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DEPENDENCE OF THE BIODEGRADABILITY OF CARBOXYMETHYLCELLULOSE ON ITS SUPERMOLECULAR STRUCTURE AND MOLECULAR PARAMETERS

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It has been established that the resorption of carboxymethylcellulose (CMC) on its implantation into a living organism depends on its molecular mass, degree of substitution, and supermolecular structure. Its mechanism includes both hydrolytic breakdown of the main chain and macrophagal pinocytosis. It has been shown that CMC is absorbed completely and is excreted from the organism without accumulation.

The process of resorption of polymers on their implantation is a little-studied sector of the chemistry of medico-biological polymers [1-4]. Any polymeric material implanted into a living organism is perceived by its cells as a foreign body, which is shown in the form of a response reaction [5, 6]. This is an aseptic inflammation the degree of which is determined by both the chemical and the physical properties of the implant.

Cellulose, being a natural polymer, is biocompatible; however, it is not resorbed on implantation. A cellulose thread implanted into an organism causes a prolonged response reaction of the surrounding tissues (six months and more) and the process is completed by the formation of a coarse scar around it [7]. Thus, the reaction of the organism to cellulose is the encapsulation of the implant. The reason for the nonresorbability of the natural polymer cellulose has not been explained. However, the presence in the cellulose macromolecule of any hydrophilic functional group whatever imparts a capacity for resorption to it. There are reports of the resorption in the living organism of carboxymethylcellulose (CMC) [8], water-soluble acetylcellulose [9], methylcellulose [10], and monocarboxycellulose [11].

In this communication we give the results of an investigation of the processes of biodegradation in the resorption of CMC in the living organism and of a determination of the dependence of the rates of its resorption on its molecular structure and molecular parameters. Table 1 gives the characteristics of the materials based on CMC that were subjected to resorbability testing. The resorbability of the CMC materials was determined by morphological and histological investigations and also by a study of the reaction of the surrounding tissue to the implant.

As can be seen from Table 1, the materials based on CMC had different degrees of polymerization (DP) and degrees of substitution of the carboxy groups (DS). They differed in their supermolecular structure, which was due to the different initial raw materials [12]. This enabled us to establish the influence of these parameters on the nature of the resorption process and its rate.

The reaction of tissue to an implant and the nature and degree of resorbability of the CMC-based materials were determined visually and under the microscope. As an example, let us give the results of an investigation of the reaction of muscle tissue to implants of CMC with DPs of 100 and 400 and a DS of 0.68 [13].

After 1-2 days from the operation, the muscle wound with sample II having DPs of 400 and 100 implanted in it had an irregular form. Its edges were rough, and there were fissures in the parenchyma formed through extension of the wound by the swollen implant. The material had risen above the surface of the wound and the fibers were swollen.

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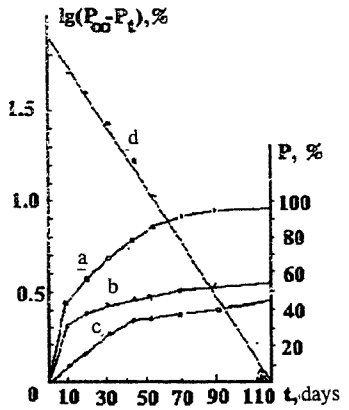

Fig. 1

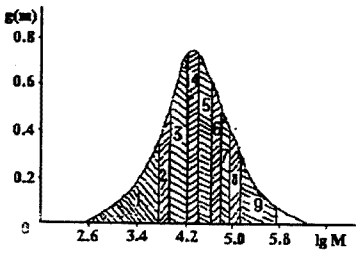

Fig. 2

Fig. 1. Elimination of [^{14}C]CMC: a) total cumulative excretion; b) excretion with the urine; c) excretionwith the feces; d) linearization curve: tan α – 0.018; Ke_1 = 0.042 day^{-1}; $T_{1/2}$ – 16.5 days.

Fig. 2. MMD of [^{14}C]CMC (with DP = 110) implanted in the liver: 1) 1 day, MM 5700; 2) 6 days, MM 8500; 3) 10 days, MM 17,300; 4) 20 days, MM 26,000; 5) 31 days, MM 40,000; 6) 43 days, MM 60,000; 7) 55 days, MM 87,000; 8) 70 days, MM 138,000; 9) 90 days, MM 436,000.

A histological investigation showed that sample II with DPs of 400 and 100 had the form of interwoven bright violet bands and oval formations; there was no suppurative inflammatory reaction. Seven days after implantation into an intermuscular pocket, material II with DP 400 was a uniform gel-like transparent mass. The muscle tissue adjacent to the wound channel had not changed visually. On histological examination no elements of a suppurative inflammatory reaction were observed.

On the 14th day after the implantation of material II with DP 400 the pattern of the muscle wound was similar to that described above. Histological examination revealed the growth of young connective tissue the fibers of which were growing between the particles of the implant, separating it into small fragments. Pronounced phagocytic–macrophagal infiltration was determined. No elements of suppurative inflammation were detected.

After 21 days, the pattern of the muscle wound with material II having DP 400 implanted in it was identical with the preceding pattern.

After 30 days, no material II with DP 400 was detected visually. The microscope showed, in the zone of the wound channel, a growth of connective tissue between the elements of which microinclusions of the implant in the form of a powder were encountered. Macrophagal infiltration was detected round the microinclusions.

After 60 days, under the microscope the zone appeared free from II. The wound channel was filled with mature connective tissue.

On the seventh day after implantation into an intermuscular pocket, the material II with DP 100 consisted of a shapeless amorphous mass with a gel-like consistency. Microscopy of the zone of the wound channel showed the growth of young connective tissue the collagen fibers of which separated the conglomerate of material II into small fragments. Phagocytic–macrophagal infiltration was seen round the particles of material II. No elements of suppurative inflammation were detected.

On the 14th day after the implantation of material II with DP 100 no hemostasis was detected visually in the zone of the wound channel. On examination under the microscope we detected in the muscle wound the growth of connective tissue, between the elements of which there were microinclusion of the material II surrounded by macrophages. There was no suppurative–inflammatory reaction.

TABLE 1. Characteristics of the CMC Preparations Subjected to Testing for Resorbability

Symbol, base, and form of the preparation	Characteristics of the preparation		Tissue	Time of complete resorption, days
	DP	DS of the COOH groups		
I. Wood cellulose, powder	530	0.80	Muscle, kidney	30–60, 60–90
II. Cotton cellulose, carboxymethylated bandage	400	0.68	Muscle, kidney	60–70, 180–270
	100	0.68	Muscle	30–40
III.	745	0.40	Muscle	180
IV.	705	0.15	Muscle	360
V. Hydrocellulose, carboxymethylated knitted fabric	270	0.38	Kidney	90–120
VI.	210	0.64	Kidney	60–90
			Liver	30–60
VII. Cotton thread, carboxymethylated thread	1940	0.12	Muscle	about 400

TABLE 2. Amount of [^{14}C]CMC in 1 g of Tissue ($\times 10^6$) (dose 2 mg, 100×10^6 pulses/min)

Organs	Days from the moment of implantation										
	10	20	25	31	35	55	65	75	90	105	120
Blood	0.25	0.20	0.17	0.15	0.09	0.028	0.015	0.009	0.0005	0.0001	0
Heart	0.03	0.025	0.043	0.03	0.017	0.018	0.004	0	0	0	0
Spleen	0.30	0.27	0.23	0.23	0.20	0.061	0.054	0.02	0.002	0	0
Lungs	0.065	0.06	0.057	0.043	0.038	0.009	0.001	0.0003	0	0	0
Liver	6.0	5.3	4.9	4.2	3.5	1.4	0.87	0.63	0.45	0.23	0
Muscle	0.032	0.021	0.012	0.008	0.006	0.001	0	0	0	0	0
Kidneys	1.5	0.99	0.98	0.94	0.85	0.31	0.21	0.12	0.013	0.009	0
Brain	0.004	0.004	0.002	0.002	0.002	0	0	0	0	0	0

On the 21st day after the implantation of material II with DP 100 into muscle tissue the pattern of the zone of the wound channel differed from the preceding one only by the fact that the amount of microinclusions in material II had decreased considerably.

On the 30th day after the implantation of material II with DP 100 into muscle tissue, microscopic examination revealed no wound channel. The muscle wound was filled with formed connective tissue.

The faster resorption of the sample with DP 100 showed that the rate of resorption of materials depends directly on the DP.

The histological and morphological investigations showed that, in contrast to cellulose, CMC-based materials are resorbed completely in the organism, and the rate of resorption depends on the molecular parameters and on the organ into which implantation is made. The reaction of the surrounding tissue to the implant is the same in all cases.

The changes in the CMC preparations taking place during resorption may be considered arbitrarily as stages of early and late changes. In the stage of early changes, the preparation retains its structure but undergoes swelling and a loss of strength. In the stage of late changes, fragmentation, homogenization, absorption of the implant by macrophagal cells, and resorption take place. The time of the early changes is almost identical for all the preparations. During this time, the material is saturated by blood and tissue fluid and swells, and the penetration of cell elements into it begins. The times of the stage of the later changes of the preparations are different, especially the times of the growth of collagen fibers in the material and resorption. It is possible to see a definite dependence of the shortening of the time of complete resorption of the material with a lowering of the DP and an increase in the DS and also with a loosening of the structure of the material.

As can be seen from Table 1, the times of resorption depend on the organ subjected to implantation. An implant is resorbed more slowly in kidney tissue than in an intermuscular pocket and the liver. With an increase in the looseness of the structure of the implant, and also with a decrease in the DP and an increase in the DS, the times of resorption shorten.

The histological and morphological investigations permit the conclusion that the process of resorption can be classified as simultaneous hydrolysis catalyzed by intercellular enzymes and partial phagocytosis through cell elements. If one of these mechanisms is excluded, it is impossible to explain the combination of processes occurring with respect both to the reactions of the surrounding tissue and to the morphological patterns.

Morphologically, the complete disappearance of new CMC-based material from the implantation site was established. However, the subsequent fate of the material that had disappeared is not clear. Histological studies of internal organs (liver, kidneys, spleen, stomach, intestine, lung, heart, brain, adrenal) carried out 14, 30, 60, 90, 180, and 270 days after implantation of the CMC materials in the organism revealed no changes whatever in them. Consequently, the materials tested cause no chronic intoxication of the organism and do not accumulate in vitally important organs.

For a pharmacological study of the process of resorption of CMC by the isotopic-indicator method we synthesized [^{14}C]CMC by the reaction of mercerized hydrocellulose with $ClCH_2[^{14}C]COOH$.

The specific radioactivity of the [^{14}C]CMC was 0.92 MBq/mg, while the DS of the carboxymethyl groups was 60+2 and the DP 110$\pm$10.

The kinetics of the distribution and excretion of the [^{14}C]CMC were studied by the operative implantation into the liver of 2 mg of [^{14}C]CMC (100×10^6 pulses/min or 45.45 μCi) per animal. The duration of the experiment was 120 days.

As can be seen from Fig. 1, a considerable part of the implant was resorbed even in the first few days after implantation. The dissolved implant passed out with the bloodstream from the liver, was distributed over the organs, and was eliminated from the organism with the urine and the feces. Judging from the cumulative curve, about 43% of the preparation was excreted in the first 10 days.

It may be assumed that in the first stage that part of the implant is resorbed in which the low-molecular-mass fraction predominates, while the dissolved high-molecular-mass fraction continues to circulate in the blood, accumulating in the organs and tissues, from which, through the RES system, it passes out into the gastrointestinal tract.

Table 2 gives the distribution of [^{14}C]CMC over the organs at various times after implantation. As we see, the CMC was excreted from the organism completely. First, the low-molecular-mass fraction of the polymer was removed from the implant, and this was eliminated from the organism with the urine. This feature of the elimination of the implant is connected with its polymeric nature, and, in particular with its molecular-mass distribution (MMD). The residue of the implant was swollen material consisting of molecules with mean and high molecular masses. On the whole, during the first ten days (phase of rapid excretion) about 43% of the dose administered was excreted with the urine and feces.

We used ultracentrifugation to investigate the MMD of the CMC obtained by the mercerization of hydrocellulose with monochloroacetic acid (DP = 110 and DC = 60±2). Calculations show that the CMC consisted of macromolecules with MMs from 6000 to 440,000.

Figure 2 gives a diagram of the elimination of an implant from the organism as a function of molecular mass. If it is considered that, on the whole, it is the complete implant that is subjected to biodegradation, then after 90 days CMC macromolecules with MMs of about 440 thousand are already being eliminated.

Of course, it is impossible on the basis of this diagram to state that, if the implant consists of macromolecules with MMs from 400 to 440,000, resorption takes place in stages with macromolecules having low molecular massses being eliminated first and only macromolecules with high molecular masses at the end. The resorption process is complex, and the whole implant is subjected to its action. The diagram must therefore be considered in such a way that at a definite moment in time, let us assume 10 days after implantation, the existence in the implant of macromolecules with MM 17-18 thousand is possible. However, macromolecules having MM 17-18 thousand in the initial implant will already have been subjected to degradation and have been eliminated from the organism quantitatively.

Thus, it has been shown that, on implantation into the animal organism, CMC is completely resorbed, and the products of its metabolism are completely excreted from the organism. The decisive condition for the resorption process is the hydrolysis of the main chain of the CMC and macrophagal pinocytosis.

EXPERIMENTAL

The carboxymethylation of cellulosic materials was carried out by immersing them in an emulsion of 45% NaOH and isopropyl alcohol (IPA) at a ratio of 1:58 and 25°C for 2-4.5 h. The pressed-out material was placed in a 10% solution of monochloroacetic acid in IPA at a ratio of 1:40-50, and the mixture was kept at 25°C for 4-5 h. The product obtained was treated with a 1:5 mixture of concentrated HCl and IPA at a ratio of product to acid mixture of 1:10 for 10 h, and it was then washed free from Cl^- ions with water and was dried in the air.

Sedimentation analysis was conducted on a 3180 centrifuge (MOM, Hungary) in one- and two-sector cells at a rotor speed of 44 thousand rpm. Sedimentation coefficients were determined from the slope of the curve of the dependence of ln X on the time τ. The degrees of polymerization of the samples were determined viscometrically [14]. Degrees of substitution in carboxy groups were determined by the method of Timokhin and Fenkel'shtein [15].

The morphological and histological investigations of the tissues with the implants were made as in [16]. The biodegradation of the CMC preparations was studied by the isotopic-label method [17].

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