

## REACTION OF TISSUES TO ALLOYS USED IN OSTEOSYNTHESIS

*Experimental, histological examination of reaction of soft  
tissues in animals.*

*By*

HANS EMNÉUS and UNNE STENRAM

Sometimes the irritative effect of metallic substances used in osteosynthesis is apt to spoil the end-result of an otherwise successful operation. Until the 1930's surgeons appeared to be little interested in the composition of the plates, screws and nails used for osteosynthesis. They contended themselves with what was offered by the metallurgists. A brief review of the metals employed is given below.

In 1911 *Strauss & Mauer* of the laboratories of the Krupp-Works applied for a patent for a Cr-Ni-steel alloy, which had fortuitously been found to be very resistant to corrosion. This alloy was improved upon and when placed on the market its approximate composition was 18 % Cr, 8 % Ni, and the rest Fe, so-called 18-8 steel. In 1913 *Brearley* in England discovered the high resistance of pure Cr-steel to oxidation, and in 1915 he applied for a patent for this composition. Most of the Cr-steels afterwards contained about 13-14 % Cr. In 1922 *Strauss* of the Krupp-Works found an addition of 2-4 % Mo to increase the resistance of the Cr-Ni-steels to acids and the Krupp-Works applied for a patent the same year. It was soon discovered that all steels could be made more resistant to corrosion by keeping the C-content below 0.07 %. Owing to technical difficulties in the manufacture of such alloys, however, it was not until some years later that they were manufactured on a large scale and thereby made available for a wide range of purposes. In the end of the 1920's Cr-Ni-Mo-steel was tried in the cellulose-industry. But it was not until the 1930's that the acid-fast,

stainless steel alloys could be made malleable and thereby suitable for a wide variety of purposes.

In Great Britain and the U.S.A. metallurgists focused interest on alloys for osteosynthesis as early as the end of the 1930's (*Venable & Stuck* 1938). In Great Britain all ferrous material for osteosynthesis has during recent years been of standard composition, namely 18 % Cr, 8 % Ni, 2.75 % Mo. During the last 2 or 3 decades vitallium (65 % Co, 30 % Cr and 5 % Mo) has also been used widely in bone surgery. Finally, the extremely non-corrosive element titanium has lately been introduced in surgery by *Leventhal* (1951, 1957).

The desirable qualities of the above-mentioned alloys, especially of the so-called stainless steels, are due not only to the low electro-affinity of the incorporated elements but also to the finish of the surface which reduces the affinity. It is believed that this procedure results in the formation of an extremely thin protective oxide film (*Kalling & Liljekvist* 1948). Stainless steel material for osteosynthesis is surface-treated by polishing and vitallium by scraping or sand-blasting. Pure Cr-steel has certain excellent physical properties, particularly malleability. As mentioned in the report of the "Metallnormkommittén" in 1948, however, it should not be used for purposes requiring highly non-corrosive material.

*Hjördis Jørgensen* (1941) made a detailed study of the irritative effect of osteosynthesis material on the tissues of animals. She found that the alloys, used in the first decade of the century, such as soft steel and bronze-aluminium, were much more irritating than the stainless 18-8-steel, which came into vogue in the 1930's. As spokesmen of the subcommittee for screws and plates *Venable & Stuck* (1948) presented a report on extensive investigations carried out in 1941-1947. According to their report, of the alloys fulfilling the requirements of a satisfactory material for the manufacture of screws and plates for surgical purposes, 18-8 Mo-steel and vitallium were the ones most widely used. *Bowden, Williamson & Laing* (1955), and *Laing* (1958) have shown that such screws and plates are apt to be damaged by the tools used for fastening them to the bone. Minute particles can be torn off and deposited on the surface of the plate or the screw. *Bowden et al.* found the surface of a plate damaged in this way to be the later site of corrosion in vitro and tried to explain the phenomenon on physical grounds. Such corrosion could also be demonstrated in animal experiments. *Clarke & Hickman* (1953) and *Hickman, Clarke & Jennings* (1958) found the irritative effect of an alloy on the tissues to decrease

with increasing back-electromotive force of the metal or alloy. At a symposium of the Section of Orthopaedic Surgery, Royal Society of Medicine, held in May 1957 (*Hicks 1957, McKee 1957, Zarek 1957*) conventional Cr-steel was condemned as a material for osteosynthesis. As pointed out above, the "Metallnormkommittén" in Sweden concluded that Cr-steel should not be used when high demands are placed on anti-corrosion properties. All of the investigations referred to above have shown that both 18-8-steel (especially 18-8-2.5) and vitallium are excellent materials for screws and plates. Vitallium is less irritative, but stainless steel is stronger.

In Sweden these British and American investigations appear to have passed unnoticed until recent years. We have therefore used imported and Swedish material as well as Cr-steel for osteosynthesis.

*Emnéus (1957)* and *Hicks (1958)* were able to demonstrate aseptic inflammation of the soft tissues around metals used for osteosynthesis. They interpreted this reaction as an electrolytic inflammation. This prompted *Emnéus & Petersen (1958)* to study the electrophysical properties of alloys used for osteosynthesis. They found among other things that the hardness of the vitallium plate varied from one part of the plate to another (range 32-18 Rc (Rockwell units)). Between such parts a difference of 0.15 to 0.20 V and of a few mA could be demonstrated. The parts were soon polarized, but could be readily depolarized by rinsing. It is tempting to assume that the potential difference was due to differences in hardness. In a vascularized, biological tissue the conditions present permit rinsing of the electrodes, which implies that the tissue may be irritated by an electrolytic process. *Emnéus & Petersen* also found that cold-drawn 18-8 steel, *i.e.* Cr-Ni-steel without Mo, has undesirable properties long known to metallurgists. In surgery the steel is cold-drawn on application of a cerclage (ring) when the twisted or screwed part of the cerclage becomes harder and electrolytically active in relation to the undrawn part (*Emnéus & Petersen 1958*).

#### BIOLOGICAL TESTS

One might imagine deposition of metal appliances in the living tissue to be able to cause the following types of irritation.

1. *Traumatic, operative effect.*--Surgical intervention may lead to the death of groups of cells with subsequent cicatrization. Fixation of the plates, screws or rings is also attended by traumatization varying in severity from case to case.

2. *Mechanical irritation.*—A screw might be passed right through a sharp bone so that its tip abuts a tendon or an articular surface. Independent of the composition of the screw, use of the limb will result in irritation of the tissue. The same situation arises if a screw or nail for osteosynthesis has lost its grip in the bone so that the fracture ends rub against one another. The screw will then wobble and press alternately against either side of the marrow cavity. The walls of the cavity atrophies with so-called pressure-atrophy as a result.

3. *Toxic irritation.*—If the body-fluids dissolve metal from the alloy and the products thus formed contain ions toxic to the tissue, irritation will result.

4. *Electrolytic inflammation.*—This is closely related to the toxic irritation, but the dissolution of the metals is due to differences in electrolytic pressures. For example, the presence of two metals in contact with each other results in electrolysis and consequent inflammation. However, electrolysis might also be due to differences in structure within one single piece of an alloy.

5. *Oligodynamic effect.*—The foreign substance, without going into solution, should exert an effect, possibly by acting as a catalyst, on the life of nearby cells.

6. *Cancerogenic effect.*—It is known that foils of certain metals and alloys, as gold, silver, platinum, vitallium and stainless steel can induce sarcoma in rats, mice and hamsters (*Schinz 1942, Oppenheimer, Danishefsky & Stout 1956, Alexander & Horning 1959, Kaplan 1959, and others*). No such effect has been seen in hens (*Oberling 1959*). Experience suggests that there is no such cancerogenic effect in man.

In any biological investigation of the irritative effect of different metals or alloys the above mentioned points must be considered. The significance of points 1 and 2 can be reduced appreciably by the use of a suitable technique. The oligodynamic effect, point 5, is not properly understood. The cancerogenic effect is, of course, an important problem, but it lies beyond the scope of the present investigation. In addition, it is probably of no significance to the present experiments described below for the following three reasons: the experimental animals were hens, the experimental period was short, and the piece of metal deposited was small.

The reactions of greatest interest in this investigation are toxic irritation and electrolytic inflammation. From a practical point of view it is of less importance what role is played by these two factors separately, the important thing is their total effect.

It is clear from the preceeding paragraphs that several materials used for osteosynthesis are unsuitable and can give rise to aseptic inflammation. The underlying mechanism of this undesired reaction is not properly understood, although some investigations suggest that it might be of electrolytic nature. *Below an account is given of the results obtained in an experimental histological study of the irritative effect of different metals and alloys on soft tissue in animals, a link in an investigation by one of us (H. E.) on the aseptic inflammation accompanying metal osteosynthesis.*

#### MATERIAL AND METHODS

With reference to what is said above under Biological Tests, chickens were selected as experimental animals. With the aid of a long, thin knife the test material was deposited in the musculature close to the bone on either side of the pecten carinatus. The conditions in this richly vascularized tissue provide possibilities for electrodes to be rinsed. The mechanical and traumatic operative irritation is minimal. The only imaginable equally good site in the rat or rabbit would be between processus spinosi and mm. erectores trunchi or between ala ossis ilei and mm. glutaeci. The possibility of producing operative traumata of varying severity with a varying amount of scar tissue would, however, be much greater by such a method. The disadvantage of fowls is that they probably differ biologically from man more than do small mammalia such as rats and mice. On the other hand, the cancerogenic effect in rats and mice might complicate interpretation of the findings.

The test material consisted of 1-2 cm. long rods or wires or, in some groups, rods welded together. The composition and appearance of the test material used in the different experimental groups are given in Tables 1 and 2. The test bits were made (H.E.) from commercially available material and under the guidance of docent Petersen at the department of Medical Physics of the Institute of Physiology, Lund. Surface treatment of the material was also done there. In some groups welded specimens were used. These test bits were prepared by spot-welding for less than 1 second, which implies that no gas was used and that the fragments were only stuck together. In view of the purpose of the experiment, this appeared to be the best method for uniting the bits of the metals.

As mentioned, a piece of test material was placed on either side of the carina so that every chicken represented two experiments. The number of experiments in each experimental group is given in Table 2. The test material was deposited in 12-week old chickens. The chickens were kept in open-air pens in the country. They appeared to do well and showed no symptoms of any side-effects of the operation. They were killed 3 months after deposition of the test material. The carina with surrounding musculature was removed. The test material was removed en bloc with the surrounding soft tissue and fixed in 10 per cent formol. The experimental material was then handed over to the other of us (U.S.).

The material deposited was carefully dissected from the surrounding, fixed tissue. The tissue was carefully inspected. Inspection with the naked eye sometimes revealed distinct changes in the form of rust-like lumps. These were always seen in

the angle between two spot-welded rods. They could not be dissected from the test-material without being separated from the rest of the tissue. This material was embedded separately in paraffin, sectioned and stained. In Table 2 this material is given under the heading "Gross lumps. Turnb. pos" (see also Figs. 7 and 8). The rest of the tissue round the material deposit could be dissected en bloc. The dissected material consisted of tissue fragments 2-3 cm. long, 1-2 cm. wide and ½-1 cm. thick. It was embedded in paraffin and sectioned at 2 different levels, *i.e.* at one end of the specimen and in the middle of the specimen, and in both cases at right angles to the longitudinal axis of the test material that had been removed. In some cases a reaction was observed in a small region with the naked eye, and then care was taken to include such change in the sections.

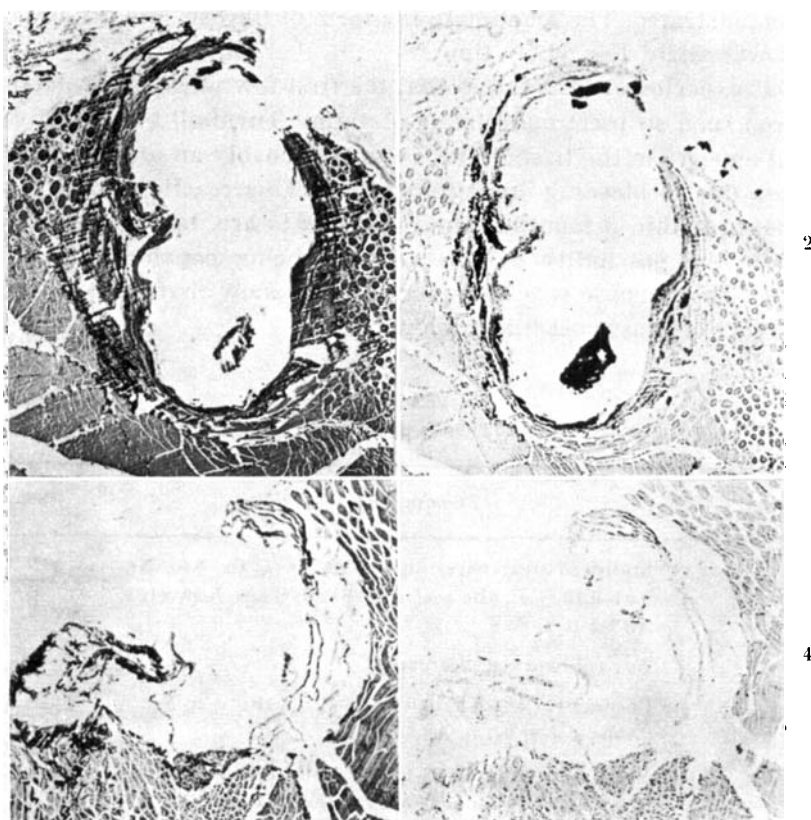
Sections from both levels were stained with Htx-eosin, Htx-van Gieson and according to the Turnbull blue-method for iron (Romeis, 1948, § 1204). Tests for formalin pigment were performed in some experiments according to Romeis, 1948, § 274. For each specimen, *i.e.* half of the animal, the results were noted in tabular form, in the same way as in Table 2, from 0 to + + + +. "Collagenous fibers" is to be understood as the amount of collagenous fibers, formed around the test-material. "Lymph. giant cells" gives the amount of lymphocytes and giant cells observed. The following columns give the amount of "pigment". Turnbull positive and negative, phagocytized and non-phagocytized, that was found on microscopic examination. The reaction was not always equally pronounced along the entire length of the test-material. This holds specially for the above mentioned lumps along the welded rods. In such cases only the most pronounced reaction was recorded in the table. The last column in Table 2 gives the macroscopically visible rust-like lumps (see above). All of them proved Turnbull-positive. The findings are illustrated in the Figs.

At the time of the histological examination the examiner was not aware of the type of material that had been deposited in the individual animals. Not until afterwards were the findings recorded in the experimental protocol and the results transferred to Table 2 which gives the mean reaction of each experimental group. In the table + (+) implies that the mean reaction for the group lay between + and + +. In none of the animals were the findings recorded as + (+), but only in whole units. The range of variation in each group was very narrow.

The following experiments not included in the tables were also performed. Platinum wires were deposited in the way described, but the animals were killed after 1, 2, 3, 4, 6, 7, 10, 11 and 12 weeks. One animal thus comprising two experiments was killed at each of the above intervals. The animal killed after 3 months is included in Table 2.

## RESULTS AND DISCUSSION

The experiments described in the last paragraph of Material and Methods gave the following results. After one week Turnbull-positive pigment occurred around the inserted material, a reaction that was quantitatively recorded as + + (cf. below and Figs. 1-5). This reaction diminished gradually with time. Between 6 and 11 weeks there was a very slight reaction in the form of a Turnbull-positive pigment in about half of the experiments. After 3 months no pigment at all could











*Figs. 1-4.*

*Fig. 1.* Animal from experiment 8. Two rods have been situated close together within the large space in the reproduction. The section passes through the site of the welded spot. Thick connective tissue bands with phagocytized material and round cells are seen around the space (see Fig. 5). The reaction in the form of collagenous fibers<sup>+++</sup>, lymph. giant cells<sup>+++</sup>, Turnbull-pos. material phagocytized<sup>+++</sup>, non-phagocytized<sup>++++</sup>. No other reactions. Htx - van Gieson.  $\times 145$ .—*Fig. 2.* Section adjacent that illustrated in Fig. 1 and stained according to the Turnbull blue method. The black substance around and in the space is Turnbull-positive.  $\times 145$ .—*Fig. 3.* Animal from experiment 17. One rod was inserted. The space is seen to the right of the picture and is opened in the left upper portion. Small connective tissue bands are seen around the space (compare Fig. 6). Reaction in the form of collagenous fibers +, lymph, giant cells + and phagocytized Turnbull-neg. material +. No other reactions. Htx - van Gieson.  $\times 145$ .—*Fig. 4.* Section adjacent that shown in Fig. 3 and stained according to the Turnbull blue method. No Turnbull-positive substance, but naturally black pigment was seen in the connective tissue bands to the right in the picture.  $\times 145$ .

be demonstrated. The reaction in the form of fibrosis and inflammatory cells was slight the whole time.

This experiment thus shows that the first few weeks after deposition of even such an inert material as platinum Turnbull blue positive material appears in the tissue. This is most probably an unspecific phenomenon due to bleeding during operation. This reaction gradually disappeared within 3 months. The experiments are, however, too few to exclude the possibility of this reaction being capable of persisting longer. The purpose was, however, only to show that at three months this reaction was most likely insignificant.




















TABLE 1  
*Survey of material.*

Designation in table 2	Composition	Shape
A	Stainless steel wire, unworked, 18 % Cr, 9 % Ni, 0.04–0.10 % C, the rest Fe. "Sandvikens Jernverks AB" 2 R 2.	
B	Do., coldworked, "cerclage".	
C	Cr-steel. 17.5 % Cr, 0.07–0.08 % C, the rest Fe. "Avesta Jernverks AB" 249 M.	
D	Vitallium, hard. 62 % Co, 30 % Cr, 5 % Mo. Hardness 34–38 Rc = 334–363 Brinell. "Dental AB".	
E	Vitallium, soft. 52 % Co, 28 % Ni, 19 % Cr, 0.5 % Mo. Hardness 131 Brinell. "Dental AB".	
F	Titanium. > 99.8 % Ti. "Avesta Jernverks AB" A Ti – 24.	
G	Platinum. "Heraeus Hanau".	
H	Contracid. 61 % Ni, 19.5 % Fe, 15 % Cr, 2.5 % Mo, 2 % Mn. "Heraeus Hanau".	

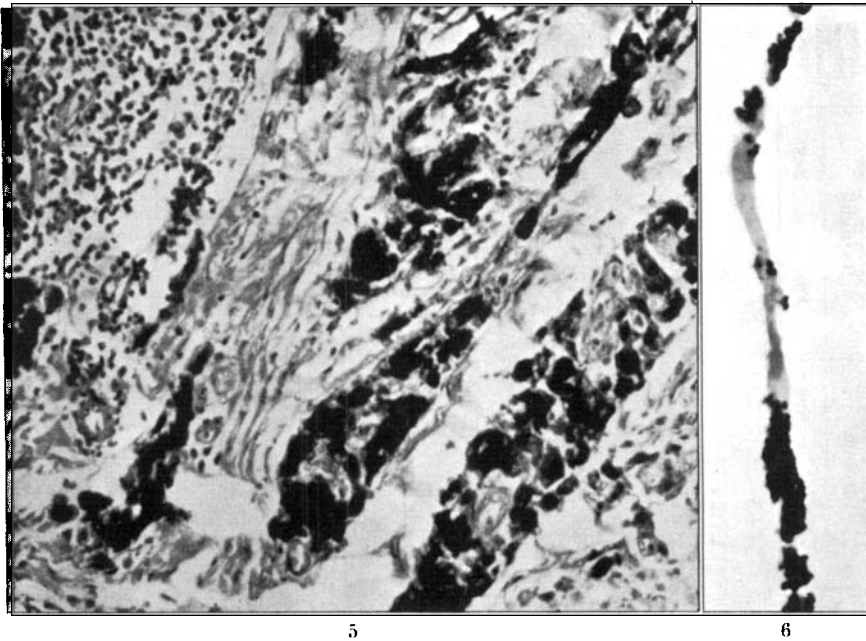
The results of the main experiments are summarized in Table 2 (for explanation see Material and Methods) and are illustrated in the Figs.

In all of the experiments a reaction was observed around the deposited material in the form of a usually thin capsule of collagenous fibers (Figs. 1 and 3). The capsule was, as a rule, poor in cells. The cells observed were mainly lymphocytes, giant cells and phagocytes. Only

TABLE 2  
Summary of experiments and results.

Exp.	Num-ber of exp.	Material (cf. table 1)	Shape of material	Collag. fibers	Lymph. giant cells	Phagoc. "pigm." Turnbull		Non-phagoc. "pigm." Turnbull		Gross lumps Turnbull pos.
						Pos.	Neg.	Pos.	Neg.	
1	5	A		+		+	(+)			
2	3	A + A pickled		+(+)	++	+(+)		+		
3	5	A + C pickled		++	++	+++		+++		++
4	5	B		++		(+)		(+)		
5	3	B pickled		+	+					
6	3	B stress-relief annealed and pickled		+	+	(+)		(+)		
7	6	C pickled		++	+(+)	+				
8	3	C + C pickled		++	++	+++		+++		+
9	3	C + D sandblasted		++	++	++		++		
10	3	C + E sandblasted		++	++	+++		+++		+
11	3	D sandblasted		+	+					
12	3	D pickled		+	+		(+)			
13	6	D + E sandblasted		++	+	+		+		+
14	3	E sandblasted		+	+	+		+		+
15	3	E pickled		++	+	(+)		(+)		(+)
16	6	F sandblasted		+	+	+		+		
17	12	F polished		+	+					
18	5	G		+	+					
19	3	H sandblasted		+	+					

Pickled means preliminary pickling in H<sub>2</sub>SO<sub>4</sub> and secondary pickling in HNO<sub>3</sub>.



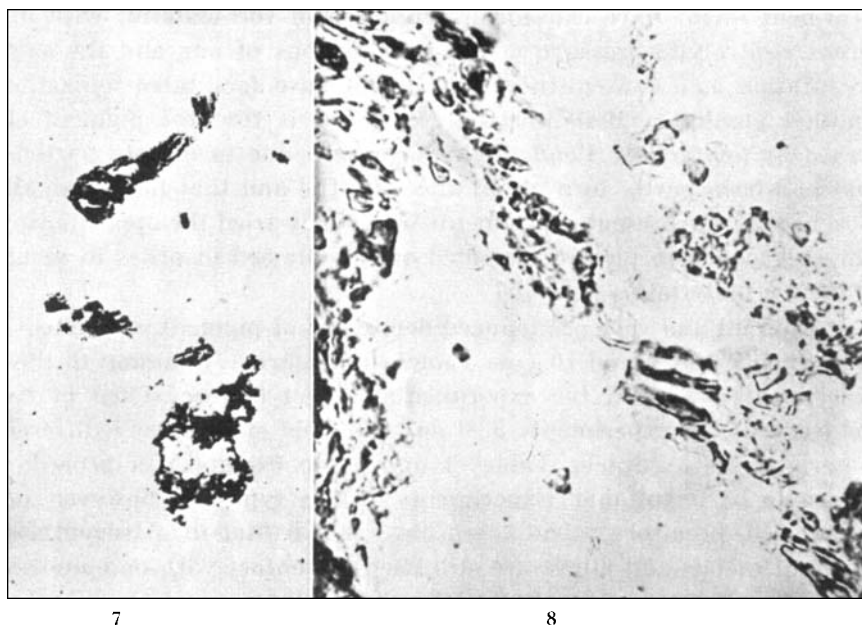
Figs. 5-6.

*Fig. 5.* Detail of Fig. 2. In top left hand corner are lymphocytes; to the right, phagocytes with Turnbull-positive substance (black in reproduction) and intermediate bands of collagenous fibers.  $\times 230$ .—*Fig. 6.* Detail of narrow connective tissue band in right upper corner in Fig. 4. Naturally black Turnbull-negative substance in phagocytes.  $\times 760$ .

very few fibrocytes, fibroblasts and polymorphonuclear leucocytes were observed and were therefore not included in the table. The greatest difference was seen in the amount of pigmented material. It was sometimes Turnbull-positive, sometimes Turnbull-negative. Figs. 3-8 illustrate the variation in the amount of pigmented material and how it was estimated.

In some experiments (Nos. 5, 11 and 18) no pigment deposits were observed. But the experimental groups were relatively small, and the tissue around the test material was not studied in serial sections, but only at 2 different levels, so that it is hardly possible to exclude the presence of any pigment at all.

The difference between these groups and those groups (1, 2, 4, 6, 7, 12, 13, 14, 15, 16, 17 and 19), in which pigmented material was observed and given in Table 2 as + or (+) was small (see Figs. 4 and 6). It is therefore not possible to say, which of these materials was more or less



Figs. 7-8.

*Fig. 7.* Same animal as in Figs. 1, 2 and 5. Section through macroscopically observed rust-like lump, dissected from angle between rods. Reaction judged as gross lumps + + +. Stained according to the Turnbull blue method. Nearly all material Turnbull-positive.  $\times 14.5$ .—*Fig. 8.* Section adjacent that shown in Fig. 7. Part of the material appeared to consist of crystals. Unstained.  $\times 760$ .

irritative. In two of the cases the test material consisted of welded rods, in one of which (experiment 2) the rods were of the same material (18-8-wire), in the other (experiment 13) the rods were made of soft and hard vitallium. In the other experiments (1, 7, 11, 12, 14, 15, 16, 17, 18 and 19) the material deposited was a single rod or wire, though it had in some cases been cold-drawn (cerclage, experiment 4, 5 and 6).

The presence or absence of any reaction in the form of phagocytized pigment on deposition of these metals and alloys may to a certain extent be dependent on the surface treatment. *Seyfarth & Voigt* (1955) thus reported that deposition of *inter alia* gold and platinum (separately) was followed by the deposition of pigment in the tissues. As mentioned, in the present investigation no such deposition was found in the experiment with platinum (experiment 18). *Seyfarth & Voigt*, however, mentioned that they had scratched the surface of their material with a steel file (personal communication) in order to augment the surface between the deposited material and the tissue. Such surface

treatment might have caused an activation of the material with different electrolytic pressure in different regions of one and the same preparation as a consequence. This might have facilitated ionization. Another possibility that must be considered is that the pigment observed by *Seyfarth & Voigt* might have been due to minute particles that had been partly torn off by the steel file and that had gradually loosened. In the present experiments we usually tried the opposite way. The surfaces were pickled, polished or sandblasted in order to secure as inert a material as possible.

A different and very pronounced deposition of pigment was noted in experiments 3, 8, 9 and 10 (see Tables and figures). Common to these experiments was that the experimental object had consisted of two welded rods. In experiments 3, 9 and 10, rods of relatively different material were used (see Tables 1 and 2) to exemplify a procedure known to be unsuitable. Experiments of this type are, however, not unjustified, because clinical cases have shown that in osteosynthesis different metals and alloys are still used in contact with one another.

It is clear from experiment 7 that although the properties of Cr-steel are inadequate for osteosynthesis, deposition of a single rod does not cause such a serious reaction as two welded Cr-steel rods (experiment 8). In surgical practice, where a certain cold processing with the same material is more or less the rule, Cr-steel should be avoided.

In most of the present experiments the material was not handled during application as is the case in osteosynthesis. The results can therefore not be explained by the transfer of metal from the tool to the plate etc., demonstrated by *Bowden, Williamson & Laing*. Exceptions to this rule, however, are experiments 1, 4 and 18, in which the test material was not subjected to surface treatment after its manufacture. Thus the difference in the results between experiments 4 and 5 may be due to transfer of metal from the tools to the test material (see Table 2).

Experiments 4, 5 and 6 are of particular interest to Swedish surgeons, cerclage often being used for osteosynthesis in Sweden but not in Great Britain or the U.S.A. As mentioned, cold treatment of 18-8-steel has the disadvantageous effect that the cold-worked part becomes electrolytically active compared with the undrawn part (*Emnéus & Petersen* 1958). It is clear from Table 2 that the reaction of the tissues also appeared to be more unfavourable in experiment 4 than in experiment 1, though the difference is too small to be considered significant.

Titanium was tested. The series was small, but it appears that unworked titanium is not more irritative than vitallium and unworked

18-8-steel. This result is also in agreement with *Leventhal's* (1957) clinical experience with titanium.

In most of the experiments pigment was seen in the tissues under the microscope. As mentioned, it was sometimes phagocytized, sometimes extracellular. Some of the pigment appeared yellow-brown in unstained sections. It proved Turnbull-positive. The extracellular pigment had, at least sometimes, the appearance of almost colourless crystals (Fig. 8). It was always Turnbull-positive. The phagocytized and non-phagocytized Turnbull-negative pigment (see Figs. 4 and 6) found in some experimental groups was black or dark in unstained sections. The phagocytized Turnbull-positive and -negative pigment was finely granular (Figs. 5 and 6).

It appears probable that the pigment that stained positive with the Turnbull blue method consisted, at least in part, of ionized iron. It is tempting to assume that this iron emanated from the deposited test material, which usually contained iron. (It should be observed that vitallium can also contain iron, about 0.5 %, as a contamination). However, when Fe-pigment is found in a biological tissue it is difficult to exclude the possibility of the iron emanating from the living tissue. One might, of course, imagine iron to be accumulated or transferred to more readily observable form around the deposited material because of some irritation, such as bleeding in association with operation. Two factors, however, argue against this possibility. To begin with, on special investigation with a platinum wire (*cf.* above) a Turnbull-positive pigment could be demonstrated only during the first few weeks after deposition of the wire and after 3 months no pigment at all could be demonstrated. Secondly, on deposition of Fe-free material a reaction often occurred in the form of a Turnbull-negative pigment. It appears reasonable to assume that in the latter case the pigment did not contain iron but titanium or elements of vitallium (Co, Cr, Mo). The possibility of Fe compounds not giving a Turnbull-positive reaction must also be considered. One might also imagine that the Turnbull-negative pigment is an artefact, so-called formalin pigment, occurring on fixation of the tissue. Formalin pigment can, however, be dissolved out (*Romeis* 1948, § 274). In all cases in which Turnbull-negative pigment was demonstrated, sections were treated in such a way. The pigment was not dissolved out. The Turnbull-negative pigment observed was therefore most probably not a formalin pigment. Further investigations are being performed on the nature of the pigments (*Emnéus, Stenram & Baecklund*, in press).

In view of the remarks set forth above it appears probable that all or almost all of the pigment demonstrated in the tissues in our experiments came from the test material. At least in those cases in which appreciable amounts of pigment were demonstrated (experiments 3, 8, 9 and 10), it had probably been dissolved electrolytically. It was in these experiments that material (Cr-steel) known to be less resistant to corrosion was used. In experiments 1 and 4, where only a minute amount of pigment was demonstrated in the tissue and where the test material had not been surface treated, it is possible that minute particles from the tools might have been to a certain extent responsible.

#### S U M M A R Y

Different metals and alloys in the form of small, usually surface-treated rods were deposited in the musculature on either side of the carina in chickens. In some cases 2 rods welded together were inserted. Three months later the animals were killed and the tissue around the rods was examined histologically. Deposition of one metal or alloy produced extremely slight inflammatory reaction of the surrounding tissue. In some cases minute accumulations of pigment in the tissue were observed. On insertion of two rods welded together a sometimes appreciable amount of pigment was observed around the material. This might be explained by an electrolytic dissolution of metal from the deposited material.

#### R E S U M E

Différents métaux et alliages sous forme de petites tiges dont la surface a été traitée selon les procédés habituels ont été déposés dans la musculature sur l'un ou l'autre côté du carène de poulets. Dans certains cas, deux tiges soudées ensemble furent insérées. Trois mois plus tard, les animaux furent sacrifiés et les tissu autour des tiges examiné histologiquement. La déposition d'un métal ou d'un alliage produit une très légère réaction inflammatoire du tissu environnant. Dans certains cas une faible accumulation de pigments dans le tissu a été observée. A l'insertion de deux tiges de métaux ou d'alliages soudées ensemble, une quantité parfois appréciable de pigment a été observée autour de la substance. Ceci peut s'expliquer par la dissolution électrolytique du métal de la substance déposée.

## ZUSAMMENFASSUNG

Verschiedene Metalle und Legierungen in der Form von kleinen, gewöhnlich oberflächenbehandelten Stäben wurden in die Muskulatur an beiden Seiten der Carina von jungen Hühnern eingelegt. In einigen Fällen wurden zwei zusammengeschweisste Stäbe angebracht. Die Tiere wurden 3 Monate später getötet und das die Stäbe umgebende Gewebe wurde histologisch untersucht. Versenkung eines Metalls oder einer Legierung rief eine äusserst leichte entzündliche Reaktion des umgebenden Gewebes hervor. In einigen Fällen wurden minimale Ansammlungen von Pigment im Gewebe bemerkt. Bei der Anbringung von zwei zusammenschweissten Stäben wurde eine manchmal ganz erhebliche Menge von Pigment in der Umgebung des Materiales wahrgenommen. Dies kann durch eine elektrolytische Auflösung von Metall aus dem versenkten Materiale erklärt werden.

## REFERENCES

- Alexander, P. & Horning, E. S.*: Ciba Found. Symp. on Carcinogenesis, p. 12, London, 1959.
- Bothe, R. T., Beaton, L. E. & Davenport, H. A.*: Surg., Gyn. & Obst., 71: 598, 1940.
- Bowden, F. P., Williamson, J. B. P. & Laing, P. G.*: J. Bone & Joint Surg., 37 B: 676, 1955.
- Clarke, E. G. C. & Hickman, J.*: Ibid., 35 B: 467, 1953.
- Emnéus, H.*: Acta Orthopaed. Scand. 26: 236, 1956/57.
- Emnéus, H. & Petersen, P.*: Nord. Med., 59: 593, 1958.
- Emnéus, H., Stenram, U. & Baecklund, J.*: Acta Orthopaed. Scand., in press.
- Hickman, J., Clarke, E. G. C. & Jennings, A. R.*: J. Bone Joint Surg., 40 B: 799, 1958.
- Hicks, J. H.*: Proc. Royal Soc. Med., 50: 842, 1957.
- Hicks, J. H.*: in Modern Trends in Surgical Materials. Ed. L. Gillis, Butterworth & Co., London, 1958.
- Jørgensen, H. J.*: Experimentelle Undersøgelser over Fracturhelingen ved Osteosyntese, Disp. København, 1941.
- Kalling, B. & Liljekvist, G.*: Cellulosa och Papper. Ed. Svenska Pappers- och Cellulosaingenjörsföreningen, Stockholm, 1948.
- Kaplan, H.*: Cancer Res., 17: 791, 1959.
- Laing, P. G.*: J. Bone Joint Surg., 40 A: 853, 1958.
- Leventhal, G. S.*: J. Bone Joint Surg., 33 A: 473, 1951.
- Amer. J. Surg., 94: 735, 1957.
- McKee, G. K.*: Proc. Royal Soc. Med., 50: 837, 1957.
- Modern Trends in Surgical Materials*, Ed. L. Gillis, Butterworth & Co., London, 1958.
- Oberling, C.*: Ann. Rev. Medicine, 10: 233, 1959.
- Oppenheimer, B. S., Oppenheimer, E. T., Danishefsky, I. & Stout, A. P.*: Cancer Res., 16: 439, 1956.

- Romeis, B.*: Mikroskopische Technik, 15. Aufl., München, 1948.  
*Schinz, H. R.*: Schweiz. med. Wochenschr., 72: 1070, 1942.  
*Seyfarth, H. & Voigt, G.*: Acta Histochem., 1: 240, 1955.  
*Venable, C. S. & Stuck, W. G.*: J.A.M.A., 111: 1349, 1938.  
– J. Bone Joint Surg., 30 A: 247, 1948.  
*Zarek, J. M.*: Proc. Royal Soc. Med., 50: 845, 1957.