THE INFLUENCE OF LACTIC ACID ON THE RATE OF ABSORPTION OF PREMETALLIZED DYES ON UNTANNED PROTEINIC TISSUE

A Thesis

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by

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I

GENERAL PROPERTIES OF THE SUBSTANCES UNDER INVESTIGATION

1. The Biochemical Role of Lactic Acid (7)

The scope of the research work undertaken and presented in this paper is related to cancer research. Warburg (10,11) first noted the abnormal acidity of malignant tissues and was able to identify the acid accumulated in tumors as lactic acid. He described an experiment carried out in vitro with tissues, normal as well as cancered, and found that the supression of glycolysis, which occurs in general in normal tissues in the presence of oxigen, failes to occur in neoplastic tissues, or is of much smaller extent than in normal tissues. As an average he calculated the lactic acid formation due to aerobic glycolysis as 0.075 mg per hour per mg of glucose added, as compared to 0.001 mg lactic acid formed in normal tissues under the same conditions. From his experiments Warburg deduced, that the mechanism of glycolysis was disturbed in malignant cells and that the tumor constitutes a foreign body within the animal or human body.

F. Bernhard (2) was able to measure the lactic acid concentration in vivo and found a content of .30 to .40 % in malignant tissue as compared with .15 - .17 % in normal tissue.

It was the idea of Noyan (6) to find nontoxic substances which would be preferentially absorbed by cancered cells and would aid in recognizing them in the body at an as early stage as possible. Screening a wide variety of possible substances Noyan found the premetallized dyes most suitable, particularly the chromium derivatives. The dyes are nontoxic, and will not precipitate in the blood. They offer the further advantage that the chromium can be irradiated before incorporation into the dye molecule and that

any accumulation can easily be dedected by measurement of the radioactivity. The advantage of chromium lies in the fact of its relatively short half life period of 26 days and the low energy of its radiation. Therefore, any hazardous side effects are unlikely to happen.

2. Premetalized Dyes (1)

The incorporation of c rtain metals in complexforming dyes with the purpose of improving particularly the light fastness was practized since long by treating acid dyes after their application to the fiber with chromium salts. Investigations to simplify the dying procedure led to the development of dyestuffs which could be applied in one bath. A complex dye molecule was prepared by coordinating one complex forming dye molecule to one metal atom (fig.1), before application to the substance to be dyed. These dyes are simple to apply to the fiber, except for the fact that a high quality even dye level is obtained only at boiling temperatures and in strongly acidic dye baths. Due to this disadvantages a further development of the premetalized dyes took place leading to the advent of products which could be applied in neutral or slightly acidic solutions at normal temperatures. They differ from the prototype by including two dye-forming molecules bonded to one chromium molecule and are frequently designated as 1:2 complex in comparison with the 1:1 complexes of the earlier type (fig.2).

The dying mechanism is assumed an ionic bonding of the dyestuff to the basic groups of the fiber as well as an absorption by the polyptide chain (1,3). The water solubility is affected by hydro-

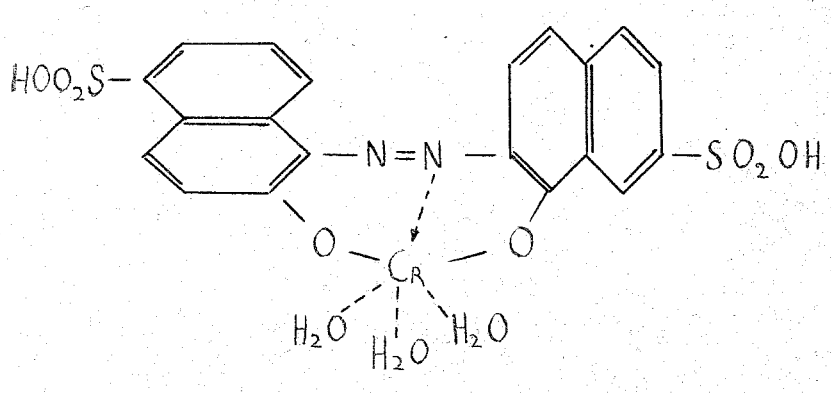


FIG. I: ULTRALAN-BLUE - 1:1 COMPLEX PREMETALIZED DYE
[DYE USED FOR EXPERIMENTATION]

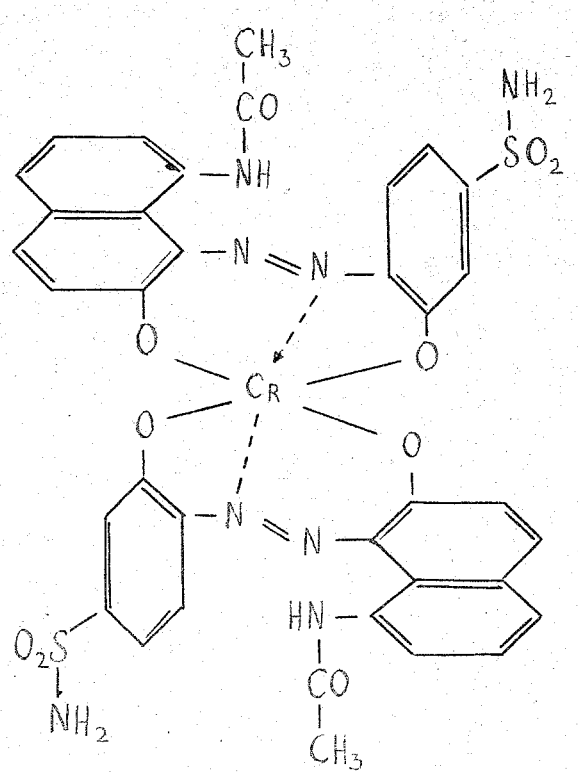


FIG. II: 1:2-COMPLEX PREMETALIZED DYE

philic, but normally not ionized groups as the methylsulfon-, sulfonamid-, and acylamino-group. The molecule undergoes solvation, probably under formation of hydrogen bridges. The premetalized dyes are prepared by reacting the sodium salts of the complexforming dyes with trivalent chromium salts or with chromates in a suitable medium under the influence of heat, in general at the boiling point of the reaction medium. The dyes formed in this way behave as sodium salts of strong acids as has been concluded by Schetty (1a) from the fact that their titration characteristics coincide with that of hydrochloric acid.

In this work the 1:2 complexes could not be used because the free acids of these complexes show a relatively low water solubility, particularly in the presence of other salts. The measurement of the "true pH" in the malignant tissue is very much subject to disagreement between various authors (7), and seems also to vary considerably with various types of tumors. Therefore, a precipitation of the dye within the living body might lead to unforeseeable complications. The dye used in this work belong to the Ultralan series (ICI), that is to the 1:1 complexes. The dyes had been tested in various organic and inorganic acids at a wide range of pH-values without a noticeble change in solubility and appearance after one week of exposure.

3. The Choice of Material for Experimentation

The proteinic material to be used in the experiments of the prevailing investigation had to fulfill certain requirements:

a - the conditions of tissue "in vivo" should be reproduced or approached as fair as possible,

b - the material should be readily available in sufficient quantities,

c - the material should be easy to be stored and handled, and

d - the material should allow a fairly constant reproduction of experiments.

The material found most suitably in this line was animal skin; throughout the experiments calfskin was used. The dehaired, but otherwise untreated skin was stored in a 10 % salt solution, to which enough phenol was added to avoid bacteriological destruction. The skin was not subjected to any degreasing treatment, because the absence of any grease was considered as a severe deviation from "in vivo" conditions in spite of the higher absorption rates which could be exspected by using degreased material.

The Chemical Structurs and Properties of Hides (4a, 9)

The dry matter of the hide consists to about 80 % of proteins which can be described as macromolecules build up from alpha-amino acids linked together by peptide bonds. Thus, the general structure of the proteins can be given in the following manner:

$$(-NH.CHR.CO-)_n$$

whereby R symbolizes a side chain extending from the back bone or polypeptide chain. The side chain may consist of a single hydrogen atom, but may be rather complex, including aliphatic chains, aromatic groups, and hetorocyclic aromatics. The groups may exert hydrophobic or hydrophillic properties, depending on characte-

ristic substituents as alcohols, ethers, thiols and thioethers. It are these side chains which determine the properties and the functions of the proteins.

Proteins can be classified as fibrous or globular according to their configuration. The leather forming portion of the hide consists of an interwoven network of fiber bundles of collagen, the most important constituent of the hide because of its reaction with the tanning agents in the course of leather manufacture. The globular proteins are essentially involved in the vital processes within the tissue. Their functioning requires solubility in water or weak salt solutions, while the fibrous or structural proteins are not soluble in these solvents.

The main step in leather manufacture is the tanning, either vegetable tanning or chrome tanning, to the later of which some consideration will be given in here due to its similarity to chrome dying.

4. <u>The Mechanism of Chrome Tanning (4b)</u>

The fixation of chromium on or in the hide during tanning is assumed as deposition of a highly basic, insoluble chromium compound. Different investigators differ in their conclusions from experimental results whether this compound is merely deposited or whether it is combined chemically with the skin proteins. Shuttleworth states several reasons complicating the development of satisfactory theories of chrome tanning; the most important of which is involved with the bonding mechanism between proteins

and chromium tanning complexes.

The multifunctional character of collagen proteins makes it possible to visualize the following bonding mechanisms, cited in order of increasing bond strength:

I - Electrovalent or salt link,

II - Nonpolar adsorption,

III - Residual valency or Van der Waal's forces,

IV - Hydrogen bonds,

V - Coordinate bonds involving shared pairs of electrons and

VI - Covalent bonds.

The earlier theories dealt with absorption, residual valences, and salt formation, but particularly, improvements in the knowledge of the chemistry of chromium compounds are responsible for the rejection of these theories to be accepted as primary bond mechanism.

Coordination theories allow three interpretations, namely coordination of nitrogen atoms of the amino - group only, bridge formation between amino and carboxyl groups, and coordination of carboxyl groups only. Several researchers have proved that hydroxyl-groups and the peptide-back-bone of the proteins is, if at all, responsible only for a very minor amount of chromium uptake and that the bonding mechanism takes place by residual valence and/or hydrogen bondage.

In view of the fact that a wide range of organic carboxyl groups are capable to form stable coordination complexes with trivalent chromium ions under chromium tannage conditions and that carboxyl

groups incapable of forming coordinated compounds with chromium do not seem to exist, Shuttleworth (4b) concludes that at least a part of the chromium fixed to collagen has undergone coordinative bonding with the carboxyl group. Deamination or protection of the amino groups before chrome tannage does not result in a remarkable decrease of chromium uptake, while esterification of the carboxyl groups before tannage reduces the chromium uptake substantially. From these facts as well as from other experimental evidences Shuttleworth concludes further that coordination of protein carboxyl groups to chromium is the main reaction in chrome tannage.

The high heat stability of chrome tanned leather, increased with respect to vegetable tanned specimens, lead to the conclusion that bridge formation between neighboring protein chains should prevail, thus setting up an interconnected three-dimensional network. However, a final proof of this theory was not yet possible, particularly because of the difficulties in interpretation of X-ray diffraction patterns. Chromium oxide in tanning solutions exists in oleated form, that is in chains of the pattern Cr - O - Cr and is has been calculated that the chain length of a four-chromium complex is about 24 A. This would be approximately the minimum distance between two free carboxyl groups from projecting side chains of neighboring peptide chains. Thus, effective bridging between adjacent proteins requires a considerable chromium take-up, while X-ray diffraction photographs become unclear with increasing chromium oxide content.

II

ABSORPTION PHENOMENA ON ANIMAL SKIN

1. The Application of Lactic Acid to Animal Skin

The swelling of hides when placed in acid or alkaline solutions is a long known fact. The swollen state of the hide may be considered as equilibrium state between the solution power of the solvent and the adhesive forces keeping the individual protein molecule in solid structure. It has been calculated (4b) that the distance between two main chains of proteins is about 10 A in the dry state and 17 A in the swollen state. Vickerstaff (9) mentions a calculation, according to which the distance of protein chains in wool is of the order of 40 A in the swollen state. The leather manufacturer makes use of the swelling action to control the rate of diffusion of chromic acid into the skin.

The swelling as described was the first effect to be observed when the hide was placed in the lactic acid bath. By visual inspection it was obvious that the effect became more pronounced with increasing concentrations of lactic acid in the bath. Measurements of the hydrogen ion concentration of the lactic acid bath indicated that an equilibrium distribution of lactic acid between hide and bath was established after about 22 hours. The lactic acid in the hide must be assumed in a state of true adsorption or fixed by ionic bonds to the amino groups of the protein. This is concluded from the observation that the pH of a freshly prepared dye bath decreased remarkably during the first part of the dying period, while the pH stayed almost constant over the entire dying period when the dye was added to the lactic acid bath. In the former case the decrease of the pH is attributed to a readjustment of a new equilibrium distribution

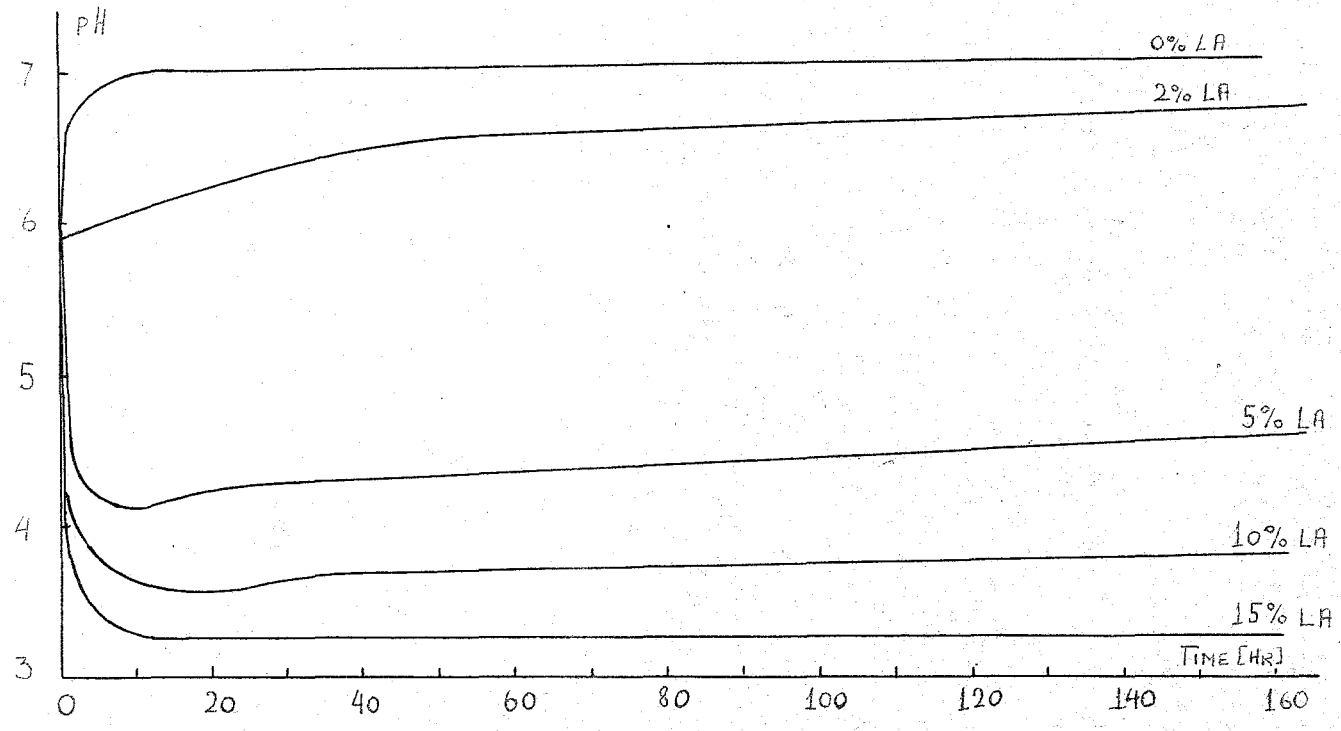


FIG. V : VARIATION OF pH IN DYE BATH
[LACTIC ACID AND DYE APPLIED IN DIFFERENT BATCHES]

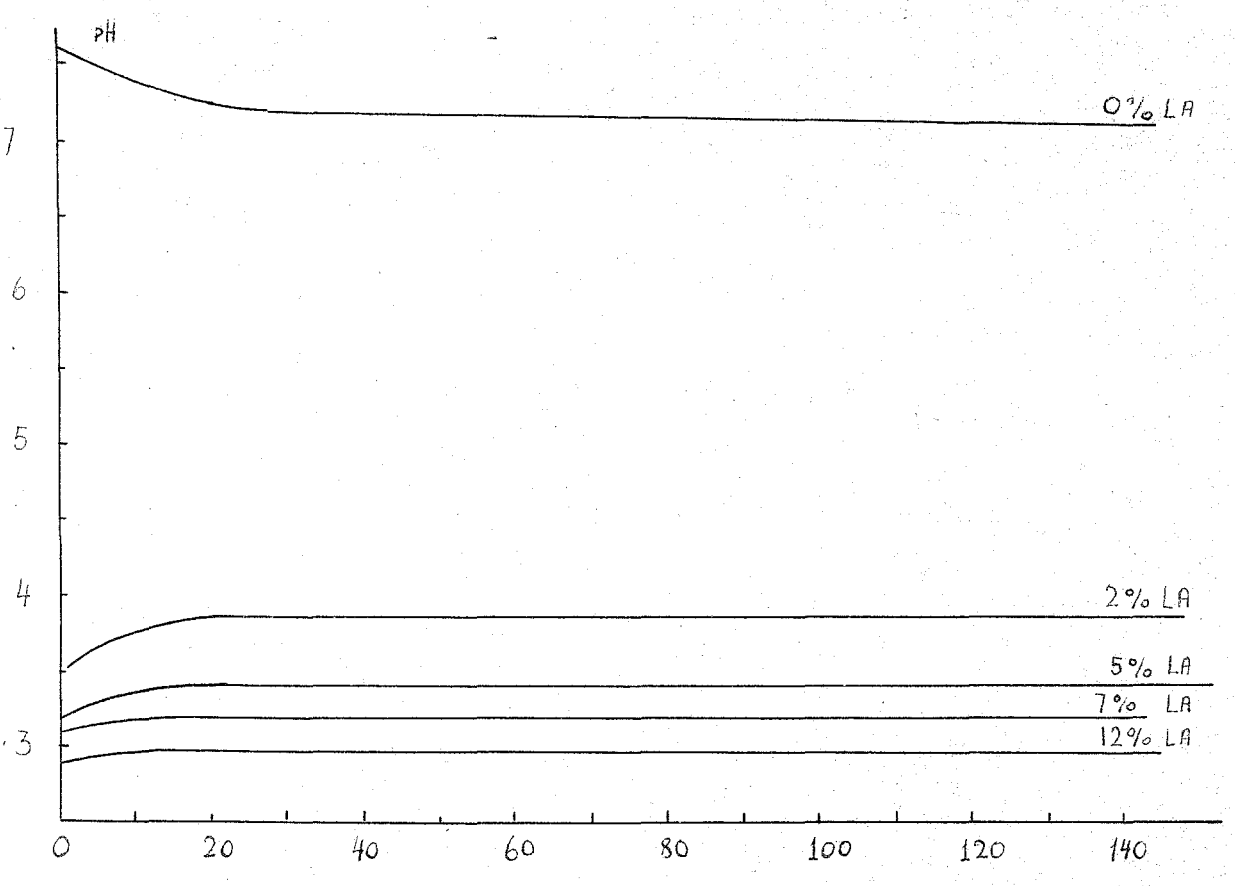


FIG. VI : VARIATION OF pH IN DYE BATH
[LACTIC ACID AND DYE APPLIED SUBSEQUENTLY IN SAME BATCH]

of lactic acid (fig.V,VI).

Dying of Animal Skin with Premetalized Dyes.

Gustavson (4b) and others are reported to have investigated absorption phenomena on hides by the use of hide powder. This practice was not followed mainly for two reasons, one of which is technical nature, the other being of theoretical consideration. The later reason is based on the observation of Smith (8), that gelatine and glue is of lower grade when prepared from dried glue stocks instead from fresh ones. Thus a chemical change of the hide might be assumed with possible influences on dye absorption rates and equilibrium distribution of the dye between hide and liquor. The technical reason appeared in the difficulty to separate the hide powder from the liquor, particularly when the dye liquor was to be included in the determination of absorption rates. Filtration by the aid of filter paper would give raise to false results as the dye would be absorbed by prereference on the paper, and a centrifuge powerful enough for sedimentation of the fine powder was not available. Also, powderization would consitute a deviation from the approach of reproducing conditions of tissue structure "in vivo".

In a previous chapter similarity between dying with chrome dyes and chrome tanning was mentioned. However, comparison should not be driven to far. While in chrome tanning an olated compound of chromic oxide is coordinated to the free carboxylic acid group of a side chain, the dyes under consideration posses strongly ionizable acid groups as the sulfonic acid group. In view of this fact Bird (3) reports the following bonding mechanism of preme-

talized dyes on wool (fig.4). If this bonding mechanism is accepted it can be seen that three different bonds participate in keeping the dye molecule at a certain position, namely ionic, coordinate, and covalent bonds. Further more, depending on steric conditions the dye molecule can be attached intra- or intermolecular.

Vickerstaff (9) has devided the amino acids found in natural proteins into four main classes according to their chemical nature:

a- amino acids yielding non-reactive side groups, into which he includes oxyproline in spite of its polar hydroxyl group on the argument that this group is much less contributive to absorption of dyes than other groups.

b- amino acids yielding basic side groups, whose number is very important as determinant of the maximum amount of acid dyes which can be combined with the fiber.

c- amino acids yielding acidic side groups as the carboxyl group which might be in the form of a free acid or as amide by combination with amonia.

d- amino acids capable of linking two polyptide chains. Cystine, the only member of this class, is analyzed in sufficient quantities only by hydrolysis of wool. Its influence on the dye absorption is indirect in as much cystine is the controlling factor of wool swelling in solvents.

The main constituents of wool and collagen, as obtained by hydrolysis are given in table (1). From there it is obvious that

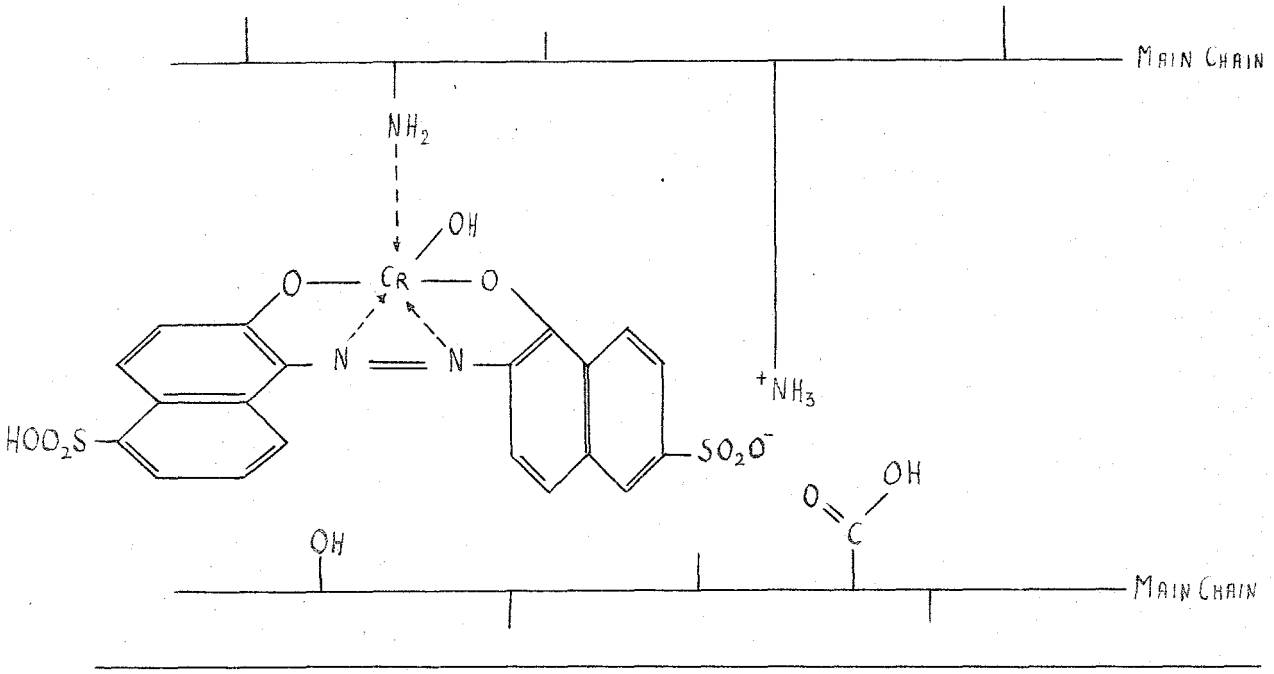


FIG. III: INTRAMOLECULAR BONDING OF PREMETALIZED DYES TO POLYPEPTIDE CHAINS

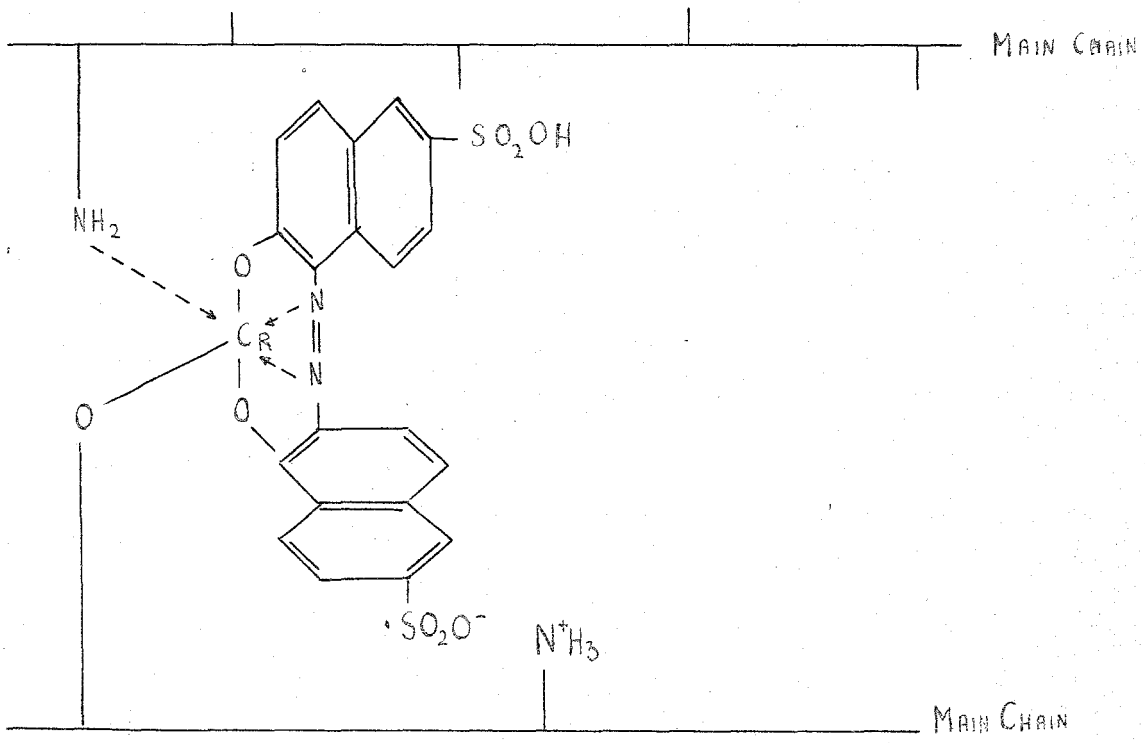
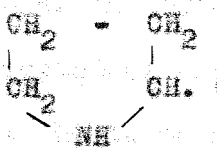
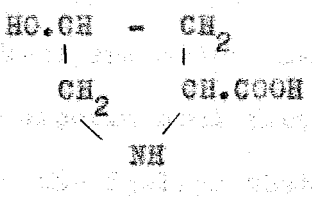


FIG. IV: INTERMOLECULAR BONDING OF PREMETALIZED DYES TO POLYPEPTIDE CHAINS

Table I: Constitution of Typical Fibrous Proteins
(g.amino acid per 100 g. (fibre)

Acid	Constitution	Wool	Collagen
Glycine	$NH_2 \cdot CH_2 \cdot COOH$	6.0	26.2
Leucine (and isoleucine)	$(CH_3)_2 \cdot CH \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	11.5	5.6
Proline		4.4	15.1
Oxyproline		-	14.0
Cystine	$HOOC \cdot CH(NH_2) \cdot CH_2 \cdot S \cdot S \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	13.1	-
Arginine	$NH_2 C(:NH) \cdot NH \cdot (CH_2)_3 \cdot CH(NH_2) \cdot COOH$	10.2	8.8
Glutamic acid	$HOOC \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	15.3	11.3

arginine (class b) is present in comparable amounts in wool and hide proteins and that the acid dye absorption properties of these two substances should not differ significantly.

The main factors influencing any absorption process are the absorptivity of the absorbant, the concentration of the absorbate, and the temperature. Different sets of experiments have been deviced in order to obtain insight into the mechanism of the absorption of the premetalized dyes on animal skin and to evaluate the impact of the factors mentioned on the rate of absorption. It should be mentioned in advance, that the uncertainty of knowledge about proteins and particularly about the proteins of skins and hides does not allow for to much generalization of the experimental results. Facing these difficulties, the author had the feeling that a mathematical treatment of the subject as the evaluation of rate constants was not justified.

The Absorptivity of the Animal Skin. The internal structure of the absorbant and the availability of spots to which the absorbate can be attached is determining the absorptive power of the absorbant.

The variations to which the skin had been subjected were the soaking in solutions of lactic acid of various concentration. The swelling of the skin caused by the lactic acid is due to an increase of the distance between individual polypeptide chains. From this fact it can be concluded that an increased degree of swelling will facilitate the penetration of soluble constituents

into the structure of the skin. Thus, an increase of lactic acid concentration is expected to increase the absorption rates of the dye, as long as it is accompanied by an increased degree of swelling. A second effect of the presence of lactic acid within the skin is the suppression of ionization of acidic groups as the carboxyl group and the favoring of the formation of polar groups of basic character and the ionization of base groups. Particularly, the amino-group ($-NH_2$) will readily accept a proton in the form of the hydrogen ion to yield an "ammonium" ion ($-NH_3^+$). In this manner the amino-group is protected from the immediate formation of a coordinate bond with the dye molecule, a fact which is utilized by the dyer by application of leveling agents (3). It is assumed that due to this effect the passage between individual polypeptide chains is less readily blocked by the deposition of a dye molecule. Experimental support for this assumption is the fact, that under otherwise equal dying conditions the depth of dye penetration into the interior of the skin was increased with higher concentration of lactic acid.

Vickerstaff (9) and Back (1) state the theory, that dying follows a two step mechanism, of which the first one is a mere surface effect being rather rapid, while the second step consists of the penetration of the dye molecule into the interior of the fiber, a relative slow process. The ionization effect as carried out above would not be in contradiction to this theory. The rate increase of dye absorption with lactic acid concentration would then be explained as an acceleration of the second step.

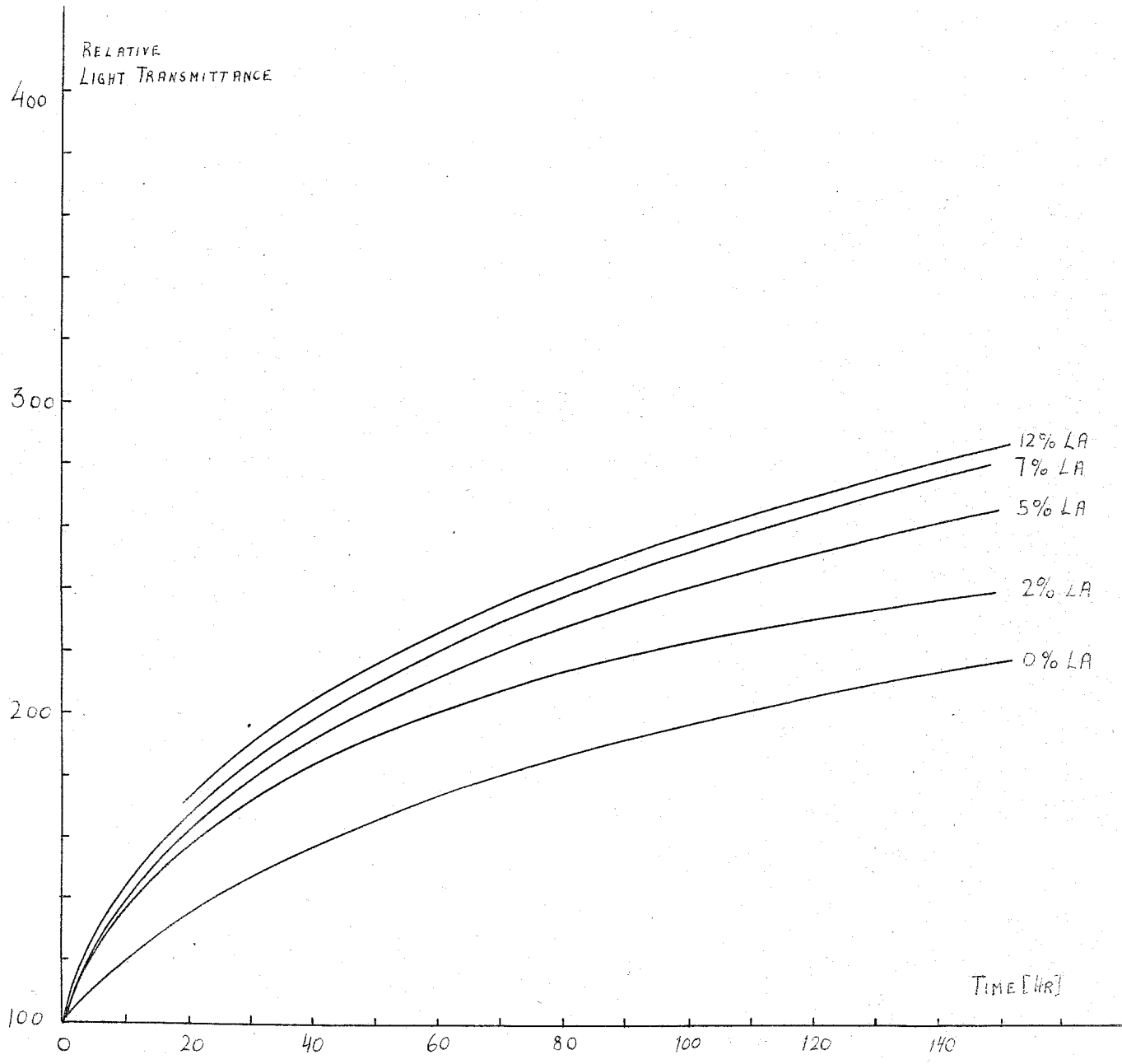


Fig. VII : Rate of Absorption of Dye on Calfskin
Lactic Acid Bath : 24h, Dye Shade: 0.25%
Parameter: Lactic Acid Concentration

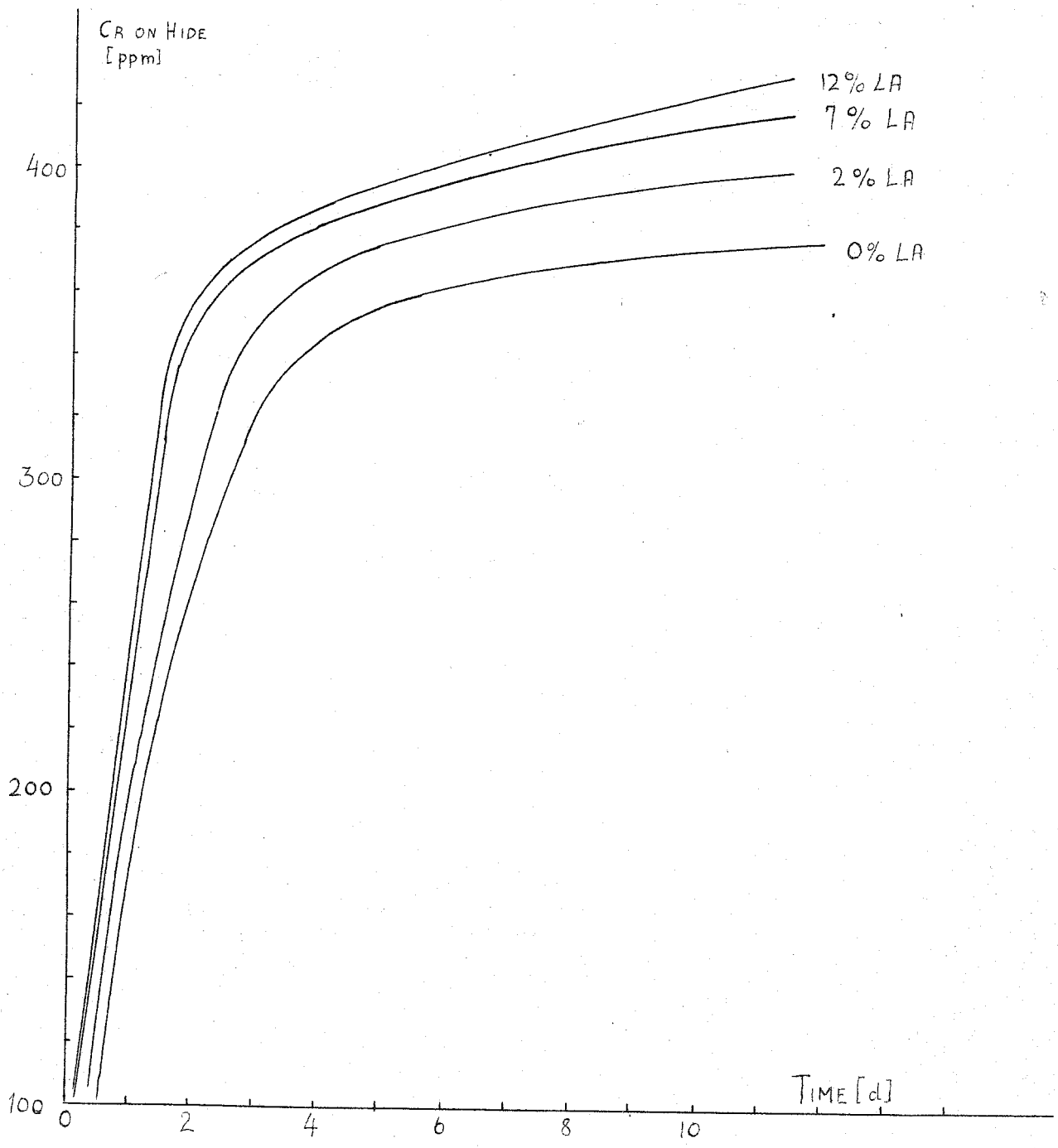


Fig VIII: Chrome Fixation on Calfskin
Lactic Acid Bath: 24h; Dye Shade: 0.25%

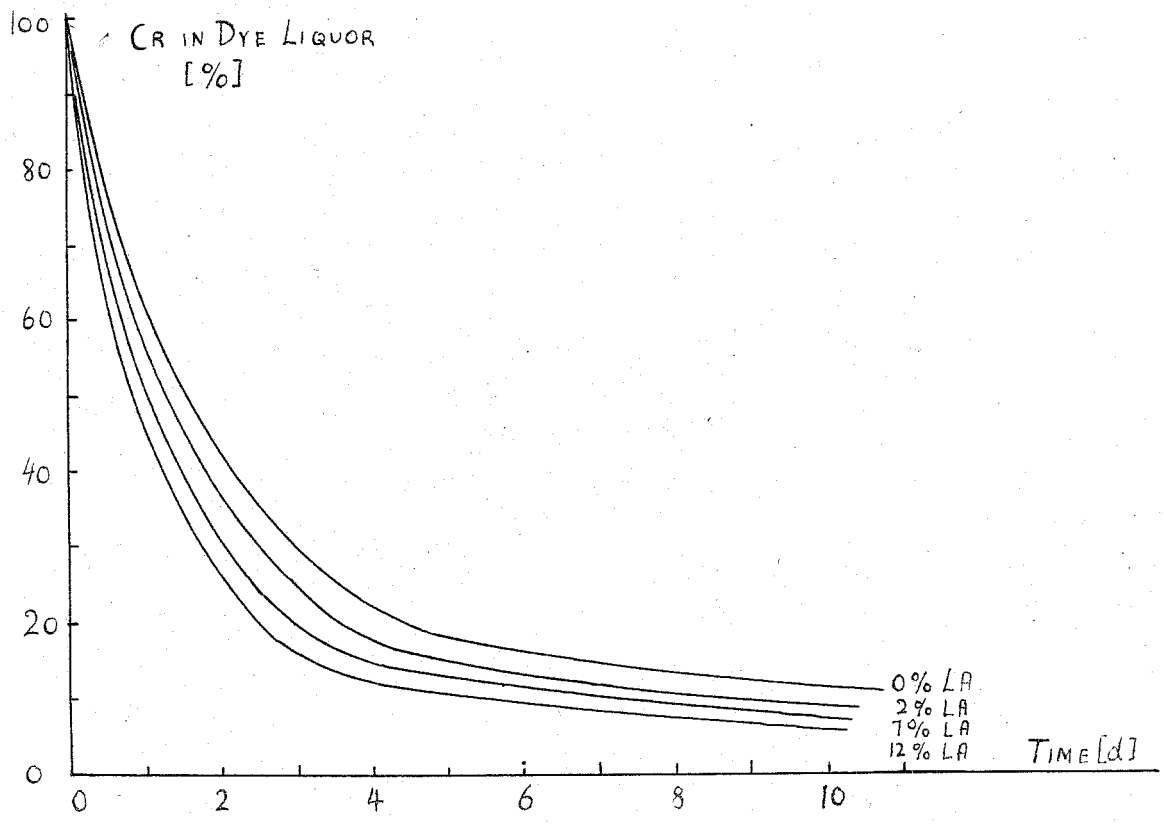


FIG IX: DYE BATH EXHAUSTION RATES
DYE SHADE: 0.25%

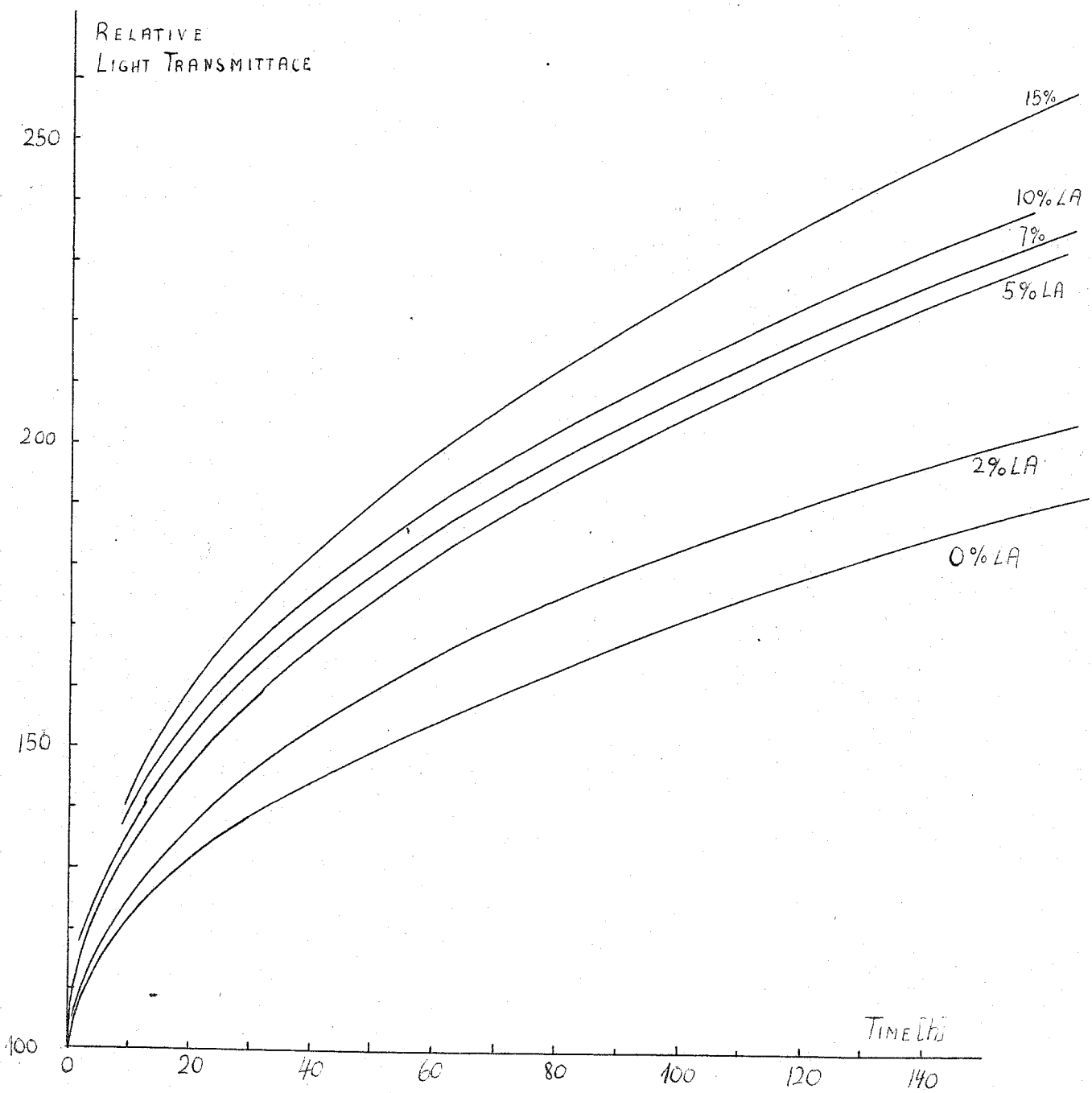


Fig. X: Rate of Dye Absorption in Dependence on Lactic Acid Concentration
Lactic Acid Bath: 24h; Dye Shade: 0.5%

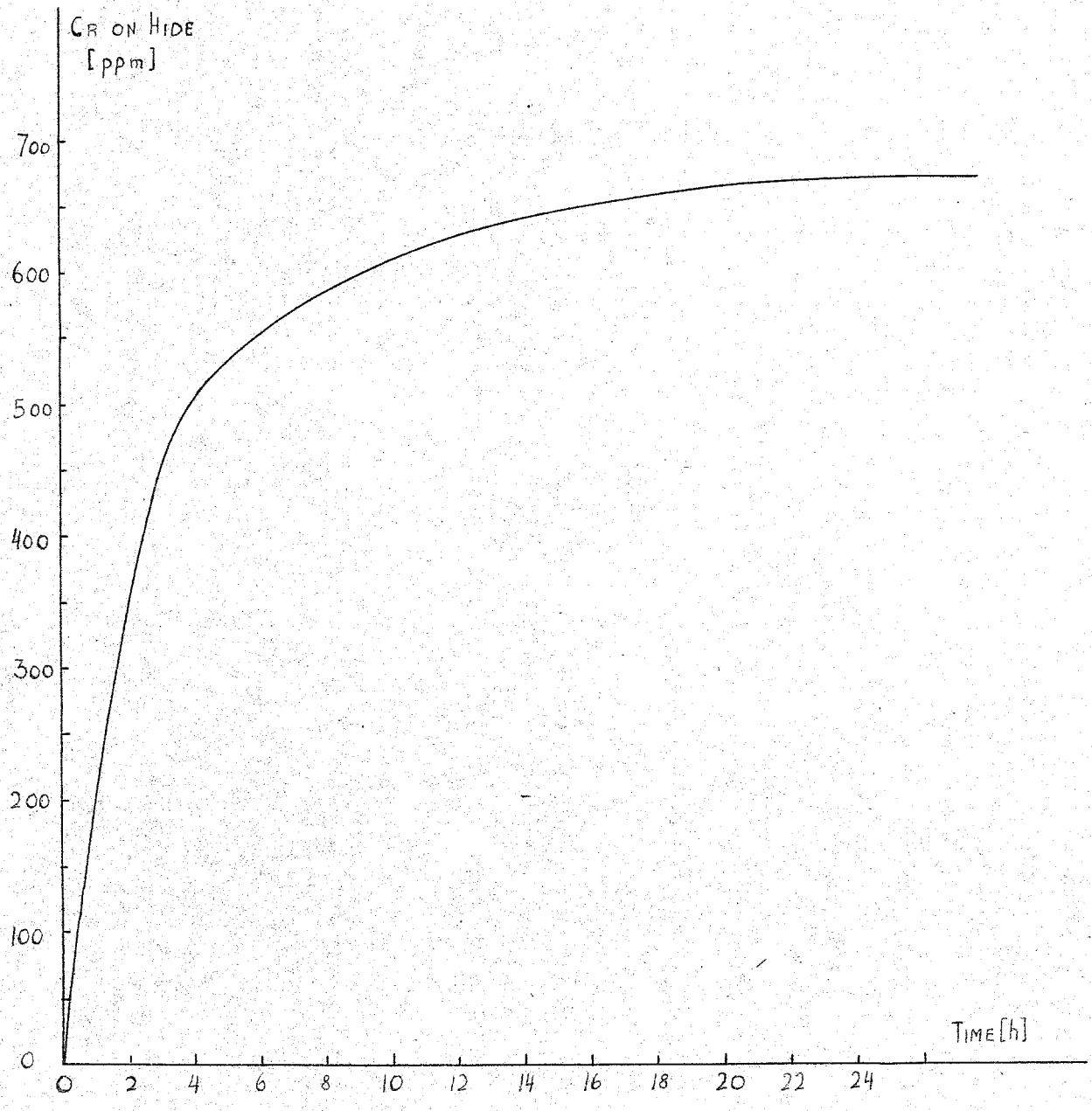


Fig. XI : Chrome Fixation on Calfskin
Lactic Acid Bath: 24h; Dye Shade: 0.5%
Lactic Acid Concentration: 4%

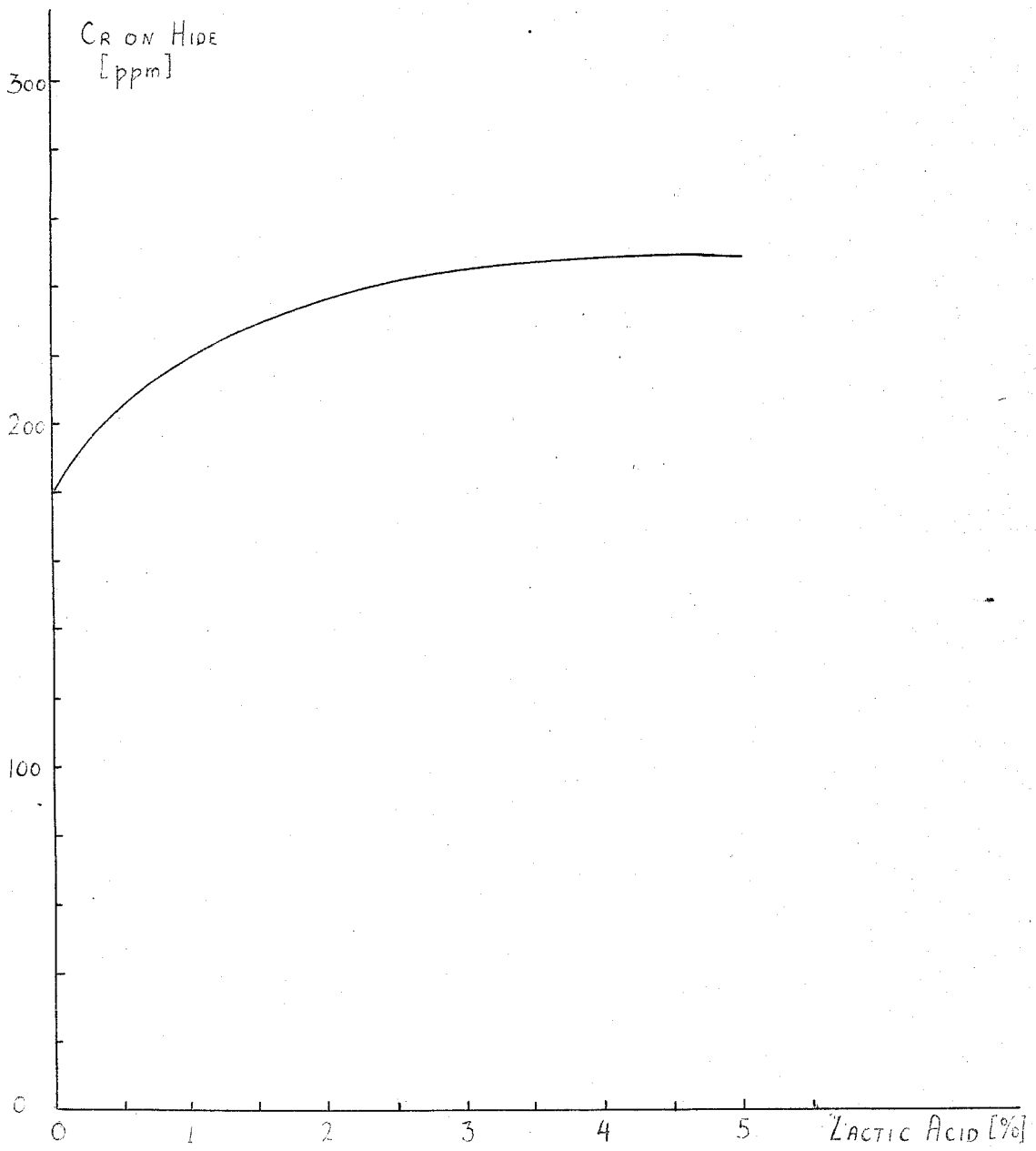


Fig XII : Chrome Absorption on Calfskin
Lactic Acid Bath: 24h; Dye Bath: 4h; Dye Shade: 0.5%

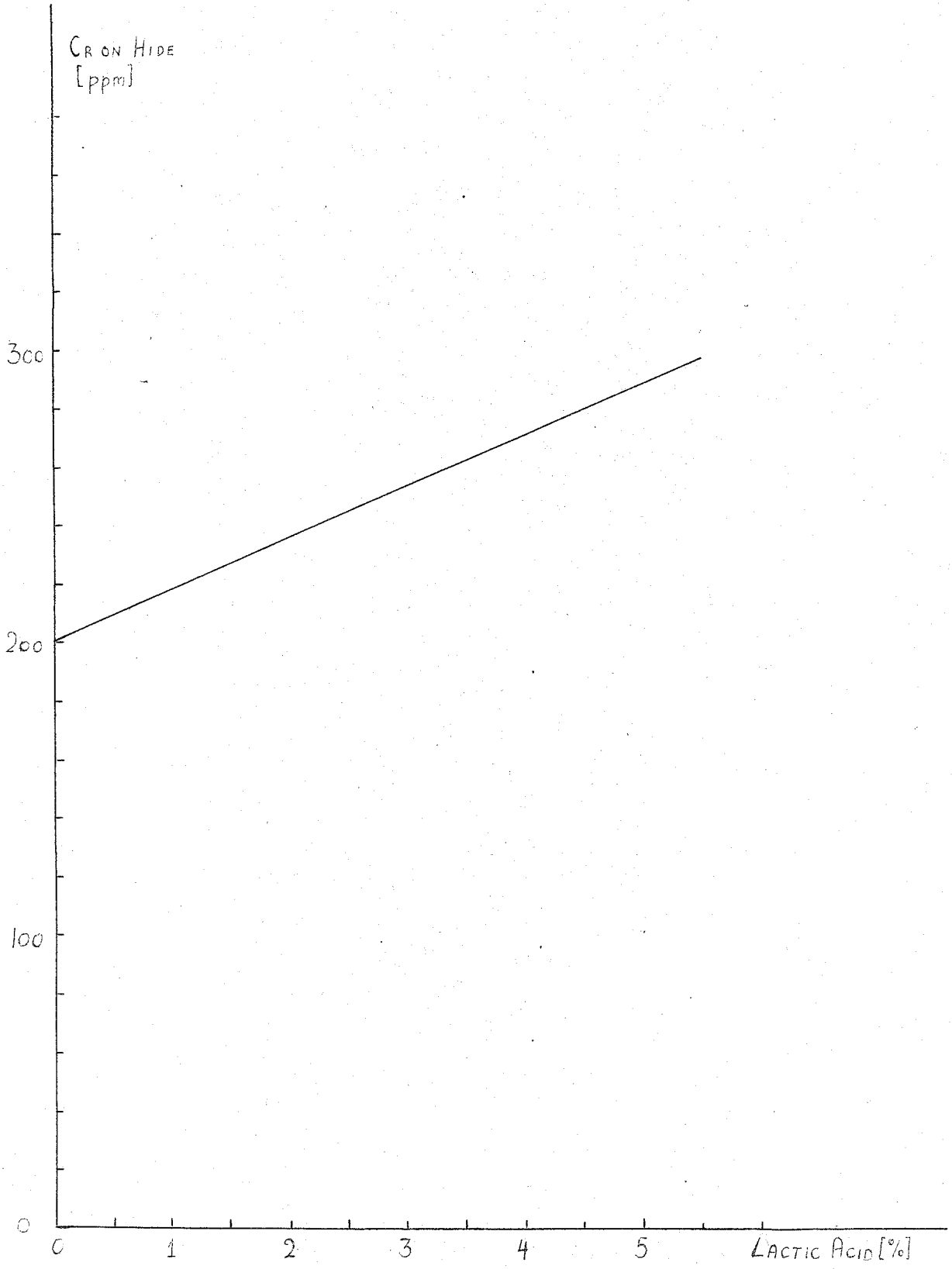


Fig. XIII : Chrome Absorption on Calfskin
Lactic Acid Bath: 24h; Dye Bath: 4h; Dye Shade: 1%

Experimental evidence has been found to support the theoretical considerations above within the range of lactic acid concentration as applied. In particular, fig. VII and fig. VIII. illustrate these aspects and show the initial rates to be increased up to 100 % as compared without lactic acid.

The Concentration of Dye in the Dye-Bath. The concentration of the dye in the dye bath has been varied in certain limits. However, the results of comparable experiments appear to be inconsistent suggesting a higher chromium fixation from baths of lower acid concentration (cf. fig. IX and fig. XII) or a large difference in chromium uptake under comparable dying conditions. The explanation must be sought in the disuniformity of the skin. Thorstensen (4c) devided an unspecified hide into five areas and determined the absorption of chromium from a chrome tanning bath on different parts of the hide. He did not only find variations of aver 50 % of chromium fixed on the sample, but he found also differing distribution patterns within samples from different areas by conducting stratigraphic analyses.

Another source of uncertainty was introduced by the fact, that the weight of the skin was determined as a wet weight. Atmospheric conditions as the air humidity might very well be assumed as being of influence on the moisture content of the sample.

Temperature. Comparative experiments have been conducted at a temperature of 37 °C and at room temperature. As might be expected, the rate of absorption was remarkedly increased at the higher temperature (fig. XIV - XVIII).

During all the experiments it had been noticed that the hides in the dye bath were subjected to structural decay with the evolution of a putrid odor. This effect was starting earlier and was more pronounced with samples containing none or very little lactic acid. At the elevated temperature of 37 °C the decay was accelerated in such a way, that the tests involving 0 % and 1 % lactic acid concentration had to be interrupted after 6 days, because the evaluation of the experiments became unreliable. This decay can be attributed to bacteriological putrifaction, and in later experiments the decay was largely suppressed by the addition of about 0.1 % of phenol to the dye bath. Any possible influence of phenol upon the absorption rate has not been studied.

The large scattering of the rate datas for the elevated temperature is attributed to the decay phenomenon, mainly for two reasons. During the cause of decay rather small hide particles were disintegrated from the main sample, thus producing an increase in the surface area of the absorbant and correspondingly a rate increase. Partly due to the disintegration, but also influenced by the elevated temperature greasy constituents were released from the sample causing the water repellency of the hide to decrease. Thus, a better penetration of the hide was established.

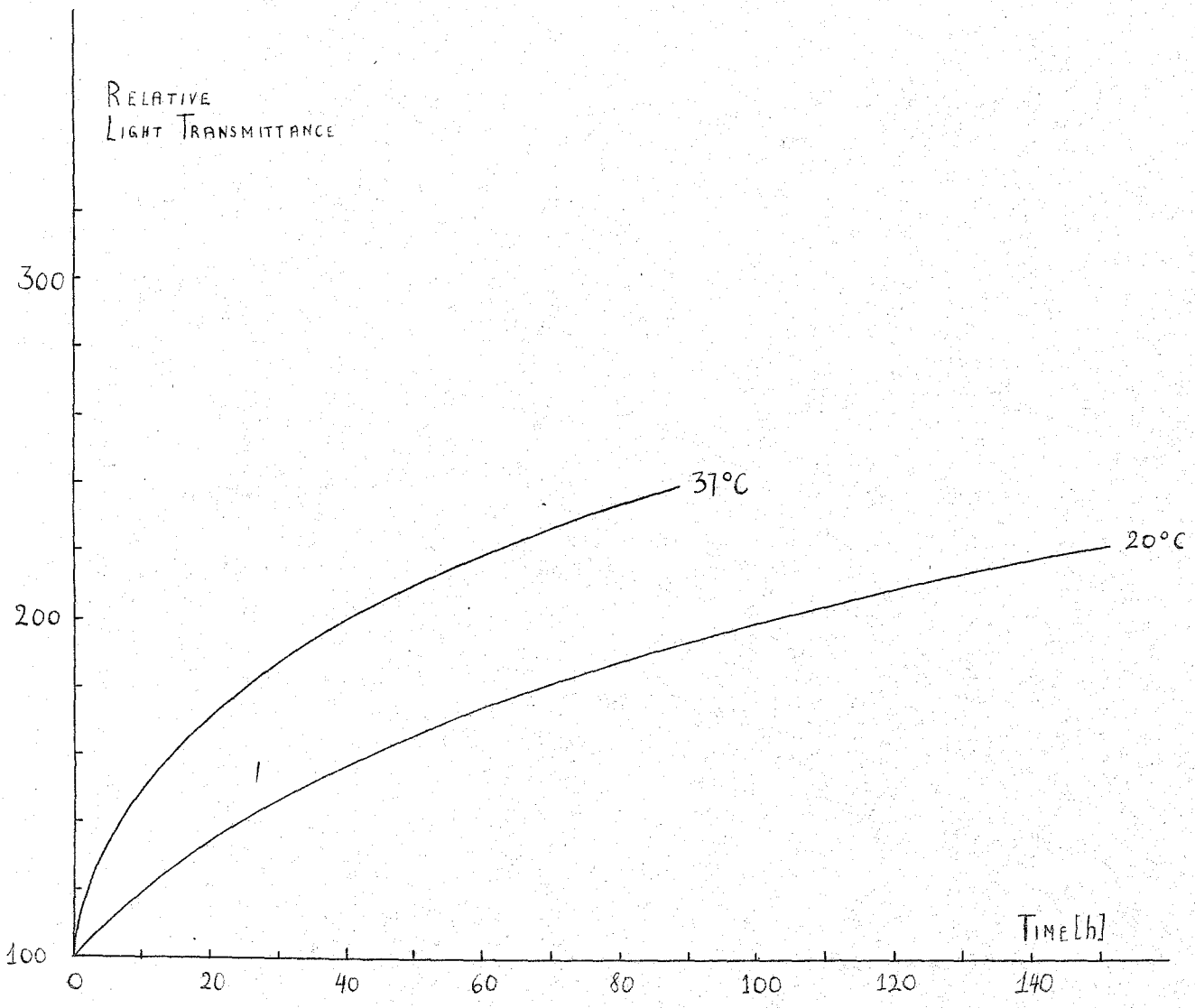


Fig. XIV: Temperature Dependence of Absorption Rate
Lactic Acid Concentration: 0 %; Dye Shade: 0.25%

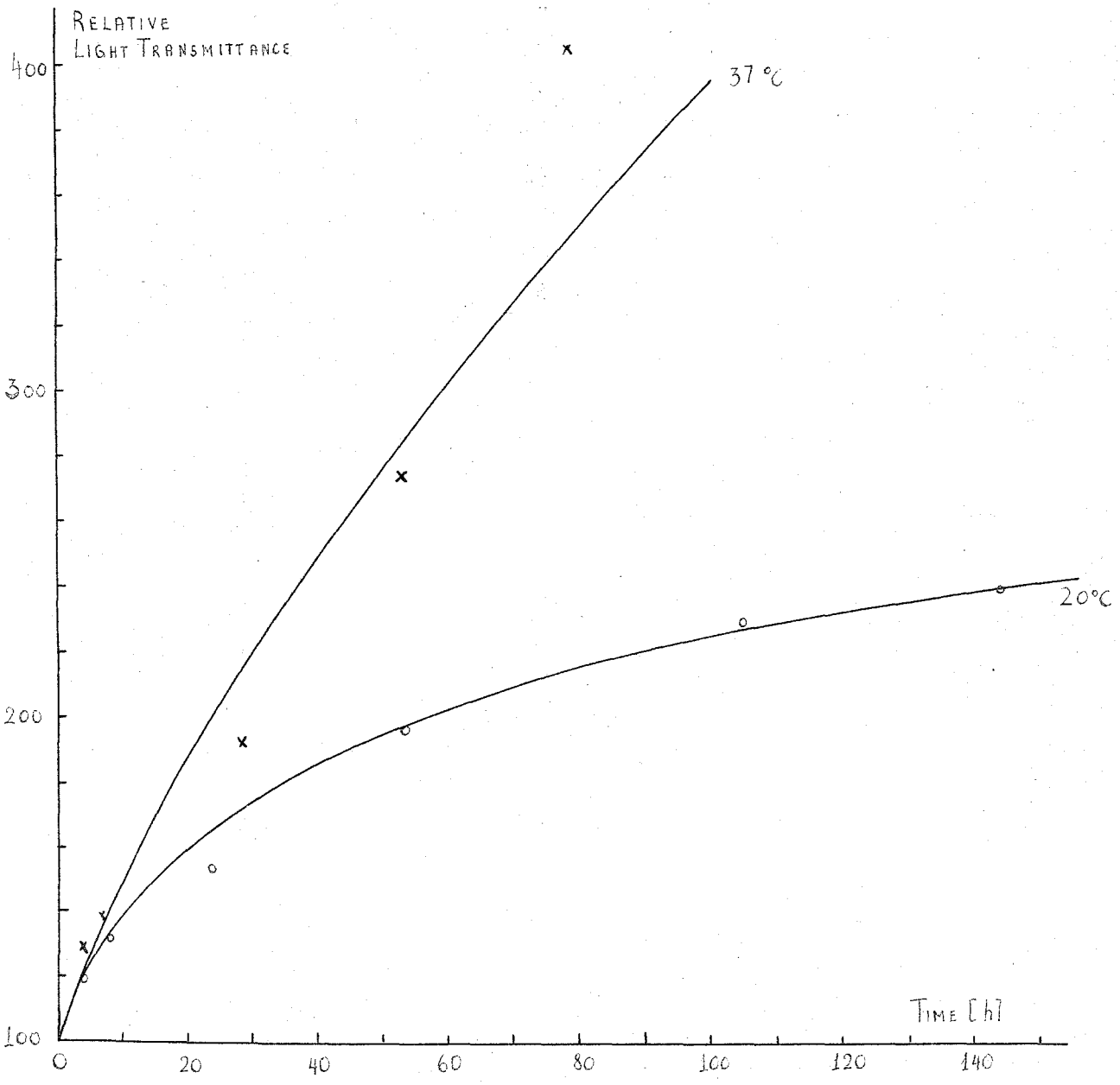


FIG. XV: TEMPERATURE DEPENDENCE OF DYE ABSORPTION RATE
LACTIC ACID CONCENTRATION: 2% ; DYE SHADE: 0.25%

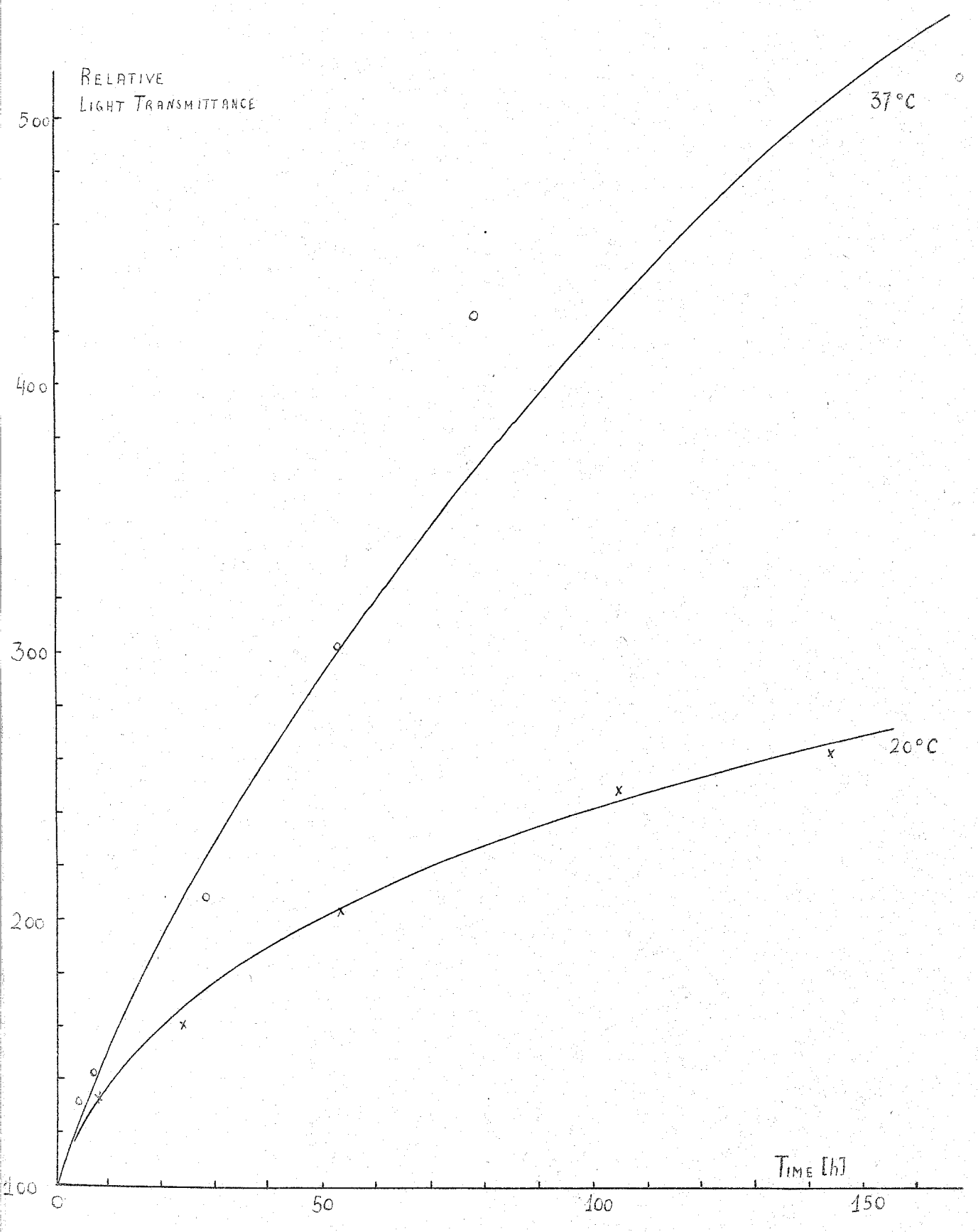


Fig. XVI: Temperature Dependence of Dye Absorption Rate
Lactic Acid Concentration: 5% ; Dye Shade: 0.25%

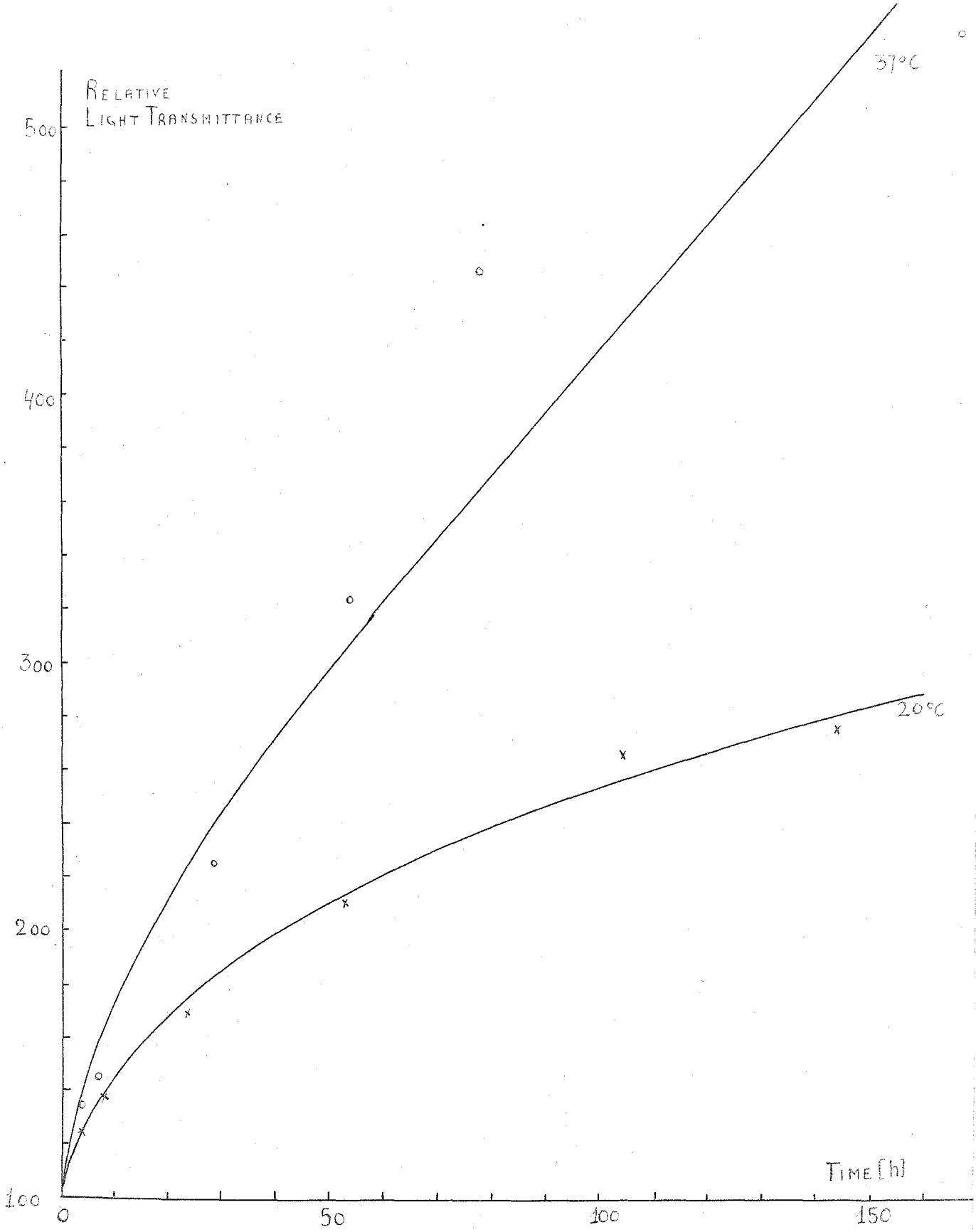


Fig. XVII: Temperature Dependence of Dye Absorption Rate
Lactic Acid Concentration: 7%; Dye Shade: 0.25%

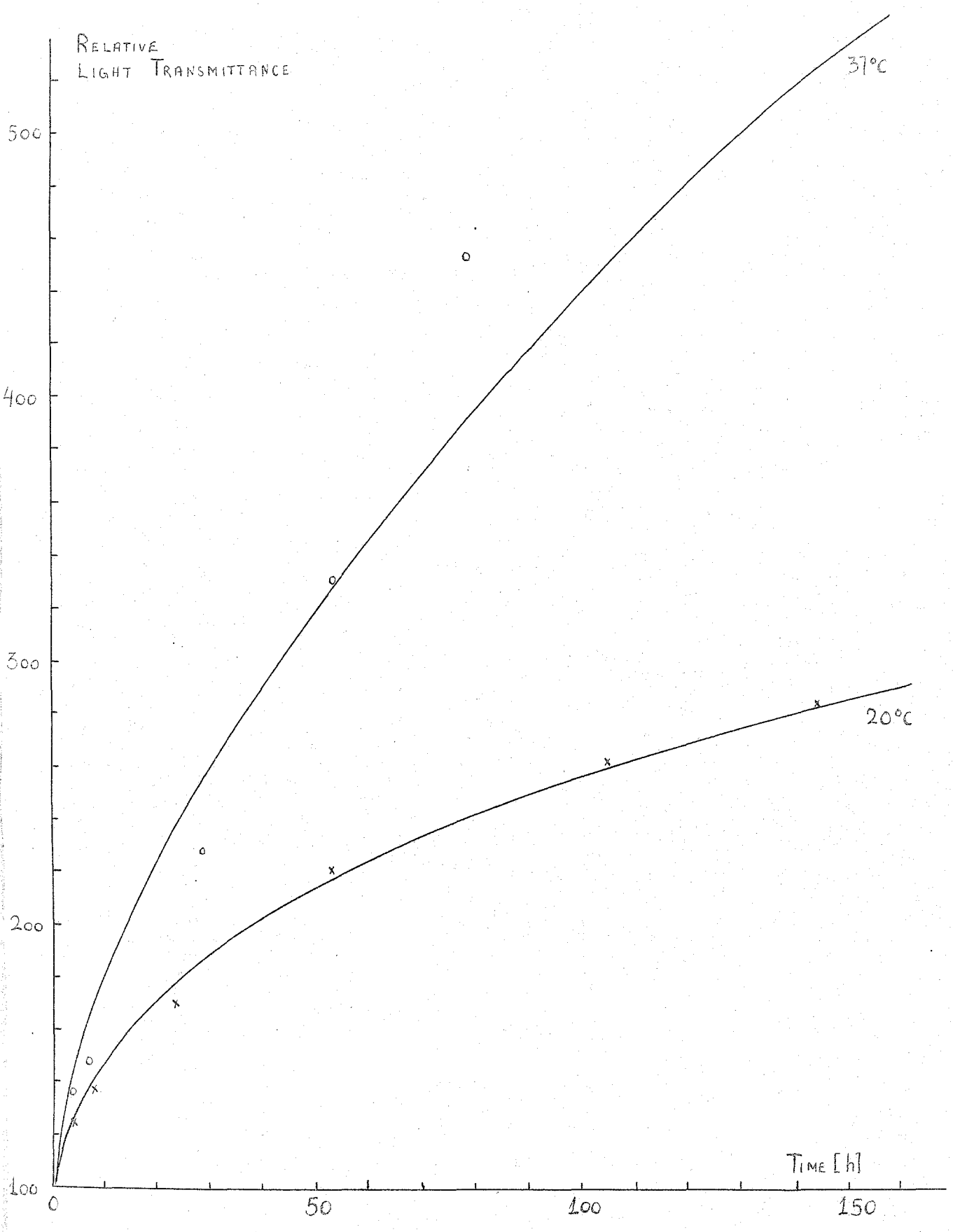


Fig. XVIII: Temperature Dependence of Dye Absorption Rate
Lactic Acid Concentration: 12% ; Dye Shade: 0.25%

The degreasing of the hide resulted in a very tough but brittle product, which could not be cut by scissors, but had to be broken by handicraft- tools.

3. Equilibrium Distribution of the Dying Material.

In determining the affinity of a dye towards a certain substrate it is conventional to determine the equilibrium distribution of the dye between substrate and solvent under various conditions. An attempt to follow this praxis has not been successful. The amount of chromium fixed on hides differing in lactic acid content is represented in table XIX. The samples had been stayed in the dying bath for three weeks before being analyzed. The previously mentioned decay makes the usefulnes of these datas very doubtful.

The samples of low lactic acid content showed the usual decay, the occurence of small particles in the dye bath lowering the availability of dye to the sample. The samples of high lactic acid content adopted a very turbid appearence of the dying liquor. This turbidity could not be sedimentated in a centrifuge rated at 6,000 rpm. The turbidity may prevail from products of hydrolysis of the proteins due to the prolonged exposure to a aqueous solutions of low pH.

Fig. IX representing the dye bath exhaustion under various conditions, allows the conclusion that the lactic acid has only a catalyzing effect, that is a rate accelerating effect, but does not influence the equilibrium distribution of the dye between the phases.

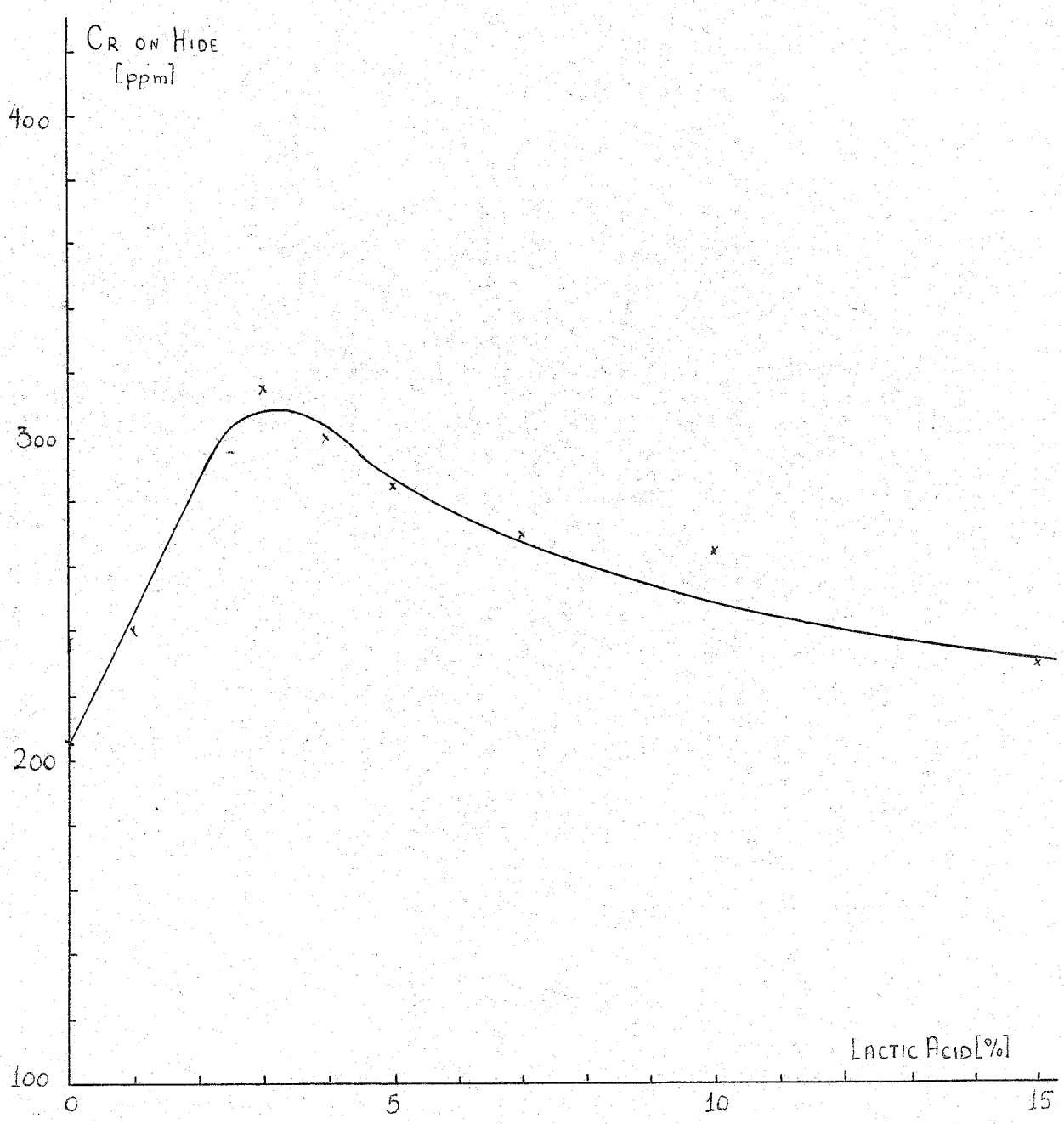


Fig. XIX: Chrome Fixation on Calfskin
Lactic Acid Bath: 24h; Dye Shade: 0.5%
Dye Bath: 3 Weeks

Thus, it appears to be necessary to carry out equilibrium determinations in a carefully controlled manner to avoid any disintegration of the specimen. The influence of addition agents on either absorption rate or on equilibrium distribution has to be established in advance.

4. The Reproducibility of Experiments

Experiments have been carried out to test the reproducibility of dye absorption under various concentrations of lactic acid by observing the rate of change of optical density of the dying liquor. The comparison is limited to samples taken from one site of the hide; samples from different areas of the hide, according to the classification of Thorstensen (4c) have not been tested directly. The results of a indirect comparison are discussed in the section dealing with the dye concentration in the dye bath (see p. 26).

The reproducibility test were conducted by cutting a piece of hide into samples of suitable size, and treating each two of them alike. The results of these tests are represented in figures XX to XXII. Except for one case, the agreement of datas is nearly perfect. The scattering of the datas of fig.XXII is still within acceptable limits. It may be attributed to small differences in experimental conditions between the two samples.

For reasons of reproducibility the experiments of this work were conducted in sets, that is for the investigation of the influence

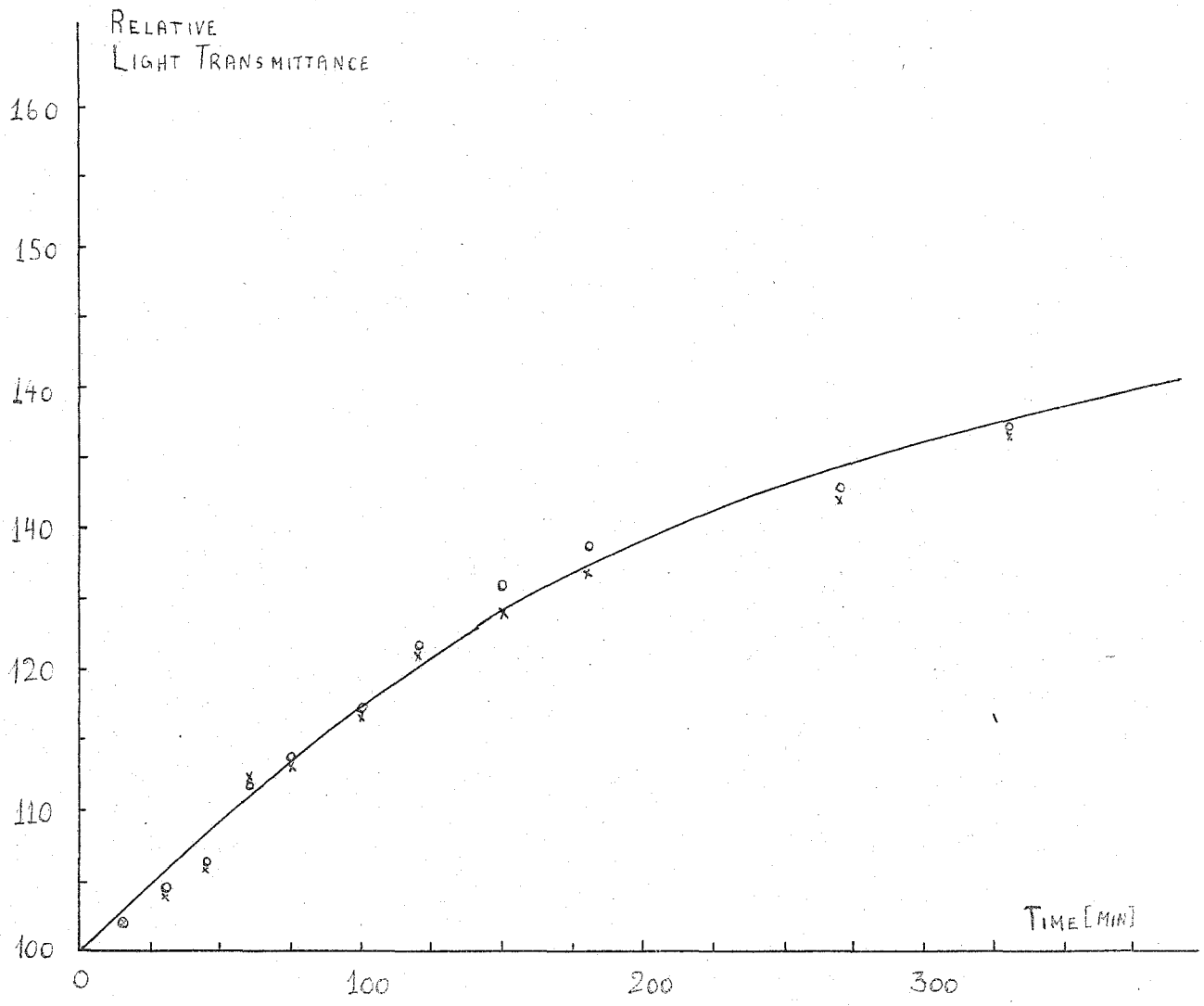


Fig XX: Reproducibility of Dye Absorption on Calfskin
Lactic Acid Bath: 24h; Dye Shade: 0.5%
Lactic Acid Concencentration: 0%

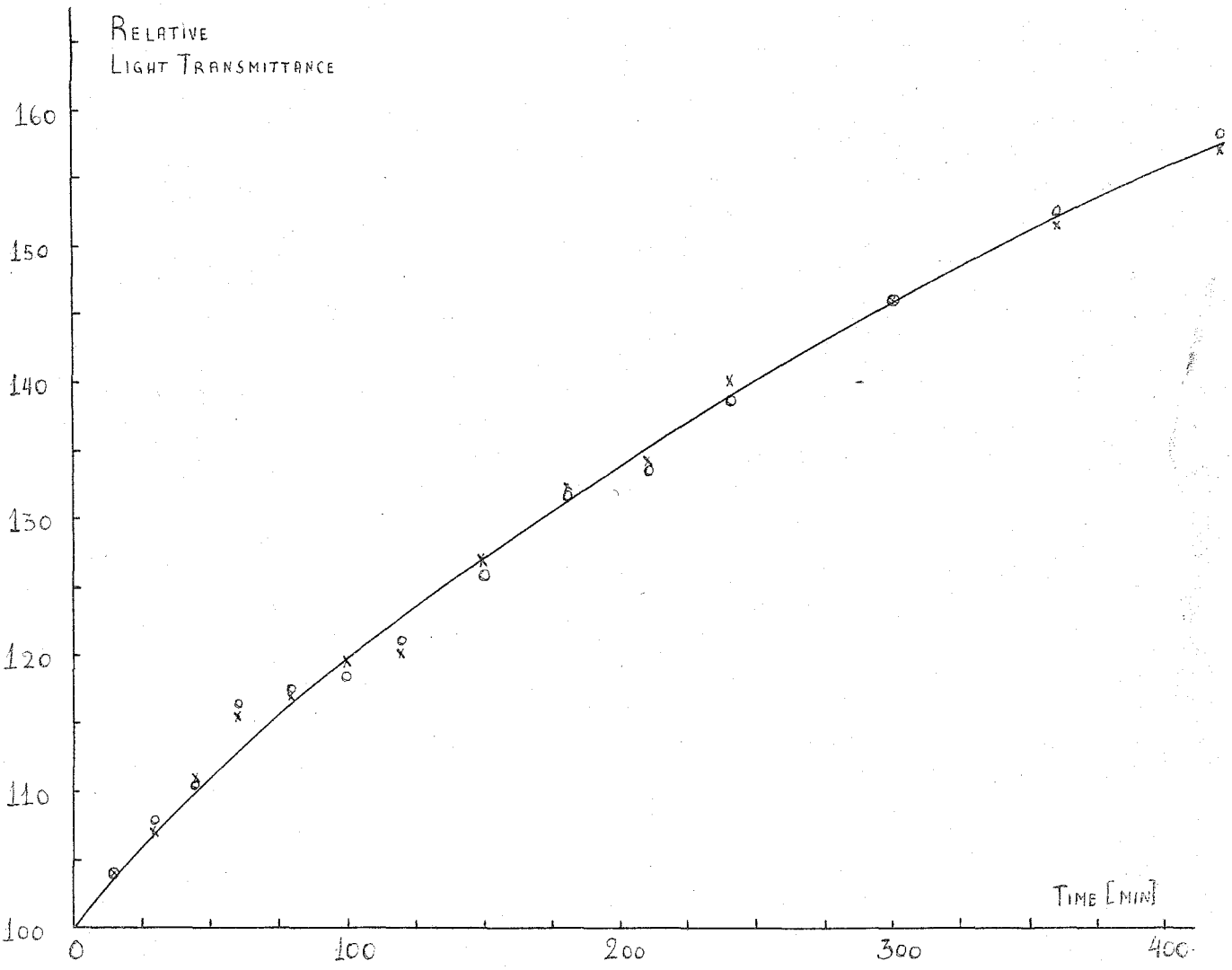


Fig. XXI: Reproducibility of Dye Absorption on Calfskin
Lactic Acid Bath: 24 h ; Dye Shade: 0.5%
Lactic Acid Concentration: 1%

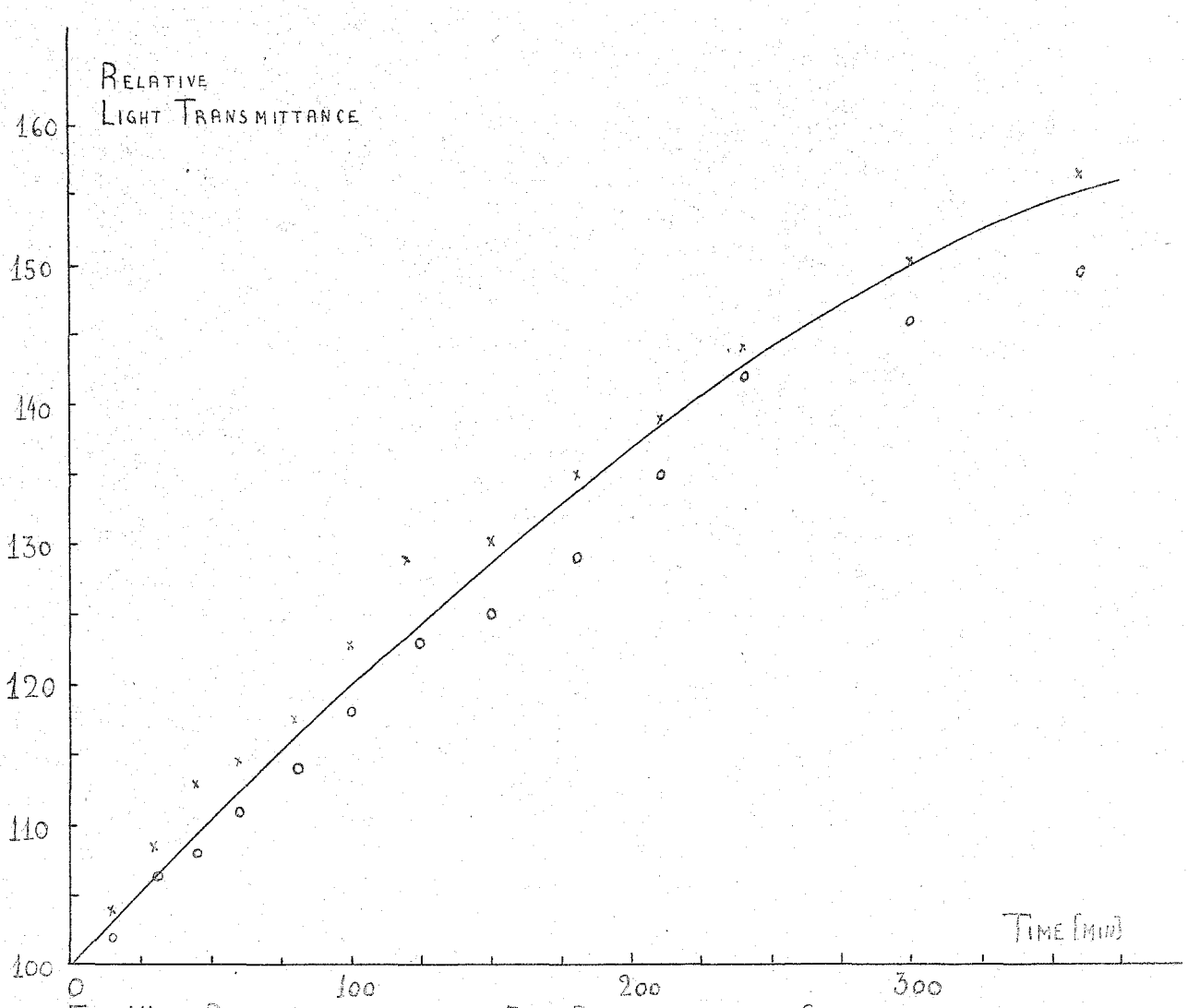


Fig. XXII: Reproducibility of Dye Absorption on Calfskin
Lactic Acid Bath: 24h; Dye Shade: 0.5%
Lactic Acid Concentration: 3%

of a certain variable on absorption rates a compact piece of hide was taken and cut into smaller samples, in order to obtain comparable results.

5. Summary and Conclusion

The presence of lactic acid in samples of hides has been found to accelerate the absorption of premetalized dyes on the hide. Within the range of experimental conditions the rate increased with the concentration of lactic acid. About the equilibrium distribution of the dye between the phases nothing conclusive could be said.

From the "in vitro" - experiments nothing contradictionary to the assumption of a preferential absorption of the premetalized dyes by malignant tumors can be duduced. Further confirmations have to be based on investigations including malignant tissue in vitro as well as in vivo.

6. Suggestions for Further Research

As it seems to be established that the presence of lactic acid has a favorable influence on the absorption rates of the premetalized dyes, research should be extended to slices of normar as well as malignant tissues in isotonic solutions. If the same conditions of preferential absorption can be confirmed, "in vivo" experiments have to be undertaken to establish distribution curves of the dye within the body. This seems to be necessary in order to assure that the dye, particularly when irradiated, will not

accumulate at essential sites in the body as the brain, the spinal mark of the back bone, etc.

The premetalized dyes, and especially the chrome derivatives, might gain some importance in the chrome tanning leather industries. Of primary importance are the light fastness and wet fastness properties on leather. Even though it seems unprobably that chrome tanning can be substituted completely by chrome dying, (because the chromium uptake in chrome tanning is of the order of 50,000 ppm chromium oxide or about 35,000 ppm chromium in contrast to the average of 300 ppm chromium in chrome dying,) the chrome dyed leather seems to posses altered physical properties with respect to the untreated leather when dried, particularly the brittleness and toughness appears to be reduced in favor of increased flexibilty. Any usefullness of these dyes would be enhanced to the leather manufacturer could they be applied together with the chromic acid in the same bath. Thus the compatibility of the premetalized dyes with the conditions of the chrome tanning bath must be investigated, and also, whether their use justifies a considerable decrease of the concentration of chromium salts in the tanning bath. Of main interest in this connection would be the influence on standard tests as the shrinkage temperature and the available gelatine content after treatment with premetalized dyes.

The investigation of the bonding mechanism between the dye molecule and polypeptide chains bears more theoretical importance, however could enable the leather manufacturer to predict more precisely optimum conditions for the development of intermolecular coordinate bonds, resulting in ultimate strength and fastness properties of the finished leather. Studies of this kind should include the determination of the groups most contributive to intermolecular bonding and the average distance between these groups, in order to enable the dye manufacturer to formulate dyes of corresponding molecular length.

III

EXPERIMENTAL PART

1. Procedures

The proteinic tissue used in these experiments was untanned hide of calf. It was stored in a 10 - 15 % NaCl - solution, to which enough phenol had been added to prevent deterioration of the hide during storage. Approximately equal pieces of hide, equality regarding surface area as well as weight, were cut off, and cleaned at the flesh side of adhering foreign materials as far as possible.

Difficulties arose in the determination of the weight of the crude hide. Attempts to dry the hide completely before treatment with lactic acid or the dye, were unsactisfactory because the bone dry hide was difficult to handle. The practice followed was to press the hide over a dry cloth by means of a cylindrical body and to place the pressed hide on a cardboard until constancy of weight was attained. The hide was then cut into pieces approximately equal in surface area and weighed. The moisture content of the hide was about 50 - 60 % based upon the weight thus obtained. All calculations as to concentrations of lactic acid and dye shade as well as bath ratio are based on this wet weight.

Lactic Acid Bath. The hide-bath ratio was 1:30 when acid treatment and dying was performed in different baths and approximately 1:29 when a 0.1 % dye solution was added to the acid bath after the acid treatment period being ellapsed. From pH-measurements it had been concluded that equilibrium distribution of lactic acid between the hide and the aqueous phase, as indicated by constancy of the pH, was established after 22 hours. The acid

treatment period was therefore taken as 24 hours in every case. Agitation was not provided to the bath.

The Dye Bath. The hide-bath ratio was 1:30. The dye bath was prepared by adding the appropiate amounts of a 0.1 % solution of the premetalized dye to the acid bath or to distilled water in a suitable glass beaker. The beaker was covered with a watch glass to avoid undue losses of water changing the concentration of the bath. When the course of absorption was followed by measuring the change of optical density of the dye bath, the bath was stirred with a glass rod just before sampling to assure uniformity of the probe. Other means of agitation were not provided. To equalize dying conditions it was observed that the sample to be dyed was lying flesh-side down, hair-side up in the beaker.

pH-Measurements. A Beckmann pH-meter was used for measuring the pH of the solutions when thought to be necessary.

Optical Density. The determination of the optical density of solutions was necessary for the chromium analysis of the dyed hide as well as for rate determinations of the absorption process. A Fisher Electrophotometer was employed for this purpose. Monochromatic light was obtained by suitable choice of a filter, depending on the color of the solution to be examined.

Colorimetric Determination of Chromium in Organic Matter (5,12)

Organic material is digested by the action of concentrated sulfuric and nitric acid, followed by hydrogen peroxide. The chromium is oxidized to hexavalent state by ammonium persulfate and determined colorimetrically by usage of diphenylcarbazide yielding a soluble violet colored compound of unknown composition. The quantitative determination is carried out by means of a calibrated photoelectric absorptiometer.

Reagent solution are diphenylcarbazide of which 0.05 g are dissolved in 5 ml of glacial acetic acid and diluted to 5 0 ml with water. A standard chromium solution is prepared by diluting 5.8 ml of N/10 potassium dichromate solution to one liter with water in a measuring flask. The resultant solution contains 0,000 010 g of chromium per ml.

The instrument used in this experiments was the Fisher Photoelectrometer. It was calibrated by determining the optical density of solutions of known chromium content containing 2.5 ml of 3M sulfuric acid and being diluted to 50 ml with water against a blanc containing no chromium.

The results are presented graphically in a calibration curve used for reading of the chromium of the sample (fig.XXIII).

The diphenylcarbazide reagent solutions has to be prepared freshly each time when required, because it is subject to alteration on storage leading to substantial errors. One gram of the representative sample is weighed into a 100 ml Kjeldahl flask and a mixture of five ml of concentrated nitric acid and three ml of concentrated sulfuric acid is added. The mixture is warmed until reaction commences

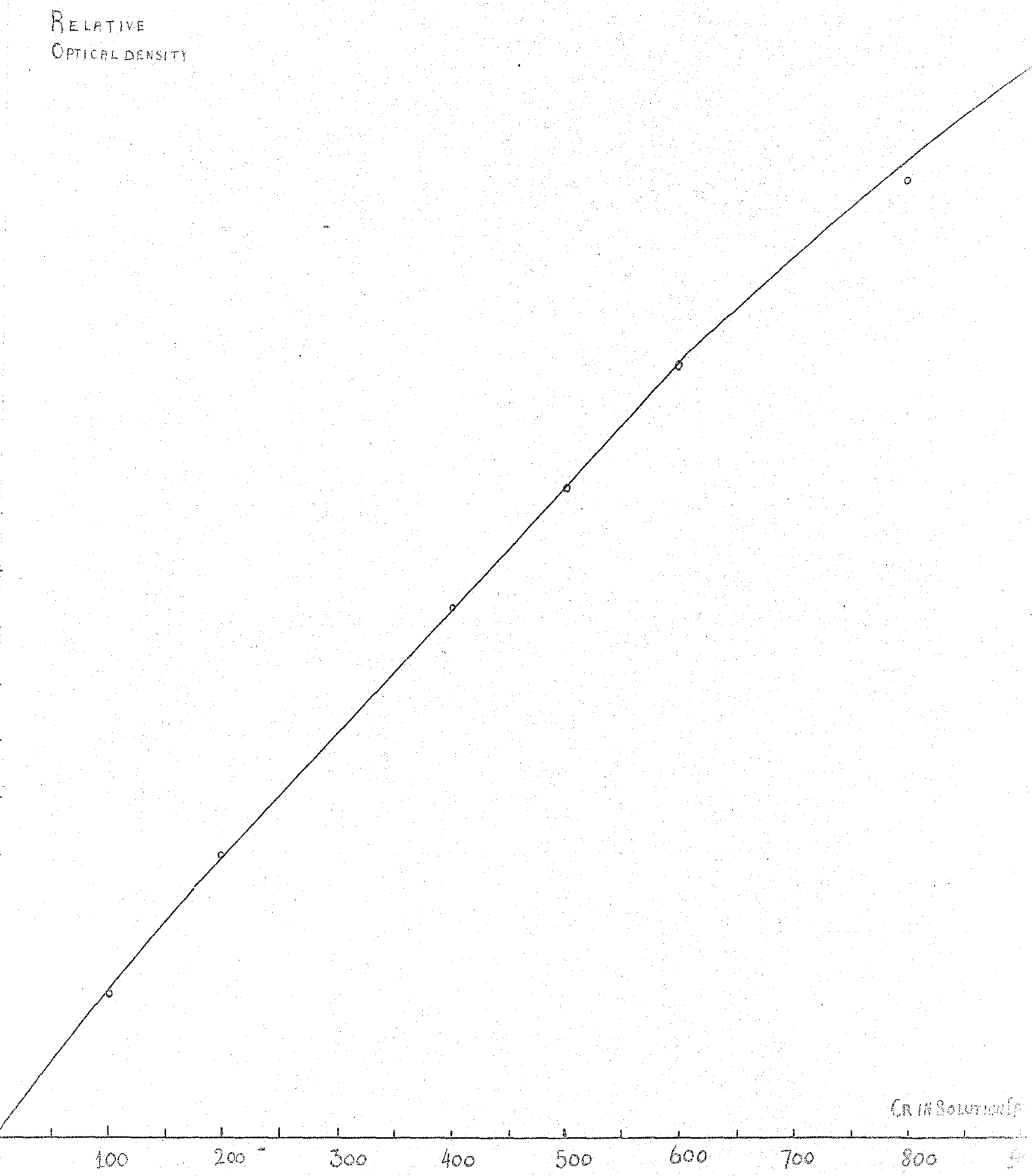


Fig. XXIII: Typical Calibration Curve For DPG-Reagent

and boiled down rapidly after the initial vigorous reaction has finished. When the solution begins to darken the flask is removed from the source of heat and small portions of concentrated nitric acid are added, followed again by strongly heating. This cycle is repeated until the solution fails to darken and remains pale yellow in color. Last traces of organic matter are destroyed by the addition of small portions of hydrogen peroxide and successive heating until the solution becomes quite colorless. After slight cooling of the solution in the flask a quantity of 10 ml of water is added and the content of the flask is heated rapidly to fuming for 15 minutes. After cooling, again 5 ml of water are added and the solution is boiled down until white fumes of sulfur trioxide appear. Then, 25 ml of water are added to the solution which is then boiled gently until clear. The final solution is transferred into a 250 ml beaker quantitatively, diluted to a volume of about 80 ml with water, and one ml of a n/10 silver nitrate solution and 2 ml of a 10 % ammonium persulfate solution are added. This mixture is boiled for about 15 minutes, allowed to cool and diluted to 100 ml in a measuring flask.

A 10 ml portion of this solution is pipetted into a 50 ml measuring cylinder and diluted to about 25 ml with water. 2 ml of the diphenylcarbazide reagent solution are added and the mixture is diluted to 50 ml with water, drained into a 100 ml conical flask which is then covered with a glass bulb. After shaking well, the solution is allowed to stand for five minutes. The optical density is then determined in a previously calibrated absorptiometer.

	pH AT LACTIC ACID CONTENT				
TIME (h)	0 %	2 %	5 %	7 %	12 %
0	7.60	3.48	3.18	3.12	2.90
4	7.50	3.62	3.25	3.15	2.92
8	7.40	3.70	3.30	3.15	2.95
24	7.18	3.90	3.45	3.25	2.95
53	-	-	-	-	2.94
105	6.72	3.97	3.40	3.21	2.95
144	7.10	3.93	3.42	3.23	-

TABLE: 2 : EXPERIMENTAL DATA TO FIG. V.

TIME (h)	pH AT LACTIC ACID CONTENT OF				
	0 %	2 %	5 %	10 %	15 %
0	5.92	5.92	5.92	5.92	5.92
1	6.42	5.90	4.40	4.00	3.72
2	6.70	5.82	4.28	3.90	3.60
4	6.83	5.99	4.20	3.80	3.50
8	6.99	6.02	4.11	3.70	3.40
12	7.00	6.02	4.09	3.55	3.30
24	7.22	6.30	4.22	3.65	3.22
48	-	6.59	4.28	3.70	3.22
96	7.07	-	4.40	-	3.22
168	7.12	6.80	-	3.85	3.28

TABLE: 3 : EXPERIMENTAL DATA TO FIG. VI.

TIME (h)	RELATIVE LIGHT TRANSMITTANCE OF DYE LIQUOR WITH SAMPLES CONTAINING LACTIC ACID OF				
	0 %	2 %	5 %	7 %	12 %
0	100	100	100	100	100
.5	101.5	103	105	106	105
1	108.2	109	110	112	112.5
2	112	113	116	117	117.5
4	116	119	123	124	125
8	122	130.5	133	137	136.5
24	131	154.3	160	169	169.5
53	160	197	203	210	220
105	200	228	248	265	260
144	218	239	261	275	282

TABLE: 4 : EXPERIMENTAL DATA TO FIG. VII.

TIME (DAYS)	CR-CONTENT OF DYE LIQUOR (%) WITH LACTIC ACID CONTENT OF			
	0 %	2 %	7 %	12 %
0	100	100	100	100
1	62.4	56.3	50	44.2
3	29.4	24.0	18.4	14.7
10	11.9	9.5	7.9	6.1

TABLE 5 : EXPERIMENTAL DATAS TO FIG. VIII.

TIME (DAYS)	CR-FIXATION ON HIDE (ppm) WITH LACTIC ACID CONTENT OF			
	0 %	2 %	7 %	12 %
0	0	0	0	0
1	174	194	229	248
3	322	352	372	377
10	374	396	414	429

TABLE 6 : EXPERIMENTAL DATA TO FIG. IX.

TIME (h)	RELATIVE LIGHT TRANSMITTANCE OF DYE LIQUOR OF SAMPLES WITH LACTIC ACID CONTENT OF					
	0 %	2 %	5 %	7 %	10 %	15 %
0	100	100	100	100	100	100
.5	101	101	106	106.2	107.2	107.5
1	105.8	107	108	109.2	110.2	111.5
2	111.2	112.2	115.8	116.3	117.8	120
4	115	116.5	122	122.7	123.0	123.8
8	121.8	125	131	132	133.2	135
12	125.3	129.3	137.2	138	139.8	143.3
24	133.5	138	150.3	151	152.5	159.6
48	147.0	157.3	178	179.2	182	190.3
96	168.5	181	202.5	206.5	228.5	238.5
168	193.0	205	234	237	243	259

TABLE: 7 :EXPERIMENTAL DATA TO FIG. X.

TIME (h)	CR-FIXATION (ppm)
0	0
5	120
1	135
1.5	250
2	340
2.5	370
3	365
4	510
6	560
7	580
8	515
17.5	650
25.5	670

TABLE: 8 : EXPERIMENTAL DATA TO FIG. XI.

LACTIC ACID (%)	CR - FIXATION (ppm)
0	200
2	240
3	250
4	270
5	290

TABLE: 9 :EXPERIMENTAL DATA TO FIG. XII.

LACTIC ACID (%)	CR - FIXATION (ppm)
0	180
1	220
3	240
4	245
5	245

TABLE: 10 :EXPERIMENTAL DATA TO FIG. XIII.

TIME (h)	RELATIVE LIGHT TRANSMITTANCE (%)	
	AT 20 ° C	AT 37 ° C
0	100	100
4	116	129
7	-	137
8	122	-
24	131	-
29	-	181
53	160	226
78	-	234
105	200	- [x]
144	218	-
168	-	-

[x] EXPERIMENT INTERRUPTED BECAUSE OF DESTRUCTION OF HIDE

TABLE 11 : EXPERIMENTAL DATA TO FIG. XIV.

TIME (h)	RELATIVE LIGHT TRANSMITTANCE	
	AT 20 ° C	AT 37 ° C
0	100	100
4	119	129.5
7	-	139.5
8	130.5	-
24	154.3	-
29	-	193
53	197	274
78	-	404
105	228	- [x]
144	239	-
168	-	-

[x] EXPERIMENT INTERRUPTED BECAUSE OF DESTRUCTION OF HIDE

TABLE 12: EXPERIMENTAL DATA TO FIG. XV.

TIME (h)	RELATIVE LIGHT TRANSMITTANCE (%)	
	AT 20 ° C	AT 37 ° C
0	100	100
4	123	131.5
7	-	142
8	133	-
24	160	-
29	-	208
53	203	300
78	-	424
105	248	-
144	261	-
168	-	514

TABLE 13: EXPERIMENTAL DATA TO FIG. XVI.

TIME (h)	RELATIVE LIGHT TRANSMITTANCE (%)	
	AT 20 $^{\circ}$ C	AT 37 $^{\circ}$ C
0	100	100
4	124	134
7	-	145
8	137	-
24	169	-
29	-	225
53	210	323
78	-	445
105	265	-
144	275	-
168	-	514

TABLE 14 : EXPERIMENTAL DATA TO FIG. XVII.

TIME (h)	RELATIVE LIGHT TRANSMITTANCE (%)	
	AT 20 $^{\circ}$ C	AT 37 $^{\circ}$ C
0	100	100
4	125	136
7	-	147.5
8	136.5	-
24	169.5	-
29	-	238
53	220	330
78	-	457
105	260	-
144	282	-
168	-	168

TABLE 15 : EXPERIMENTAL DATA TO FIG. XVIII.

LACTIC ACID CONTENT (%)	CR - FIXATION (ppm)
0	206
1	240
2	285
3	315
4	300
5	285
7	270
10	265
15	230

TABLE: 16, EXPERIMENTAL DATA TO FIG. XTX.

TIME (MIN)	RELATIVE LIGHT TRANSMITTANCE (%)	
	SAMPLE I	SAMPLE II
0	100	100
15	102	102
30	104.2	104.6
45	105.8	106.4
60	112.2	111.7
75	113.1	113.8
100	116.8	117.4
120	121	121.8
150	124	126.
180	126.8	128.8
270	132	133
330	137	137.3
390	140.8	141.0
CR-CONTENT AFTER 5 DAYS (ppm)	375	367

TABLE 17: EXPERIMENTAL DATA FOR FIG. XX.

TIME (MIN)	RELATIVE LIGHT TRANSMITTANCE	
	SAMPLE I	SAMPLE II
0	100	100
15	104	104
30	107	108
45	111	110.7
60	115.5	116.5
80	117	117.5
100	119.5	118.5
120	120	121
150	127	126
180	132	131.5
210	134	133.5
240	140	139
300	146	146
360	151.5	152.5
420	157.5	158.5
CR-CONTENT		
AFTER 5 DAYS (ppm)	435	450

TABLE 18: EXPERIMENTAL DATA TO FIG. XXI.

TIME (MIN)	RELATIVE LIGHT TRANSMITTANCE (%)	
	SAMPLE I	SAMPLE II
0	100	100
15	104	102
30	108.5	106.5
45	113	108
60	114.5	111
80	117.5	114
100	123	118
120	129	123
150	130	125
180	135	129
210	139	135
240	144	142
300	150	146
360	156.5	149.5
CR-CONTENT		
AFTER 5 DAYS (ppm)	480	440

TABLE19: EXPERIMENTAL DATA TO FIG. XXII.

OIL-CONTENT (ppm)	RELATIVE OPTICAL DENSITY
0	0
100	5.0
200	9.5
400	16.7
500	22.9
600	27.3
800	35.8
1000	40.4

TABLE 20: TYPICAL DATAS OBTAINED IN CALIBRATING ELECTRO-PHOTOMETER WITH DEO-STANDARD SOLUTION (FIG. EXTIT)

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