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REACTOGENICITY OF BIOMATERIALS AS STUDIED BY BIOCHEMICAL, MORPHOLOGICAL AND ULTRASTRUCTURAL TECHNIQUES

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Abstract

Reactogenicity is a characteristic of biocompatible materials that provokes the reparative and proliferative reaction of connective tissues, a compulsory stage of which is inflammation. Thus, reactogenicity studies should include experiments in vivo. A quantitative assessment of reactogenicity can be obtained by subcutaneous implantation of standard olive-shaped specimens covered by the biomaterial under study followed by biochemical, histological and scanning electron microscopical studies of the capsule developing around the implant. Reactogenicity of surgical threads is evaluated by semiquantitative histological analysis of the wound healing process after suture application. Biomaterial reactogenicity can be modified by changing the structure of the surface and its chemical characteristics, and in particular, by applying different biologically active substances including atrane-containing compounds. The reactogenicity indices suggested present the results of the interaction between the biomaterials and the cells.

Key words: Biomaterials, Implants, Reactogenicity, Connective Tissue Reaction, Inflammation, Granulofibrous Tissue, Surgical Threads, Methods of Study.

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Introduction

The basic concept used for the characteristics of biomaterials, i.e., materials implanted in a living body is that of biocompatibility (Bagnall, 1980; Marchant *et al.*, 1984; Murabayashi and Nose, 1986; Park, 1979). This concept comprises a dual meaning. On the one hand the concept of biocompatibility is defined as a safe contiguity between the material and living tissues, presuming the absence of toxicity, cancerogenicity and infection of the material. Such a concept of biocompatibility cannot call forth objections. However, on the other hand biocompatibility is often interpreted as bioinertness, as an ability of the material not to provoke local reactions on the part of the organism. This second viewpoint of biocompatibility has only a conventional significance, since the implantation of a foreign body, as will be demonstrated, is principally impossible without local reaction. The question is the intensity and the quality of the implant-caused reaction.

Thus, the characteristics of biocompatible materials should be supplemented by the concept of reactogenicity (Sevastjanova *et al.*, 1987). The present review is devoted to the definition of reactogenicity of biomaterials and to the methods of its investigation.

General Rules for the Reaction of Implant

When implanting devices or products made of biomaterials into the body, our first concern was the tissue destruction caused by surgical intervention, i.e., implantation, irrespective of its purpose and site. It is well-known, that any tissue damage and, more precisely, necrosis taking place due to the biological activity of products of cellular destruction (Lindner and Huber, 1983; Henson and Johnston, 1989) causes a universal reaction by the connective tissue. This reaction is the expression of the proliferative and reparative function of the tissue.

The biological significance of a broader protective reaction (Slutskii, 1969) is, that it provides filling of the tissue defect caused by the injury, and consequently restoration of the integrity and in some cases the vitality of the whole organism. Wound healing serves as the most

typical and well-studied example of the connective tissue reaction (Falanga *et al.*, 1988).

The main stages of this process are:

a) Necrosis of cellular elements; the following stage cannot and does not start without cell destruction;

b) Inflammation during which the so-called inflammatory tissue is formed (Houck, 1976; Lundberg, 1983; Merker, 1984; Peterson *et al.*, 1987) from humoral components of the exudate, which is a result of blood vessel injury and increased permeability of the vascular wall (Ward, 1980; Clerc *et al.*, 1990), and which consists of blood coming out of the injured vessels and of cells beginning to proliferate;

c) Inflammatory tissue develops into granulo-fibrous tissue, characterized by prevailing anabolic reactions directed at accumulation and organization of macromolecular components of extracellular connective tissue matrix (Diegelmann and Lindblad, 1985; Pricolo *et al.*, 1990). A particularly active role in this transformation process is played by the macrophages and their growth factors (Jalkanen *et al.*, 1988; Diegelman *et al.*, 1986b; Browder *et al.*, 1988; Fukasava *et al.*, 1988; Danon *et al.*, 1989; Miller and Anderson, 1989; Lunch *et al.*, 1989).

d) The process is completed by the development of the granulo-fibrous tissue into a collagen-rich fibrous (scar) tissue repairing damage caused by the injury, both in its anatomical and biomechanical aspects (Doillon *et al.*, 1985).

Current concepts of the dynamics and mechanics of the inflammation, the formation of granulation-fibrous tissue and scar tissue have been reported in recent series of reviews (Schechter *et al.*, 1984; Clark and Denver, 1985; Kanzler *et al.*, 1986, Martinez, 1987; Pessa *et al.*, 1987; Eckersley and Dudley, 1988; Goslen, 1988) and a detailed consideration of them is outside the scope of the present paper. However, it should be emphasized that inflammation is a necessary though transitional linking stage between necrosis and reparative proliferation of the connective tissue, i.e., wound healing. Inflammation can easily and frequently result in complications (in the first place when infections occur), distorting the course of healing optimized during phylogenesis. But in itself this optimized course includes inflammation developing within strictly determined limits. Particularly, active components of the inflammatory exudate form a starting mechanism for proliferation of connective tissue cells and activation of their biosynthetic potentials. Fibroplasia and healing are impossible without inflammation (Agelli and Wahl, 1989; Barbul *et al.*, 1989; Barnes, 1988).

All the above concerns the general response to trauma inflicted during implantation of devices made from biomaterials. However, also additional, specific implant-dependent factors are present: the implant plays the role of a foreign body (Krizek, 1983) inflicting additional injury to the surrounding tissue. The intensity and quality of this effect depend on the mechanical, physico-chemical and chemical properties of the implant, but the general rules of the process remain unchanged. Just as in any other type of injury the same chain of sequential events is observed here: necrosis, inflammation, development of granulo-fibrous tissue, completed in the final stage by the formation of fibrous (scar) tissue that eventually turns into an implant-isolating capsule.

When the implanted material is not subjected to biodegradation, its presence remains a constant source of traumatization to adjacent tissues, and a complete regression of the capsule caused by the collagenase and non-specific proteases (Figueras and Pardo, 1986) does not take place. Thus the surgical suture threads of non-absorbable materials remain within the capsule (Salthouse, 1983). However, also absorbable materials, including absorbable threads prolong the action of the trauma caused by implantation depending on their destruction rate and extend the course of the granulo-fibrous process; thus resulting in a long-term presence of scar tissue at the implantation site (Postlethwaite, 1979).

Of all the implant properties influencing the inflammation course and the subsequent fibrous reaction, the size and geometrical shape of the implant are most important since the biomechanical conditions (compression, distraction) created by the implant in the adjacent tissues are directly dependent on both these properties. In turn, the biomechanical conditions influence the cells participating in the inflammatory stage of the reaction. For example, the activity of lysosomal acid phosphatase turned out to be highest in the cells of the granulo-fibrous tissue around different shape implants of the same material (Matlaga *et al.*, 1976; Salthouse, 1976). The results of enzyme histochemical studies show that standardization of the size and geometrical shape of implants is absolutely necessary during comparative testing of different biomaterials.

A priori, it seems that implant hardness may also play a significant part in the development of reaction but this dependency has not been studied in detail. Another significant physical factor undoubtedly is surface smoothness or roughness of the implant (Knöfler *et al.*, 1984). This factor can play a double role: firstly, surface roughness increases the contact area of the implanted material with adjacent tissue, thus enhancing the intensity of physicochemical and chemical interactions; and secondly, the presence of notches and sharp facets makes the contact with tissues more traumatic.

The role of the texture of the implant was convincingly demonstrated in the experiment (Taylor and Gibbons, 1983) where polytetrafluoroethylene implants with a smooth surface were compared with those having a surface processed in vacuum by an argon laser. Such processing led to the formation of different height and thickness thorns on the surface. The biological reaction to these implants was different: surface texturing intensified cell adhesion, increased the cellular activity of succinate dehydrogenase and acid phosphatase, and also increased the number of giant cells of the foreign bodies and macrophages in the cellular population. Texturing

also induced changes in the kinetics of the development of a fibrous capsule around the implant and resulted in earlier involution. The significance of the surface texturing of the implant has been also confirmed by Whalen (1988) and Picha *et al.* (1990).

Alongside with the mechanical peculiarities of the implant, one of the critical factors determining the course of the connective tissue reaction are the physicochemical properties, and in particular, surface energy that is different for hydrophilic and hydrophobic materials (Murray *et al.*, 1989). For metallic implants, their subjection to surface corrosion is of utmost importance and leads to a direct chemical interaction of the implant with surrounding tissues (Lemons and Lucas, 1986; Exbrayat *et al.*, 1987; Vitale and Fallat, 1988).

Electrostatic and electrokinetic interactions play a significant role in the tissue reaction to synthetic polymers and influence the adsorption of proteins from the inflammatory exudate (Chiu *et al.*, 1981), and in particular, lipoproteins (Jayakumari *et al.*, 1989). The types of adhesive proteins influence, in turn, the dynamics of cells joining the reaction (Absolom *et al.*, 1987; Strong *et al.*, 1987).

Physicochemical and among them electrochemical properties of biomaterials are, finally, the products of the chemical nature, and we cannot neglect the direct chemical influence of the implant on the adjacent tissues (Clark *et al.*, 1976). In metals, such an influence is exerted by the products of corrosion. As to the synthetic polymers, all become chemically inert following completion of the polymerization process (Lipatova, 1979; Becker, 1985); therefore, we speak about their "bioinertness" as a precondition for their use in medicine. However, this is only valid for "ideal" polymers; and in reality the majority of polymers are contaminated by low-molecular substances that remain following polymerization, e.g., initial monomers, catalysts, solvents, that can diffuse from the polymer and chemically react with biological molecules. Moreover, and this is of the utmost importance, all polymers that are placed in the organism for a long period of time are subjected to biodegradation. As the result of this process carried out by three mechanisms: hydrolysis, phagocytic activity of macrophages and lytic activity of giant cells of foreign bodies (Kopeček and Ulbrich, 1983), release of low-molecular fragments and free molecules that are frequently chemically active, takes place.

A number of biomaterials and synthetic polymers, in particular, have been especially developed with a view to a controlled biodegradation during which a gradual predetermined release of low-molecular biologically active substances, for instance, pharmacological agents added to polymer, takes place (Langer and Pappas, 1981; Schakenraad *et al.*, 1988b). This field of study has great significance in the development of biomaterials if considered from the viewpoint of reactogenicity control that we shall revert to afterwards.

In this respect, a method to affect the reactogenicity of biomaterials by copolymerization of synthetic polymers with biopolymers, in particular collagen (Amudeswari *et al.*, 1986; Gilbert and Lyman, 1987) should be mentioned. If collagen is firmly connected to a synthetic polymer by crosslinking, it is preserved for a considerable period of time. First, it significantly inhibits cellular reaction, but later the reaction, on the contrary, intensifies (Gilbert and Lyman, 1987).

Cells participating in the inflammation reaction to the implant (Salthouse, 1984; Lentz *et al.*, 1985; Schakenraad, 1987; Strong *et al.*, 1987; Schakenraad *et al.*, 1988a) are subject to all influences of biomaterials considered above. The secretion by the cells of those active factors that determine their interaction is changed. In particular, the physiologically optimized rate of the cellular interactions controlling the proliferative and biosynthetic activity of cells, with fibroblasts completing the process, is affected (Bateman *et al.*, 1986; Matsuda *et al.*, 1987; Bretcher, 1988; Ziats *et al.*, 1988; McCauley *et al.*, 1990; Miller and Anderson, 1989; Pricolo *et al.*, 1990). This controlled activity results in the final structure of the extracellular connective tissue capsule that has developed around the implant.

As noted before, the development of this capsule is generally subjected to the same rules as those regulating the development of scar tissue during wound healing (Rigg, 1982; Shah *et al.*, private communication). This principal similarity can very well be seen in studies of capsules caused by soft silicone rubber which is widely used in plastic surgery. In experiments on rats it was stated that accumulation of glycosaminoglycans similar to that seen in wound healing initially takes place in the tissue of the capsule. The total concentration of glycosaminoglycans increases until the 30th day and decreases afterwards. Concentration peaks of individual glycosaminoglycans did not coincide: for chondroitin-4 sulphate the maximal concentration peak is observed between days 10 and 15, for hyaluronate between days 25-30, and for dermatan sulphate around the 30th day. Collagen accumulation starts with soluble fractions, but on the 2nd day post-implantation the basic collagen mass turns into an insoluble (fibrillar) form and the total collagen concentration reaches 15 g/ 100 g of dried tissue. Later the rate of collagen accumulation gradually slows down according to an asymptotic curve; in a completely mature capsule studied on the 200th day the collagen contents were 42 g/100 g of dried tissue (Ksander and Vistnes, 1981). The same type III and V collagens as found in wound healing appear earlier, but with the maturation of capsules type I collagen prevails. The type I fibers are distinguished by their great mechanical strength (McCoy *et al.*, 1984; Marshall *et al.*, 1989).

One more principal similarity between wound healing processes and implant ingrowth is the development of granulo-fibrous tissue as the result of inflammation. When this tissue has reached the maximal degree of development it is subjected to a more or less pronounced involution (Asman *et al.*, 1988). This final stage of scar formation is considered as a remodelling stage and is performed by the same enzymatic mecha-

nisms (collagenases in particular) (Pardo *et al.*, 1983) and with the participation of the same cellular elements, macrophages and fibroblasts (Pasyk *et al.*, 1984). If the implant is made of non-absorbable biomaterial, the capsule surrounding it later acquires a stationary character.

Reactogenicity of Biomaterials and its Control Possibilities

From this it follows that reactogenicity of biomaterials is a characteristic expressed during implantation into the body, and provoking the reparative and proliferative connective tissue reaction (Sevastjanova *et al.*, 1987). In its essence the concept of reactogenicity is a quantitative one and reflects two quantitative parameters of the connective tissue reaction, firstly, its intensity. The mass (volume) of the inflammation tissue developing during inflammation and afterwards becoming the granulo-fibrous, and finally the fibrous tissue, serves as intensity index. Secondly, the rate at which the implant-caused reaction passes all stages of the process. Both quantitative parameters are closely interrelated. Moreover, even variants of the interrelation are possible, as when the intensive reaction proceeds slowly acquiring the character of chronic inflammation, or on the contrary, as when as a result of a rapid process with minimally produced exudate and proliferative manifestations, a very small amount of mature fibrous tissue develops.

Together with all this, the concept of reactogenicity has also a qualitative aspect. This concerns qualitative characteristics of the reaction caused by a particular biomaterial, such as the prevalence of different cellular populations in the reaction, differences in the functional conditions of cells, and in the biochemical contents of the inflammation exudate and extracellular matrix of the granulo-fibrous tissue.

The data presented above on the development of the connective tissue reaction to implant and its physical and physicochemical characteristics point to the fact that the reactogenicity of biomaterials is controllable. In addition to what was said above about the role of physical properties, it should be noted that the implant reactogenicity can be changed, and as a rule increased, by imparting it porosity. Even the presence of micropores, i.e., pores impassable for cells, lead to the development of a more massive capsules around the implant in comparison with a non-porous implant of the same chemical nature. Moreover, the effect is proportional to the degree of porosity (Wildevuur *et al.*, 1987).

Truly porous (sponge) biomaterials are those, in which the size of pores allows free penetration of cells, and where these pores are intercommunicating throughout the whole thickness of the material (Bobyn *et al.*, 1982). Such porous implants are completely ingrown by the granulo-fibrous tissue (Yan *et al.*, 1989, Lipsky and Lamberton, 1990) and this characteristic made them despite some objections (Schreuders *et al.*, 1988) one of the most attractive experimental models for a detailed investigation of the reparative processes of connective tissue (Boyle and Mangan, 1980; Diegelmann *et al.*, 1986a), including a quantitative assessment of the influence of different control factors and pharmacological means. The larger the size of pores, the more intensive was the filling by granulo-fibrous tissue; in this respect the use of cellulose sponges was most effective (Andreassen and Jorgensen, 1985; Hørslev-Petersen *et al.*, 1988).

Since the most significant parameter of the reaction to biomaterials is cell adhesion to their surface, a promising approach to reactogenicity control is the use of natural "molecular glues". These are adhesive glycoproteins, of which fibronectin is most common. It has been established that spreading of the plasmatic form of fibronectin on bioceramics increases the in vitro cell adhesion, and significantly accelerates cell spreading, (Seitz *et al.*, 1982). The use of fibronectin for this purpose is justified, since it takes active part in the formation of granulo-fibrous tissue where its fibers form a primary framework (Knox *et al.*, 1986; Igisu, 1986; Clark, 1988), which is why this glycoprotein improves wound healing (Nagelschmidt *et al.*, 1987; Cheng *et al.*, 1988). It is possible that another adhesive glycoprotein, vitronectin, may be an even more potent regulator of interaction at the biomaterial surface, since it promotes cell adhesion to artificial materials, for instance, glass (Preissner *et al.*, 1988; Reilly and Nash, 1988).

An interesting suggestion on regulative influence on biomaterials was made based on data on the role of cyclic adenosine monophosphate (cAMP) (Faga and Merlino, 1985). The concentration of this nucleotide in tissue surrounding the polymer implant, which is the same as the granulo-fibrous tissue of a healing wound, is dramatically reduced in early stages of the process in comparison with normal tissue (dermis). cAMP concentration becomes lower with the maturation of the capsule (or wound healing). Therefore, it could be expected that the encapsulation rate of the implant is accelerated by increase of the local cAMP concentration by phosphodiesterase inhibitors.

A topical question regarding implantation of biomaterials in clinical practice is the prophylaxis of infection. To achieve this the application of different antibacterial agents on the implant surface is recommended. It was noted that a number of these agents together with their anti-infection activity also can influence the granulo-fibrous tissue of healing wounds. This influence is indeed revealed during a completely aseptic course of the process (Kenyon *et al.*, 1986; Leaper and Simpson, 1986; Niedner and Schöpf, 1986)); it can be directed both in the direction of suppression of fibroplastic reaction (obviously due to cytostatic activity) and in the direction of a pronounced stimulation.

Owing to this property the antibacterial agents can be considered as modifiers of biomaterial reactogenicity, as confirmed by our experiments. Of a number of antibiotics, two: Lyncomicin and Ampioxum (a combination of Ampicillin Natrium and Oxacillin Natrium), that were applied on the surface of subcutaneously implanted olive

covered by a layer of a biomaterial (this experimental method is described below) increased the mass of the granulofibrous capsule developing around the implant. This increase was accompanied by biochemical changes in the tissue; an increase in the concentration of nucleic acids and sialyloglyoproteins with a simultaneous decrease of collagen contents.

New possibilities of the modification of the reactogenicity of biomaterials were opened up by the use of low-molecular organic compounds containing an atom of silicon or germanium forming the content of the so called atrane cycle (Voronkov, 1979). The mechanism of the high biological activity of these compounds (silatranes and germatranes) remains unrevealed, but one can presume that it lies in the interference with the biosynthetic activity of fibroblasts (Slutskii, 1981; Taylor *et al.*, 1986).

According to our data chlormethylsilatrane forming the contents of polyurethane and applied on the surface of the implanted olives (the same experimental model) increased the mass of the granulofibrous capsule by 50%; the concentration of nucleic acids did not change but that of the collagen decreased by 14% in comparison with the control group. In other words, we can speak about the absence of cellular proliferation and suppression of collagen synthesis by fibroblasts.

Another silatrane, ethoxysilatrane, caused the reduction of the sialoglycoprotein concentration in the tissue of the capsule; a characteristic component of the inflammatory exudate. One more representative of the atrane group, germatranol, did not influence the capsular mass, however, the concentration of nucleic acids reduced under its influence, but the accumulation of collagen increased considerably (by 28%) which means that the degree of tissue fibrosis increased (unpublished results).

All data presented demonstrate that the final results of the interaction between the implanted materials and the cells, and the characteristics of the capsule forming around the implant in particular, can be changed by modifying the surface of the biomaterial by biologically active substances. Generally, the capsular mass is increased as a consequence of an increased reactogenicity of the biomaterial. The biochemical changes taking place in the tissue of the capsule and resulted by the above process can be isolated or combined, as well as differently directed.

Artificial increase of reactogenicity of biomaterials widens the prospects of their use in medicine. At least five fields can be outlined where it is useful to have highly reactogenic materials.

Firstly, there are various operations for the replacement of vast soft tissue defects.

Secondly, the filling of bone tissue defects in bone plastics. In this field porous biomaterials, metallic (Pilliar, 1987; van Mullem *et al.*, 1988; van Mullem and de Wijn, 1988; Verburg *et al.*, 1988) and ceramic biomaterials (Krajevski *et al.*, 1988) are becoming increasingly popular. The ingrowth of the porous material by bone tissue provides better biomechanical results.

Thirdly, there is the implantation of prostheses in blood vessels. It is known that development of endothelial lining on the internal surface of vascular prosthesis is a prerequisite for its functioning. In its turn, this lining will occur if a good adventitial cuff has developed around the prosthesis due to increased reactogenicity of the external surface of the prosthesis (Bernhard *et al.*, 1980; Beahan and Hull, 1982).

Fourthly, the development of new materials to cover wound and burn surfaces. Imparting these materials the ability to stimulate the reparative processes in skin should improve the interrelation between mesenchyme and epithelium, thus accelerating epithelialization and healing (Doillon and Silver, 1986; Queen *et al.*, 1987; Kaufman *et al.*, 1985; Bruin *et al.*, 1990; Chrintz *et al.*, 1989). A certain success in this respect has been achieved in experiments with biologically active modifications of cellulose materials, of which the efficiency was increased by the addition of zinc (Slutskii and Dombrovska, 1982).

And finally, fifthly, there is the development of new surgical suture materials. We present an absorbable suture material that has been obtained by oxidizing and aminating the cellulose (cotton) thread. This modification of cellulose stimulates the accumulation of glycosaminoglycans in the granulofibrous tissue; glycosaminoglycans control collagen fibrillogenesis (Parry *et al.*, 1982). We speculate that the biochemical effect of the new thread (rimine) provides an accelerated restoration of biomechanical parameters of the wound scar so that these at a certain healing stage even surpass the parameters of intact skin. Such a characteristic of rimine allows suture absorption prior to wound healing, but an earlier disappearance of the thread from the wound promotes a significant improvement of the cosmetic aspect of wound healing when sutures have been applied on the skin (Kalnberzs *et al.*, 1983; Slutskii *et al.*, 1984).

When highly reactogenic biomaterials are used in these fields of application the danger of excess stimulation of connective tissue reaction should be taken into account. The consequences of the excess stimulation can be hyperproduction of fibrous tissue with the formation of hypertrophic scars or subsequent wrinkling: a direct similarity with keloid formation (Ksander, 1988; Caffee, 1990; Datubo-Brown, 1990; Muir, 1990) due to disturbances in the function of cells participating in wound healing (McCauley *et al.*, 1990). Even pathological calcification of capsules around the implanted biomaterials is possible (Schoen *et al.*, 1988). These possible complications should be taken into account when practical recommendations are developed with regard to the clinical application of biomaterials with increased reactogenicity.

Methods of Investigation of Biomaterial Reactogenicity

Since the central factors determining the course of

the reaction caused by biomaterials are the properties of the implant surface, great attention is paid to a detailed characteristics of these properties: surface chemistry, surface energy and morphology. Suitable methods as a necessary condition for the development of improved biomaterials are reviewed by Ratner *et al.* (1987).

However, reactogenicity, as defined earlier, is a property of biomaterials, which finds its expression in the interrelations with biological tissue, and therefore, should be investigated mainly by biological methods. Of these methods, the *in vitro* methods, that are mostly efficient for the assessment of the toxicity of biomaterials are very common. Most often cytological and histological criteria are used. Matsuda *et al.* (1987) used as a criterion the growth rate of L fibroblast culture. Kotoura *et al.* (1985) judged the toxicity of biomaterials from their influence on the formation of colonies in a V 79 fibroblast culture. To obtain comparative assessment of a number of polymers, morphological analysis was performed on explants of the epithelium of the middle ear mucosa cultured on these polymers (Bakker *et al.*, 1988).

Cell migration and adhesion to the surface of the biomaterials serve as rather specific indices of the influence of biomaterials on cellular functions; the degree of cellular spreading over the surface was also assessed (Lydon *et al.*, 1985; Pizzoferrato *et al.*, 1985; Schakenraad *et al.*, 1986). Also qualitative biomechanical methods for the assessment of cellular function found their application in experiments *in vitro*. Thus, the influence of polymetallic (cobalt-chromium-molybdenum) powder on cultured fibroblasts was determined from the collagen accumulation in the culture (Thomas and Evans, 1986). When applying the same fusion (used in stomatological practice) on the culture of the explant of mucous gums, the production changes of the Type I and III collagens and fibronectin were immunologically evaluated ((Exbrayat *et al.*, 1987).

To overcome the principal restrictions of the analyses of *in vitro* experiments, Schakenraad *et al.* (1987) suggested and original method for the studies of cellular adhesion and spreading over the surface of cultured cells *in vivo*. The method involves intraperitoneal implantation of polymer tubular porous prosthesis, filled by a culture of smooth muscle cells. The prostheses were removed after definite time intervals, and the fate of cells adhering on the internal surface of the tube was determined. In this way, the dependency of cellular adhesion and spreading on the surface free energy of the biomaterials was confirmed.

The most widespread method to characterize the reaction to biomaterials in *in vivo* experiments is the histological method. Histological pictures of tissue developing on the implant surface characterize the cellular contents and extracellular matrix (Keller *et al.*, 1984; Mendes *et al.*, 1985; Ito *et al.*, 1987; Lange and Donath, 1989; Makisalo *et al.*, 1989; Miyamoto *et al.*, 1989). Recently, the routine histological staining during the investigations have been supplemented by enzyme histochemical methods. Thus, when studying the bone tissue reaction to materials used for joint replacement it is advisable to determine the activity of alkaline and acid phosphatase in cells around the implanted prosthesis (Reichelt *et al.*, 1988).

There have been attempts to impart a quantitative direction to histological studies to supplement the descriptive evaluation by morphometric data (Keller *et al.*, 1985; Schubert *et al.*, 1988). It should be noted that scanning electron microscopy has been applied in the studies of tissue and cellular reaction to biomaterials (Neupert *et al.*, 1987; Chegini *et al.*, 1988). The comparatively few publications devoted to the use of quantitative biochemical methods for the characteristics of connective tissue reaction to biomaterials have been mentioned above.

We have studied the reactogenicity of biomaterials *in vivo* in conditions that maximally resembled the practical conditions during implantation; and a new complex quantitative method is suggested. The implant is imparted the shape of an olive (as least traumatic) either totally made of the biomaterial under study or coated by a layer of this material. Olives are implanted in rats through a skin incision on their back, between the skin and subcutaneous fascia; the incision is sutured. After a definite period of time, the olives surrounded by the already formed granulo-fibrous tissue or fibrous capsule are extracted by bluntly separating the capsule from the surrounding tissue. The standard size of the olives makes the conditions of experiment reproducible. The capsule is totally removed from the olive, its mass is weighed, and afterwards the capsular tissue is subjected to biochemical analysis. The latter is supplemented by morphological studies (general histological picture of the capsular cut and scanning electron microscopy).

The investigations performed 90 days following implantation of olives that were coated by two different polymers for medical use: polyethylene and polyurethane PU-155 revealed dense capsules of approximately the same mass (0.13 ± 0.013 g and 0.14 ± 0.022 g, respectively). Biochemical analysis (Fig. 1) demonstrated that the capsules are intensively fibrotic approaching the concentration of collagen in aponeurotic tissue or mature wound scars of the skin. All biochemical parameters studied were rather similar (evidently in late periods of capsular evolution), the differences in reactogenicity, if they exist at all, of the polymers under study lose their significance, and the final result of the process is the consequence of the long-term presence of foreign bodies in the tissues.

However, it turned out that already on the 7th day post implantation the olives were surrounded by well-formed capsules, that could be easily separated from the surrounding tissue (their mass constitutes 0.46 ± 0.059 g for polyethylene and 0.40 ± 0.039 g for polyurethane), and in this case statistically significant differences in biochemical indices were revealed (Fig. 2). If the 90 days old capsules by references to their biochemical data could be characterized as fibrous, then the tissue of 7

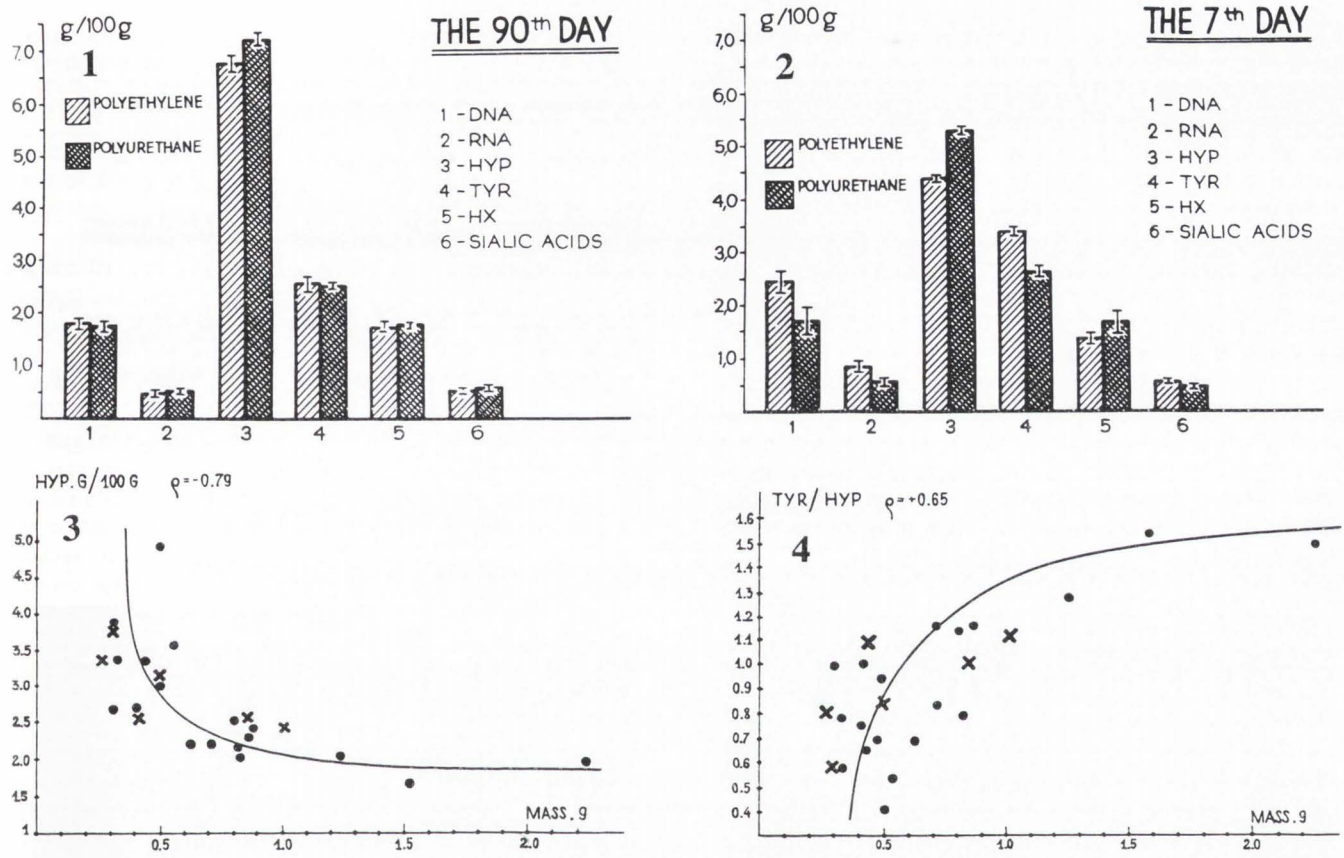

Figure 1. Biochemical composition of 90-days-old capsules: 1 - DNA (NS), 2 - RNA (NS), 3 - Hydroxyproline ($p < 0.05$), 4 - Tyrosine (NS), 5 - Hexoses (NS), 6 - Sialic acids (NS). NS = not significant.

Figure 2. Biochemical composition of 7-days-old capsules: 1 - DNA ($p < 0.05$), 2 - RNA ($p < 0.05$), 3 - Hydroxyproline ($p < 0.01$), 4 - Tyrosine ($p < 0.01$), 5 - Hexoses (NS), 6 - Sialic acids ($p < 0.05$).

Figures 3-4. Correlation between capsular mass and hydroxyproline concentration (**Figure 3**), and capsular mass and tyrosine/hydroxyproline coefficient (**Figure 4**) in 7-days-old capsules. Crosses represent different brands of polyethylene and polyurethane; dots, different modifications of polyurethane.

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days old capsules, judging by their relatively low concentration of collagen (hydroxyproline) and the high concentration of non-collagenous proteins and glycoconjugates, were nothing else but granulo-fibrous tissue, and at this time period the characteristics of the connective tissue reaction, i.e., differences in the reactogenicity of biomaterials can be distinctly determined. When testing a large number of chemical modifications of synthetic polymers, including polymers with biologically active substances introduced into them, we found pronounced differences in the capsular mass. The examples of such differences caused by the use of some antibiotics and atranes were presented before. Thus, such a simple techniques as the determination of the mass of the capsule (weighing) developing during a standard time interval around a standard implant, conveys essential information for the comparative quantitative evaluation of the connective tissue reaction to the biomaterial.

The considerable variability of the capsular mass is combined with the even more considerable variability of the quantitative indices of its biochemical content, moreover, between the mass, on the one hand, and biochemical indices on the other interesting correlations exist. According to the data of studies on 26 modifications of implanted biomaterials, that the Spearman's Rank correlation test between the mass and collagen concentration equals -0.79. This means that at the early stages of development of capsules, the collagen concentration is inversely proportional to their mass (Fig. 3).

In addition, a positive correlation has been discovered between the mass of the capsule and the coefficient tyrosine/hydroxyproline (+0.65) (Fig. 4) that reflects the correlation between non-collagen proteins (mainly glyco- and mucoproteins) and collagen. This correlation means that the larger the mass of the capsules, the higher their content of inflammatory exudate (which is the prevailing part of the granulo-fibrous tissue at the early stages of its development). Hence, the considerable capsular mass established on the 7th post-implantation day, can point either to an intensified development of the inflammatory reaction or its retarded course.

The existence of these correlations in our experimental model permits a fast screening of biomaterials and their modifications with reference to their

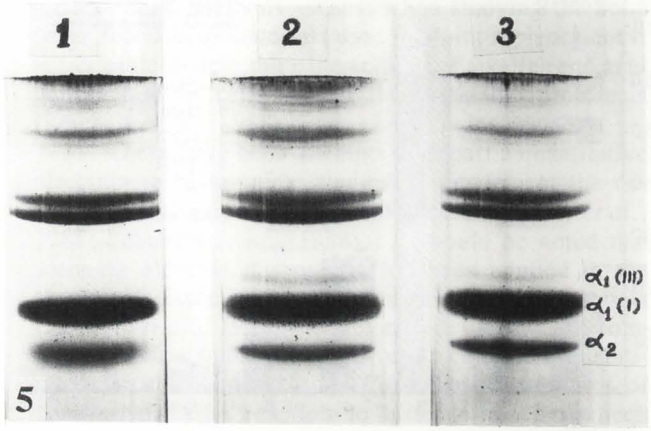
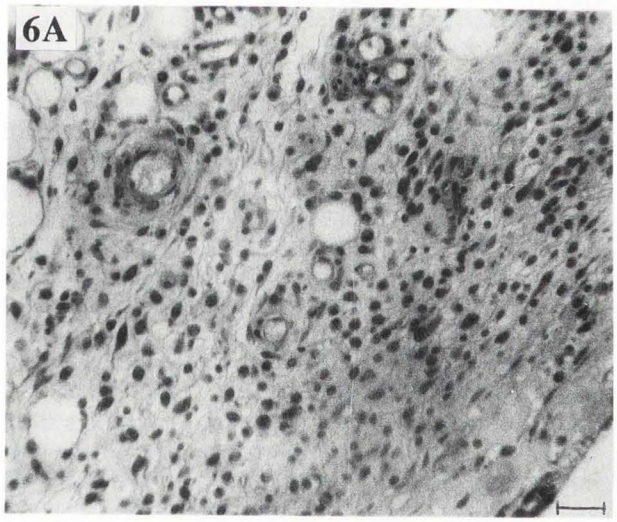
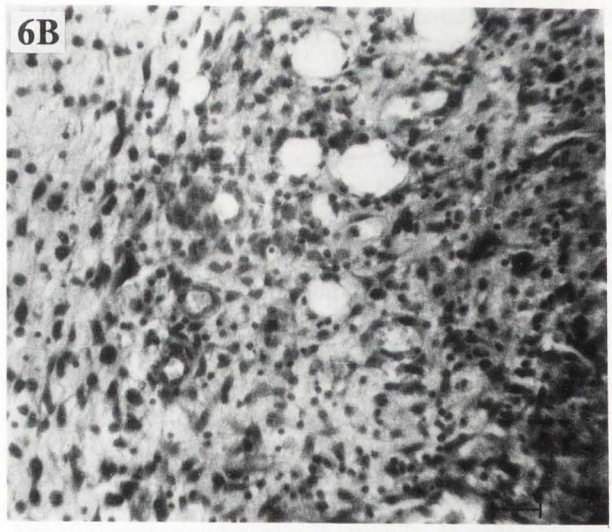


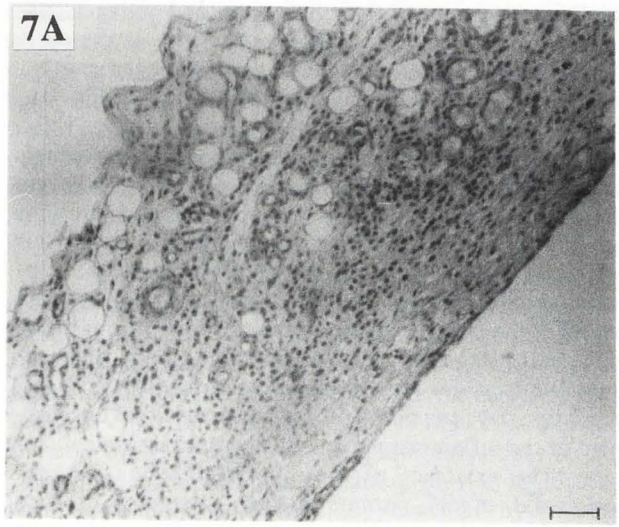
Figure 5 (at top right). SDS-PAGE (sodium dodecylsulfate polyacrylamide gel electrophoresis) of pepsin-solubilized collagens from capsules around polyurethane (PU) implants: 1 - PU unmodified; 2 - PU + ampioxum natrium 2%; 3 - PU + ampioxum natrium 2% + chlormethylsilatrane 1%.

Figure 6. **A.** 7-days-old capsule around polyurethane implant, modified by vinditate. Haematoxyline-eosin staining. **B.** 7-days-old capsule around unmodified polyurethane implant. Haematoxyline-eosin staining. Bars = 100 μm.

Figure 7. **A.** Mature collagen fibres in the 7-days-old capsule around polyurethane implant, modified by vinditate. Van Gieson staining. **B.** Less mature collagen fibres and more granulation tissue around 7-days-old unmodified polyurethane implant. Van Gieson staining. Bars = 100 μm.







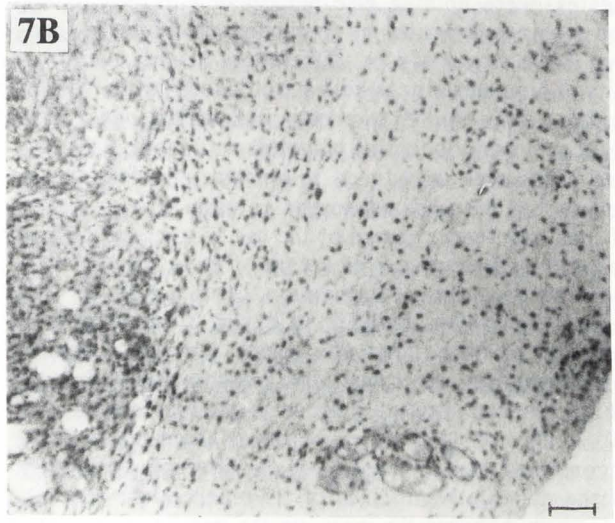


Figure 8. Scanning electron micrograph of the inner layer of capsule around polyurethane implant. Bar = 71.6 μm.

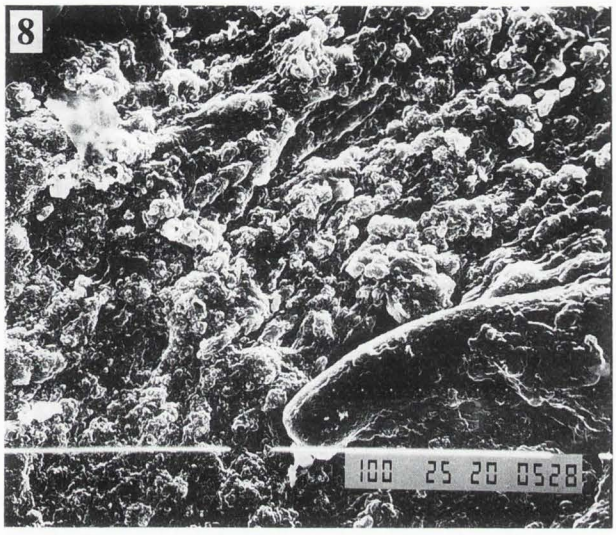


Figure 9. Scanning electron micrograph of the middle layer of the same capsule as in Figure 8. Bar = 73.8 μm.

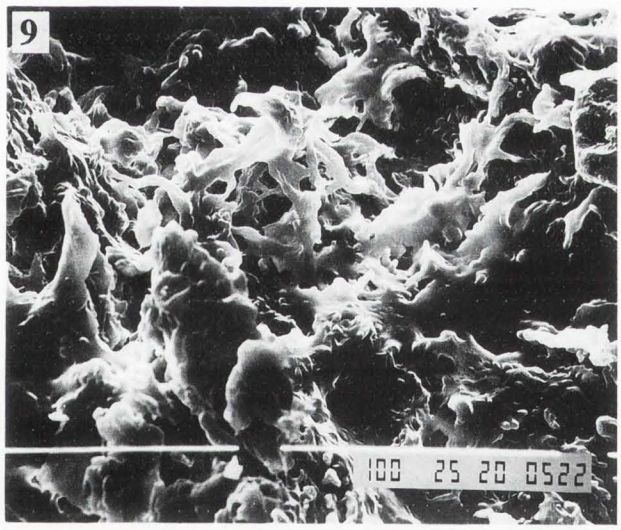
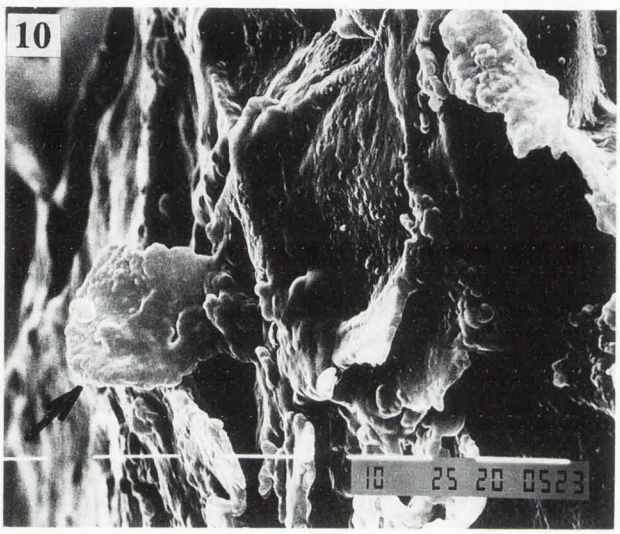


Figure 10. Scanning electron micrograph of structure and surface of the fibroblast in the same capsule as in Figure 8. Bar = 7.3 μm.



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reactogenicity. However, this is only a preliminary evaluation, since it cannot reveal the tendency of the subsequent evolution of the capsule and the prognosis of the outcome. Thus, high reactogenicity determined by this simplified method, can provide an improved integration of the implant, but it can under certain conditions provoke the development of an inflammation into a chronic form by retaining "immature" granulo-fibrous tissue for a long time period around the implant. And, on the contrary, the discovery of a thin and collagen-rich capsule evaluated as the manifestation of a low reactogenicity of the biomaterial, can be the result of the reaction rapidly passing from the stage of inflammatory exudation to the stage of fibrosis and involution. However, the same picture can be observed in the selective suppression of exudative phenomena due to which the process acquires proliferative and fibrous character already from the very beginning. Therefore, we recommend a more detailed investigation of the capsular tissue by making use of the complex of biochemical (as shown above in Figs. 1-4) and morphological methods.

The degree of maturity of capsular tissue at a selected time period can be established if the general collagen concentration (according to hydroxyproline) is supplemented by collagen type fractions. According to our data, obtained by means of electrophoresis of collagen α- chains on polyacrylamide gel (Hayashi and Nagai, 1979) the capsules around different implants differ from each other by the absence or the presence (Fig. 5) and also the quantity of Type III collagen, the presence of which is characteristic for relatively early stages of development of granulo-fibrous tissue (Gay *et al.*, 1978).

Additional information on this problem can be obtained from the histological pictures of the capsule. When performing biochemical studies of capsules around the implant made of polyurethane modified by a new anti-inflammatory compound, vinditate (The Irkutsk Institute of Organic Chemistry), we found a reduced concentration of sialoglycoproteins and increased contents of collagens when compared to the capsule around the same unmodified polymer. According to the histological data, vinditat sharply reduces the number of inflammatory cells (macrophages and lymphoid cells) in the internal layer of the capsule (Figs. 6a, b) and increases the tinctorial maturity of collagen fibers when the capsular thickness is reduced (Figs. 7a, b).

A more detailed analysis of the architecture of capsular tissue is possible with scanning electron microscopy (Bell and Revel, 1980). It reveals the prevalence of slightly differentiated and young fibroblasts in the internal layers of the capsule where the collagen fibers and blood vessels (Fig. 8) are found; a more intensive development of collagen fibers connected with the surface of mature fibroblasts takes place in the middle and external layers (Fig. 9). The structure and surface of separate fibroblasts (Fig. 10) also can be characterized in detail. It is obvious that other methodological approaches can also be used in the work with the experimental model suggested for the studies of the reactogenicity of the biomaterial.

Special attention is given in the present paper to reactogenicity problems in surgical suture materials. The above-mentioned experimental studies of rimine, a new thread of modified cellulose, demonstrated the prospects of the use of surgical threads in which an accelerated absorption rate is compensated by the ability to activate wound healing. Thus, the problem of comparative evaluation of reactogenicity of suture materials acquires practical significance.

It should be repeated that the connective tissue reaction comprises within itself as compulsory stages cellular necrosis and inflammation (Salamon *et al.*, 1980). Hence, evaluation of surgical threads based on *in vitro* experiments with cell and tissue cultures is theoretically erroneous. The effect of the thread on only one of the cell types participating in the process or on the growth of tissue culture does not disclose the complex reaction to trauma caused by the wound itself and by needle and thread.

The methodological approach suggested by us is a modification of the method originally developed by Sewell *et al.* (1955) and successfully used in the last years by van Rijssel *et al.* (1988) and Sanz *et al.* (1988). Linear skin wounds of standard length and depth in rats are sutured by the thread to be tested. Wound tissue together with the sutures is excised at different time periods and subjected to histological studies. The histological pictures are evaluated semi-quantitatively; an 0 to 4 point scoring system is applied. In preparations stained by hematoxylin-eosin, the amount of different cells is evaluated as well as the extent of edema and the degree of epithelialization of the wound defect; attention was paid to the regularity of arrangement of the regenerating epidermal layer. In preparations with picrofuchsin staining, fibroplasia in the wound scar and around the thread was assessed; the amount of collagen flares, their degree of maturity, and the regularity of the arrangement of the collagen network were taken into account. In preparations with Alcian blue staining, glycosaminoglycan accumulation intensity was assessed as an important factor of control of collagen fibrillogenesis. In all preparations attention was paid to thread absorption.

All indices were divided into negative (related to alterative and exudative reaction components) and positive (related to beneficial aspects of the reaction on sutures and wound healing process). A negative score was attached to edema, the quantity of neutrophile leukocytes, lymphocytes, plasmocytes and giant cells. The positive indices included: number of macrophages and fibroblasts, accumulation of glycosaminoglycans, fibroplasia and epithelialization, and also thread absorption. Negative and positive scores are summed separately; thus, two scores characterizing "negative" and "positive" reactogenicity are obtained. The algebraic sum of these two scores expresses the final semi-quantitative assessment of the reaction to the suture material in the healing wound at the moment of study.

To test whether this method was informative, three surgical suture materials were compared: the new rimine thread; traditional absorbing thread, catgut; and surgical silk. The results obtained are presented in Table 1, and in Fig. 11, the final assessment of the three threads is shown in the shape of curves representing the dynamics of the reaction as a whole.

The absorption of the rimine thread proceeds very rapidly and is practically completed on the seventh day. Such an accelerated absorption could provoke danger of wound dehiscence. However, since the thread absorption is combined with a general beneficial picture of healing, we give the absorption a positive score. The reaction to the rimine thread is only weak and is characterized by a rapid involution of negative components, on the one hand, and a rather early appearance and progression of positive components on the other. The generalized index (algebraic sum of the points) of the reaction to rimine in all periods of the experiment has a positive value, and reaches a higher maximum value than that for silk and catgut. The reaction develops more rapidly and the decrease in the score noted on the 14th post-operative day obviously reflects the completion of the process and the onset of the normalization of the histological picture. Thus semiquantitative assessment allows us to consider rimine as a highly reactogenic suture material, where in addition, the reactogenicity has positive character. Transient productive aseptic inflammation is accompanied by an accelerated fibroplasia and wound epithelization.

Absorption of the catgut takes place at a considerably lower speed. A marked edema around sutures, intensive and slowly reducing infiltration by neutrophile leukocytes, a significant amount of lymphocytes and plasmocytes are noted. Positive indices develop slowly and the dynamics curve of the final index crosses the border between negative and positive values only on the 7th day. But even on the 14th day the final index remains low, which reflects the incomplete character of the wound healing process. Accumulation of glycosaminoglycans is almost not observed. Judging by these data catgut can be characterized as a highly reactogenic suture material, but with a predominantly negative reactogenicity: the intensive inflammatory reaction is prolonged, and therefore wound healing is more slow.

The dynamics curve of the semi-quantitative evaluation of the reaction to surgical silk occupies an

Table 1. Semi quantitative estimation of reactogenicity of surgical threads.

Indices	Time (Days)											
	Rimine				Silk				Catgut			
	1	3	7	14	1	3	7	14	1	3	7	14
Oedema	-1.0	-0.5	0	0	-2.0	-1.0	0	0	-4.0	-3.0	-1.0	0
Neutrophiles	-2.0	-1.5	0	0	-3.0	-2.0	-1.0	-1.0	-4.0	-3.0	-2.0	-1.0
Lymphocytes	-2.0	-1.5	-1.0	0	-1.0	-2.0	-2.0	-1.0	-1.0	-2.0	-2.0	-2.0
Plasmocytes	0	-0.5	-0.5	-0.5	0	-1.5	-2.0	-2.0	-1.0	-2.0	-3.0	-2.0
Giant cells	0	0	-0.5	0	0	0	-0.5	-1.0	0	0	0	-1.0
Macrophages	+1.5	+4.0	+3.0	+0.5	+1.5	+1.5	+3.5	+3.5	0	+1.0	+2.0	+2.0
Fibroblasts	+1.0	+2.5	+4.0	+2.5	+0.5	+2.0	+3.0	+4.0	0	+0.5	+2.0	+3.0
Glycosaminoglycans	+3.0	+4.0	+1.0	0	0	+0.5	0	0	0	+0.5	+1.0	+0.5
Fibrillogenesis	+1.0	+2.0	+4.0	+4.0	0	+0.5	+2.0	+4.0	0	+0.5	+2.0	+2.0
Epithelization	+1.0	+2.5	+4.0	+4.0	0	+1.0	+3.0	+4.0	0	+0.5	+1.0	+2.0
Thread resorption	+2.0	+3.0	+4.0	+4.0	0	0	0	0	0	+0.5	+1.5	+2.0
Final evaluation	+4.5	+14.0	+18.0	+15.0	-4.0	-1.0	+6.5	+10.5	-10.0	-7.0	+1.5	+7.5

intermediate position between rimine and catgut curves. Such a position is characteristic of silk with regard to the majority of separate indices. This relates to the intensity and absorption rate of the edema, and to the number of all cellular elements except giant cells. Fibroplasia in the wound scar and epithelization start later and proceed slower than when rimine thread is used, but contrary to the catgut it is completed on the 14th day. According to the criteria suggested, silk is a suture material of low reactogenicity: it does not induce dramatic inflammation, and influences wound healing mainly by its presence.

Results similar to those of silk were obtained in experiments with synthetic absorbable suture threads, dexon and vicryl, that are significantly slower absorbed than rimine, and traditionally are considered as "inert" materials. The data obtained show the high scientific significance of the suggested morphological and semi-quantitative assessment method of reactogenicity evaluation of surgical suture materials as a special kind of biomaterials.

In conclusion, when the methodological aspects of the problem of reactogenicity are discussed it should be noted that species differences in the dynamics of connective tissue reaction to implants are noted in experimental studies (Christensen *et al.*, 1989). Nevertheless, a careful comparison of the histological picture of this reaction performed by Schreiber *et al.* (1990) allows the conclusion that there is a principal likeliness of the process in laboratory rats, guinea pigs and humans. This justifies the transfer of experimental data from animals to humans and gives sound grounds for the direct significance of experimental studies of reactogenicity for practical use of biomaterials in clinical medicine.

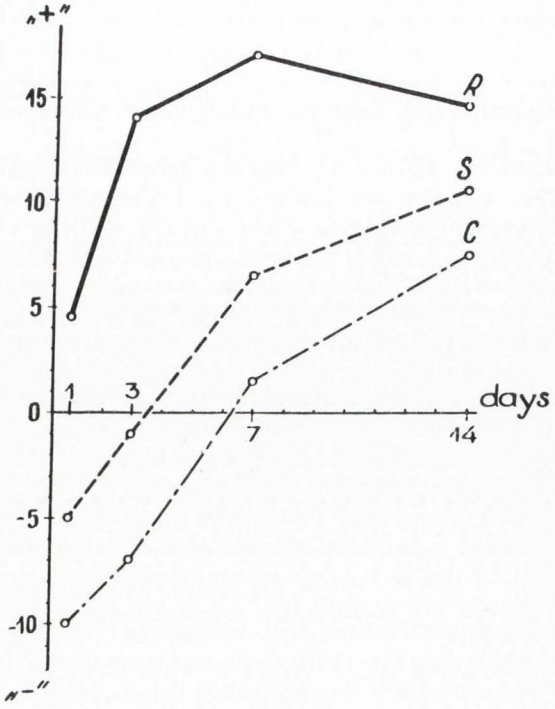

Figure 11. Dynamics of the semiquantitative index (see text and Table 1) of tissue reaction and wound healing with three suture materials: **R** - rimine; **S** - silk; and **C** - catgut.

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Discussion with Reviewers

Reviewer III: The term "reactogenicity" is a new creation and not necessary because there are already terms that can be used!

Authors: We hold the opinion that the term "bioactivity" is semantically incomplete: it implies the existence of bioinert materials. The latter as we tried to prove in our paper, is principally impossible, and the term "bioinertness" has not been recommended for use (See Williams DF, Definitions in Biomaterials, Amsterdam: Elsevier, 1987). The term that we have suggested is "reactogenicity", and it does not imply the aforementioned drawback and is oriented for a quantitative approach of the assessment.

H.J. Busscher: After having read the manuscript, I believe that such surfaces are also very apt to attract microbial infections. Gristina and co-workers describe the ultimate fate of a biomaterial in the human body as the result of "a race for the surface between bacteria and cells". Please comment.

Authors: There are definite theoretical grounds underlying the concept about the danger of possible intensified attachment of microbial infection in case of increased reactogenicity of biomaterials. However, the beneficial results obtained by the use of implants with increased reactogenicity owing to their porous structure in bone plastic surgeries, testify otherwise. In addition to that, reactogenicity increase can be achieved by introducing antibiotics over the implant surface, that promotes avoidance of infection. There are corresponding examples provided in the article. Increased reactogenicity was also noted when introducing immunostimulators in the polymer materials (See Pkhakadze GA. Biodegradable polymers, Kiev: Ukrainian Academy of Science Publ., 1990, 99-124, in Russian).