in the sample. See diagram 1.

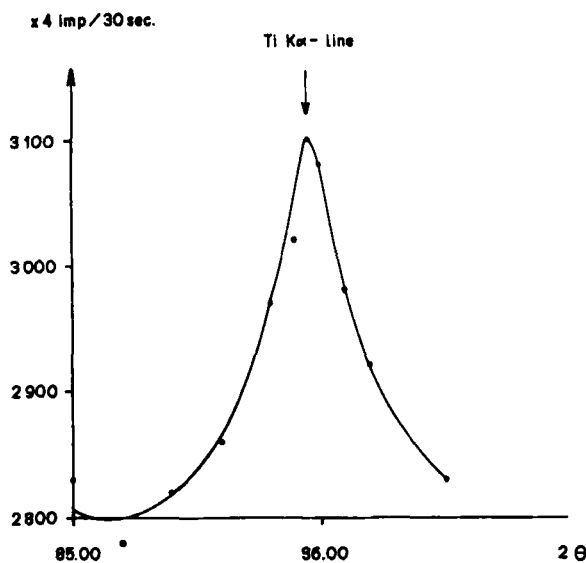


Diagram 1.

TABLE 2

Experimental conditions as in Table 1, except that the measurements were made by counting 40,000 impulses each time and by registration of the time necessary for this count. Sectioned tissue from animal 27.

2θ	Time in sec. for 40,000 imp.	Imp./s. (aprox.)
85.60	155.46	260
70	137.26	290
80	114.60	350
90	97.89	410
95	94.00	430
86.00	92.23	430
10	100.00	400
20	116.71	340

Standard deviation ± 0.5 sec.

In this case also the presence of titanium could be demonstrated. See diagram 2.

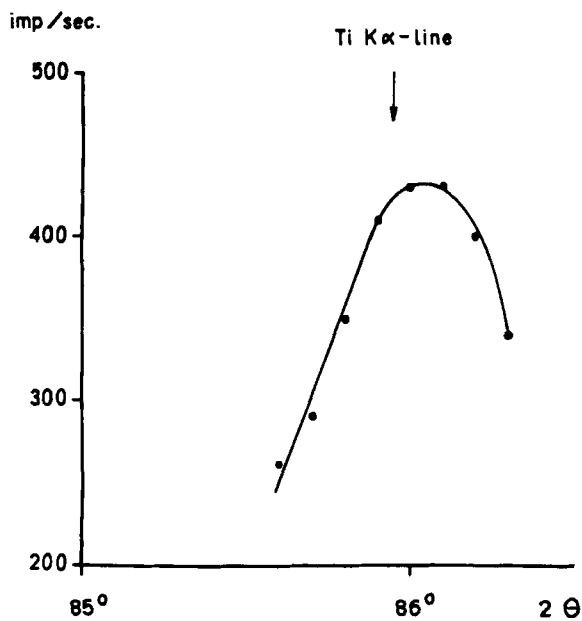


Diagram 2.

TABLE 3
Experimental conditions as in Table 1. Tissue not sectioned. Paraffin removed. Impulses/30 sec.

		Chicken (l = left, r = right).															
		27 l	28 r	29 r	29 l	30 r	30 l	31 r	31 l	32 r	32 l	33 l	34 r	35 l	36 l	37 l	38 l
20		2966	4027	2917	3173	3339	2394	3550	2790	3776	3611	3870	3571	3261	3404	2567	3469
85.80		2935	4082	2892	3140	3412	2423	3580	2782	3759	3604	3854	3578	3249	3389	2462	3493
85		3016	4074	2906	3151	3373	2379	3537	2832	3750	3645	3865	3510	3240	3426	3551	3509
90		2966	4022	2965	3139	3389	2399	3506	2817	3805	3556	3913	3554	3252	3455	2526	3454
86.00		2983	4064	2879	3185	3398	2364	3522	2837	3764	3564	3890	3619	3286	3439	2523	3469
05		3028	4034	2905	3139	3318	2365	3552	2837	3691	3520	3905	3510	3207	3423	2576	3457
10		3012	4044	2908	3081	3358	2394	3609	2911	3629	3565	3878	3516	3212	3441	2495	3525

Standard deviation $\pm$ 30 imp./sec.

In none of these cases was titanium demonstrable in the tissue. See diagram 3.

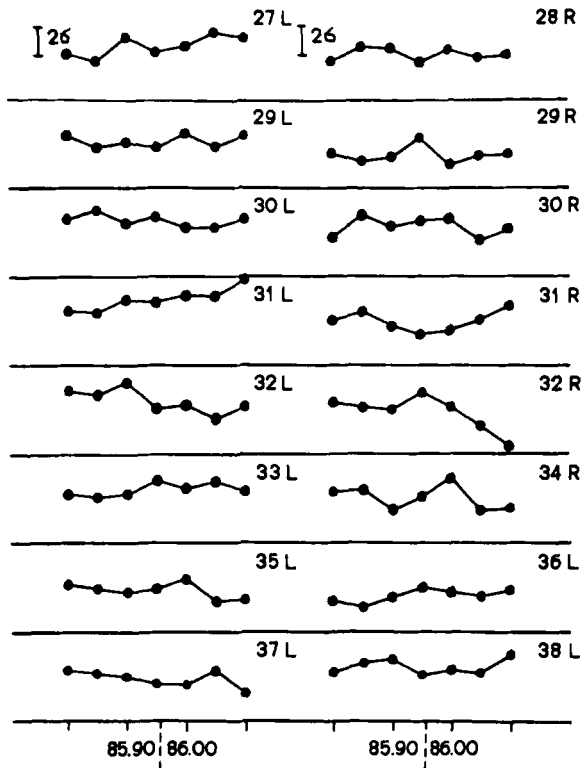


Diagram 3.

Investigation on cobalt.

Cobalt could not be demonstrated in $7\ \mu$ thick sections from 6 experiments (Emnéus and Stenram, 1960, exp. no. 13), although pigment had been histologically demonstrated in adjacent sections. When paraffin was removed and the rest of the tissue was taken as a whole, cobalt was demonstrable in 12 cases out of 18 experiments (Emnéus and Stenram, 1960, exp. no. 11–15) (see further Discussion).

TABLE 4

Anode material: gold. Tube voltage 54 kV. Current 30 mA. Scintillation detector 1250 V. LiF-crystal. Slit 14 mm. Grid. no. 2. Division factor 4. Spectrograph evacuated. Damping 36 dB. Tissue not sectioned. Paraffin removed. Measuring time 60 sec. The maximum of the Co K α -line is at 52.65° Standard dev. $\pm$ 30 imp.

2 θ	15 R	16 L	16 R	17 R	18 R	19 R
52.50	3270	2774	2373	3879	3176	1860
55	3347	2789	2403	4008	3210	1901
60	3424	2808	2422	4108	3256	1883
65	3429	2791	2443	4179	3279	1912
70	3467	2838	2423	4184	3257	1876
75	3432	2788	2445	4123	3246	1862
80	3391	2760	2352	4118	3242	1874
90	3284	2748	2328	3816	3170	1805

In these 6 cases cobalt was demonstrable. See diagram 4.

TABLE 5*

Anode material: W. Tube voltage 54 kV. Current 13 mA. Scintillation detector 1250 V. LiF-crystal. Slit 14 mm. Grid no. 2. Division factor 4. Spectrograph evacuated. Damping 36 dB. Tissue not sectioned. Paraffin removed. Measuring time 20 sec. The maximum of the Co K α -line is at 52.81°. Standard dev. $\pm$ 60 imp.

2 θ	17 L	20 R	21 L	22 L	23 L	26 L
52.50	1244	1106	1371	1555	1619	1019
55	1256	1151	1441	1589	1743	1000
60	1351	1200	1481	1580	1624	1056
65	1558	1281	1478	1703	1682	1123
70	1552	1351	1476	1863	1771	1171
75	1585	1464	1529	1885	1883	1162
80	1568	1446	1521	1979	1804	1349
85	1625	1488	1614	1965	1896	1373
90	1468	1408	1567	1949	1975	1390
95	1342	1314	1548	1896	1921	1326
53.00	1262	1197	1470	1714	1849	1245
05	1082	1116	1277	1632	1749	1196
10	1060	1047	1337	1596	1707	1115
15	985	985	1203	1528	1628	950

In these 6 cases also cobalt was demonstrable. See diagram 5.

* The spectrograph was changed between the analysis given in table 4 and 5.

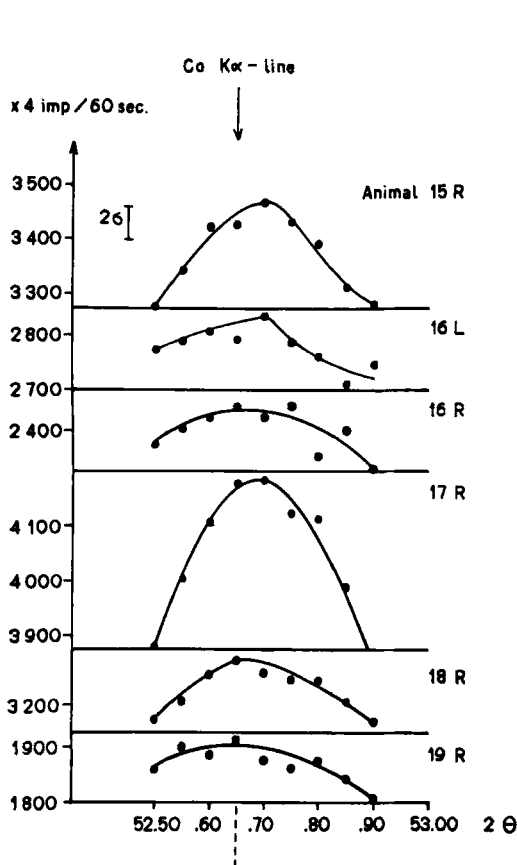


Diagram 4.

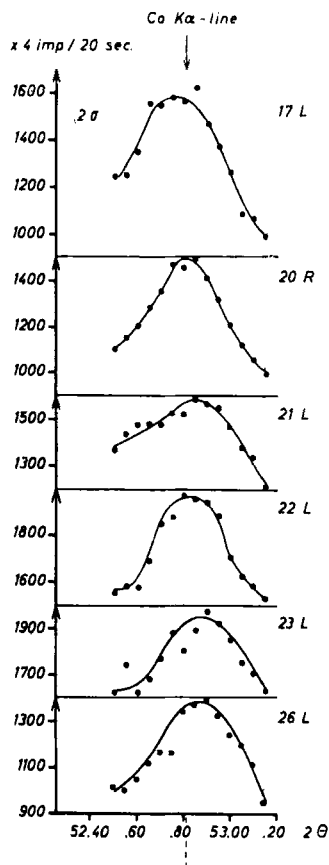


Diagram 5.

DISCUSSION

The x-ray spectrograph analysis of paraffin sections has thus shown that in 2 cases titanium was present in the tissue around the titanium implants. Therefore the black pigment demonstrated previously in adjacent sections by Emnéus & Stenram (1960) most likely consisted in part or entirely of titanium compounds, Fig. 1. Histologic sections showed black pigment also in 8 cases in which the x-ray-spectrographic analysis of the entire piece of tissue could not reveal the presence of titanium. It may be assumed that this pigment was also titanium, although it occurred in such small quantities that it could not be demonstrated by the technique used.

Cobalt was demonstrated spectrographically in 11 cases out of 12

where implants consisting of soft vitallium and pieces of soft and hard vitallium, welded together, has been deposited. In 9 of these cases previous histologic examination had shown a relatively large amount of pigment, which was dark brown after reaction with potassium ferricyanide, *i.e.* a colour obtained with cobalt ions. In the light of the x-ray-spectrographic analyses it therefore appears to be established that the pigment observed contains cobalt. Cobalt could be demonstrated x-ray-spectrographically in one case out of 6 (26 L, Table 5) where only hard vitallium had been deposited. In this case and one other the histologic examination showed dark brown pigment in Turnbull staining. It appears probable that this pigment also contains cobalt.

The investigation thus showed the presence of cobalt and titanium in tissue around some implants of cobalt alloy and titanium respectively. The elements demonstrated must emanate from the metallic implant.

Discussion took place whether the pigment observed might be a fragment torn off the rods (Emnéus & Stenram, 1960). The surface treatment applied, however, invalidates such an assumption. It is more reasonable to suppose that the pigment had arisen by electrolytic dissolution of the metal and had then been deposited in the tissue (see also Emnéus, *in press*).

S U M M A R Y

In a previous investigation Emnéus & Stenram (1960) demonstrated the appearance of Turnbull negative pigment in the soft tissue around titanium and cobalt alloy implants in chickens. In the present paper, an x-ray-spectrographic analysis is described, which revealed the presence of titanium and cobalt respectively in the tissue. It seems therefore established that the pigment contained these elements. It is assumed that the pigment had arisen by electrolytic dissolution of the metal.

R E S U M E

Dans une enquête précédente, Emnéus et Stenram (1960) ont démontré l'apparition de pigment Turnbull négatif dans le tissu mou autour des alliages de titane et de cobalt implantés chez les poulets. Dans la présente publication, il est rendu compte d'une analyse spectrographique aux rayons X qui révèle la présence respectivement de titane et de cobalt dans le tissu. C'est pourquoi il paraît établi que le

pigment contient ces éléments. Il est présumé que le pigment est produit par une dissolution électrolytique du métal.

ZUSAMMENFASSUNG

In einer vorausgehenden Untersuchung wiesen Emnéus und Stenram (1960) das Auftreten von Turnbull negativem Pigment in dem Weichteilgewebe nach, das Einplantungen von Titanium und Kobaltlegierungen bei Hühnern umgab. In der vorliegenden Arbeit wird über eine röntgenologisch-spectrographische Analyse berichtet, die das Vorhandensein von Titanium und Kobalt im Gewebe aufgezeigt hat. Es scheint daher festgestellt zu sein, dass das Pigment diese Elemente enthielt. Man nimmt an, dass das Pigment durch Elektrolyse des Metalls entstanden ist.

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