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# BIODETERIORATION STUDIES ON PARCHMENT - AND LEATHER-ATTACKING BACTERIA IN THE COMMONWEALTH OF SOCIALIST STATES

ABSTRACT - *The results of microbiological studies on leather and parchment objects of historical and cultural importance to the heritage of Russia, Estonia, Latvia, Ukraine, Armenia, and Georgia are summarized.*
*The ability of isolated microorganisms to hydrolyze collagen fibers and other proteinaceous components of leather and parchment was revealed after screening for exoenzyme production as well as by electron microscopy studies and by specific experiments. The microbial communities including collagenolytic, caseinolytic and non-proteolytic species have been found to be involved in biodeterioration of leather and parchment. Four of the most active species belonging to the genus Bacillus were identified and used as test-organisms.*
*The survival rate of the test bacteria, at controlled humidity, was determined by nuclear magnetic resonance technique using water-proton-relaxation characteristics in the parchment and leather samples.*
*The effectiveness of two quaternary ammonium compounds (QAC) - Catamin AB (Russia) and Preventol R-80 (Bayer, Germany), known as disinfectants, were assessed for the bacteria isolated from leather and parchment objects. When applied in combination, QAC and gamma-irradiation exhibited a synergistic activity against radioresistant bacteria.*
*Minimal sterilizing doses for treatment of parchment and archaeological leather objects heavily contaminated by bacteria were determined. Decontamination of parchment required lower gamma-irradiation doses than that of leather. No changes in the surface layer microstructure and physical-chemical properties of the treated material were observed as a result of the treatments. Preventol R-80 proved to be more effective than Catamin AB. An effective treatment procedure for the protection of ancient leather and parchment objects was achieved.*

## Introduction

Historical and cultural objects made from parchment and leather consisting of fibrillar protein collagen as a structural base were selected for study. Most of the literature dealing with microbial contamination of these types of materials has examined fungal contamination and not problems caused by bacteria. This paper is focused on problems of bacterial damage on these types of objects.
The first, and perhaps most important issue, concerning microbial contamination of parchment and leather is establishing a causative relationship between the microbe(s) isolated from the material and damage from their metabolic activities.
Once the microbes have been shown to be destructive, it is important to investigate the conditions which affect their survival, growth, and destructive activity.
An independent, but vitally important research question, is the selection and demonstration of disinfection and sterilization techniques to remove or neutralize the damaging microbes without further harming the culturally important material.
The main goals of this research on parchment and leather were: isolation of bacteria from these classes of objects; determining which bacteria could cause deterioration of the objects; determination of proper storage conditions to inhibit the bacteria; and determination of appropriate disinfection and sterilization procedures for infested objects.

## Materials and Methods

Parchment and leather objects were selected from museums, archives, and libraries of different regions of the former Soviet Union: Estonia, Latvia, Lithuania, Belarus, Ukraine, Moldavia, Georgia, Armenia, and Russia (Fig. 1). Among the objects were parchment manuscript books, Acts, and Deeds, as well as leather articles from the applied arts.

The following growth media were used to isolate bacteria from the selected parchment and leather objects: beef extract agar; potato agar; gelatin agar; Czapec medium; wort agar; and media containing NH4, NO3 (0.07%), KH2 PO4 (0.25%), MgSO4 . 7H2O (0.05%), FeCl3 . 6H2O (traces), NaCl (2.34%), tap water, pH 7.5 -7.6 with either glucose or modern parchment or insoluble denatured collagen (Reahim, Russia).
Viable microorganisms were enumerated by plate counts. Proteolytic and collagenolytic activity of bacteria were determined in the culture fluid (Petushkova et al., 1984a and b, respectively). Light optical and electron microscopy studies were carried out according to Poglazova et al., (1988).

**Isolation and Investigation of Bacteria**
Viable colony counts, ability to grow, measurements of proteolytic and collagenolytic characteristics, and electron microscopy studies were used to detect and quantify any microbial activities on the above material.
Plate counts and the "bacprint" method were used for enumeration of the microorganisms.
"Bacprint" is a sterile camera, 3 cm in diameter, with a hollow in a cover where agar medium is transferred. The surface of the agar medium was touched to the surface of an object and then incubated for 6-7 days. Peptone medium and nutrient wort, as well as more selective media containing insoluble denatured collagen or modern parchment, were used.
The "bacprint" technique may reveal up to 12 colonies of bacteria from a 2- $cm^2$ parchment or leather area. Fig. 2 presents results of bacterial counts obtained with the "bacprint" method. In some tests micrococci were present and frequently bacteria of the genus Bacillus.

It should be noted that the "bacprint" procedure is suitable for viable counts of aerobic microorganisms localized on the surface layer of an object. This method is simple, rapid and "nondestructive" to the object. This latter fact is extremely important when handling works of art. The "bacprint" technique, however, does not provide information on any microorganisms that may be sequestered inside the objects. The plate method, on the other hand, permits isolation of bacteria from inner layers, but it is rather labor intensive as it requires crushing samples into pieces and then making the necessary dilutions. The plate count method has assayed up to 1 million cells in badly decayed parchment manuscripts.

**Results and Discussion**
Thirty-six bacterial strains isolated from parchment (Fig. 3) and 21 from leather (Fig. 4) were screened for their ability to grow on collagen-containing media and to hydrolyze collagen fibers and other protein components of these materials. Most of the strains were able to grow on media with collagen or parchment as the sole source of carbon and nitrogen. Proteolytic activity measurements showed that 26 of the 36 strains isolated from parchment were active to casein hydrolysis (Fig. 5), while 19 of 21 strains isolated from leather demonstrated proteolytic activity (Fig.6).

High numbers of bacteria did not always correlate with extensive deterioration of an object. Geographic locations also did not correlate with the extent of deterioration.
Four strains that could synthesize highly active exoproteases and that intensively grew on parchment and collagen media were selected for further study (Fig. 7). These were identified as Bacillus subtilis, B. licheniformis, B. megaterium and B. pumilus. Collagenolytic activity of these 4 bacteria was investigated. The highest activity for hydrolysis of native collagen was detected in B. subtilis (strain R 3-1, see Fig. 7) isolated from a parchment deed, XVI C. This same strain proved to be the least active to casein hydrolysis. B. licheniformis (Fig. 7). Strain T MC, on the other hand, had the least collagenolytic activity towards native collagen and the greatest caseinolytic activity.

Thus, the investigated bacteria possessing proteolytic activity are capable of utilizing casein, denatured collagen, and parchment as substrates, and some of them hydrolyze native collagen. These strains can be assumed to be responsible for decomposition of the parchment and leather objects. These results were confirmed by direct electron microscopic investigations.
Electron microscopic examination of medieval parchment fragments has shown a high degree of bacterial contamination, both Gram-positive and Gram-negative species, in the test samples. The bacteria were localized in subsurface layers of parchment, along the collagen fibers. A fibrolytic effect is clearly seen on ultra-thin sections (Figs. 8a & b).
Only a few of the species in this study were found within the parchment (the rest were on the outside). It therefore seems probable that only a limited number of bacterial species are capable of existing under these conditions (of low moisture content) and of using parchment as a substrate.

**Preventive control measures: the effect of humidity on bacteria**

The level of contamination found was, to a great extent, correlated with storage conditions for the objects–relative humidity (RH) seemed to be the most important parameter. For example, a decrease in numbers of bacteria occurred in parchment manuscripts of The Scientific Library of Tartu State University (Estonia) after they were moved to a new building with air-conditioning. Also, no microorganisms were detected on parchment manuscripts in the largest depository of ancient Matheiadaran manuscripts in Erivan (Armenia) where optimum conditions of storage were maintained.

A knowledge of both the content and the state of water in parchment and leather of different origins is important for control of biodeterioration. Hydration of parchment and leather depends not only on ambient humidity and temperature, but also on the structure of their protein macromolecules, primarily the collagen fibers.

**NMR and hydration measurement**

To determine the content and state of water nuclear magnetic resonance (NMR) was used. Relaxation characteristics of the outer hydration layer in medieval and modern parchment, XVI C. book bindings, as well as in XIX C. leather were measured. According to the water sorption isotherms (Fig. 9) the water content in parchment doubles as the RH increases from 55% to 98%. Leather moistening also increases, but to a lesser degree.

Relaxation characteristics of water protons are a direct reflection of their mobility in a material **ref?** They are different for water fractions with different degrees of binding to protein macromolecules. For example, the proton spin-spin relaxation time (T2) for free water is 2-3 ms and for frozen water about 1æs. In parchment and leather, at 55% RH, the T2 value is about 0.2 ms, indicating that strongly bound water is an integral part of collagen structure. Values with this interval correspond to water fractions that exhibit different degrees of interaction with the polar groups of the proteins, and therefore with the degree of water mobility.

In all of the samples in the experiments reported herein, the relaxation time of protons responsible for resonance was found to be about 0.2 ms at 55% to 65% RH (Fig. 10). This corresponds to bound water with restricted mobility. In modern parchment and leather an 0.2 ms T2 was obtained up to an RH of 95%. The maximum values for medieval parchment and XVII C. binding parchment was 0.8 ms at 98-100% RH and the maximum value for modern leather and parchment was about 0.5 and 0.4, respectively, at 98-100% RH.

**Water mobility and bacterial cell counts**

It should be noted that the degree of water mobility determines accessibility of water for microbial cells. As noted with NMR, the higher the humidity, the greater the mobility of the water and presumably, the more microbial growth one should get. To test this, a series of experiments were undertaken assessing cell count versus parchment and leather hydration, as measured by humidity. Experiments that assessed for the effect of humidity on B. subtilis in parchment samples (Fig. 11); B. licheniformis in parchment (Fig. 12); and bacilli in leather samples (Fig. 13) are summarized.

A definite correlation was apparent between the water state and growth of bacteria in parchment and leather (Figs. 11-13). The largest increase in cell number occurred with B. licheniformis in medieval parchment at 90% RH and above. The appearance of water with relatively high mobility that has been observed in the medieval parchment is one of the causes of its vulnerability to biodeterioration. The data indicate that medieval parchment and leather are more susceptible to biodeterioration, at a significantly lower RH, than is modern parchment. In modern parchment and leather, the growth of these bacteria was weak until about 98% and 96% RH, respectively. Lower growth in modern material may be connected with lower water mobility and lower nutrient value of modern material.

The survival rate of bacilli was lowest at 65% RH. Electron micrographs (e.g., Fig. 14) of bacilli taken from air-dried parchment grown at 65% RH revealed condensed nucleoids of Gram-positive cells. This is the resting state of bacilli. Thus maintaining parchment and leather objects at no more than 65% RH induces the resting state for bacilli and prevents deterioration. Since temperature and humidity fluctuations induce hydration alterations and older, already partially decayed, parchment has the greatest water absorbing capacity, it is necessary to ensure that optimum indoor humidity conditions are always prevalent. If not, biodecay may set in.

**Techniques of Protection of Biodeterioration of Parchment and Leather Historical Objects**

Since bacteria may be in the resting state in parchments and leathers stored at RHs less than 65%, it may be desirable to eradicate them altogether, rather than risk growth if RH controls should fail. This section reports on the effects of chemical (2 compounds) and physical (gamma irradiation) agents, either alone or in combination, on bacteria.

The quaternary ammonium compounds, Preventol R-80, which was graciously supplied by Bayer AG (Germany), as well as Catamin AB (Russia) were selected as biocides. The latter is widely used for the protection of various museum objects by restorers in Russia.
Four of the bacteria isolated above were tested for MIC (minimum inhibitory concentration) for Catamin AB and Preventol R-80 (Fig. 15, rows 1 and 2). Preventol R-80 proved to be more active. MIC for Preventol R-80 was 310 ug/ml compared with 620 ug/ml for Catamin AB.
Further tests with Catamin AB (readily available in Russia) on bacilli-infested-parchment have shown that even a concentration of 3%-5% was not effective against some bacilli.
It was decided to test if a combination of various concentrations of Catamin AB and doses of gamma-irradiation could provide a more effective treatment than either alone. Radiosensitivity testing of the bacterial isolates revealed the most radioresistant bacilli. These were then used in combination experiments.
The minimum lethal dose against the radioresistant cultures was determined to be 25 kGy. However, doses of more than 20 kGy are known to induce destructive alterations of collagen fibers (Fig. 16) **ref.** Therefore it would normally be impossible to use radiation for treatment of parchment. A series of tests of different concentrations of Catamin AB and different dose of gamma radiation were tried. The minimum effective doses were determined for parchment samples infected by bacilli resistant both to Catamin AB and to gamma-irradiation (Fig. 17).
A synergistic effect of the above-mentioned agents has been found as the result. Antimicrobial effectiveness was achieved with gamma-irradiation doses of 2.5-5.0 kGy when combined with a pre-treatment of 3% Catamin AB. No changes in the surface layer microstructure and physical-chemical properties of the treated parchment were observed.
This techniques has been used on heavily biodeteriorated fragments of the Greek medieval parchment manuscript of the Central State Archives of Ancient Acts in Moscow and for archaeological leather objects from museums of the Ukraine, Belarus, and Moldavia.

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JULIA P. PETUSHKOVA graduated from the Department of Biology of Moscow State University in 1969. Since 1969 to 1977 she worked at the Chair of Microbiology where she studied and sulphur metabolism of the purple bacteria. Since 1977 she was working in the State Research Institute for Restoration as Senior Research Associate. In 1983 she defended the theses for Ph. D. /Kandidat in Biology on the problems of biodeterioration of parchment and leather works of art. Since 1994 she is a head of the Laboratory of Biodiagnostic and Conservation of Cultural Property at AO "Biotechnologia". She is an author of more than 60 papers.

ROBERT J. KOESTLER received his Ph. D. in the field of cellular biology from the City University of New York. He has worked in the museum field for 24 years, eight of those managing a scanning electron microscope facility at the American Museum of Natural History in New York, and the balance at the Metropolitan Museum of Art, New York, where he is currently Research Scientist. In addition, Dr. Koestler is Adjunct Professor of Conservation at New York University's Institute of Fine Arts, Conservation Center, in New York. His more than 80 publications, and two edited volumes, cover a variety of aspects of the biological and conservation sciences. In the

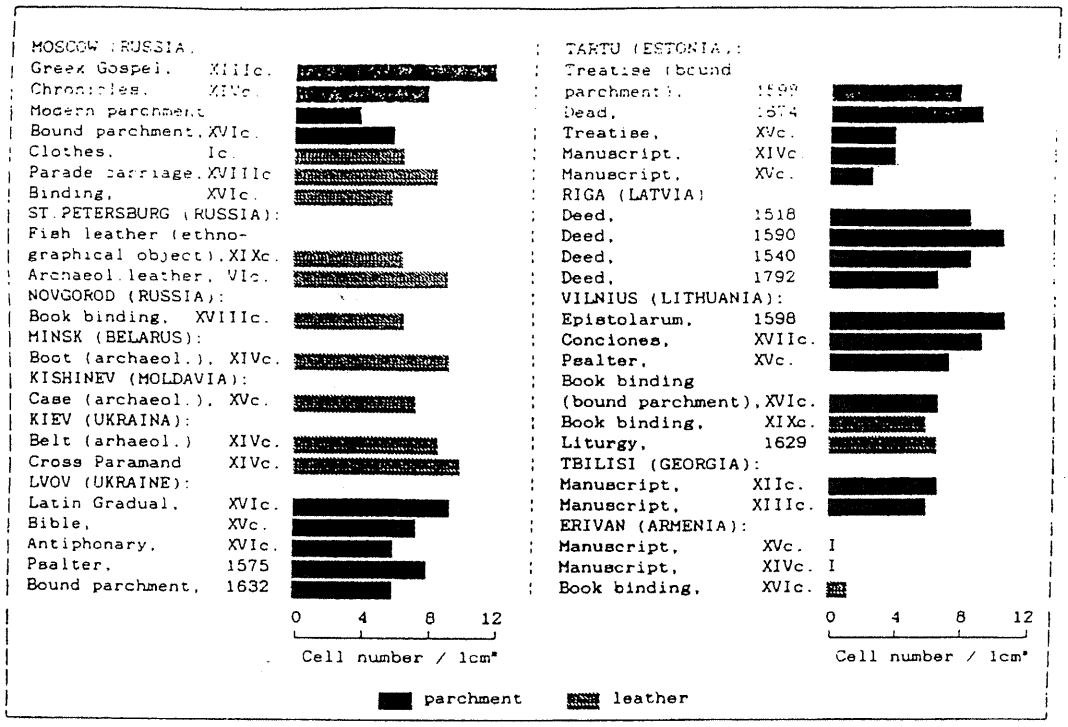


FIG. 2 - COUNT OF VIABLE BACTERIA FROM PARCHMENT AND LEATHER OBJECTS OF MUSEUMS AND ARCHIVES OF THE FORMER SOVIET UNION.

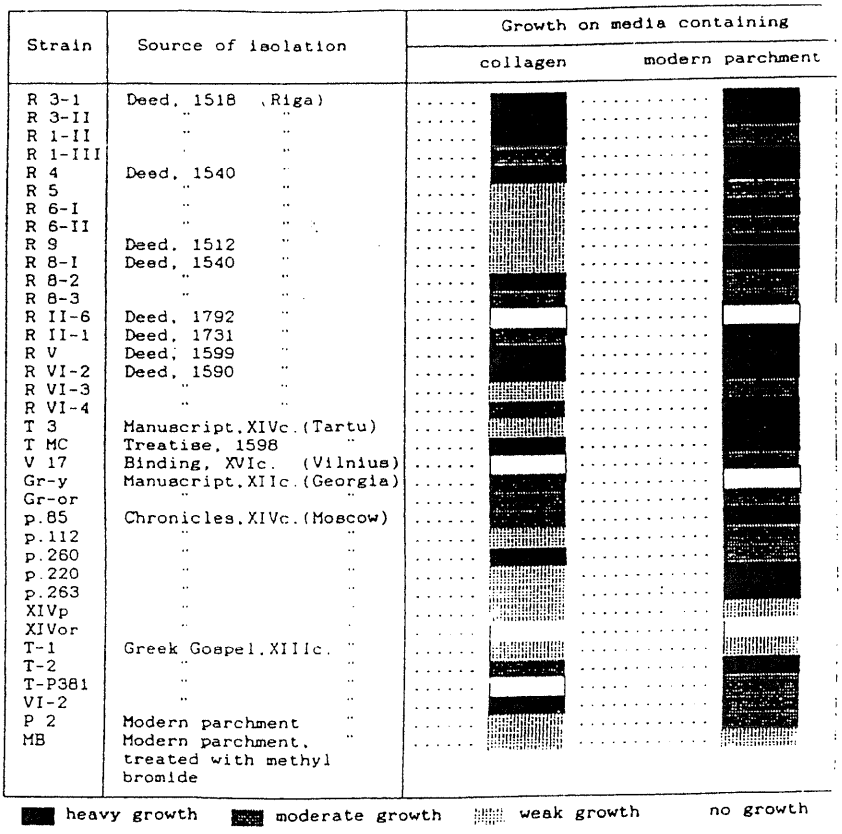


FIG. 3 - GROWTH OF BACTERIA FROM PARCHMENT ON COLLAGEN-CONTAINING MEDIA

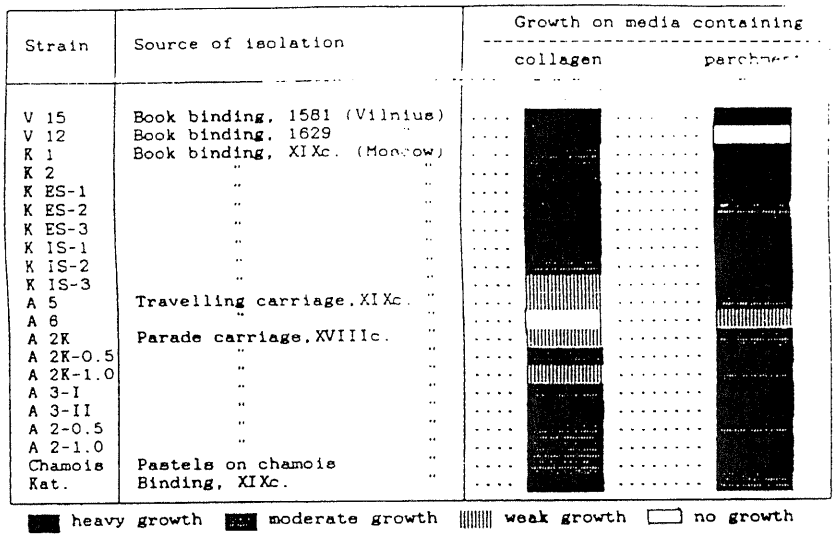


FIG. 4 - GROWTH OF BACTERIA FROM LEATHER ON COLLAGEN-CONTAINING MEDIA

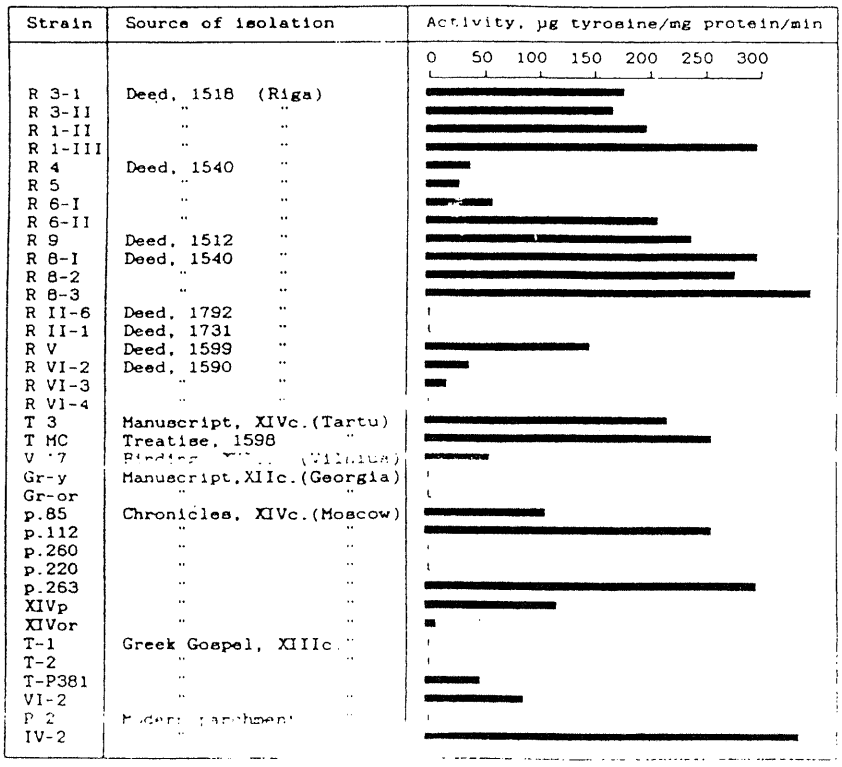


FIG. 5 - CASEINOLYTIC ACTIVITY OF BACTERIA FROM PARCHMENT OBJECTS.

conservation field he has studied biodeterioration of stone, glass, and treatment materials and analysed the component materials of fine art, such as those composed of paper, textiles, glass, stone, and metals. He is also an editor for *International Biodeterioration and Biodegradation*, a, Elsevier journal.

His most recent research concerns invention of new measurement procedures for identifying the presence of organism hidden within fine art, and development of low-risk treatment strategies to eradicate microbial and insect infestations found in fine art objects.

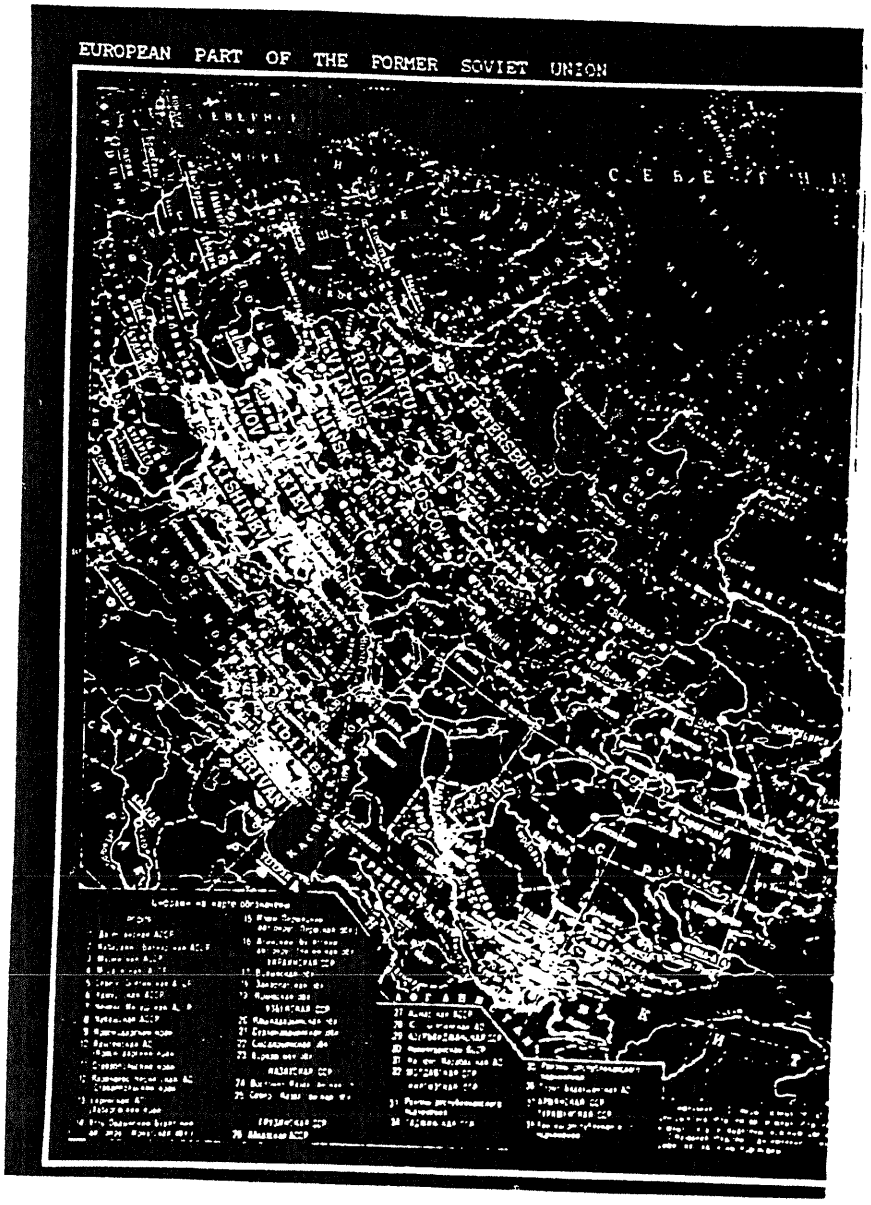
EUROPEAN PART OF THE FORMER SOVIET UNION
RIGA
TARTU
ST. PETERSBURG
MOSCOW
MINSK
KIEV
LVOV
VOLGOGRAD

FIGURE NO. 1

| Strain | Source of isolation |
|---|---|
| V 15 | Book binding, 1581 (Vilnius) |
| V 12 | Book binding, 1629 " |
| K 1 | Book binding, XIXc.(Moscow) |
| K 2 | " " |
| K ES-1 | " " |
| K ES-2 | " " |
| K ES-3 | " " |
| K IS-1 | " " |
| K IS-2 | " " |
| K IS-3 | " " |
| A 5 | Travelling carriage, XIXc. " |
| A 6 | " " |
| A 2K | Parade carriage, XVIIIc. " |
| A 2K-0.5 | " " |
| A 2K-1.0 | " " |
| A 3-I | " " |
| A 3-II | " " |
| A 2-0.5 | " " |
| A 2-1.0 | " " |
| Chamois | Pastels on chamois " |
| Kat. | Binding, XIXc. " |

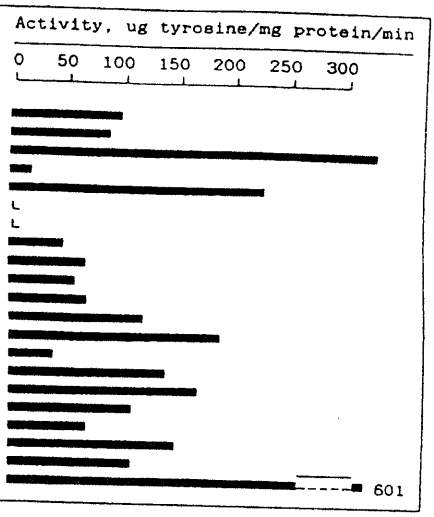


FIG. 6 - CASEINOLYTIC ACTIVITY OF BACTERIA FROM LEATHER OBJECTS.

| Strain | Identified species | Source of isolation | Biomass (mg/ml) | Collagenolytic activity* | Casenolytic activity* |
|---|---|---|---|---|---|
| R 3-1 | B. subtilis | Deed, 1518 (parchment) | 1.3 | 2.04 | 20.6 |
| T MC | B. licheniformis | Treatise, 1598 (bound parchment) | 1.7 | 0.00 | 74.0 |
| ES 1 | B. megaterium | Book binding, XIXc (leather) | 1.5 | 0.92 | 42.5 |
| A 3-II | B. pumilus | Parade carriage XVIIIc (leather) | 1.5 | 0.75 | 52.3 |

FIG. 7 - COLLAGENOLYTIC AND CASENOLYTIC ACTIVITY OF BACILLI FROM PARCHMENT AND LEATHER.

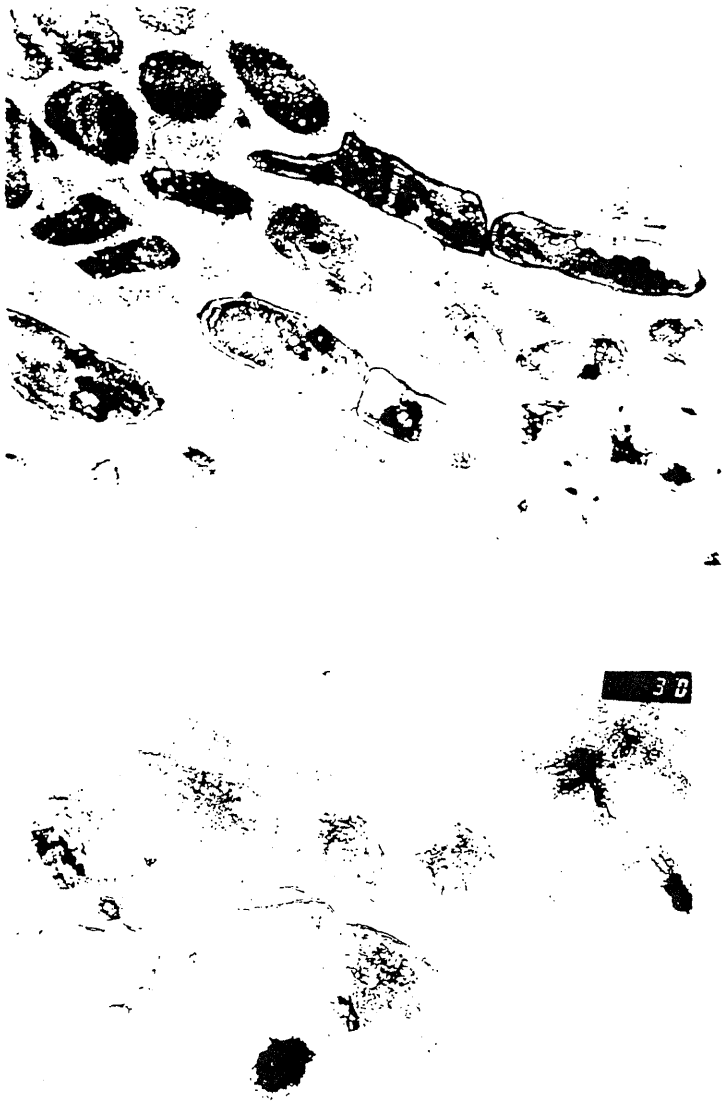

FIGURE NO. 8

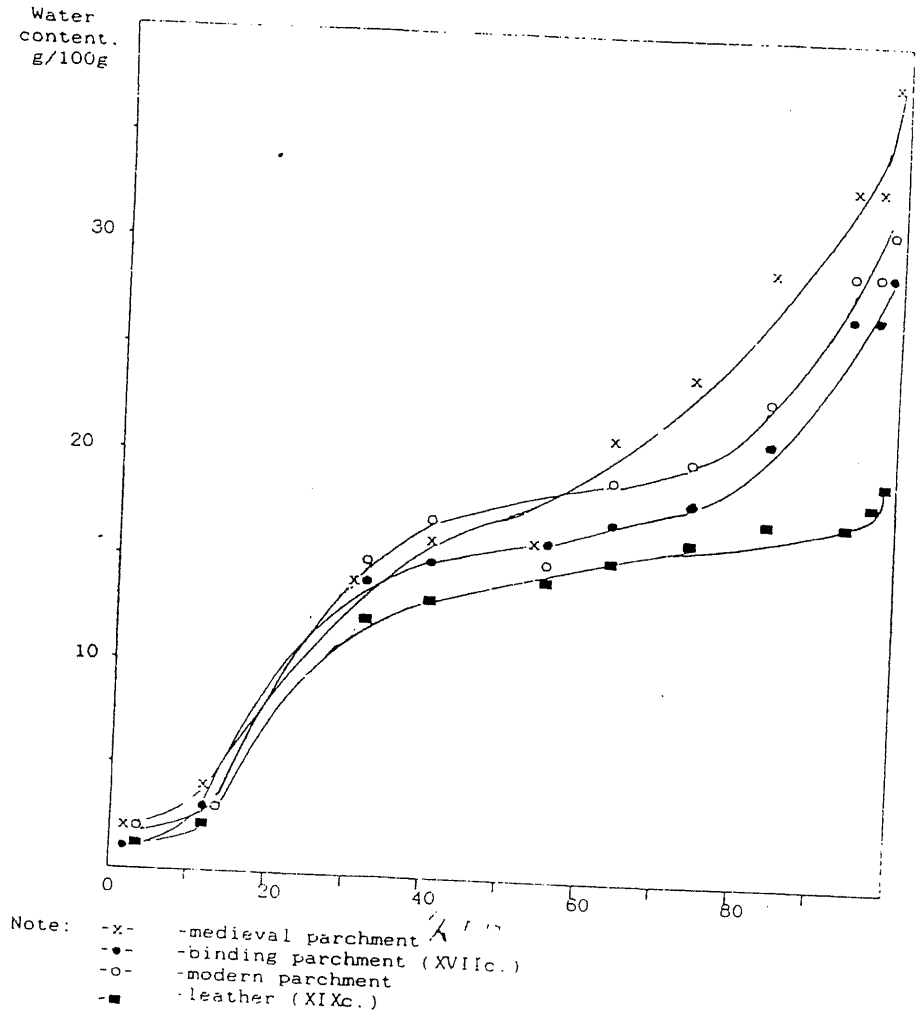


FIG. 9 - WATER SORPTION ISOTHERMS OF PARCHMENT AND LEATHER SAMPLES AT 4 °C.

12

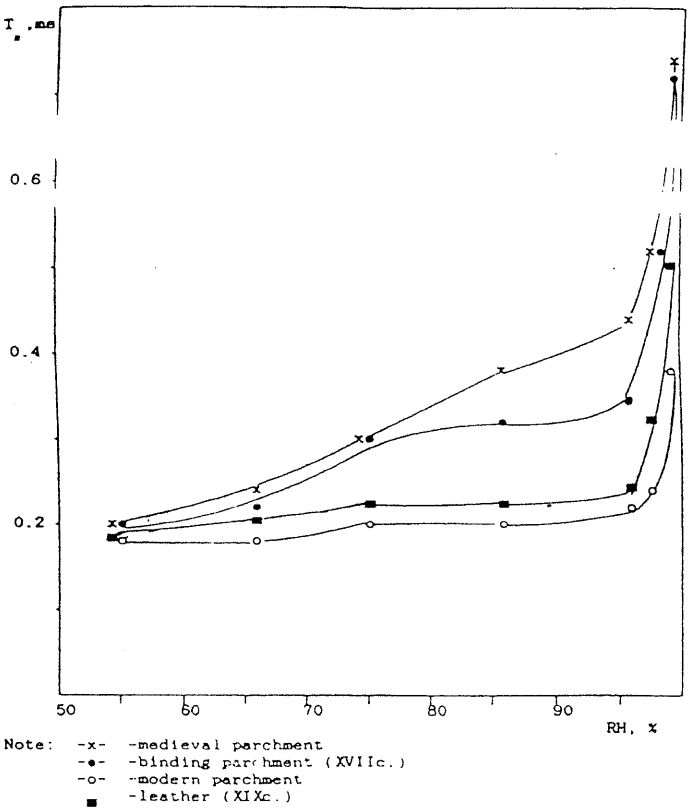


FIG. 10 - SPIN-SPIN RELAXATION TIME OF WATER PROTONS IN PARCHMENT AND LEATHER.

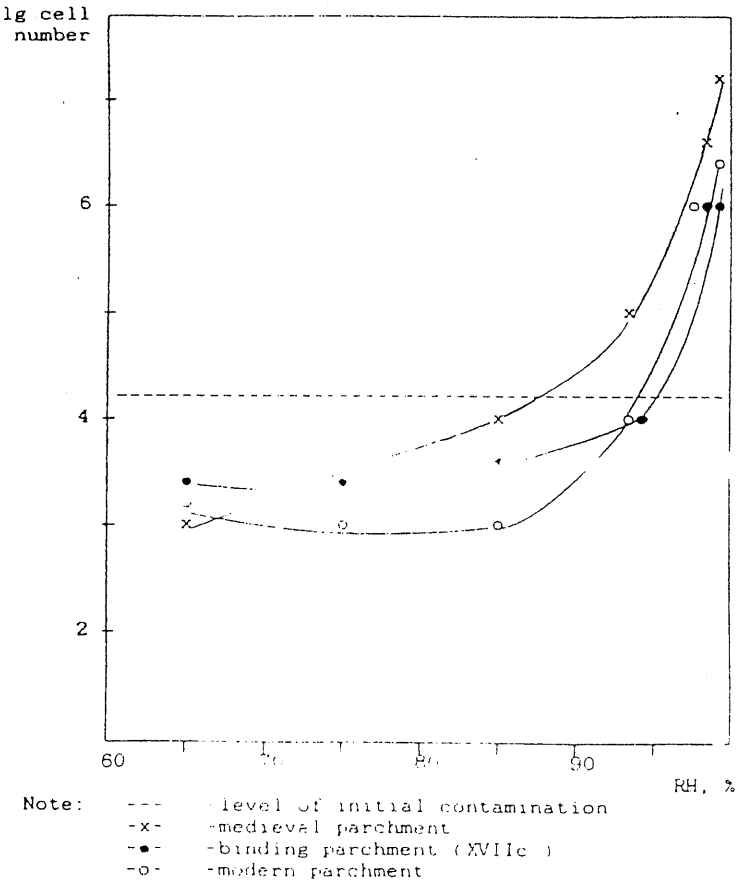


FIG. 11 - EFFECT OF UMIDITY ON B. SUBTILIS IN PARCHMENT SAMPLES

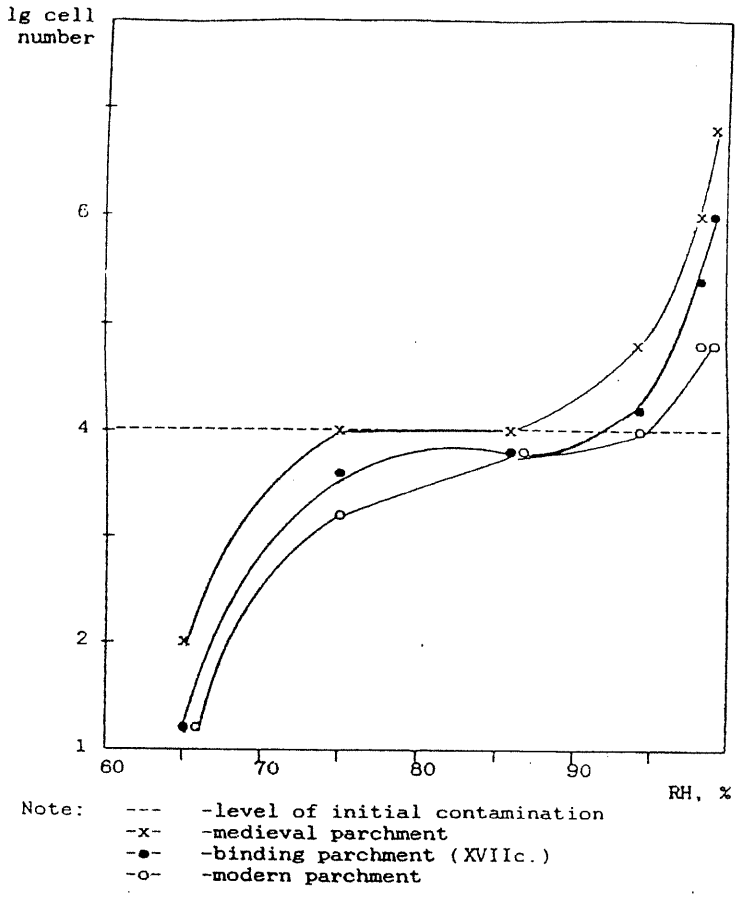


FIG. 12 - EFFECT OF UMIDITY ON B. LICHENIFORMIS IN PARCHMENT SAMPLES.

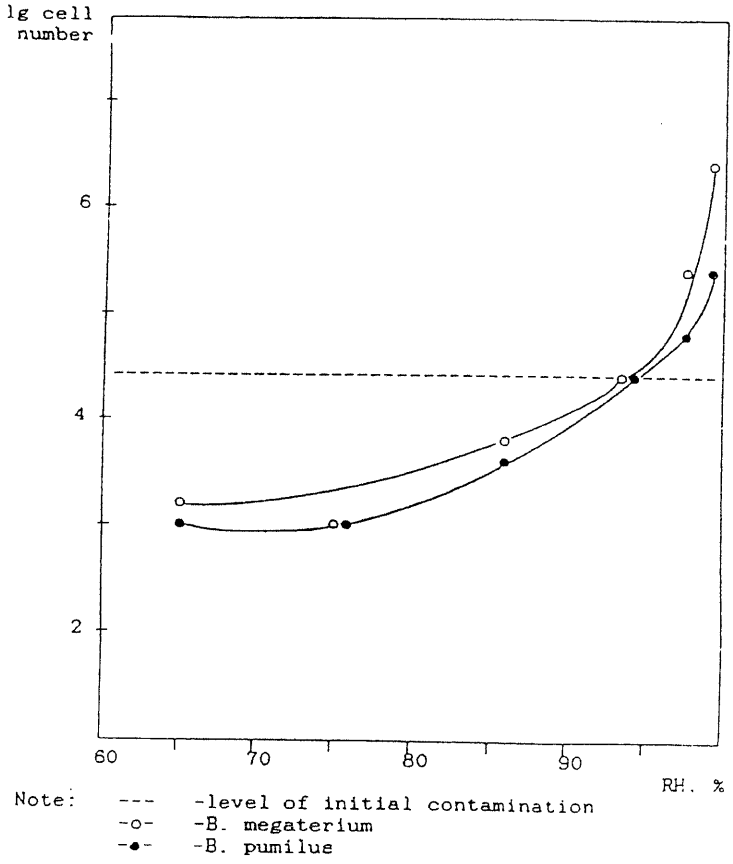


FIG. 13 - EFFECT OF UMIDITY ON BACILLI IN LEATHER SAMPLES.

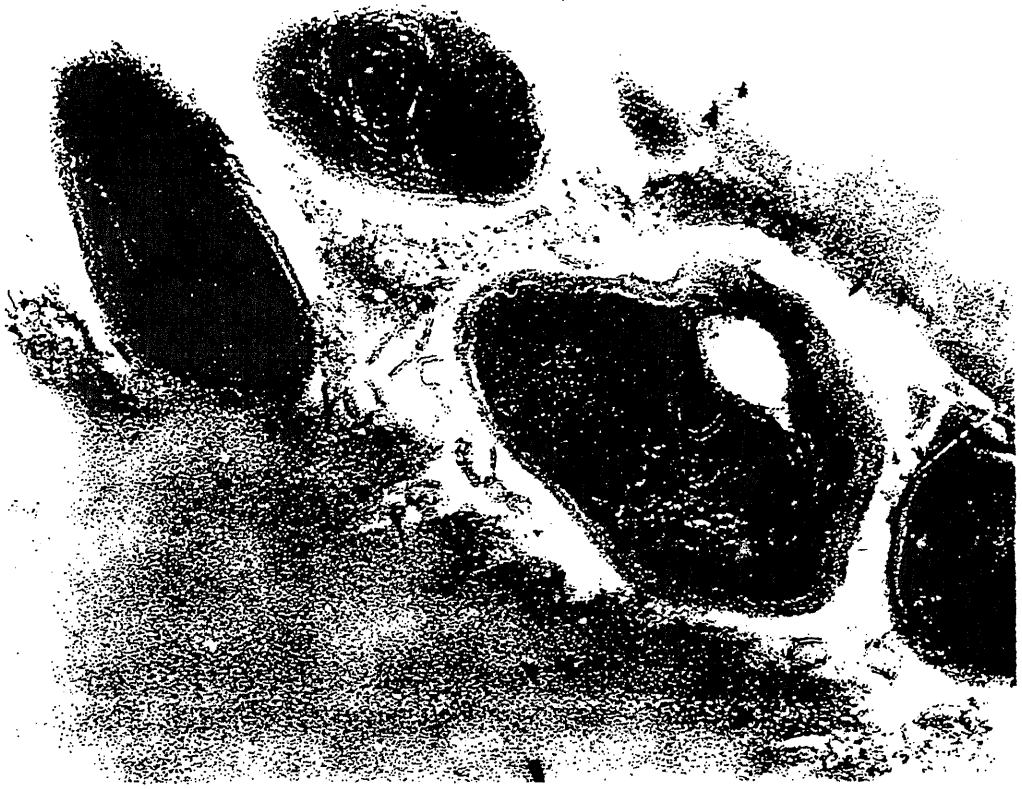

FIGURE NO. 14

| Strain | Source of isolation | CATAMIN AB | | PREVENTOL R-80 | |
|---|---|---|---|---|---|
| | | I (ug/ml) | II (ug/ml) | I (ug/ml) | II (ug/ml) |
| A 3-II | Leather (XVIIIc.) | 10 | 1250 | n.t. | n.t. |
| GM 1 | Parchment (XIIIc.) | 10 | 620 | 10 | 310 |
| GM-0,25 | Parchment (XIIIc.), treated irradiation | 5 | 620 | 10 | 310 |
| LA-cat | Leather (XIV c.), treated catamin AB | 40 | 1250 | 10 | 620 |

Fig.15. MINIMUM BACTERIOSTATIC (I) AND BACTERICIDAL (II) CONCENTRATIONS OF QAC

| Strain | Source of isolation | LD10*kGy | LD10**kGy | Lethaldose, kGy |
|---|---|---|---|---|
| | PARCHMENT | | | |
| R II-1 | Deed, 1731 (Riga) | 1.57 | 1.94 | 20 |
| R II-3 | Deed, 1792 " | 2.21 | 2.50 | 25 |
| R 6 | Deed, 1540 " | 1.86 | 2.22 | 20 |
| R 8 | " " | 1.65 | 2.22 | 20 |
| R 9 | Deed, 1540 " | 1.72 | 2.22 | 20 |
| R V | Deed, 1599 " | 2.15 | 2.22 | 20 |
| R VI | Deed, 1590 " | 0.56 | - | 0.5 |
| T 3 | Manuscript, XIVc. (Tartu) | 2.15 | 2.22 | 20 |
| T MC | Treatise, 1598 " | 1.81 | 2.50 | 25 |
| Gr-y | Manuscript, XIIc. (Georgia) | 1.17 | 1.11 | 5 |
| Gr-or | " " | 1.24 | 1.66 | 15 |
| IVp | Modern parchment, Moscow | 2.30 | 2.22 | 20 |
| II | " | - | 2.22 | 20 |
| T-1 | Greek Gospel, XIIIc. (Moscow) | 0.56 | - | 5 |
| T-2 | " " | 2.00 | 2.22 | 20 |
| T-3 | " " | 2.08 | 2.22 | 20 |
| T-P381 | " " | 2.17 | 2.50 | 25 |
| VI-2 | " " | 2.08 | 2.78 | 25 |
| Co | " " | | | |
| | after gamma-irrad. (2.5 kGy) | 2.19 | 2.22 | 20 |
| | LEATHER | | | |
| K 1 | Book binding, XIXc. (Moscow) | 1.38 | 1.66 | 15 |
| K IS-1 | " " after pretreatment with iso-propylsorbate (in ENGLISH?) | 3.03 | 2.78 | 30 |
| K ES-1 | " " after pretreatment with ethylsorbate (?) | 2.69 | 2.78 | 25 |
| A 5 | Travelling carriage, XIXc. " | 1.51 | - | 10 |
| A 6 | " " | 0.56 | - | 5 |
| A 1 | Parade carriage, XVIIIc. " | 2.57 | 2.78 | 25 |
| A 2 | " " | 2.36 | 2.50 | 25 |
| A 3-I | " " | 1.74 | 2.50 | 25 |
| A 3-II | " " | 2.27 | 2.33 | 30 |
| Chamois | Pastels on chamois " | 1.51 | 2.22 | 20 |
| Kat. | Canvas, XIXc., after pretreatment with Catamin AB " | 0.56 | - | 5 |

**Fig.16** RADIOSENSITIVITY OF BACTERIA FROM PARCHMENT AND LEATHER OBJECTS

**Notes:** *LD10 - dose that causes a ten-fold reduction in the size of the microbial population. This was determined by serial dilutions and plating on casein agar of microbial suspensions irradiated with doses up to 10 kGy.
LD10 is formulated as LD10 = D / lgSo - lg S1
where, D = experimental dose of gamma irradiation, So = size of initial population (number of CFU) and S1 = size of population after irradiation.
**This LD10 was determined by the method of direct inoculation of irradiated with above 15 kGy doses suspension in nutrient broth with 1% glucose.
LD10 is formulated as LD10=D/9, where D = the dose at which no microbial growth occured in any 3 test tubes.