HOW ENZYMES WORK AND WHEN THEY CAN BE USED IN PAPER RESTORATION

by

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The enzymes derive their greatest importance from the fact that life itself is intimately bound up with enzymatic reactions. But for these key substances, there would be no life on this earth. Man himself, as we know him, would not exist.

A hundred years ago little was known about the enzymes and how they work. Today we know a great deal about their structure and mode of action, but quite many fundamental questions are still left to be answered. We know, for instance, that they are inanimate chemical compounds, formed within all living cells, in man, in plants, in fungi and even in the smallest single-celled microorganism. We know what purpose they serve in nature: they are catalysts whose characteristic property is their ability of *accelerating definite chemical reactions* (1000 billions to 1 trillion times). By this ability to speed up fundamental biological processes, the enzymes are essential to life.

For thousands of years primitive man knew how to utilize enzymatic reactions. By accidental discoveries he observed that the juice of grapes became wine, when spores of yeast fungus fell into their jars. For the restorers it might be interesting to know that for hundreds of years, up to the beginning of this century, dung (faeces) from dogs, birds, a.o. was used to make animal hides and skins soft and pliable. Parchment, made for writing, was treated in this way. In the dung we find proteolytic enzymes, such as trypsin a.o., which give the skins the desired quality.

The enzymes are proteins and are large molecules, composed by one or more amino acid chains. They can be divided into two groups:

1) Simple proteinenzymes, consisting only of one protein
2) Conjugated enzymes, which in addition to the enzym-protein part has connected a low molecular component (prosthetic group).
   The enzymprotein alone (the apoenzyme), is inactive without the prosthetic group (or coenzyme). The active complex is usually named "holoenzyme".

A chemical reaction proceeds to equilibrium only if the molecules have sufficient energy of activation to form an activated complex. From this complex products can be derived. Enzymes greatly increase the chances for reactions to occur by making specific

molecules more reactive. This is done by forming intermediate compounds with them. These quickly break down to form stable products, and the enzymes released are able to speed the formation of additional products.

Theoretically one would expect the enzymes to catalyze a reversible chemical reaction, going both ways. In pratice they usually go in one way, probably due to the various ways the enzymes are connected to the substrate, partially because the energy conditions favour the one way drive.

The enzymes react basically like all other biocatalysts, but because they are proteins, they differ in some respects. They are specific in that they catalyze only one chemical reaction. They are sensitive to factors that influence protein reactions a.o.

1) They are influenced by changes in pH. An enzyme will be most active at a certain pH, at its pH-optimum. This is mostly around 7-8 in the organism. Exception from that is e.g. pepsin with a pH-optimum at 2-3. The enzyme loses very much of its activity as the pH changes widely from the optimum on both sides, fig.1.

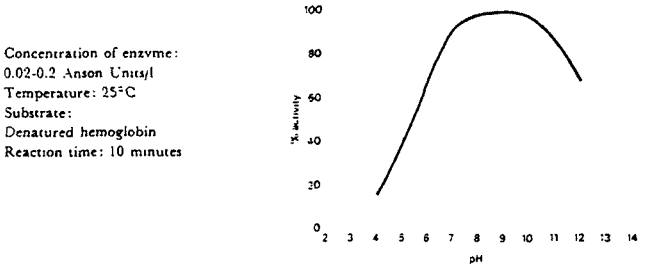

Fig.1. Activity of proteolytic enzyme Alcalase "Novo" at different Ph-values

2) The enzymatic activity is much influenced by temperature. The optimum is for many enzymes around +40-50 degrees Celsius. They lose much of their activity as temperatures decline or rise from the optimum, fig.2.

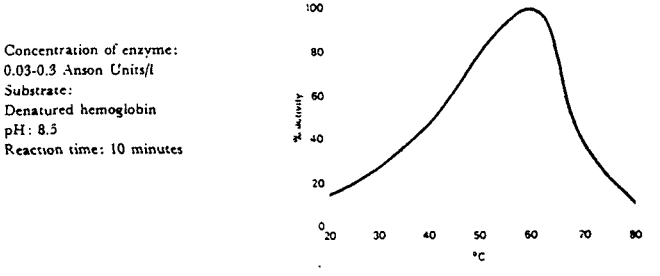

Fig.2. Activity of proteolytic enzyme Alcalase "Novo" at different temperatures

In general an enzyme work by reducing the activation energy required for that specific chemical reaction to occur, fig.3.

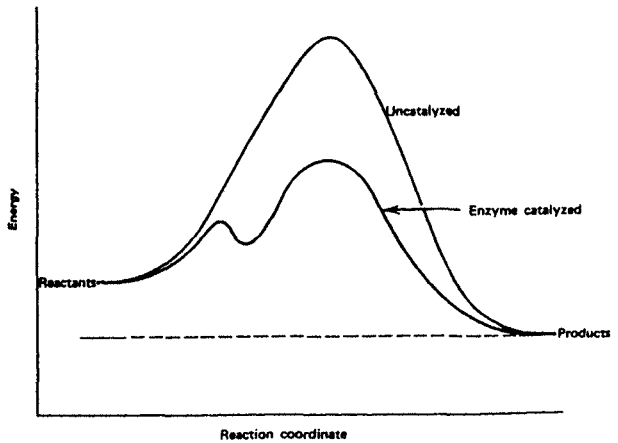

Fig.3. Energy diagrams for catalyzed vs noncatalyzed reactions

It starts by an enzyme combines with a substrate at a specific site on the surface of the enzyme molecule, at *the active site*. A basic requirement for this is that the substrate molecules fit into the enzyme like a "key-and-lock". This counts for the specificity of the reaction. When this happens an enzyme-substrate complex is formed, fig 3.

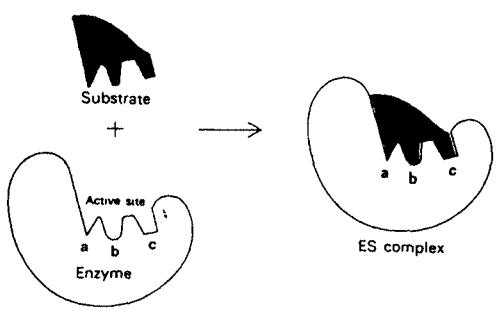

Fig.4. Lock-and-key model of the interaction of substrates and enzymes

The reason for the great efficiency of enzymes is not fully understood. It is due partly to the precise positioning of substrate molecules and catalytic groups at the active site. This serves to increase the probability of collisions between the reacting atoms.

There are lots more to be said about how enzymes work, but the short period of 25 minutes to lecture about it does not permit the author to go more into details about the subject. The interested reader is advised to consult one of the many excellent textbooks in this field.

subject. The interested reader is advised to consult one of the many excellent textbooks in this field.

A water leakage into one of the stacks of the University Library of Bergen during the summer of 1967 prompted the initiation of the author's restoration studies. These led in the end to the use of enzymes as the most suitable tool to obtain the satisfactory results sought for.

In 1969 the author was confronted with the question as to whether the waterdamaged books should be thrown away, or new efforts made to have them restored. In the intervening years expert restorers had been consulted and they all concluded that the task of restoration was an impossible one, as most of the books consisted of art paper. Within a few hours of being exposed to water the books of this composition had become compact blocks. This is due to the casein adhesive in the art paper which makes the sheets extremely liable to stick together when wet. Subsequently they resisted every attempt to reopen them.

As a former reader at the University of Oslo with a background in clinical biochemistry, the author felt that modern chemistry ought to have an answer to this problem, which at first appeared to be a minor one. The path to its solution was not direct; it was only after testing many chemical principles that the enzymatic approach finally yielded the results hoped for by Wendelbo (1).

Paper is a thin tissue of fibrous material. Most commonly employed are plant fibres from cotton or wood. To make the paper more suitable for writing , the early papermakers used sizers to make the surface harder and less penetrable to ink. Animal glue was most commonly used as a sizer; later rosin (a natural resin) and alum (aluminium potassium sulphate) came into more frequent use. To give the paper even better properties, fillers (mineral pigments) such as China clay (Kaolin) was added and coating processes developed. An adhesive, e.g. casein and mineral pigments, is used as coating agent. Approximately 90 % of art paper contains casein as adhesive in the coating layer, the resting 10% being other proteins or synthetics. Paper of this kind is high grade quality paper, often called art paper. It is excellent for fine printing and for meeting the demands of the modern printing industry with regard to the half tone processes and the printing of illustrations in colour.

Experiments were carried out, testing three different chemical principles:

1. Substances lowering the surface tension of water. No useful effects were observed.

2. Foamproducing solutions. In casu books soaked in hydrogen peroxide were exposed to solutions containing the enzyme catalase. Some separation of the leaves was obtained, but the paper became vulnerable because of the formation of gas bubbles in the individual leaves. Damage was done to the text and illustrations when attemps were made to open the sealed pages.

3. "The enzymatic scalpel".The idea of using the enzyme trypsin came to the author while he was attending the 32nd Nordic Congress of Internal Medicine in Bergen, 25.-7.6.1970. For many months previously, other enzymes had been tested, but in vain. At one of the stands displaying medicaments and drugs, the author was offered a new preparation for the removal of wound debris. The preparation contained the enzyme trypsin as its active ingredient. It then struck the author that if trypsin was able to "digest" the proteinaceous wound debris, it would very likely do the same to the protein coating of the art paper, which in very many books consists a.o. of casein. Casein glue, as well as animal glue, are readily split

by trypsin as they contain the amino acids argine and lysine. The explanation for this is that trypsin hydrolyses peptides, amides, esters etc. at bonds involving the carboxyl group of argine and lysine.

The results of the enzymatic seperation of the leaves are shown in Appendix 1 (plates 1 - 2).

Another example of enzymatic restoration from 1974, on papers, which is more than 450 years old, is shown in Appendix 2 (plates 3 and 4). In the covers of old books, especially from the 16th century A.D., one may find boards consisting of waste paper, glued together by bone glue and starch paste to make stiff support for the cover. This waste papers is sometimes of considerable importance as a literary source for books and manuscripts, lost long ago through events such as flooding, the auto-da-fés of bookburning, neglect of proper storage etc. It is a delicate operation to remove these boards from the covers without damaging the texts. The examples shown in Appendix 2 are from a book published in Basel in 1529 AD, which consists of works of Galen (129-200 A.D.). After the enzymatic seperation, the boards proved to contain ten leaves each, revealing parts of *David's Psalter in the Bible*, "Gedruckt zu Nürnberg durch Jobst Gutknecht", and parts of Richard de St. Victoir's *De Trinitate*. Both printings may be dated to the second decade of the 16th century A.D. Wendelbo (2,6,9). Both protelytic- and carbohydrate splitting enzymes were used during this restoration.. The author has used the carbohydratesplitting enzyme alpha-amylase since 1972, but has not published his findings. The interested reader is referred to the work of Segal and Cooper (11).

A presentation of a new protease (*A. saitoi*), was published by Pia De Santis in 1983 (13). Although this was obviously intended to be a science study, it unfortunately falls short of its aim. It may lead the average restorer to draw the wrong conclusions after reading the findings. In the discussion about the eventual possibility of reactivation of enzyme residues, one of the arguments is "according to one microbiologist, an enzyme dried on a paper could retain the ability to be reactivated for several years" (personal communication). Which enzyme, in what quantity, having what activity at the starting point and what activity when later measured? Or one reads, "from an unsophisticated experiment, performed *on one piece of paper*,... paper that had been coated with a thick layer of gelatine and oven aged for over three days at + 100°C, ...after being immersed in an a protease solution,...given a cursory rinse with tapwater, ...exposed for five weeks to dust, light and room tp,...enzyme residues could be reactivated." Any conclusions about reactivation drawn from such a study is highly irrevelant.

At this juncture the author feels it appropiate to remind the reader about the important saying of Lord Kelvin about science " when you can measure what you are speaking about and express it in numbers you then know something about it; when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre kind ". What is surprising about all the published studies on the reactivation of enzyme residues, is that not a single one of those encountered by the author gives any information about the quantities of enzyme residues left in the paper. As the authors of these articles admit that the experiments for testing the eventual detrimental effects of these residues are inconclusive, one must conclude that neither theory nor practice lend support to the warnings of delayed adverse effects from the use of enzymes for paper restoration.

The enzymatic approach was used in 1974 to solve the problem of the extraction of old papyri documents from gesso cartonnage. The use of proteolytic enzymes once again gave the same good results as in the previous works on paper. This topic will not be covered in this lecture;- the interested reader is referred to the author's publications, Wendelbo (3,4,5,6,7,8,9,10), Appendix 3 (plates 5 and 6).

In 1981 B.Fosse, F.C.Størmer, K.Kleve published their results on a cheap and easy method of removing papyrus from gesso cartonnage (12). They were able to extract the papyri from the cartonnage by using a phosphate buffer solution only. The conclusion of this study is that the use of enzymes in the removal of papyrus from gesso cartonnage is unnecessary.The method used does not describe any active chemical principle, apart from the use of *water*. As stated by the authors, the aim of the phosphate buffer solution is to maintain a constant hydrogene ion concentration of pH 7.5. "The condition of the water can thus be made equal everywhere in the world". The author of this article finds that there is a

more probable explanation of these findings, where over a period of more than 2100 years, the original glue of the tiny fragments of gesso cartonnage has been gradually broken down. In future work in this field, an estimate of the quantity of nitrogenous organic matter in the gesso cartonnage will be a guide in the decision to use water or an enzyme in the restoration process.

The author, in his previously published works, refers to his use of the enzyme trypsine, which was chosen for practical resasons, in 1970. The idea then of using enzymes for restoration purposes was quite new at the time, and it was only by introducing the old enzyme classic trypsine in its purest form one could hope to pave the way for the new enzymatic approach.

For the same reasons the author undertook the strictest precautions in disposing of the enzyme residues left in the paper at the end of the restoration processes. The theoretical arguments, against the use of enzymes, encountered by the author obliged him to introduce the most scrupulous methods in disposing of these residues by rinsing and deactivation.

This has certainly been too much of a good thing and has probably deterred restorers with limited chemical training from using enzymes at all. It is not very likely that the reactivation of enzymes in the concentrations used for restoration purposes will occur, as the enzyme residues are degraded during normal storage conditions as part of an ongoing process. For a substantial reactivation to take place, sufficient quantities of water would need to keep the eventual residues" in motion". Water in these quantities is not present at the usual storage conditions in library stacks. Even during disasters where water is involved, there must be other concomitant factors present at the same place, at the same time, before anything can happen. The chances that all these factors being present simultaneously are very small indeed. As any chemist knows.

The author sees a danger in the arguments regularly offered by advanced restorers about reactivation and delayed, harmful effects. It may be a temptation for the cunning to overemphasize for their own benefit any possible harmful effects, while at he same time using enzymes for their own restoration work, without admitting to their use. One cannot ignore the economical aspects of restorations in the art market.

It has to be understood that enzymes are far more lenient to the restoration object than most of the other chemicals commonly used by the restorers, as oxidants, reducing agents, acids, alkalies etc. This is due to the fact that a proteolytic enzyme *participates in only one* chemical reaction, while the others *are active at several* and consequently increases the risk of harmful effects on an old vulnerable restoration object.

In conclusion the author advocates the widespread use of proteolytic- and carbohydratesplitting enzymes for paper restoration. They are cheap and easy to use without dealyed harmful effects. Instead of using the expensive, highly purified enzymes, one should benefit from the cheaper technical preparations available today from industrial firms such as the Danish enzyme manufacturer NOVO (Copenhagen), SIGMA etc. These enzyme preparations can be dissolved in water instead of buffers, and used at room temperatures. As they do not contain cellulase, they do not attack the paper fibres (cellulose). The previously described rinsing processes are to be avoided. Instead, rinsing the paper in water in the same quantities as that used after using bleaching agents, acids or alkalies, is suitable. Resizing of the paper is to be recommended.

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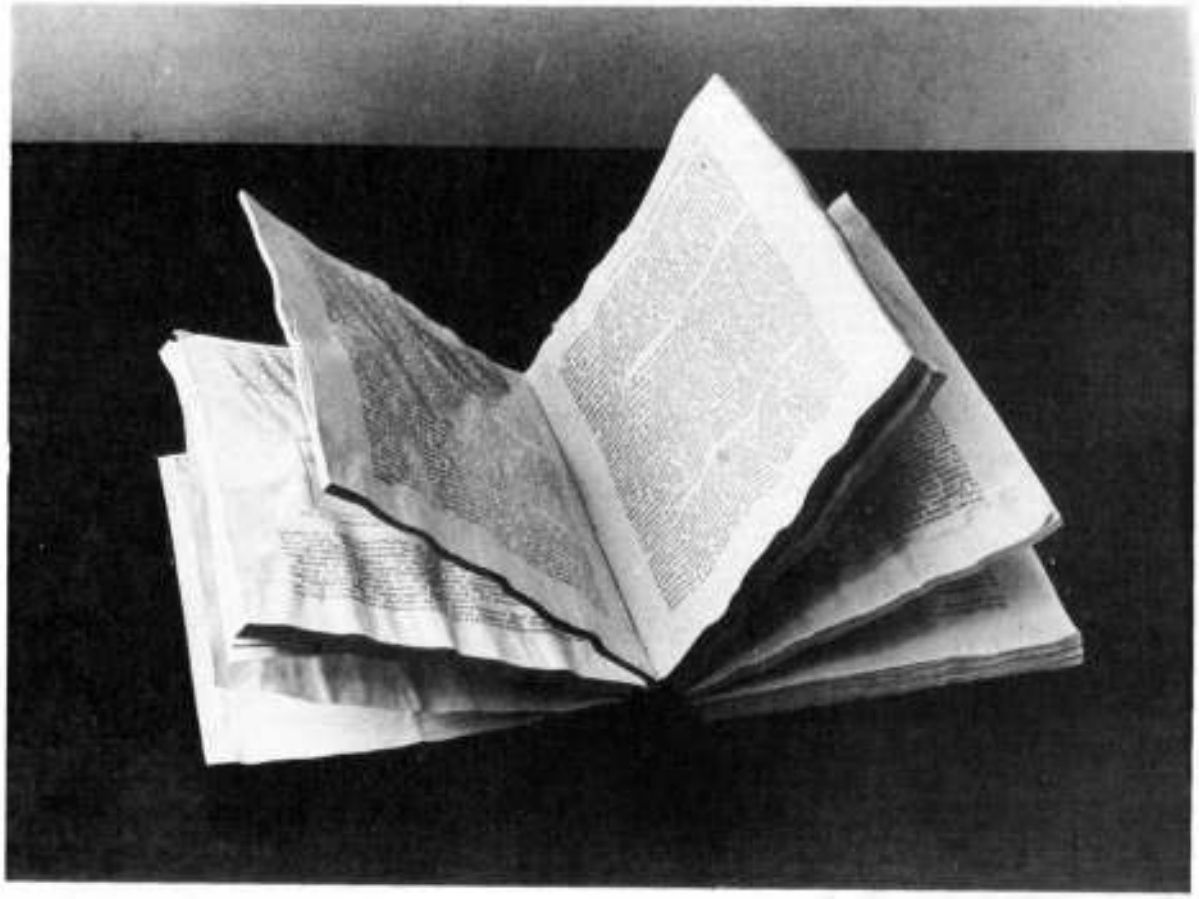
ABSTRACT: Enzymes are biocatalysts. Their unique property is their ability to accelerate chemical reactions, from 10^{15} to 10^{18} times (1000 billion to 1 trillion times). Being proteins, they differ from most other catalysts in two ways; they are specific for one chemical reaction only and they are influenced by factors critical to other protein reactions e.g. pH, temperature a.o. They act by combining to substrate molecules, forming an enzyme-substrate complex. A part of the enzyme molecule, the active site, starts the reaction with the substrate in a 'key-and lock' position. This lowers the activation energy for the reaction and is the very basis for the reaction to start so swiftly.

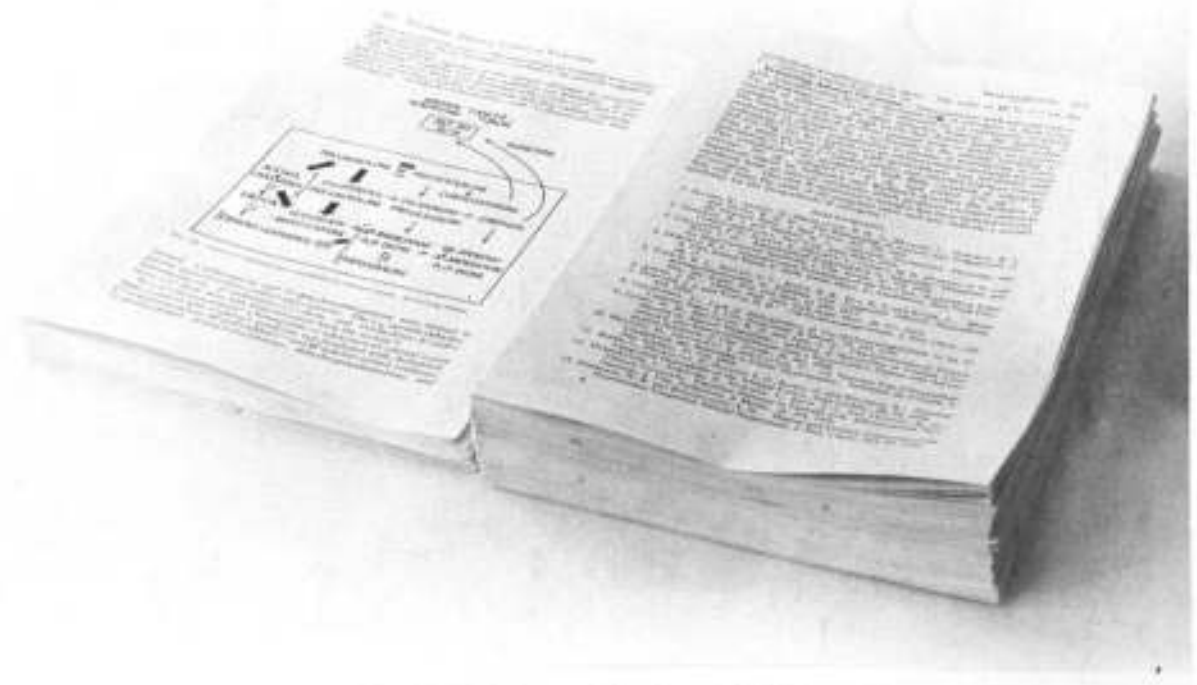
The author started to work with enzymes for restoration purposes in 1970, due to an accident with waterdamage to books in the University Library of Bergen. The enzyme trypsin was used as an "enzymatic scalpel" to reopen the stiff waterdamaged book blocks. Over the years the enzymatic approach has been found useful to solve other restoration problems as well. Sealed papers by glue or starch paste can safely be separated either by proteolytic or carbohydrate splitting enzymes. The same good results have been demonstrated for the removal of bookplates from valuable books, for the freeing glued historical documents from bookcovers used in the 16th-17th centuries, the extraction of old Egyptian papyri from gesso cartonnage a.o.

It is the author's opinion that "the enzymatic scalpel", properly used, is an indispensable tool in the the restorer's armamentarium.

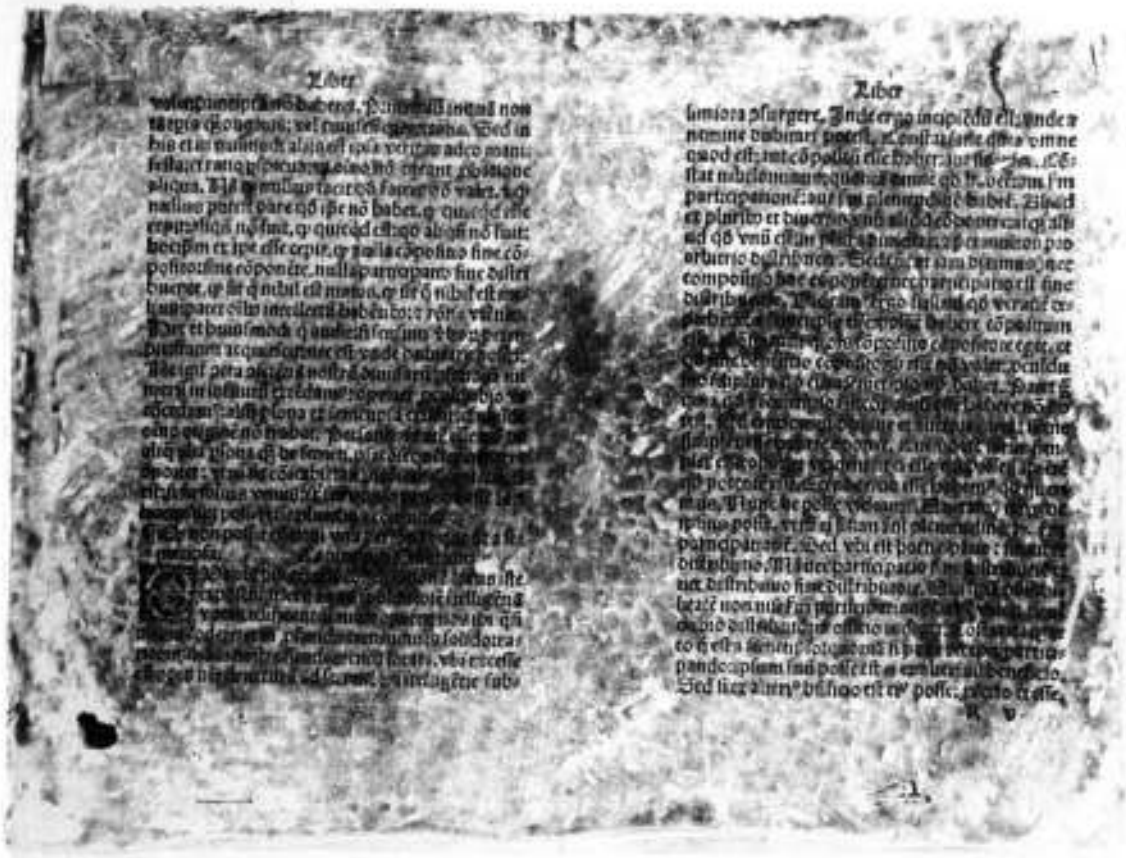
KEY WORDS: ENZYMES RESTORATION CONSERVATION PAPER PAPYRUS CARTONNAGE ADHESIVES GLUE STARCH



APPENDIX 1. Plate 1: The waterdamaged book: J. L. Soffer et al.: The human adrenal gland. Philadelphia 1961. 591 pp ill.



APPENDIX 1. Plate 2: The same book as shown in Plate 1 after enzymatic separation of the leaves



APPENDIX II. Plate 3: Latin waste paper from late Gothic period. The block proved to contain 10 leaves.

Appendix II. Plate 4: The block shown in plate 3 after enzymatic separation of 10 leaves. Remnants of flour paste are still left.

APPENDIX III. Plate 5: A papyrus fragment (ca. 12 cm. x 16 cm.), about 2,100 years old, covered with gesso.

APPENDIX III. Plate 6: Same papyrus as shown in plate 5 after the enzymatic removal of the papyrus from the cartonnage. (A small piece in the right hand corner removed for test purposes).