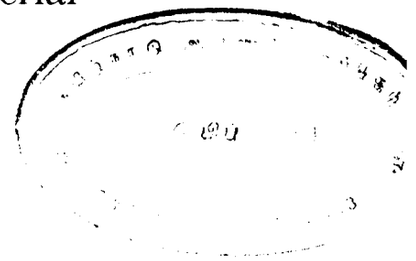
ISSN 0034-5806

RESTAURATOR

International Journal for the Preservation of Library and Archival Material



Volume 14 · Number 4 · 1993

M[illegible]SGAARD · COPENHAGEN

Restaurator, 1993, 14, 199–216
Printed in Denmark – All rights reserved

RESTAURATOR
ISSN 0034-5806

Proteinase Inhibitors from Vegetables and their Application in Enzymatic Conservation Treatments

by DIANA NIKOLOVA

INTRODUCTION

Work with enzymes in conservation has become quite popular since Wendelbo & Fosse[1] first published their results of using enzymes for opening documents, sealed by sudden water damage and to remove papyrus from gessocartonage.[2] Later enzymes have been used successfully in painting, photography and textile conservation.[3] Nowadays there are numerous uses of enzymes in paper conservation processes in which their action has facilitated safe treatments that could not have been executed without them.[4]

When enzymes are used in conservation, after the desired result is reached, an enzyme inactivation procedure is required in most cases.[5] The methods traditionally applied for enzyme inactivation so far include organic solvents,[6] prolonged washing with tap water and a denaturing effect of higher temperature and pH on the enzymatic activity.[7–9] According to Makes & Pühringer,[10] enzymes can be inactivated by means of naphthoquinones, especially juglone, vitamin K_3 and most effectively by vitamin K_4 (menadione).

Besides, the enzymatic preparations, even the highly purified ones, except for the main enzyme also contain extraneous enzymes. In most cases their presence is undesirable since they can cause damage. This imposes the necessity of substances in the enzymatic preparations that suppress the action of the obstructive enzymes only. The application of such substances could provide an opportunity to obtain absolutely pure solutions as regards the main enzymatic activity. It could also mean a new possibility for eliminating risky situations when enzymatic treatments are used in conservation.

A general solution to these problems is the application of protein inhibitors of enzymes. The strongest argument in favour of their usage is their character-

istics:[11] exceptional specificity of the activity of protein inhibitors. The enzymes are inactivated very quickly – right after the inhibitor is added into the enzymatic solution. Protein inhibitors are easily dissolved in water, thus forming colourless solutions. These substances have a high degree of stability regarding denaturing agents. Every single protein inhibitor reacts only to the corresponding enzyme and is not chemically active to the material content of the objects of conservation. They are not dangerous to the health of the restorer.

It has long been known that animals and plants produce protein inhibitors. In Japan microorganisms have been studied for enzyme inhibitors since 1965 and world-wide beginning more recently.[12] The interest in protein inhibitors is connected with their biological action and the possibilities for their application in different fields. Their isolation, purification, examination and application are one of the modern tendencies in contemporary biochemistry. Nevertheless, nowadays companies for biochemicals offer just a few protein inhibitors of enzymes for research purposes. Thus, the application of protein inhibitors in enzymatic processes in conservation begins with the search for suitable sources and schemes for their isolation. The percentage of protein inhibitors is greater in vegetable sources than in animal or microbial ones. Besides, the vegetable sources are easily accessible and inexpensive. That is why they are most suitable as a source of protein inhibitors of enzymes for conservation treatments.

The purposes of the study as a whole were:

- to find protein inhibitors of proteases from a vegetable source typical of Bulgarian flora;
- to find a vegetable source that is rich in protein inhibitors;
- to find an inhibitor (or inhibitors) of Bulgarian preparations of proteolytic enzymes or trypsin that is generally available;
- to elaborate a scheme for isolation and purification that is easy to apply in conservation laboratories; and
- to demonstrate the use of the protein inhibitors in enzymatic conservation treatments.

Material and Methods

Seeds of Kohlrabi (*Brassica napus* var. *rapifera*) were purchased from the company Sortovy Semena (Sofia, Bulgaria). Pancreatic trypsin Novo (6 Anson units/g) was a product of Novo Nordisk (Bagsværd, Denmark). Trypsin purified from bovine pancrease was a product of Fluka AG (Buchs, Switzerland), cat. no. 93612. Pure α-chymotrypsin from bovine pancrease was supplied by

Sigma Chemical Company (St. Louis, MO, USA), cat. no. 7762. Preparations of α-amylase from *Bacillus subtilis*, α-amylase from *Aspergillus oryzae* and alkaline proteinase B-72 were products of Chimcompany (Botevgrad, Bulgaria). Sephadex G-25 was obtained from Pharmacia (Uppsala, Sweden).

Two sorts of papers were used to study the salt content of the solutions for washing away the coloured components in the extract out of the treated objects A from 1990 and B from 1960. The brightness of the paper samples was measured on a Specol equipped with reflection meter (Carl Zeiss, Jena, Germany). A scanning electron microscope Philips, model 515 (the Netherlands) was used.

Two photographs with purely gelatine emulsion were taken for the investigation. They were dated from 1930 and 1960. Both photographs were private and without historical value. The protein content of the extract after the purification procedures was determined on the basis of amino acid composition. It was calculated by using a BIOTRONIK automatic analyser model 6001 (Germany) after 24 h of hydrolysis in 6 M HCl in evacuated sealed tubes at 110 °C.

Method of isolation and purification of the protein inhibitors from kohlrabi seeds

One hundred grams of kohlrabi seeds was ground and extracted for 90 min at room temperature with 500 ml of distilled water. The extract was filtered through gauze or similar cotton textile. The sediment was re-extracted with 250 ml of distilled water under the same conditions. The two filtrates were combined and the pH was adjusted to 5.00. After one night at ± 4 °C, the fats and precipitated proteins were removed by centrifugation at 4500 rpm for 15 min. The pH of the supernatant was adjusted to 7.4 and the precipitated pH-labile proteins were discarded by centrifugation. The supernatant was heated gradually to 70 °C, kept for 5 min at this temperature and cooled to room temperature. The mixture was centrifuged and the sediment containing thermo-labile proteins was discarded. The supernatant obtained was the purified extract applied in conservation. The extract can be stored frozen. If it is possible, the extract can be lyophilized. The obtained powder is stored at 4 °C.

As the extract is yellow, it could be treated with activated carbon (shaped rods, ∅ 1.2 mm – VEB Jenapharm – Laborchemie, Germany) for eliminating the dyestuffs. The carbon was used in the proportion 15 g per 100 ml of extract for 30 min at room temperature. The mixture was filtered through a Büchner funnel and a blue tape filter.

Determination of inhibitory activity

The inhibitory activity was tested according to the caseinolytic method of Ogiso et al.[13] with a slight modification.

The following procedure was applied: to 0.25 ml of enzyme solution in 0.1 M phosphate buffer, pH 7.4, was added 0.25 ml of inhibitory extract into both test and control tube. The mixtures were incubated for 5 min at 36 C. Then 2.5 ml of 1% casein solution, pH 7.4 was added to the test tube and 2.5 ml of 0.5 M $HClO_4$ to the control tube. After 10 min the enzymatic reaction in the test tube was stopped with 2.5 ml of 0.5 M $HClO_4$. Then 2.5 ml of 1% casein solution was pipetted into the control tube. After filtration through a blue tape filter, the absorption of the filtrates was measured at 280 nm. The difference between the absorption of the test tube and the control tube as a percentage from the full enzymatic activity was the percentage of the remaining enzymatic activity. Full enzymatic activity was established in the same way, but instead of 0.25 ml of inhibitory extract, a buffer solution was dropped into the tubes. It was accepted as 100%.

The difference between the full enzymatic activity and the remaining enzymatic activity was the inhibitory activity of the extract. One unit of inhibitory activity is 1% suppression of the enzymatic activity. This unit was accepted as a conditional one because the present study was not connected with a standardized inhibitory product. The remaining activity of enzymes in the presence of inhibitors was presented not only as a percentage but also as absorbance at 280 nm. A recording spectrophotometer (Shimadzu model 3000, Japan) was used to measure the absorption.

Results and Discussion

Testing different vegetable seeds established a very high inhibitory activity of the extract from Kohlrabi seeds towards trypsin, alkaline proteinase B-72 and proteinase in α-amylase preparations from *Bacillus subtilis* and *Aspergillus oryzae* and no activity to chymotrypsin.

The scheme for isolation and purification included changes of pH and heating of the extract for removing pH- and thermo-labile proteins. These procedures were possible because the protein inhibitors of enzymes are very resistant to pH changes and high temperatures as well as to other denaturing agents.[11] That is why the scheme without the stage of discoloration could be also used as a test in screening vegetable sources for protein inhibitors of enzymes.

A summary of the purification procedure of the fully extracted inhibitors is presented in Table 1.

Gel filtration was a step of purification for obtaining pure protein inhibitors. The elution profiles (Fig. 1) show two protein peaks – A and B. They have inhibitory activity towards the above-mentioned enzymes. The first peak contains a typical trypsin inhibitor.[11] The data in Table 1 were used to calculate that the content of the inhibitors in peak A and peak B was 7.6% and 2.6% respectively or 10.2% in total, which was a great quantity of protein inhibitors. The explanation was that these proteins belong to the *Brassica napus* family of storage proteins.[11] At seed maturity, napins comprise approximately 20% of total seed protein.[15]

The third peak (Fig. 1) corresponded to the dyestuffs in the extract. The elution profiles before (curve 1) and after (curve 2) the treatment with activated carbon gave information on the effect of discoloration. Carbon was used because the dyestuffs could not be extracted satisfactorily with volatile organic solvents, as is often done in such cases. The treatment with activated carbon is connected with considerable losses of inhibitors and inhibitory activity – about 35% (Table 1). But it was the easiest way to achieve discoloration. Of course, there are possibilities for absolute discoloration of the extract. They are ultrafiltration or gel chromatography. But they require the specific equipment of biochemical laboratories and biochemical manufacturing.

The final extract, discoloured by means of activated carbon, had a very

Table 1. Purification of protein inhibitors of proteinases from kohlrabi seeds

Filtrates		Volume (ml)	Inhibitory activity (units/ml)	Total activity (units)	Protein content (mg/ml)	Specific activity (units/mg)	Recovery (%)
E_1: extract, pH 5.00		450	588	262,600	24.40	24.10	100
E_2: extract, pH 7.40		430	576	247,680	22.10	26.10	94
E_3: extract, pH 7.40 after heating at 70 C for 5 min		420	552	231,840	20.90	26.41	88
E_4: discoloration of E_3 with activated carbon		380	384	145,920	16.10	23.85	55
E_5: gel chromatography of 18 ml of E_3	peak A	29	172	5,000	4.20	40.95	44
	peak B	67	68	4,556	1.30	52.31	40
E_6: gel chromatography of 18 ml of E_3	peak A	26	144	3,744	3.30	43.64	30
	peak B	57	53	3,021	0.73	72.60	24

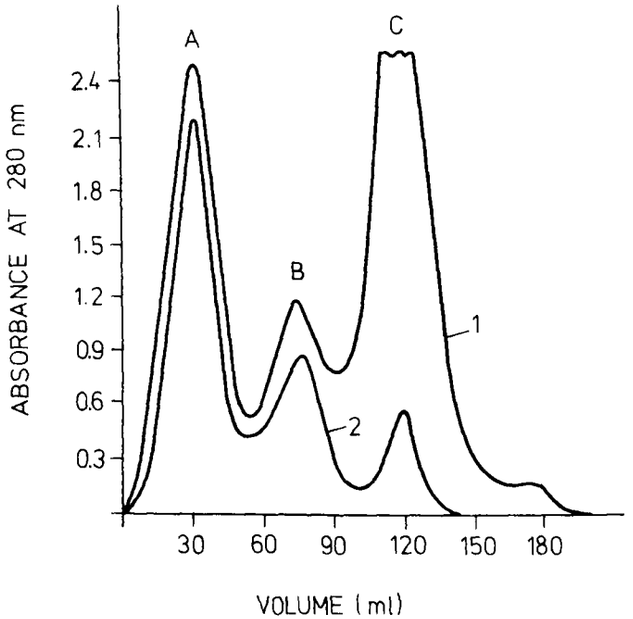


Fig. 1. Gel filtration of extract of protein inhibitors from Kohlrabi seeds

A column of Sephadex G-25 (1.5 × 80 cm) was used, equilibrated and eluted with 0.05 M ammonium bicarbonate buffer, pH 7.4, at a flow rate of 12 ml/h. Elution profile (1) before and (2) after treatment of the extract with activated carbon.

pale yellow colour that was already suitable for application in conservation practice.

There was another possibility for the elimination of the dyestuffs in the objects after their treatment with the inhibitor extract. There were 2 coloured components in the extract. They both had a high degree of polarity and negative charges.[16] Besides, the colouring substances that remained partly on Sephadex G-25 in the process of gel chromatography were removed by buffer with high ionic strength. Usually this is achieved by means of a higher concentration of NaCl. On this basis it was established that, after treatment of paper samples with a coloured extract, washing of the samples with a solution of high NaCl content (1 teaspoonful in 500 ml of water) and rinsing after that with water led to the removal of the coloured components (Table 2).

The speed of action is one of the most important characteristics of the protein inhibitors. It proves them as suitable for conservation purposes. The protein inhibitors act on the enzymes immediately after they are added to the enzymatic solution. This was demonstrated through a suitable scheme for

Table 2. Influence of the salt content of the solutions on the washing away of the coloured components in the extract out of the treated objects

Sample	Brightness (%)	
	Paper A	Paper B
Control	78	44
Treatment 1	73	43
Treatment 2	77	44

Control: paper soaked for 30 min in water; treatment 1: soaking in water for 30 min; then for 10 min in the extract, washing for approximately 10 min with tap water; treatment 2: soaking in water for 30 min, extract from kohlrabi seeds for 10 min, then washing, soaking for 5 min in a solution of NaCl, washing with water.

spectrophotometric determination of the enzymatic and inhibitory activity (Table 3). The addition of the inhibitory extract into the mixture of the enzyme trypsin (Fluka, 5 mg in 100 ml of 0.1 M phosphate buffer, pH 7.4) and the substrate (1% casein, pH 7.4) after 10 min reaction gave the same effect as the stopping the enzymatic reaction after 10 min by means of 0.5 M $HClO_4$. Visually, this was demonstrated by the following experiment.

Four samples were taken from a photograph with purely gelatine emulsion. On each of the first 2 samples were dropped 60 µl of trypsin solution in a phosphate buffer at pH 7.4 (5 mg of trypsin (Fluka) in 100 ml). On the other 2 samples were dropped 60 µl of trypsin solution in an extract from kohlrabi seeds, which was prepared as a buffer at pH 7.4. The solutions acted for 20 and 45 min respectively at room temperature. At the end of the periods of time the samples were washed with cold water, then left for 2 h in a refrigerator and finally dried with a hair-dryer. The changes in the morphology of the test samples were traced by means of a scanning electron microscope. Fig. 2–5 give the results. They confirm the effective action of the protein inhibitors.

The next stage in the investigation was to prove the effectiveness of the extract towards trypsin Novo. Solutions of trypsin Novo were prepared in

Table 3. Speed of the inhibitory action of the enzymes

Scheme of determination of enzymatic or inhibitory activities	Absorbance at 280 nm
Trypsin + buffer + casein 10 min + $HClO_4$	0.450
Trypsin + casein 10 min + buffer 10 min + $HClO_4$	0.780
Trypsin + casein 10 min + inhibitor 10 min + $HClO_4$	0.455

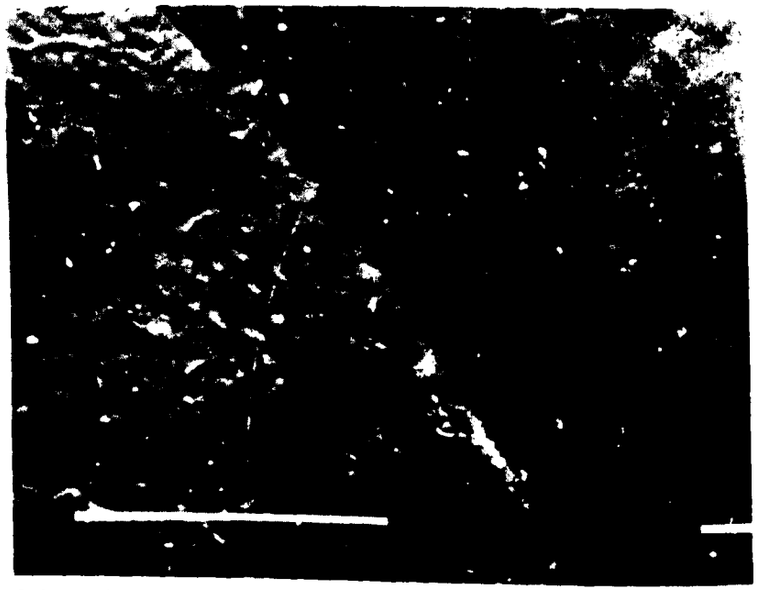


Fig. 2. Action of a trypsin solution (5 mg/100 ml) in a buffer on the gelatine layer of a photograph after 20 min (magnification × 50)

different concentrations ranging from 0.01% to 0.1%. After 10 min of enzymatic reaction, the absorption at 280 nm was measured. It corresponded to the remaining proteolytic activity. The extract can suppress the activity of trypsin Novo up to 0.04% solution of the enzyme in the extract (Fig. 6).

It is known that the pancreatic trypsin Novo contains α-chymotrypsin as an extraneous enzyme.[2] The inhibitors from kohlrabi seeds are not effective towards chymotrypsin, and its proteolytic activity can be detected after an enzymatic reaction longer than 10 min.

The action of the α-chymotrypsin in 0.02% solution of trypsin Novo in the extract was detected after 20 min (Fig. 7, curve 1). In comparison with the full activity of 0.02% trypsin Novo in a buffer (Fig. 7, curve 2), the α-chymotryptic one was about 2%. But for conservation treatments, this minimal proteolytic activity could be dangerous in the course of time.

The action of the α-chymotrypsin could be suppressed again by means of protein inhibitors from vegetables. It is known from the specialized literature that one such source is potatoes.[17,18] The scheme, described in the present study, was applied again. But the first extraction was with 150 ml and the second one with 50 ml of distilled water for 50 g of peeled and grated potatoes. No

Fig. 3. Action of a trypsin solution (5 mg/100 ml) in a buffer on the gelatine layer of a photograph after 45 min (magnification × 50)

difficulties arose with the colour in this case. The solution of pure α-chymotrypsin (Sigma) proved that the extract of potatoes had a good inhibitory activity. After that, a 0.02% solution of trypsin Novo in an extract of kohlrabi seeds was mixed with an extract of potatoes in proportions from 5 : 1 to 5 : 5. The remaining proteolytic activity of the above mixtures was determined after 60 min. It was established that the first proportion was sufficient to stop the entire activity of the extraneous chymotrypsin in the pancreatic trypsin Novo. Based on the conditions of the reaction, it was determined that every 6 ml from the mixture of the extracts of kohlrabi seeds and potatoes in a ratio of 5 : 1 suppressed the action of 5 ml of 0.02% (or 2.5 ml of 0.04%) solution of trypsin Novo in a buffer. This conclusion is of importance for the practical conservation work.

In accordance with the experiments, 4 pairs of samples were taken from a photograph with a pure gelatine emulsion (Fig. 8). The samples from the upper line were soaked in a 0.02% solution of trypsin Novo in a 0.1 M phosphate buffer, pH 7.4 at 35 °C for 20, 30, 40 and 50 min for samples 1, 2, 3 and 4. The corresponding samples from the bottom line were soaked under the same conditions in a 0.02% solution of trypsin Novo in a mixture of

Fig. 4. Action of a trypsin solution (5 mg/100 ml) in an extract from kohlrabi seeds on the gelatine layer of a photograph after 20 min (magnification × 50)

kohlrabi seed and potato extracts (5 : 1). The mixture of the extracts was prepared as a buffer. For this purpose were added the quantities of substances necessary for a 0.1 M phosphate buffer. After they dissolved the pH was adjusted to 7.4 by means of a pH meter. After 50 min (sample 4, upper line) the pure enzyme solution almost destroyed the gelatine layer (Fig. 9). At the same time, the effective inactivation of trypsin Novo by the protein inhibitors preserved the gelatine layer from damage (bottom line samples).

The following example could be given for the practical application of the extracts of protein inhibitors:

Problem: separation of 5 leaves of a book printed on glue-sized paper and scaled by water damage.

Method of separation: by means of a 0.02% solution of trypsin Novo (6 Anson units/g).

Procedure

1. Preparation of the solution for stopping the enzymatic reaction after the desired conservation effect is achieved:

Fig. 5. Action of a trypsin solution (5 mg/100 ml) in an extract from kohlrabi seeds on the gelatine layer of a photograph after 45 min (magnification × 50)

- Obtain an extract of kohlrabi seeds according to the scheme.
- Obtain an extract of potatoes according to the scheme.
- Mix the extract from kohlrabi seeds with the extract of potatoes in a ratio of 5 : 1 so that the volume of the mixture will be sufficient to cover the 5 leaves.
- Prepare the mixture of the extracts as a 0.1 M phosphate buffer at pH 7.4.

2. Preparation of the enzymatic solutions:
   Take 13.8 g of $Na_2HPO_4 \cdot 2H_2O$ and 8.8 g NaCl and add them to 1000 ml of distilled water. Adjust the pH to 7.4 with 0.5 M NaOH by means of a pH meter. Prepare a 0.02% solution of trypsin Novo with this buffer.

3. Treatment of the leaves:
   - Soak the leaves into the 0.02% buffer solution of trypsin Novo.
   - Take the leaves after their separation upon the enzymatic action and put them into another vessel.

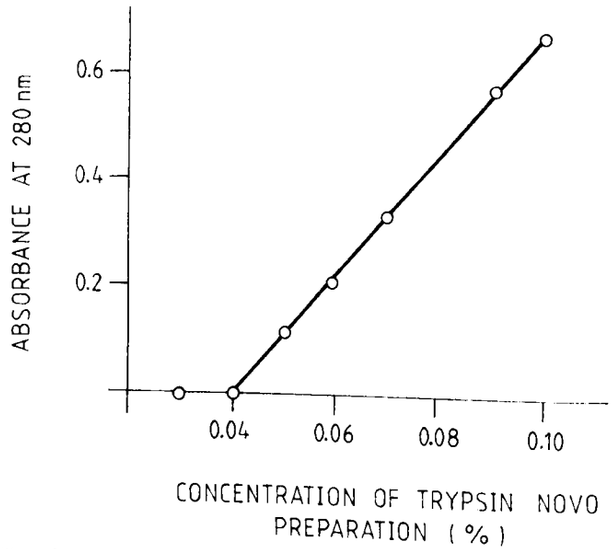


Fig. 6. Inhibition of solutions of trypsin Novo in different concentrations in an extract from kohlrabi seeds

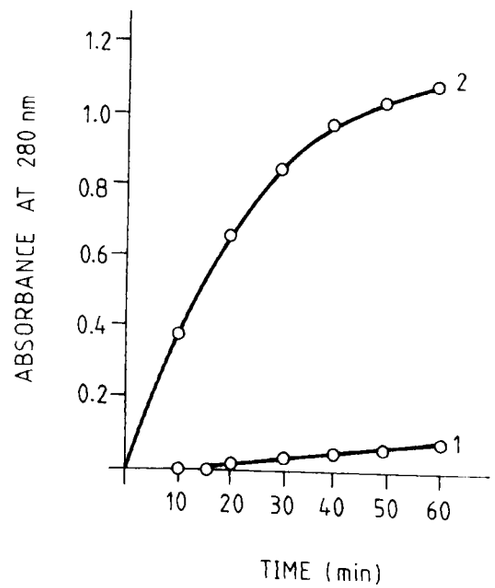


Fig. 7. Activities of trypsin and chymotrypsin in a 0.02% solution of trypsin Novo. Curve 1: in extract, pH 7.4; curve 2: in phosphate buffer, pH 7.4

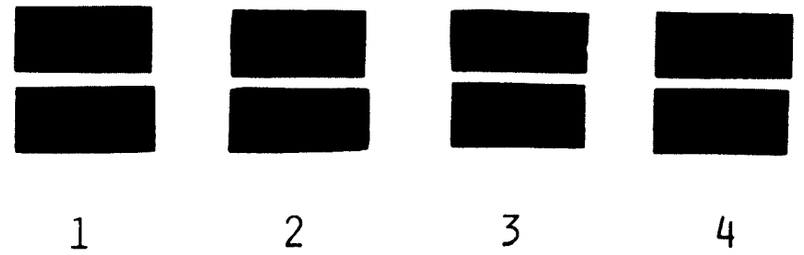


Fig. 8. Samples from a photograph before enzymatic treatment

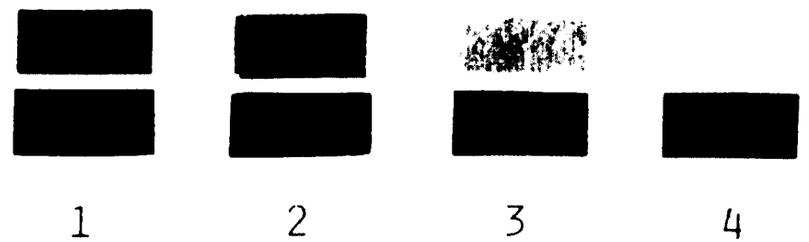


Fig. 9. Samples from Fig. 8 after enzymatic treatment in the absence (upper line) and in the presence (bottom line) of protein inhibitors

- Pour the prepared solution of the protein inhibitors over the leaves and let it stay for some minutes, shaking the vessel.
- Discard the mixture of the inhibitors and put the leaves into NaCl solution (1 teaspoonful for 500 ml of distilled water), again shaking the vessel.
- Wash away the leaves with water and keep them for the next conservation treatments.

If you work with discoloured activated carbon extract from kohlrabi seeds, the extract should be mixed with the potatoes extract in a ratio of 7.0 : 1.

The next part of the study demonstrates how important it is to use an absolutely pure solution of enzymes as well as obtaining them by means of protein inhibitors as regards the main activity of technical enzymatic products. The necessity of such purity of the enzymatic solutions for conservation purposes was substantiated by Preiss & Cook.[19] The extraneous proteolytic activity in a highly purified preparation of α-amylase from *Aspergillus oryzae* was arrested.

The proteolytic enzyme was in a very small quantity. It was detected by measuring the proteolytic activity of a 0.1% solution of the α-amylase preparation after 10, 20, 30, 40, 50 and 60 min.

The next step was to prepare solutions of the α-amylase preparation in a buffered extract of kohlrabi seeds, pH 7.4, in concentrations of 0.1%, 0.2%, 0.4%, 0.5%, 0.7% and 1.0%. Their proteolytic activity was determined after 30 min of reaction at 36 °C. The results proved that the extract could suppress the proteolytic activity of the α-amylase preparation from 0 to 0.2% (Fig. 10). The α-amylase product was used for backing removal of starch-pasted photograph. The test samples were taken from a photograph from 1930 with a purely gelatine emulsion. The photograph was stuck with starch glue on cardboard. On the underside of the photograph over the cardboard was stuck, again with starch glue, dark brown paper.

The sample on the left side of Fig. 11 was soaked in a 0.2% solution of the α-amylase in an extract of the inhibitors from kohlrabi seeds at 35 °C and pH 7.4. The extract was prepared as a 0.1 M phosphate buffer. The concentration was higher because a treatment at pH 7.4 was chosen, whereas the optimal pH of this α-amylase was 4.8. After 55 min, the photograph was separated from the backing. The picture was preserved due to the effective inactivation of the proteinase impurity by the inhibitors (Fig. 12, left). Thus, an absolutely pure solution of α-amylase was used.

The sample on the right side of Fig. 11 was treated with a 0.02% solution of the same preparation but in 0.1 M phosphate buffer at 35 °C and pH 7.4.

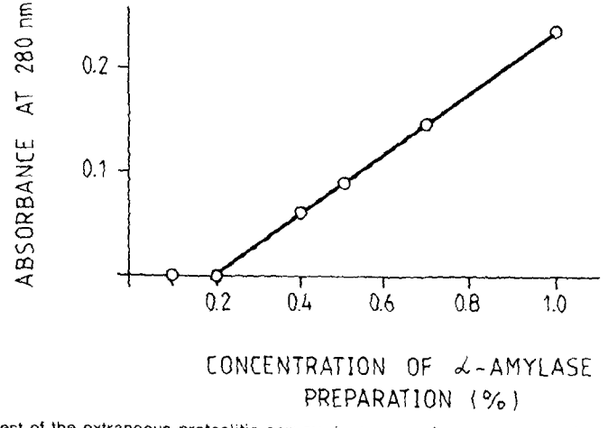


Fig. 10. Arrest of the extraneous proteolitic enzyme in an α-amylase preparation from *Aspergillus oryzae* with the inhibitors in an extract from kohlrabi seeds

Fig. 11. Samples from a photograph before enzymatic treatment

After 55 min the same effect of separation was reached. But the picture was damaged because of the destruction of the gelatine emulsion by the negligible quantities of proteinase in the α-amylase preparation (Fig. 12, right).

Conclusion

The inhibitor extracted from kohlrabi seeds is an excellent method for arresting trypsin and some other proteinases as well as for obtaining an absolutely pure solution with α-amylase activity from a microbial product of α-amylase containing an obstructive proteolytic enzyme. It entirely suppresses the action of pancreatic trypsin Novo in a mixture with the protein inhibitor of chymotrypsin in an extract from potatoes. The scheme for the isolation and the partial purification of protein inhibitors, which is given, can be successfully applied as a test to find and to obtain protein inhibitors from vegetable sources. It is also easy to follow in a conservation laboratory. Protein inhibitors are very effective to stop the enzymatic action after the desired conservation result is reached. There are protein inhibitors of amylases and other enzymes as well. It is clear that they should be applied similarly. Protein inhibitors are suitable for conservation purposes. Their application gives a finished state to the enzy-

Fig. 12. Samples from Fig. 11 after enzymatic treatment – left with 0.2% solution of α-amylase preparation in an extract from kohlrabi seeds; right with 0.2% solution of the same preparation in a buffer

matic conservation treatments from the point of view of contemporary biochemistry.

Acknowledgements

I am indebted to Professor Dr. Chem. Nikolay Genov, Head of the Department of Structural Analysis of Biopolymers, Institute of Organic Chemistry, Bulgarian Academy of Sciences, for his kind permission to use the laboratories of the Department and the necessary equipment. I want to express my sincere gratitude to Dr. Banko Bankov, operator of the amino-acid analyser, for the respective determinations as well as to Dr. Chem. Ivan Goshev for encouragement and help in some time-consuming experiments.

Summaries

Proteinase inhibitors from vegetables and their application in enzymatic conservation treatments

A general scheme for isolation and partial purification of protein inhibitors is described. It is applied for obtaining inhibitors of trypsin and some microbial proteinases from kohlrabi seeds and of chymotrypsin from potatoes. The effective action of the inhibitors has been proved spectrophotometrically by the change in enzymatic activity in the presence and in the absence of the inhibitors. The most suitable way to use the inhibitor extracts in enzymatic conservation treatments is demonstrated. Thus, the possibility for the inactivation of obstructive extraneous enzymes in enzymatic preparations with the help of protein inhibitors is shown. This provides the opportunity to apply technical enzymatic products in conservation, as these products are cheap and easily available.

Inhibiteurs de protéinase extraits de végétaux et leur application dans des traitements de restauration avec enzymes

On décrit un procédé pour l'isolation et la purification partielle d'inhibiteurs de protéines. Il est appliqué pour obtenir des inhibiteurs de trypsine et de quelques protéinases microbiens à partir de semences de kohlrabi et de chymotrypsine provenant de pommes de terre. L'efficacité des inhibiteurs a été démontrée par spectrophotométrie par changement d'activité enzymatique avec ou sans inhibiteurs. Le meilleur moyen d'utiliser ces extraits inhibiteurs dans des traitements de restauration avec enzymes est exposé comme par exemple l'inactivation d'enzymes étrangers gênants. L'application de ces techniques enzymatiques est intéressante étant donné que ces produits sont bon marché et facilement disponibles.

Inhibitoren pflanzlichen Ursprungs für Proteinasen und ihre Anwendung beim Einsatz von Enzymen in der Restaurierung

Zuerst wird eine allgemeine Methode zur Isolierung und partiellen Reinigung von Inhibitoren für proteolytische Enzyme beschrieben. Sie wurde angewandt, um Inhibitoren für Trypsin und andere Proteinasen mikrobiellen Ursprungs aus Kohlrabisamen und für Chymotrypsin aus Kartoffeln zu gewinnen. Ihre Wirkung wurde als Änderung der Aktivität des Enzymes bei Ab- und bei Anwesenheit des Inhibitors spektrophotometrisch nachgewiesen. Sodann wird die bestgeeignete Methode zur Anwendung der Enzyminhibitoren in der Restaurierung beschrieben. Sie kann eingesetzt werden, um das Enzym nach Erreichen des gewünschten Zweckes zu inaktivieren und um die schädliche Wirkung von verunreinigenden Mit-Enzymen zu verhindern. Das mag dazu dienen, in der Restaurierung Enzyme von technischer Reinheit (statt Reinheit p.a.) einzusetzen, welche wesentlich billiger und leichter zu beschaffen sind.

References

1. Wendelbo, O. & Fosse, B.: *Protein "surgery": a restoring procedure applied on paper.* Restaurator 1 (1970): 245–8.

2. Wendelbo, O.: *The removal of papyrus from gessocartonage with some remarks on the separation of glued papyri*. Symbolae Osloenses 50 (1975): 155–7.
3. Morse, E.: *Enzyme treatments for conserving artistic/historic works: a selected bibliography 1940–1990*. Technology and Conservation of Art, Architecture and Antiquities 1 (1992): 20–4.
4. De Santis Pell, P.: Talk delivered at the Enzyme Conference for Conservators, M.I.T., Cambridge, MA, Oct. 27–28, 1990. McKay Lodge Conservation Report 3 (1991): 16–7.
5. Caldararo, N. & Sheldon Ann, R.: *The discovery of hidden drawings by backing removal: three examples including a Charles Keene drawing*. Restaurator 13 (1991): 1–22.
6. Makes, F.: *Enzymatic removal of lining paste from paintings*. ICOM Committee for Conservation, 7th Triennial Meeting, Copenhagen 1984: 2/26–2/30.
7. Wendelbo, O.: *The freeing of papyri from cartonage*. Restaurator 2 (1975): 41–52.
8. Wendelbo, O.: *General discussion and conclusion*. In: *The use of proteolytic enzymes in the restoration of paper and papyrus. A contribution to interdisciplinary applied research*. Bergen (Norway): University Library 1976: 1–9.
9. Segal, J. & Cooper, D.: *The use of enzymes to release adhesives*. The Paper Conservator 2 (1977): 47–50.
10. Makes, F., Pühringer, J.: *The protection of wood against moulds from hydrophobics to inhibitors of enzymes – some strategies*. Wiener Berichte uber Naturwissenschaft in der Kunst 4,5 (1987/88): 451–79.
11. Mossolov, W. W.: *Pyrrodny inhibitory proteolitichesskv phermenty* [Natural inhibitors of proteolytic enzymes]. Usspehy Biologischesskoy Chimii 22 (1982): 100–18 (in Russian).
12. Crueger, W. & Crueger, A.: *Other fermentation processes and further prospects*. In: Brock, T. D. ed. *Biotechnology: a textbook of industrial microbiology*. Madison, WI: Science Technology Inc., 1984: 292–7.
13. Ogiso, T., Noda, T., Sako, Y., Kato, Y. & Aoyama, M.: *Studies of trypsin inhibitor in barley. I. Purification and some properties*. Journal of Biochemistry 78 (1975): 9–11.
14. Svendsen, I., Nikolova, D., Goshev, I. & Genov, N.: *Isolation and characterization of a trypsin inhibitor from the seeds of kohlrabi* (Brassica napus, *var.* rapifera) *belonging to the napin family of storage proteins*. Carlsberg Research Communications 54 (1989): 231–9.
15. Crouch, M. L., Tenbarge, K. M., Simon, A. E. & Ferl, R.: *cDNA clones for* Brassica napus *seed storage proteins: evidence from nucleotide sequence analysis that both subunits of napin are cleaved from a precursor polypeptide*. Journal of Molecular and Applied Genetics 2 (1983): 273–83.
16. Previous investigations connected with the determination of the isoelectric point of the protein inhibitor of trypsin in peak I of the elution profile.
17. Melville, J. C. & Ryan, C. A.: *Chymotrypsin inhibitor I from potatoes*. Journal of Biological Chemistry 247 (1972): 3445–53.
18. Richardson, M. & Cossins, L.: *Chymotrypsin inhibitor I from potatoes: the amino acid sequence of subunits B, C and D*. FEBS Letters 45 (1974): 11–3.
19. Preiss, L. & Cook, L.: *A warning of the use of alpha-amylase in the backing removal of starch pasted photographs*. Bulletin ICCM 1 (1983): 52–3.

Diana Nikolova
Conservation Scientist
Laboratory of Conservation
National Museum of History
2 Vitosha Str.
Sofia-1000
Bulgaria

Restaurator, 1993, 14, 217–253
Printed in Denmark – All rights reserved

RESTAURATOR
ISSN 0034-5806

Tracing Paper: Methods of Study and Restoration

by MARINA BICCHIERI, PAOLA BRUSA & GIOVANNA PASQUARIELLO

Introduction

The National Institute for Graphic Techniques (Istituto Nazionale per la Grafica), in collaboration with the Centre for Reproduction, Binding and Conservation of the National Archives in Rome (Centro di Fotoriproduzione Legatoria e Restauro degli Archivi di Stato), has recently faced the problem of restoring some architectural drawings belonging to the Virgilian Academy of Mantua. These were done in the first 30 years of this century by Duilio Cambellotti (Rome 1886-1960), an all-purpose artist, sculptor, set designer, illustrator, engraver, decorator, interior designer and poster maker.

The drawings are marked with the numbers 4, 7 and 9, and represent the following subjects:

- 4: perspective of temple façade (570 mm × 405 mm);
- 7: perspective of temple side (460 mm × 665 mm);
- 9: perspective of temple rear (570 mm × 410 mm).

The tracing-paper drawings, each supported by paperboard (from which they were partly detached), showed various signs of mechanical damage: tears, deformation, curling edges and deep creases in which there were traces of dust and insect excrement. The creases were found primarily in the central area of the sheets (which still adhered well to the paperboard) and ran the entire length of the tracing paper.

All three drawings showed unmistakable signs of biological attack in the form of rust-coloured stains (foxing) or rose and violet-black stains that had penetrated deeply into the paper. Only one of the drawings (no. 7) also had

Fig. 1. Drawing 7 before restoration

superficial black stains that could not be attributed to biological agents, the stains occurring in a rectangular strip (Fig. 1).

The conceptual interest involved in the restoration of the three Cambellotti perspectives centres around the challenge of restoring a tracing-paper support, a material that has been little studied and even more rarely restored. In order to approach the problem correctly, chemical and biological analyses were performed and simulated restoration trials were made on similar modern material, artificially aged before testing.

Chemical Studies

The aim of the analyses was to obtain the best possible knowledge about the material under study: the type of adhesive, any possible additives, the composition of the tracing paper and its interaction with water and all the products that might be used in the restoration.

A logical scheme of analysis calls for the following sequence of events:

- identifying the adhesives, to choose an appropriate means of detaching paper from paperboard;
- examining all additives or coatings on the paper, to decide what solvents can be used in the restoration without damaging the support or patina, if any;
- measuring the pH of the paper, to evaluate whether deacidification is necessary; and
- evaluating the presence of lignin and detection of fibrous matter, once all non-cellulose materials have been removed.

The dyes and pigments are also tested for their solubility in the products that will be used for restoration.

No bleaching method was considered for the areas attacked by biological agents: the cellulose chain structure of the fibres in these areas is already weakened and any chemical action that involves using strong oxidants for stain removal would cause further degradation of the paper support and compromise its conservation.

Adhesives

The analyses were performed on the paperboard to avoid damaging the documents themselves.

Before the advent of synthetic adhesives, the products most commonly used in paper manufacture were based on starch, gelatin (refined or from animal glue) and casein. The first test was for the presence of starch, which is composed of two different polysaccharides: amylose and amylopectin.

Amylose has a linear chain structure of D-glucose units joined by α-D (1-4) linkages. It is soluble in water and gives an intense blue colour with iodine. The amylose complexes have a crystalline structure and X-ray analysis shows that the amylose chain has a helix structure within which there is enough space to contain one complexed molecule.

The iodine complex seems to be formed by one amylose molecule, with a helix of six D-glucose units for one iodine molecule.

The blue colour would derive from the iodine molecules encapsulated in the helix: with a period of D-glucose units less than six, the complex formed has a red colour. Amylopectin, insoluble in water, has a branched structure of D-glucose units joined α-D (1-4) in the linear chain, whereas at the branch point the linkage is α-D (1-6).

The percentages of amylose and amylopectin in starch depend on the plant from which it derives. The majority of starches contain from 15% to 30% of

soluble fraction and are used in paper manufacture as both internal or surface size and as binders for coatings.

To detect the presence of starch[1] in the tracing-paper drawings under study, the reaction between amylose and iodine was exploited. The test was performed on the aqueous extract obtained by boiling fragments of the paperboard in distilled water. The absence of blue colour allowed us to rule out the use of starch as an adhesive.

The next step was a search for proteinaceous substances[2] characteristic of adhesives of biological origin, such as animal glue, gelatin and casein. The most commonly used test for detecting the amino acids in the proteins is based on the reaction between amino acid and ninhydrin (1,2,3, triketohydrindene monohydrate).

The colour formation mechanism is not yet well understood. According to an accepted hypothesis, an oxidation-reduction reaction occurs in which the amino acid is oxidized to aldehyde with the formation of ammonia and carbon dioxide, and the ninhydrin is reduced to hydrindantin.

The ammonia obtained forms a complex with one molecule of ninhydrin and one molecule of hydrindantin, yielding the Ruheman purple (Fig. 2). The ninhydrin test was positive.

To determine whether the proteinaceous substances derived from animal glue or casein, two analyses were performed: one specific for gelatin and the other specific for tyrosine, an amino acid present in casein (6.6%), but only in small quantities in gelatin (0.51%).

The test[3] for identification of animal glue and gelatin is based on the reaction between p-dimethylaminobenzaldehyde in propyl alcohol and hydroxyproline,

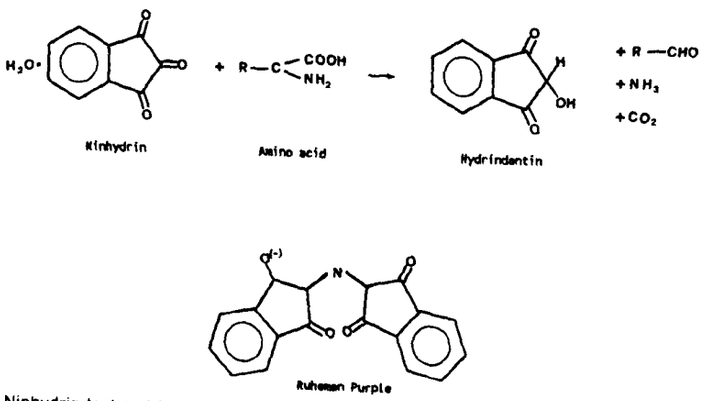


Fig. 2. Ninhydrin test and formation of the Ruheman purple

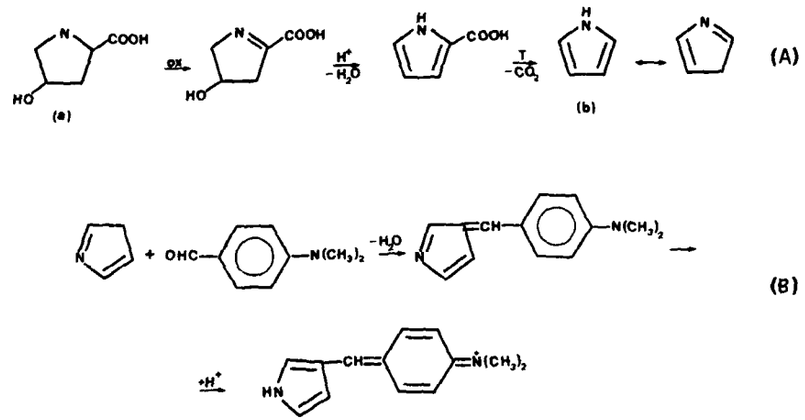


Fig. 3. p-dimethylaminobenzaldehyde test. **A**. Oxidation of hydroxyproline (a) to pyrrole (b). **B**. Formation of a complex between pyrrole and p-dimethylaminobenzaldehyde

obtained by alkaline hydrolysis of collagen, present in animal glue and gelatin. The specific colorimetric reaction consists of the oxidation of hydroxyproline to pyrrole (Fig. 3A) and subsequent formation of a pink-red complex between pyrrole and p-dimethylaminobenzaldehyde (Fig. 3B).

Other nitrogenous materials do not interfere with this test which, in this case, had negative results.

The Millon reagent was used to search for casein. It is composed of mercuric nitrate dissolved in nitric acid and potassium nitrite solution[4] which, in the presence of tyrosine (Fig. 4), gives rise to the formation of a red-brick coloured compound.

The Millon test is based on the formation of a nitro-compound, which then reacts with p-substitute phenols such as tyrosine, while the di o- and di m-substituted phenols do not react.

Neither gelatin nor animal glue interferes with this test (the result of which was positive), because their tyrosine content is below the sensitivity of the method.

The combined analytical results showed that casein provided the adhesion between the tracing paper and the paperboard.

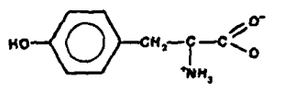


Fig. 4. Structure of tyrosine

Paper additives

Natural waxes, rubber, resins, oils and petroleum derivatives can be added to paper to improve its transparency and increase its wet strength.

A series of extractions and separations with suitable solvents is necessary to recognize the presence of such compounds.

The tests described in the literature require a significant amount of sample material, which is impossible to obtain when original works of historical and artistic interest must be examined. For this reason, in the study of Cambellotti's drawings, the tests were partially modified to obtain the best results even with minuscule sample quantities. Quantitative information could not be obtained.

The presence of rosin was detected by the Raspail test,[5] based on the development of an intense red colour due to the reaction between saccharose, sulfuric acid and rosin. An intense blue fluorescence was obtained under ultraviolet light, revealing the presence of paraffins or waxes. We then proceeded to separate the rosin from the additives by extraction and saponification of the resinic acid. The unsaponifiable fraction was then submitted to the Dunlop test,[6] based on the solubility of paraffins in acetic anhydride and their subsequent precipitation on cooling.

The small quantity of sample examined did not permit more accurate instrumental tests to determine the true composition of the additive, but an infrared spectrum of a first residue of extraction with 1-1-1-trichloroethane,[7] albeit difficult to interpret (as is always the case when complex mixtures are examined), made it possible to confirm the presence of typical peaks of hydrocarbons, rosin and rosin oil.

pH of the paper

The small quantity of material available for destructive tests led us to measure only the surface pH. A value ranging from 5.3 to 5.5 was obtained. This result allowed us to avoid any deacidification.

If deacidification could not be avoided, only the alcoholic treatment should be used, but it would not significantly change the paper pH. Aqueous deacidification could not be used in any case because of the presence of soluble dyes and pigments and because of the nature of the paper under study.

Composition of the paper

Determination of fibre composition is indispensable in characterizing the paper and its method of manufacture, conservation and history.

Technical drawing paper, also called tracing paper, is translucent, has high density and transparency; its basis weight ranges from 30 to 100 g/m^2. It is manufactured with specially refined sulfite cellulose (up to 85°SR, i.e., Schopper Riegler degrees of refining), in which the effect of fibre-cutting action is reduced and the defibering is accentuated. The pulp has no fillers and little or no size. The paper obtained is hard, with a high apparent density, but it is fragile and has poor tearing resistance.

An important quality of tracing paper is its formation, defined as the uniformity with which the fibres are distributed in the paper. Formation is a physical property of the paper and it is usually measured by the degree of uniformity of light transmission through the paper. Paper is said to have a closed formation if the distribution of the fibres is uniform and a poor or wild formation if the fibres are distributed so that they give the sheet a cloudy appearance in transmitted light. Tracing paper must be highly uniform and its peeling resistance must be great enough to permit erasures without diminishing transparency.

Tracing paper is not calendared, i.e, it has not suffered a mechanical treatment to increase its smoothness and shine. Calendering is done by passing the sheet between very smooth cylinders (the calendar or rolling press), one above the other, which exerts a strong pressure on the paper (140-700 kg/cm^2). These cylinders may or may not be heated. Calendering may have the result that pencils and ink can flow too easily over the sheet, leaving an insufficiently intense trace, which must be avoided with tracing paper.

Another type of translucent paper, now rarely used, is obtained by impregnating pure cellulose paper with a liquid that has a refraction index similar to that of the cellulose fibres.

Such impregnating agents are composed of mixtures of drying oil, natural rubber, resin, waxes, petroleum derivatives and turpentine. They yellow on aging, yielding a brownish colour on the support. The fibres are identified after the paper has been kneaded and microscope slides, stained with suitable reagents, have been prepared for observation.

A preliminary test of lignin content (absent in the drawings under study) is always performed to choose the most suitable stain. For water-resistant paper, an alkaline treatment is executed before the usual process used for disintegrating the paper.

In this particular case, this treatment did not produce a satisfactory effect.

We then proceeded, as for vegetable parchment paper,[8,9] with sulfuric acid to begin defibering. It was, however, not easy to open the fibres, so they were further opened manually on the slide.

Under microscopic observation,[10] after addition of the Herzberg stain,[11] it was possible to ascertain that the paper contains hardwood fibres from chemical

pulp, well conserved and hardly fibrillated. Long fibres make up 60% of the mixture; very broken fibres make up the remaining 40%.

Inks and pigments

No samples of the graphic media were taken to prevent damage to the pictorial image. The media were examined under the binocular microscope in order to ascertain their conservation, compactness and penetration into the paper.

The dark areas were realized in India ink and India-ink wash. The design was sketched by the artist in graphite, as seen under infrared light.

With regard to the white tempera, the use of white lead can be ruled out because this pigment darkens easily, a feature that was not found in the drawings under examination. Other white pigments used in tempera technique during the first decades of the twentieth century were Chinese white, based on zinc oxide (known since 1783 but commercially available only towards the mid-nineteenth century) and titanium white, available since 1920.

A possible attribution, without the use of destructive techniques, exploits the fluorescence of the zinc-based pigment under ultraviolet light. This occurred when the drawings were illuminated under a Wood lamp.

The solubility of each graphic medium was tested in both water and ethyl alcohol. The pigments were found to be soluble in water and stable in ethyl alcohol, even if mixed with up to 30% of water.

Biological Investigations

Biological analysis was used both to determine the agents responsible for chromatic alterations in the three tracing-paper drawings and to search for live micro-organisms.

Pink and violet-black stains and brown or yellowish stains (foxing) can be noted on perspectives 4, 7, 9 and on their corresponding paperboard supports.

The colour of a stain of suspected microbiological origin does not indicate a specific type of micro-organism but is related to the colour-generating power of the attacking microflora, the composition of the paper and the age of the vegetation. Moreover, the shape of a stain, its extent and the appearance of the edges are all strictly related to environmental conditions and to the presence of a primary and/or secondary infection of several types of micro-organisms.

For these reasons, it was felt that a first operative phase should involve the observation of the various types of stains under the binocular microscope at low magnifications ($\times 40$ and $\times 60$) with an incident light, followed by some culture tests.

Based on this first examination by binocular microscope, it was possible to formulate an hypothesis regarding the nature of the stains: those of rose to violet pigmentation are of fungal nature, given that some fungal hyphae were found in the fibres of the tracing paper. The foxing-type alterations are caused by isolated stains of limited dimensions, some with well-defined edges, others with serrated edges, on which no microbial colonization was found.

Before the biological analysis performed and the corresponding results obtained are described, it should be briefly noted that research on the phenomenon of foxing is still an open question.

The nature and mechanisms that lead to the formation and appearance of the characteristic foxed stains are still not well defined; indeed, there are various types that differ from each other in morphological and chromatic characteristics and in their fluorescence under ultraviolet light. The studies carried out over the past 50 years by researchers of various disciplines have led some of them to propose a biological origin of the phenomenon and others to suggest a chemical one, even though they do not exclude other, concomitant factors.[12-16]

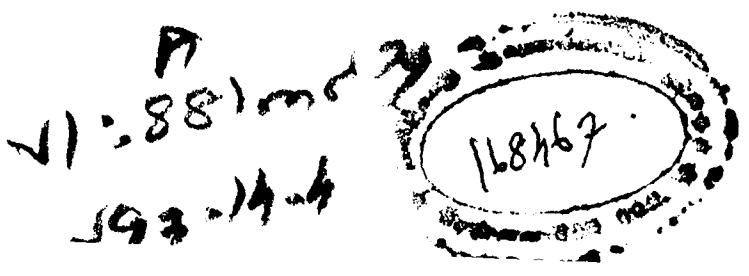
On the tracing-paper drawings, various testing methods were used to evaluate whether microbial attack was under way. Experimental testing was carried out as follows:

- samples were taken from various types of stains and in the areas surrounding them, apparently unaffected;
- culture tests; and
- microscopic tests.

These methods were used because only non-destructive tests could be performed, in the absence of some fragments of the material to examine. Two test methods were used to isolate possible microflora present on the samples:[17]

With sterile swabs: specimens were taken both from various types of stains and from adjacent areas that were seemingly undamaged. Subsequently, swabs were inoculated both into a liquid medium (Sabouraud maltose broth, Difco) and onto agar (Sabouraud maltose agar, Difco, Cooke Rose Bengal, Difco and Mycological agar, Merck);

With sterile needles: specimens of a few fibres of the surface of the three tracing-paper drawings were taken from both variegated and foxed stains and apparently unstained surrounding areas. After several washings with sterile distilled water, minute fibres of the sample were inoculated onto Sabouraud maltose agar (Difco) and Cooke Rose Bengal agar (Difco). Each sample from



various types of stain was used to make several inoculum points on each culture medium. The cultures were incubated at 28° C for 25 days.

At the same time, a few paper fibres collected with sterile needles from the three drawings (in places where the different types of stains were found) were viewed under the light microscope. Micro-cultures were prepared on slides, using Sabouraud maltose agar (Difco) as the culture medium.

Observation under a light microscope makes it possible to control and follow any microbial development without modifying the morphological structures.[18]

The methods described above were also used for tests on the paperboards.

The results of the biological investigations were all negative: no statistically significant microbial development was found from the culture tests and the microscopic tests examined. This result allows us to suppose that the alterations of biological origin on both tracing paper and paperboard occurred some time previously and had already died out. Disinfection treatment, therefore, was not scheduled, as no micro-organisms were still active.

To identify the insect excrement that was found in the creases of the three drawings, samples were taken with needles and examined under the binocular microscope. The excrement was found to belong to the genera Anobiidae, order Coleoptera.

Simulated Restoration Trials

All the simulated trials were performed on modern parchment paper (previously aged) with composition and manufacture similar to the tracing paper under study.

Two types of accelerated aging were carried out, one dry heat treatment at 105° C for three days and one moist heat treatment at 75° C and 80% relative humidity for seven days.

Some of the trial samples were glued onto paperboard similar to the originals, after a casein-based glue was prepared according to one of the most common recipes: 15 g of casein was beaten with 80 g of water for 30 min; then a solution of 0.75 g of borax in 10 g of water and 1.5 ml of ammonia was added, and, as usually given in the relevant recipe, a few thymol crystals to combat the development of mould.

On the aged samples, detachment trials were made with the following mixtures:

ethyl alcohol/water	70/30
ethyl alcohol/water/ethylene glycol	50/25/25
ethyl alcohol/water/ethylene glycol	50/10/40.

Only the two last mixtures were found to be effective for detachment purposes.

The subsequent flattening trials were performed both on samples already detached from the paperboard and on paper immersed in water, handled so as to produce deep creases, air-dried to accentuate the deformation effects and then dry-aged at 105° C for three days.

The deformed and folded sheets were flattened with a mixture of water and ethyl alcohol in a proportion of 30/70.

Some of the samples were brushed with the chosen mixture; some were flattened by having only the back of the paper absorb the mixture through capillary action, avoiding total permeation, and some by immersion.

All the samples were then pressed, after conditioning between sheets of non-woven cloth, filter paper and paperboard, and the results of the flattening were very satisfactory.

For the lining, performed on flattened but not quite dry samples, adhesives of different chemical compositions were tested:

Klucel G	4% ethanol solution
Tylose MH300P	3% water solution
Primal AC 33	commercial, undiluted emulsion
Primal AC 33	35% water emulsion
Primal AC 33	5% ethanol emulsion

Before the lining, the sizes and shapes of the sheets were reproduced on graph paper, to verify whether the dimensions of the samples had changed afterward.

The lining was done on the vacuum table, and the samples were dried in the press for 48 h, the filter paper being changed periodically.

The sheets treated with Tylose MH300P and Primal AC 33 water emulsion show notable deformation, due to the interaction of paper and water, mainly in the direction orthogonal to the preferential orientation of the fibres.

The alcoholic emulsion of Primal AC 33 does not guarantee sufficient adhesion. The samples treated with Klucel G and pure Primal AC 33 show good adhesion values; no deformation of the parchment paper support was noted.

For restoration purposes, Klucel G in ethanol solution was preferred: having a choice of products, it seemed preferable to work with an adhesive structurally similar to the support under treatment. In this case, Klucel G is a hydroxypropylcellulose, derived from cellulose.[19-21]

The adhesive, moreover, appeared suitable from the biological point of view

as indicated by some biological resistance tests performed in aqueous and alcoholic media at various concentrations (2%, 3% and 4%) and on various supports.

The adhesive was applied on glass to verify its vulnerability to micro-organisms,[22] on various types of Japanese paper commonly used in restoration and on Whatman no. 1 paper for chromatography. An additional test[23] was performed to evaluate the relationship between the hygroscopicity of the glued papers and the development of micro-organisms on them, using microclimates representing various environmental situations.

The results of the analyses showed that ethanol solutions at the tested percentages have an acceptable biological stability and resistance to microbial attack when applied to the various supports (paper and glass). Aqueous solutions are potentially vulnerable under environmental conditions with relative humidity values above 85% and temperature of 30 °C.

Restoration

The first restoration operation consisted of detaching the tracing paper from the paperboard.

Although the previously studied mixtures (water, ethyl alcohol, ethylene

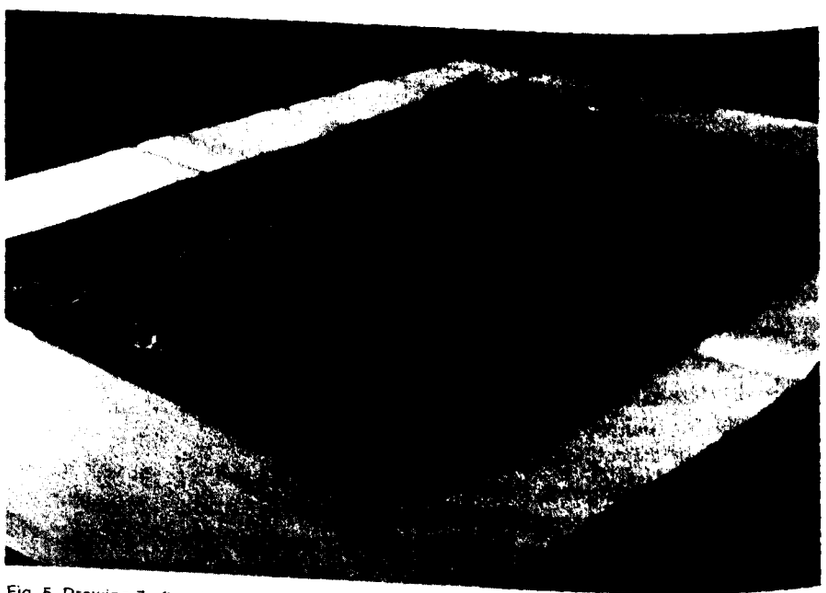


Fig. 5. Drawing 7 after detachment from paperboard

Fig. 6. Drawing 7 after detachment from paperboard, detail of upper zone

glycol) could have been used for this operation, a dry procedure was preferred: the paperboard proved to be layered and could be peeled off gradually. This long and patient operation finally reached the last paperboard layer, which was eliminated by lightly swabbing it with water and removing it with scalpels.

The choice of dry detachment was motivated by the need to avoid, wherever possible, the use of not strictly necessary chemical products that might not be completely removed.

The drawings, freed from their paperboard, were softened (like the laboratory samples) with water and ethyl alcohol (30/70) (Fig. 5, 6). The total immersion, which lasted 5 min, was followed by manual flattening on glass to eliminate contractions and to make the drawing regain its original size and shape.

After conditioning between paperboard, filter paper and veils of non-woven cloth, the drawings were then pressed for 10 min at 2.02 MPa.

The subsequent total lining with Japanese paper 508 and 4% ethanol solution of Klucel G was performed on the vacuum table, before the pressed sheet was completely dry. The vacuum table guaranteed rapid absorption of the adhesive and simultaneously kept the paper from forming new creases or deformations between areas that differed in dampness or degree of deterioration.

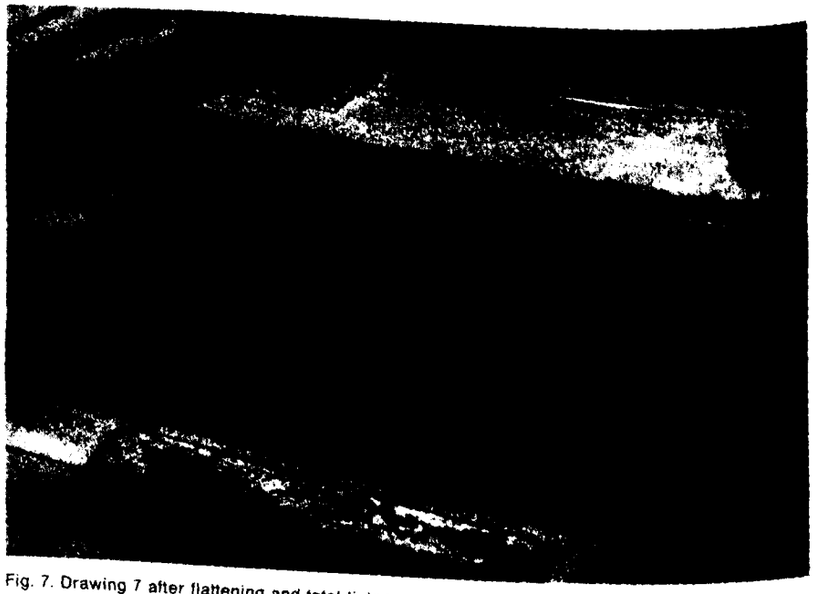


Fig. 7. Drawing 7 after flattening and total lining

At the end of this first phase of flattening and consolidation, the drawings were again pressed for 24 h at 2.02 MPa (Fig. 7, 8).

The final phase of the restoration consisted of the reintegration of lacunae, mending of tears and suture of cuts with Japanese paper of appropriate thickness and a 4% ethanol solution of Klucel G.

The Japanese paper used for reintegration was slightly retouched with Winsor and Newton watercolours in order to harmonize the background color with that of the original.

Conclusion

The problem posed by the restoration of tracing paper mainly consists of the need to work in the almost total absence of water, owing to the dimensional instability of the paper. Tracing paper cannot be immersed in water and cannot tolerate the use of aqueous glues and adhesives.

Nevertheless, the combination of tests performed prior to the restoration and the patient execution of the restoration itself made it possible to devise a method capable of producing results that can be considered quite satisfactory, as can be seen in the photographic documentation.

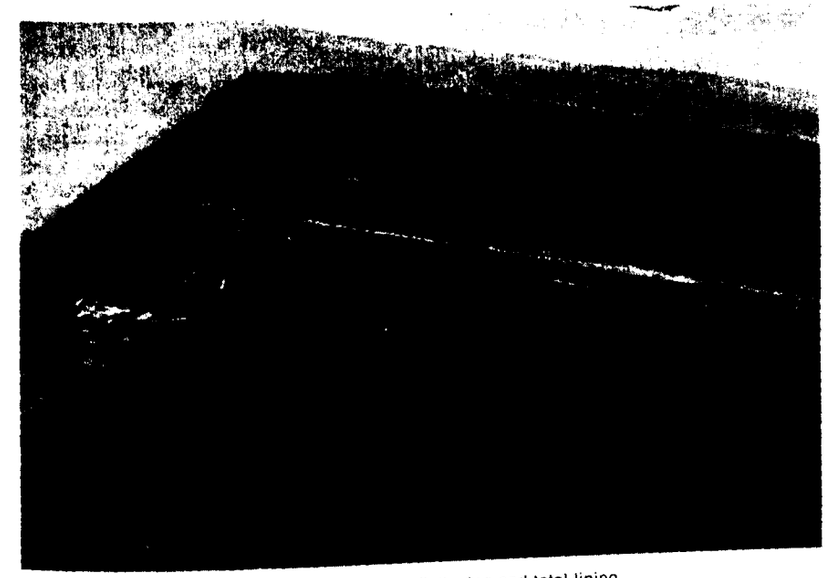


Fig. 8. Drawing 7, same detail as in Fig. 6, after flattening and total lining

Summaries

Tracing paper: methods of study and restoration

In order to find appropriate methods to restore some architectural drawings from the beginning of the twentieth century that were mounted on paperboard, these drawings were analysed for fibre, adhesive, additives, inks, colours and biological infestation. Possible methods of restoration were tested with artificially aged papers of our time. The final method that seemed to be most suitable was dry detaching, flattening by pressure (2.02 MPa) and mending on the suction table using solvent-soluble cellulose derivatives (Klucel G).

Papiers calques: Etude et restauration

Afin de trouver les méthodes adéquates pour restaurer quelques dessins architecturaux du début du XXe siècle montés sur carton, on a analysé la composition fibreuse, la colle, les additifs, les encres, les couleurs et évalué la contamination biologique. Les méthodes de restauration envisageables ont été testées avec des papiers actuels vieillis artificiellement. La méthode qui semble la plus appropriée est le détachage à sec, l'aplanissement sous pression (2.02 MPa) et la réparation sur table aspirante avec des dérivés cellulosiques solubles dans des solvents (Klucel G).

Transparentpapier: Methoden der Analyse und der Restaurierung

Zur Vorbereitung der Restaurierung einiger Bauzeichnungen vom Anfang unseres Jahrhunderts, die auf Pappe kaschiert waren, wurden diese Zeichnungen im Hinblick auf die Faserzusammensetzung, auf Klebstoffe, Zusätze, Tinte, Farben und auf biologischen Befall hin analysiert. Die denkbaren Restaurierungsmethoden wurden zunächst an beschleunigt gealtertem Transparentpapier aus heutiger Produktion ausprobiert. Am besten geeignet erwies sich das trockene Ablösen, Glätten unter Druck (2.02 MPa) und das Ausbessern auf dem Saugtisch unter Verwendung von lösemittellöslichem Cellulosederivat (Klucel G).

References

1. Grandis, E.: *Enciclopedia della stampa, aggiornamento n° 14 Prove sulla carta.* 1973: 343-4 Torino: Società Editrice Internazionale.
2. Greenstein, J.P. & Winitz, M.: *Chemistry of the amino acids 1-3.* New York: John Wiley, 1961.
3. TAPPI Standard T505. Atlanta, GA: Technical Association of the Pulp and Paper Industry, 1985.
4. TAPPI Standard T415. Atlanta, GA: Technical Association of the Pulp and Paper Industry, 1985.
5. Browning, B.L.: *Analysis of paper.* New York: Marcel Dekker, 1977: 81.
6. Ibid, p. 123.
7. Ibid, p. 125.
8. Ibid, pp. 49-52.
9. Gianni, E.: *Carte, cartoni e cartoncini.* Milan: Hoepli, 1959: 573-7.
10. Poles, G. & Della Giovanna, A.: *Analisi microscopica delle fibre naturali nella carta.* Milano: Aticelca, 1974.
11. Browning, op. cit. (ref.5): pp. 60-1.
12. Cain, E. & Miller, B.A.: *Photographic, spectral and chromatographic searches into the nature of foxing.* Preprints, American Institute for Conservation 10th Annual Meeting, Milwaukee, 1982: 54-62. Washington, DC: AIC, 1982.
13. Carter, J.: *Iron stains on textiles: a study to determine their nature and to evaluate current treatments.* Preprints, ICOM Conservation Committee, 7th Triennial Meeting, Copenhagen, 1984: 9-11-14. Copenhagen: ICOM, 1984.
14. Arai, H., Matsui, N., Matsumura, N. & Murakita, H.: *Biochemical investigations on the formation mechanisms of foxing.* The Conservation of Far Eastern Art. Preprints of IIC Kyoto Congress 19-23 September 1988: 11-12.
15. Gallo, F. & Pasquariello G.: *Foxing: ipotesi sull'origine biologica.* Bollettino I.C.P.L. 43 (1989): 139-76.
16. Hey, M., Pasquariello, G., Gallo, F., Guidi, G. & Pierdominici, F.: *Paper analysis in relation to foxing.* 2nd Int. Conf. on Non-Destructive Testing, Perugia 1988: Preprint III.9.
17. Normal-9/82 Gruppo Normal-B: *Metodologie biologiche, raccomandazioni normal.* Rome: CNR-ICR, 1983.
18. Rambelli, A.: *Fondamenti di micologia.* Bologna: Zanichelli, 1985
19. Hofenk de Graaff, J.: *Hydroxypropyl cellulose, a multipurpose conservation material.* Preprints, ICOM Conservation Committee, 6th Triennial Meeting, Ottawa (1981): 14.9.1-7.
20. Evetts, D.M., Lockwood, A. & Indictor, N.: *Evaluation of some impregnating agents for use in paper conservation.* Restaurator 10 (1989): 1-5.
21. Hercules Inc.: Klucel hydroxypropyl cellulose. 1981.
22. Australian Standard 1157, Part 10 (1979). Sydney: Standard Association of Australian Standards, 1979.
23. Block, S.S.: *Humidity requirements for mould growth.* Florida Engineering and Industrial Experimentation Station 7,10 (1953): 289-93.

Marina Bicchieri
Paola Brusa
Giovanna Pasquariello
Centro di Fotoriproduzione
Legatoria Erestauro
De Gli Archivi Stato
Via Constanza Baudana 14
I-00153 Roma
Italy

Restaurator, 1993, 14, 234–252
Printed in Denmark · All rights reserved

RESTAURATOR
ISSN 0034-5806

Paper-splitting: A Problematic but Indispensable Method in Paper Restoration

by MONIKA GAST

Paper reinforcement techniques

Various methods may be considered to consolidate brittle, degraded and damaged paper objects when *no local treatment or simple fibre-strengthening* methods, such as washing and sizing, are sufficient.

The decision about whether to consolidate and restore in these cases depends on several factors. First, the object itself:

- What kind is it, a manuscript or a printed or illustrated artefact?
- Is the script on one side only or on both sides of the paper?
- What function does it have: a source of information or a work of art?

Second: What is the scale of the damage and what are its causes?

I propose to outline five techniques available to the conservator for reinforcing paper:

- lining
- embedding
- lamination
- leafcasting
- paper-splitting.

Lining

The first choice for consolidation of one-sided objects will be lining the back. This is a common technique for most workshops using a variety of different

working methods and materials, such as Japanese or European paper; starch paste adhesive, methylcellulose, a mixture of them, or occasionally a nonaqueous adhesive, for example, a solvent soluble cellulose derivative such as Klucel®; drying under pressure (Western technique) or under tension (Chinese and Japanese technique). Naturally the object will be slightly thicker, but the texture of the original is mostly retained and nothing will be seen on the front.

Embedding

This is a process mostly used in archival conservation. Mouldy and damaged records are often treated in this way. The purpose of this method is to save the paper from further deterioration so that the information is available to users when aesthetic considerations are of secondary importance. This procedure implies that a fine tissue paper is put on both sides of the object using a thin starch paste or an aqueous methylcellulose as an adhesive. This sandwich is then put into the press and a lot of pressure is applied to make the tissue transparent. The structure of the paper is completely lost and the tissue paper that lies on top of the writing will always be visible.

Embedding as well as lining are reversible in water, but like all treatments of very degraded paper, it might only be a theoretical possibility to actually reverse it. In most cases the object will suffer damage if the tissue paper and the adhesive are removed from its surface and/or back.

Lamination

The term lamination might be used in the same sense as embedding. Here it is used restricted to a nonaqueous conservation procedure using a fully synthetic adhesive. It is applicable if the object has been damaged through the use of iron gall inks or shows copper green degradation. This process of degradation is further catalyzed by an aqueous treatment. Nonaqueous deacidification and consolidation is therefore preferable. Furthermore, starch paste and/or methylcellulose will be degraded in the same way as the old paper and must therefore be replaced by an adhesive more stable against hydrolysis and heavy metal-catalysed oxidation. During lamination a heat-sensitive synthetic adhesive, preferably an acrylic coated onto tissue paper, is activated by heat and bonded to the object. The lamination tissue lies on top of the writing and the layer will always be slightly visible. Furthermore, much of the original paper structure is lost, as substantial pressure and heat are required in the process. Lamination is reversible with an appropriate solvent in the same restricted sense as defined above with respect to embedding.

Leafcasting

Leafcasting can be a process for either mass treatment or single items, either with a machine or a unit or on a suction table. It can be appropriate not only when missing areas have to be filled in, but also when a frail object requires some strengthening. A very fine layer of fibres is deposited on both sides of the object without using strong adhesive. Leafcasting is a superb method. Tears, however, are not strengthened sufficiently and require additional repair. Missing areas have overlapping fibres, which results in the loss of some definition.

Paper-splitting

The last method is paper-splitting. The paper is split in two and a thin repair tissue is inserted as consolidation into the middle. It can be applied as another choice in cases when the object requires embedding or lamination. After paper-splitting, no tissue is visible on both surfaces of the object, which is different than the other consolidation techniques. It can also be perfectly combined with leafcasting if missing areas are present.

Methods of Paper-splitting in Eastern Germany

Paper-splitting has been done occasionally in several workshops since the beginning of paper restoration. Descriptions of experiments with this technique can be found in French, English and Austrian technical literature of the nineteenth century.[1] Restorers seem to have used many kinds of different techniques and materials. However, during the last 25 years workshops in the eastern Germany have been developing this technique into a real method, based on previous experiments in Prague.[1] One of the reasons paper-splitting came to the fore as a repair technique especially in the German Democratic Republic may be due to lack of material and equipment there. It was necessary to develop alternatives.

Wolfgang Wächter of the Deutsche Bücherei in Leipzig and especially Günther Müller of the University Library in Jena became skilled with this treatment. A mass restoration programme of paper-splitting, according to the needs of the library material to be conserved and restored, was set up in their respective institutions. Günther Müller accomplished an impressive sequence of operation and his workshop is designed to split several hundred pages a day.[1,2] After preliminary treatment, for example dry cleaning, washing, and in some cases bleaching, the objects are covered on both sides with a support

paper glued out by machine. The adhesive used is hot gelatine. The sandwiched objects are placed between polyethylene and blotting paper, put in a hydraulic press for a short time and then left under a weight overnight.

The next day the treated objects are split and the two halves pasted out with an adhesive mixture of methylcellulose, carboxy methylcellulose, a buffer of magnesium carbonate and an acrylic emulsion. A thin tissue paper is inserted for reinforcement. The objects are dried and the support papers released in a proteolytic enzyme bath the following day. Finally the objects are dried.

The whole process is based on the fact that the core adhesive is more stable and not as readily dissolved in the same solvent (by the proteolytic enzyme) used to remove the adhesive of the support paper.

Günther Müller, the pioneer of this method, is still further developing it, especially now with the access to more equipment. In the last year his workshop started to use a leafcaster in combination with paper-splitting.

In Leipzig a machine for paper-splitting is being constructed[5] to cope with the bulk of deteriorated material. Such a machine might speed up the procedure considerably. A restorer with practical experience in paper-splitting will expect that such a mechanized mass process can only deal with objects of similar damage and homogeneous structure.

Method of Paper-splitting

I shall now report on my own experience with paper-splitting. It must be introduced with the statement that the laboratory where I am working, the laboratory of the Bavarian State Library, does not deal with any mass conservation treatment. Other considerations than those in Jena and Leipzig have to be taken into account, as paper-splitting is required as a method for conserving single items. Most cases we have come across throughout the years in our institution are single rare and valuable objects or sometimes several pages of a book. Paper-splitting in combination with leafcasting is sometimes a necessary treatment.

If we come across damaged items interesting only for their content, we prefer microfilming as the cheapest and most apt means of information preservation.[6] We slightly modified the process described in the paragraph before, but the basic principle was taken over from Günther Müllers technique.

Materials

Most suitable as a facing or support paper is a wet strength filter paper. The gelatine applied to the paper must not penetrate through but sit on the surface

of the paper. However, for removing the support paper afterwards, water should easily get through the fibres to facilitate a quicker release.

Strips of filter paper cut to approximately 3 cm width are used as a margin around the object to be split. The filter paper should split easily.

A food gelatine of medium viscosity (Bloom grade about 150) is suitable as an adhesive for the support paper in most cases. The following recipe has been proved to be suitable: 400 g of gelatine per litre of distilled water together with 20 ml of glycerin. The glycerin seems to be necessary to prolong the gelling time of the gelatine. The mixture should be kept hot at about 40°C in a double saucepan.

The core paper is inserted into the middle of the split object. A lens tissue (9 g/m^2) or a thin Japanese paper will be sufficient for consolidation in most cases.

As an adhesive for the core, a mixture of methylcellulose (Tylose® MH 300) and a thin wheat starch paste provides the best results. Some magnesium carbonate can be added. I did not find an acrylic necessary during my work.

To release the support papers, hot water alone might be sufficient. However, the layer of gelatine dissolves more quickly when a protein degrading enzyme is used. I have been using Stabilozym®, a liquid enzyme made for the brewery industry by the company BAYROL (Munich, Germany). Concentration: 3 ml per 1 litre warm water (50°C). It is not necessary to use a buffer salt mixture to keep a certain pH.[7]

Process

Before a damaged and decayed paper can be split it must be cleaned first, as in most other processes for paper restoration: dried with a soft brush, possibly with draft clean powder, and then washed. Washing is an essential step when paper-splitting is intended. The more degraded the paper, the easier the paper-splitting will be. A hand-made paper with strongly linked fibres is difficult to split. In such cases, however, paper-splitting would not be required. Size also consolidates the fibre bonding and has to be washed out at the outset of the treatment. If a paper has areas of different strength, as was the case with the sheet given in Fig. 1 (front) and 2 (back), the degraded area can fall into two halves almost by itself, whereas the intact paper around it is more difficult to split. If there are missing parts they are best filled in by leafcasting. It is very important that there be as little overlapping as possible of fibres onto the object, as in these areas the object will not split, but the leafcast fibres will separate from the object. This is achieved by arranging the object in the leafcasting machine in a way that there are greater areas not covered by old

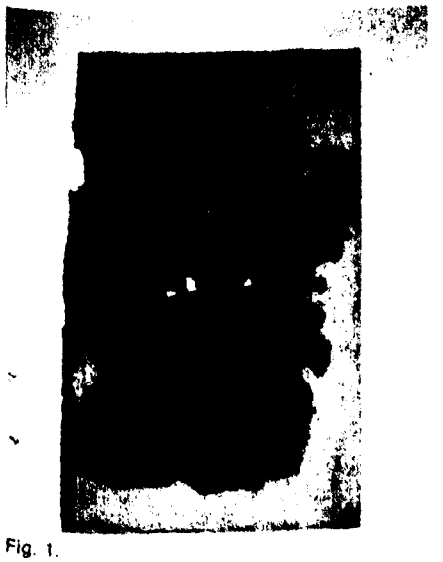


Fig. 1.

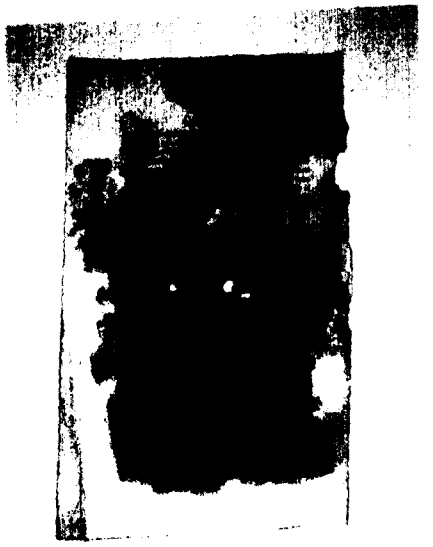


Fig. 2.

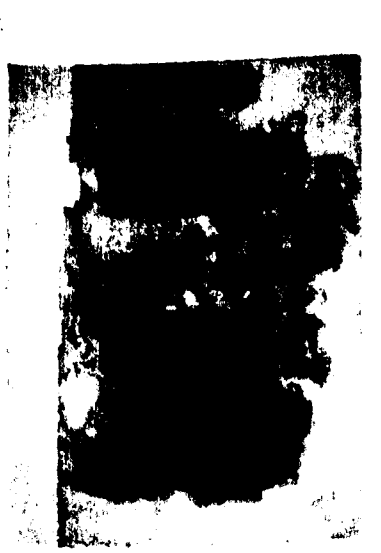


Fig. 3.

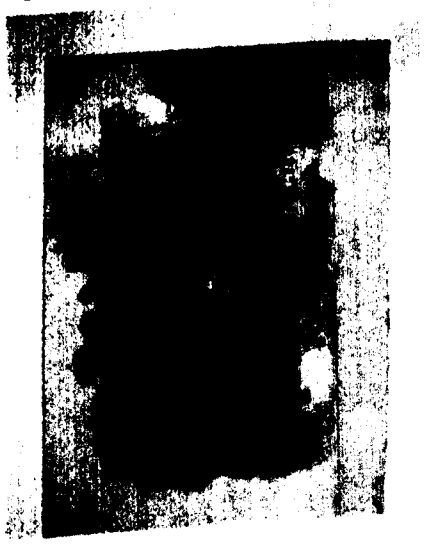


Fig. 4.

Fig. 1, 2. A paper heavily decayed in the middle area by an apparently anaerobic biological attack.
Fig. 3, 4. The same sheet as in Fig. 1 and 2, after leafcasting.

paper and by using high suction, so that the water runs off very quickly. The contrast leafcasting technique, with low suction and small areas not covered by old paper, would consolidate the paper. When the splitting process is intended, this is not necessary (Fig. 3, 4).

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 5. Brushing out the support paper with hot gelatine. Fig. 6. Placing the object to be split onto the support paper coated with gelatine. Fig. 7. Placing the second facing paper onto the object to be split.

At the beginning of the procedure of paper-splitting, the support paper is brushed out with the hot gelatine. The coating has to be very even (a bit difficult without a gluing machine), but if the layer is irregular, the paper will split unevenly. The brushing out has to be done quite quickly; the gelatine must not set before the object is laid on. For bigger sheets one might consider working on a warm metal plate (Fig. 5).

The object is then placed onto the support paper (Fig. 6). In order to facilitate the starting of the splitting of the paper, a margin around three sides of the object is helpful. If the object has been leafcast this margin already exists (Fig. 3, 4). Otherwise it can be made out of strips of filter paper.

The support paper is a little bit smaller than the object plus the margins on three sides, but bigger than the object (about 3 cm) along one side.

Now the second facing paper is glued out and placed on the other side of the object (Fig. 7). The resulting sandwich is placed between sheets of a polyester foil (Melinex® or Hostaphan®) and blotting paper and then put into the press. The gelatine should penetrate about one third into the paper from either side. The splitting takes place in the middle third.

After 10-30 min of pressure, the sandwiched object is taken out of the press and put under a weight for 4-6 h, sometimes longer, depending on the type of paper. The object and the support paper together with the gelatine must have formed a strong bonding. The whole sandwich should still be damp to facilitate the separation of the fibres.

The splitting then starts from one corner diagonally to the opposite corner. When the paper is loose along one edge, the splitting has to be done in a 90 angle and the pulling must be quite jerky. It is important to split the page in this T-form position; otherwise the paper will not split exactly in the middle. A device to keep up this position when pulling might be used (Fig. 8, 9). Although one tends to do all these operations cautiously and slowly, the splitting results are much better when the pull is hard. The fourth edge (without filter strip or leafcast paper) must stay fixed. Here the facing paper is stuck directly on to each other. It serves as a hinge and helps in putting the two halves together again. In this way laid and chain lines and watermarks are exactly lined up again and are as clear as before (Fig. 10).

The split paper is then pasted out on both sides with thin adhesive, a mixture of starch paste and methylcellulose as mentioned before. A tissue paper is placed on one side and, with the aid of the hinge, the two halves can be put directly onto each other again (Fig. 11-13). The whole object is put between polyester nonwoven fabric (Hollytex®) and blotting paper, pressed slightly and dried under a weight. The facing paper is then released in an enzyme bath. A heated basin with the solution is kept at around 50°C and the

Fig. 8.

Fig. 9.

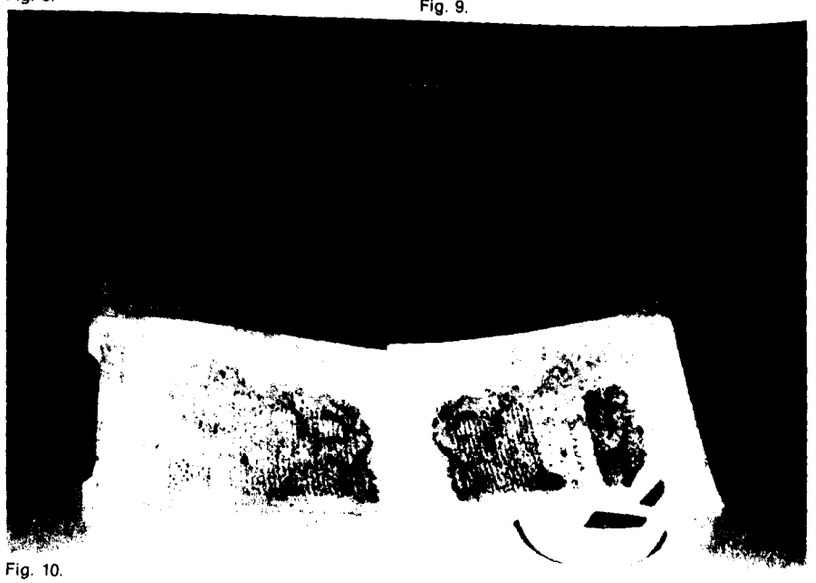


Fig. 10.

Fig. 8, 9. The splitting process. Fig. 10. The fully split object, fixed together at one corner by the support papers.

Fig. 11.

Fig. 12.

ig. 13.

ig. 11. Pasting the split paper. Fig. 12. Placing tissue paper on one of the offsplits. Fig. 13. Putting ıe two halves back together.

sandwich is placed inside. After a few minutes the gelatine is degraded and the support paper released. The object is then transferred into a bath of water above 70°C to deactivate the enzyme. The facing papers can be taken off (Fig. 14). The object is drained and then completely dried between blotters. Fig. 15 and 16 show the finished page.

Examples

A paper restorer must be aware that paper-splitting is a very drastic method that should not be applied without some consideration. In the following I should like to give some examples when paper-splitting seemed to be the most appropriate if not the only appropriate restoration method.

The first is an archival record consisting of thin laid paper with iron gall ink writing breaking through. As the ink was stable, the missing areas were filled in the leafcasting unit. The consolidation paper was fixed with methyl cellulose only. This was done with regard to the owner of the object, the National Archives of Brazil, where any natural paste or size is avoided because these materials seem to be more likely attacked by insects in a hot and humid climate (Fig. 17, 18).

The second object is a very brittle and thin newspaper of the end of the

Fig. 14. Releasing the support papers.

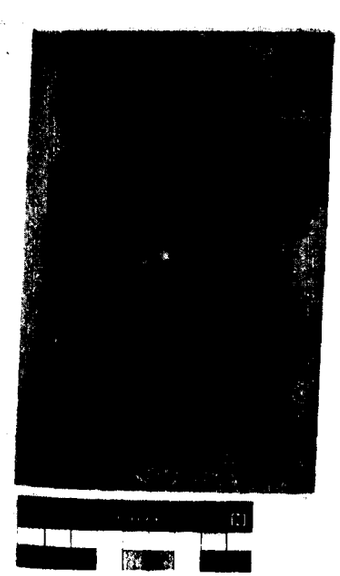


Fig. 15

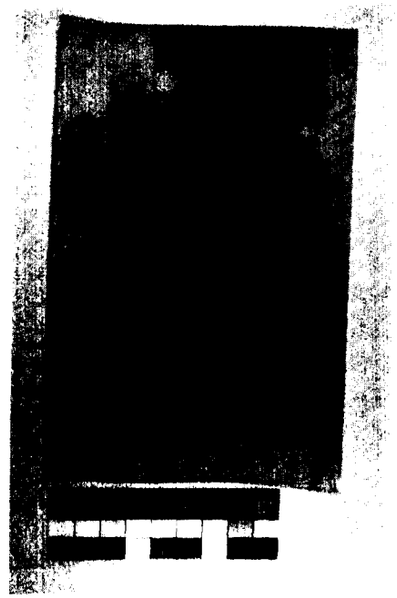


Fig. 16.

Fig. 15, 16. The object after splitting.

nineteenth century printed on both sides. Here we have no missing areas. However, consolidation is necessary and any tissue on the surface of the object would disturb considerably. The finished split object is slightly thicker than before, but it still has the feel and look of a newspaper (Fig. 19, 20).

The third example is a Coptic manuscript of the seventeenth century consisting of strong, burnished, Arabic paper. In the area of the ink writing, the paper is extremely degraded and in many places the ink has been breaking through the paper; there were some old repairs, a thin tissue already brown and degraded itself, over some losses in the writing (Fig. 21). The old repairs were removed with a mixture of alcohol and water. Care must be taken that old repairs, cellotape, residues of adhesives, wax spots and the like are removed completely before paper-splitting. In all these areas the gelatine would not penetrate into the fibres and the paper would not split. The loose pieces of the object were fixed with tiny strips of lamination tissue (Japanese tissue coated with an acrylic) using a warm tacking iron (Fig. 22). The ink was water-sensitive; it was decided to fill the missing areas with pulp on a suction table.

Fig. 17.

Fig. 18.

Fig. 17, 18. An archival object with corrosive ink.

Fig. 19.

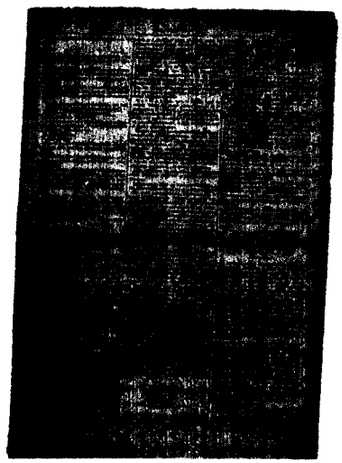


Fig. 20.

Fig. 19, 20. Page of a newspaper with illustrations, extremely brittle.

No margin around the object was formed during this process. It was therefore necessary to put strips of filter paper around 3 sides (Fig. 23). After 30 min in the press and then 6 h under a weight, the object was split as described (Fig. 24). The core paper was inserted and the two halves put back together. After drying, the support papers were released in the enzyme bath. As the object was water-sensitive, it seems strange to keep it in contact with water for some time. However, the facing with gelatine seems to fix the inks to some extent, although there was some offsetting on the support paper. Paper-splitting in this case might be disputable, as any aqueous method. The alternative treatment in this case would be laminating the page. However, the finished object shows a satisfactory result of the splitting method (Fig. 25).

MISHAPS

In an attempt to adopt the paper-splitting method into the whole of the means a paper restorer should master, I have been experimenting for some months with this method: trying out different sorts of facing papers, covering them with different types of adhesives, applying different pressures and drying times and splitting masses of different sorts of waste papers. There were occasions when I thought that hairsplitting must be easier than paper-splitting. There

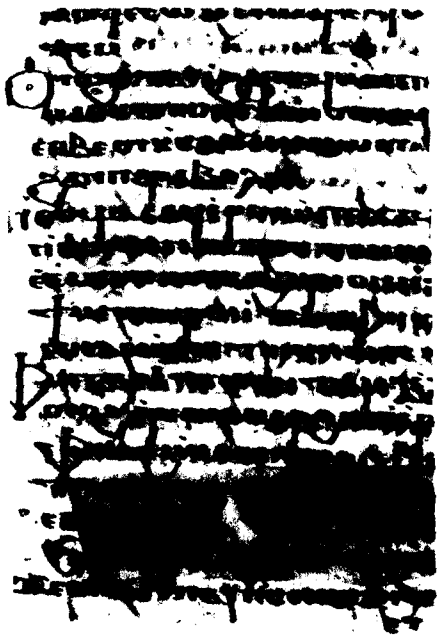


Fig. 21.

Fig. 22.

Fig. 21. Page of a Coptic manuscript with corrosive ink and old ugly repairs.
Fig. 22. Fixing loose pieces of the object to be split with tiny strip of lamination tissue.

are terrible disasters that can happen, and sometimes it is difficult to find the causes. I relate a few.

- The facing paper does not adhere to the object, or worse, it only adheres in

Fig. 23.

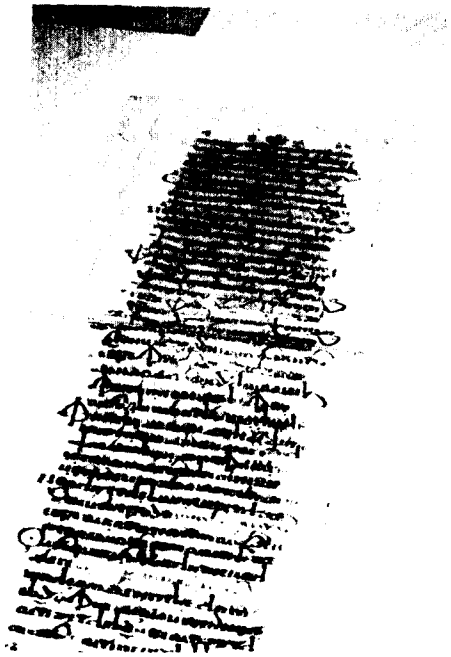


Fig. 24.

Fig. 23. Putting strips of filter paper around three sides of the object. Fig. 24. The object given in Fig. 21 after splitting.

parts, so that the object will not split in all areas. The causes might be in the object: heavily sized paper or remaining adhesive on the surface. They might also be in the working procedure: the pressure might not have been enough, or one had worked too slowly.

- The facing paper forms a tight bonding with the object and cannot be split at all. Possibly causes: the gelatine was too thin, the pressure was too high and/or the drying time was too long. The object was penetrated completely by gelatine and the fibres in the middle could not be separated.
- The facing paper cannot come apart from the object. The reasons for this could be: the enzymes not working or too much pressure after having stuck down the core, so that the gelatine and the adhesive from the inside have mixed.
- In one of the worse cases, not only the facing paper but the halves of the frail object come apart in the enzyme bath. This might happen when the adhesive for the core was not strong enough. Also, an unpurified type of enzyme containing some amylase instead of pure protease might be the cause.

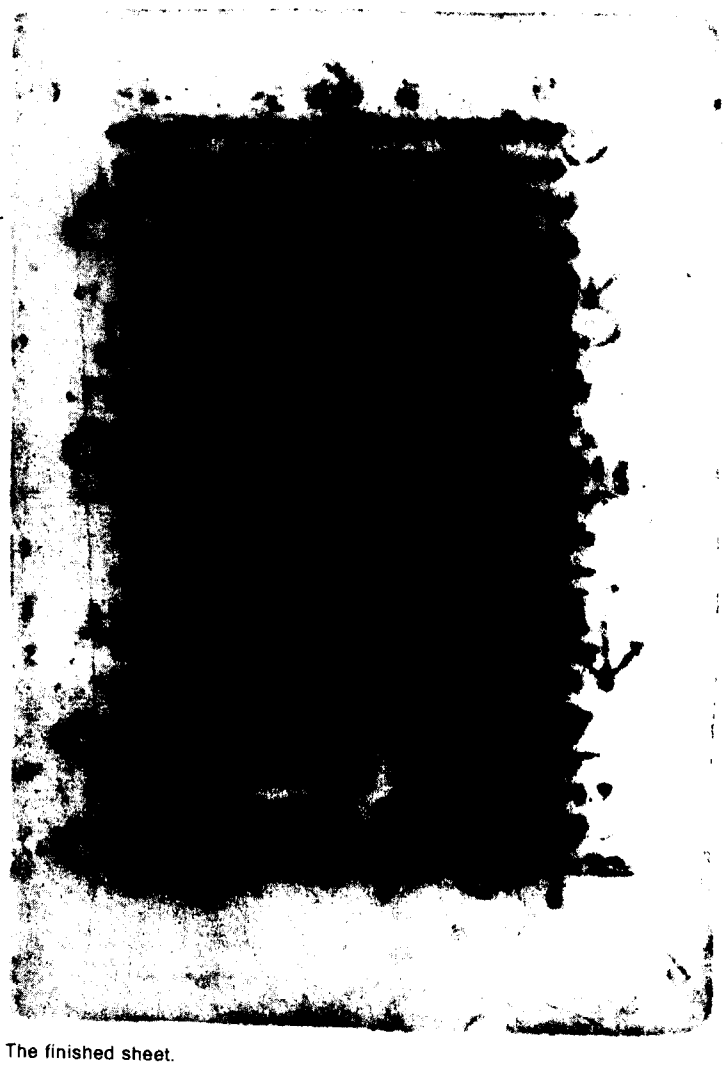


Fig. 25. The finished sheet.

I also did not have much success experimenting with precoated papers. The support papers were coated with a modified gelatine and then activated by moisture. The objects prepared in such way did not seem to get the right degree of penetration of the adhesive.

To come to terms with the process of paper-splitting much experimenting is required. At the end, a feel for the materials and a sound judgement of how

different papers will behave differently is built up. I want to warn restorers not to be too keen to apply paper-splitting without enough practice.

Ethics

It is not because of the difficult handling of this method that I want to warn about applying paper-splitting too readily. With some training every good restorer can handle it. Other considerations, however, particular ethical ones, are applicable.

Paper-splitting is a very drastic manipulation of the integrity of the object. The piece of paper with all its intrinsic value is actually torn apart, although it appears unchanged after the treatment. Inside this sheet of paper, one not only separates lined up fibres, but tears fibres apart. I understand why this method causes so much controversy, quite justified in my opinion, as the restorer or conservator actually damages the object before further treating it.

However, I think there are occasions when this method is justified. These are objects of last resort; hardly any other method will help to save them.

What can never be justified is the creating of two separate objects out of one, when a valuable drawing or print is on both sides of a work of art. In such instances the artefact must not be split and each halves lined separately. I wish to mention it as I know dealers ask for it and restorers sometimes tend to fulfil these requirements.

Acknowledgements

I should like to thank Günther Müller and his workshop in Jena who kindly introduced me to this method and showed me their work. I am also very grateful to all my colleagues in the Department of Conservation of the Bavarian State Library and to Vanessa Marshall, who made a lot of helpful suggestions to improve this article.

Summaries

Paper-splitting: A Problematic but Indispensable Method in Paper Restoration

After a survey of the methods to reinforce brittle paper, the author describes one of them, paper-splitting, in detail. Two sheets of support paper coated with gelatine are put onto both surfaces of the object to be split and separated at the right moment, when there is sufficient bonding between support papers and the object and sufficient loosening, caused by humidity, in the inner part of the object. A core of thin paper is inserted, using starch paste or methyl cellulose, and the support

papers are removed using a proteolytic enzyme. The dangers of the process are discussed as well as ethical reservations against it.

Paper Splitting: Une Méthode Problématique Mais Indispensable en Restauration du Papier

Après un aperçu des méthodes de renforcement des papiers fragiles, l'auteur en décrit une plus en détail: de *paper splitting* (division du papier). Deux feuilles d'un papier support enduites de gélatine sont appliquées sur les faces de l'objet à diviser et séparées au bon moment lorsque le lien entre les deux feuilles support et l'objet ainsi que le relâchement provoqué par l'humidité à l'intérieur de l'objet sont suffisants. Une âme de fin papier est insérée en utilisant de la colle d'amidon ou de la méthylcellulose et les papiers supports sont enlevés en utilisant une enzyme protéolytique. On discute des dangers de ce procédé ainsi que des réserves éthiques à son égard.

Papierspalten: Eine Problematische, aber Unentbehrliche Methode der Papierrestaurierung

Nach einem Überblick über die verschiedenen Methoden zum Verstärken brüchigen Papiers wird eine von ihnen, nämlich das Papierspalten, im Detail beschrieben. Zwei Blätter eines mit Gelatine beschichteten Trägerpapiers werden auf die beiden Oberflächen des zu spaltenden Objektes gebracht und im rechten Moment auseinandergezogen, d.h. wenn der Kontakt zwischen Träger und Objektoberfläche fest genug und der Zusammenhalt im Innern des Spaltgutes wegen der eingebrachten Feuchtigkeit locker genug ist. Ein Kern aus dünnem Papier wird eingeklebt, und zwar mit Stärke oder Methylcellulose, und die Träger werden mit Hilfe von proteolytischem Enzym abgelöst. Die Gefahren des Verfahrens werden ebenso diskutiert wie restaurierungsethische Bedenken, die man dagegen haben kann.

References

1. Wächter, W.: *Buchrestaurierung.* Leipzig: VEB Fachbuchverlag, 1981: 128.
2. Höge, B.: *Spalten von Papier.* Maltechnik 87 (1981): 189-91.
3. Müller, G.: *Unersetzliches Schriftgut originalgetreu für die Zukunft bewahren.* Biblos 37 (1988): 184-93.
4. Müller, G.: *Papierspalten von zerfallenem Schriftgut – Risiko oder Perfektion.* Maltechnik 95 (1989): 56-63.
5. Weber, H.: *Die Mechanisierung des Papierspaltverfahrens.* Dialog mit Bibliotheken 4 (1992): 20-2.
6. Bansa, H.: *Selection for conservation.* Restaurator 13 (1992): 193-7.
7. For the use of enzymes in paper conservation, see Segal, J. & Cooper, D.: *The use of enzymes to release adhesive.* Paper Conservator 2 (1977): 47-51 with further references.

Monika Gast
Restorer
Institut für Buch- und Handschriftenrestaurierung der Bayerischen Staatsbibliothek
Ludwigstrasse 16
D-80539 Munich
Germany

Restaurator, 1993, 14, 253–254
Printed in Denmark · All rights reserved

RESTAURATOR
ISSN 0034-5806

International Standard for Permanent Paper

In August 1993, the draft for an international standard for permanent paper was approved by ISO national member bodies in a ballot according to the ISO directives. The new standard is called ISO 9706: Information and documentation – Paper for documents – Requirements for permanence.

At a signing ceremony at the Royal School of Librarianship in Copenhagen, the chairman of ISO/TC 46/SC 10, Mr. Rolf Dahlø, made the formal decision to publish the standard. There will be no change in the technical content of the draft ISO/DIS 9706 that was sent for ballot in September 1992. This draft international standard may therefore be used provisionally until the final text of ISO 9706 has been published by ISO, as only some minor editorial changes will be included.

ISO 9706 was developed on the basis of the well-known American National Standard, ANSI Z39.48-1984. That standard was revised in 1992 and is now entitled "American National Standard for Permanence of Paper for Publications and Documents in Libraries and Archives, ANSI/NISO Z39.48-1992". The technical requirements of ISO 9706 are in conformity with ANSI/NISO Z39.48-1992, and there was a close and fruitful cooperation between the NISO and ISO committees.

Both standards cover both coated and uncoated papers. The requirements concerning pH value and alkaline reserve are the same; the requirements for tear resistance and resistance to oxidation differ slightly. Since the standards are very similar, ISO has been permitted to use, as part of the ISO standard, NISO's symbol for papers that comply with the requirements.

ISO TC 46/SC 10 is now discussing another standard. Its intention is to describe the qualities necessary not only for permanence but also for durability in the most demanding meaning of this word: a paper for documents of high historical, legal or other significant value, which can be predicted to be used

Restaurator 14: 253–254 © 1993 Munksgaard, Copenhagen

intensively in the future and must preserve their original representative outfit during this use. The text for this standard is nearly ready; there is nearly no disagreement in the technical content, i.e., the requirements of ISO 9706 and, in addition, some requirements regarding the visual inspection (free from defects such as specks, holes and wrinkles; a watermark), the fibre composition (mainly rag) and certain strength properties (tearing resistance 350 mN, folding endurance between 2 and 2.x depending on the testing instrument). The only matter still under discussion is the wording for the title. During the discussions on the technical content there was some silent agreement on the term archival paper. But testing it for its true sense, it was found not to meet the real area of use for a paper as described in the standard. For an archives any paper is archival. But a paper as described in the standard can, for economic reasons, never be used for any documents that one day may happen to become archival. It will be restricted for very specific documents as defined above: for documents on state and legal affairs.

Possibly there will be a third standard describing the requirements for paper permanence. It is an inevitable development in papermaking that fibre with a higher lignin content – wood fibre prepared in whatever existing or future technology – will be used also for paper of higher quality. For a paper restorer it is obvious that groundwood paper can be improved in its ageing behaviour when it is deacidified and when a certain amount of alkaline buffer is added. All the expensive methods of deacidification – mass treatment or single sheet – are based on this fact. From the conservator's point of view there is no reason not to accept a standard that ascribes to such a paper better ageing behaviour. The only problem might be the wording. It is not acceptable to define such a paper as permanent. Just its tendency to yellow forbids this. But to define a paper made of not or only partly purified fibre from wood as having "enhanced ageing behaviour" may be acceptable.

RESTAURATOR

International Journal for the Preservation of Library and Archival Material

Volume 14 · 1993

MUNKSGAARD · COPENHAGEN

RESTAURATOR

International Journal for the Preservation of Library and Archival Material

ISSN 0034-5806

Printed in Denmark by P. J. Schmidt A/S, Vojens.

Index 1993

Editorial Notice

RESTAURATOR, *International Journal for the Preservation of Library and Archival Material,* presents original papers, as well as reviews and news of interest to scientists, conservators, archivists, and librarians.

Authors submitting a paper do so on the understanding that the work has not been published before, is not being considered for publication elsewhere and has been read and approved by all authors. The submission of the manuscript by the authors means that the authors automatically agree to assign exclusive copyright to Munksgaard International Publishers if and when the manuscript is accepted for publication.
The work shall not be published elsewhere in any language without the written consent of the publisher. The articles published in this journal are protected by copyright, which covers translation rights and the exclusive right to reproduce and distribute all of the articles printed in the journal. No material published in the journal may be stored on microfilm or videocassettes or in electronic databases and the like or reproduced photographically without the prior written permission of the publisher.

Articles for RESTAURATOR are published in English, with Summaries (Abstracts) in French and German. However, manuscripts written in French or German are welcome, and will be professionally translated by Munksgaard. Contributors are particularly asked for linguistic and stylistic accuracy. All articles are linguistically revised by Munksgaard before publication. Authors alone are responsible for the opinions expressed in their articles.

Manuscripts should be provided on a floppy disk if possible; a printed, double-spaced copy must accompany any disk. Copy the files onto newly formatted disks and clearly label the journal, author and title, file content, originating machine and the word-processing program used. Manuscripts must be carefully revised, as alterations are expensive. The paper must be non-absorbent. The author's name must be accompanied by the title, full address, telephone and telefax number (if any) of the workplace or institution. Summaries should be written on a separate page, included with the manuscript; should not exceed 200 words; and should be written in the same language as the articles.

All graphs, drawings, or photographs are to be referred to as figures (abbr: Fig.) and should be numbered in sequence with Arabic numerals. Each figure should be identified with a figure number on the back. Any lines, numbers, or lettering that are to appear in the illustrations should be large enough for the necessary reduction. Line drawings should be drawn with black India ink on white paper and should be about twice the size of the finished blocks. Graphs can be plotted on plain white or blue squared paper; grid lines should be inked in black. In general, photographs should be about $1^1/_2$ times the size of the final block and must be submitted as mounted glossy enlargements showing good detail and adequate, but not excessive, contrast. Authors should indicate in the text the approximate position of figures.

Italics are used for emphasis, especially for distinguishing titles of books and papers.

References within the text should be indicated by numbers corresponding to those in the list of references. The list should be placed at the end of the paper. For example:

1. Miles, C.: *Wood coatings for display and storage cases.* Studies in Conservation 31 (1986):-114-24.
2. Hon, D. N.-S.: *Yellowing of modern papers.* In: Williams, J. C., ed. *Preservation of paper and textiles of historic and artistic value II.* Advances in Chemistry Series, 193. Washington, DC: American Chemical Society, 1981: 119–42.

Fifty offprints of each article will be sent to the author free of charge. Extra copies, if desired, will be supplied at a special rate.

0034-5806/93/USD 6.00+0.00.

Editorial Address

Manuscripts and review copies of books should be sent to the editorial office: RESTAURATOR, Dr. Helmut Bansa, Elisabethstraße 23, D-80796 Munich, Germany.

RESTAURATOR

Contents 14:4

Indexed in:
Chemical Abstracts
Current Contents
Information Science Abstracts
Library Literature
Library Science
Social and Educational Science
Citation Index

Printed in Denmark by P. J. Schmidt A/S, Vojens.

ISSN 0034-5806