Bioavailability of Therapeutically Used Hydrolytic Enzymes

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State of the Art

Humoral efficacy of orally administered hydrolytic enzymes used as systemic therapeutic agents requires that they should be absorbed. However, measurements of absorption in enzymes based on the substance itself result in varying results. Different measuring methods produce hydrolytic enzymes absorption rates which do not shift within the normal range but differ with regard to the dose administered between thousandth parts and more than 50% (Table 1): immunological tests show the lowest rates (< 1%), enzyme substrate tests values of less than 10%, and, radioactivity measurements using labeled enzymes (by means of radioactivity attached secondarily to biocatalysts) the most highest transfer values. The differences arise from viewing different peculiarities, i.e., substantial and effective qualities. One must bear in mind that enzymes are catalytically active substances. This means

Table 1. Special features of various enzyme absorption assays

Assay	Enzyme quantification and absorption rates
Histological assay	Qualitative evidence
Biochemical assay	Special chemical reactions missing in many secretory proteinases
Immunological assay	Qualitative and quantitative evidence (< 1%)
Enzymatic assay	Depending on the enzyme, kinetic evidence
Radiological assay, with or without chromatographic separation	Dissociation may cause high absorption (even > 50%)

Table 2. Turnover numbers of various enzymes

Biocatalytic substances	Turnover numbers (K_{cat} in s^{-1})
Lysozyme	5×10^{-1}
Papain	1×10^{-1}
Chymotrypsin	1×10^{-2}
Trypsin	$1 \times 10^{-2} - 1 \times 10^{-3}$
Kinases/dehydrogenases	up to 10^{-3}
Penicillinase	2×10^{-3}
Amylase	$1 \times 10^{-3} - < 2 \times 10^{-4}$
Acetylcholinesterase	$1 \times 10^{-3} - 5 \times 10^{-4}$
Superoxide dismutase	1×10^{-6}
Catalases	$> 8 \times 10^{-4} - 1 \times 10^{-7}$

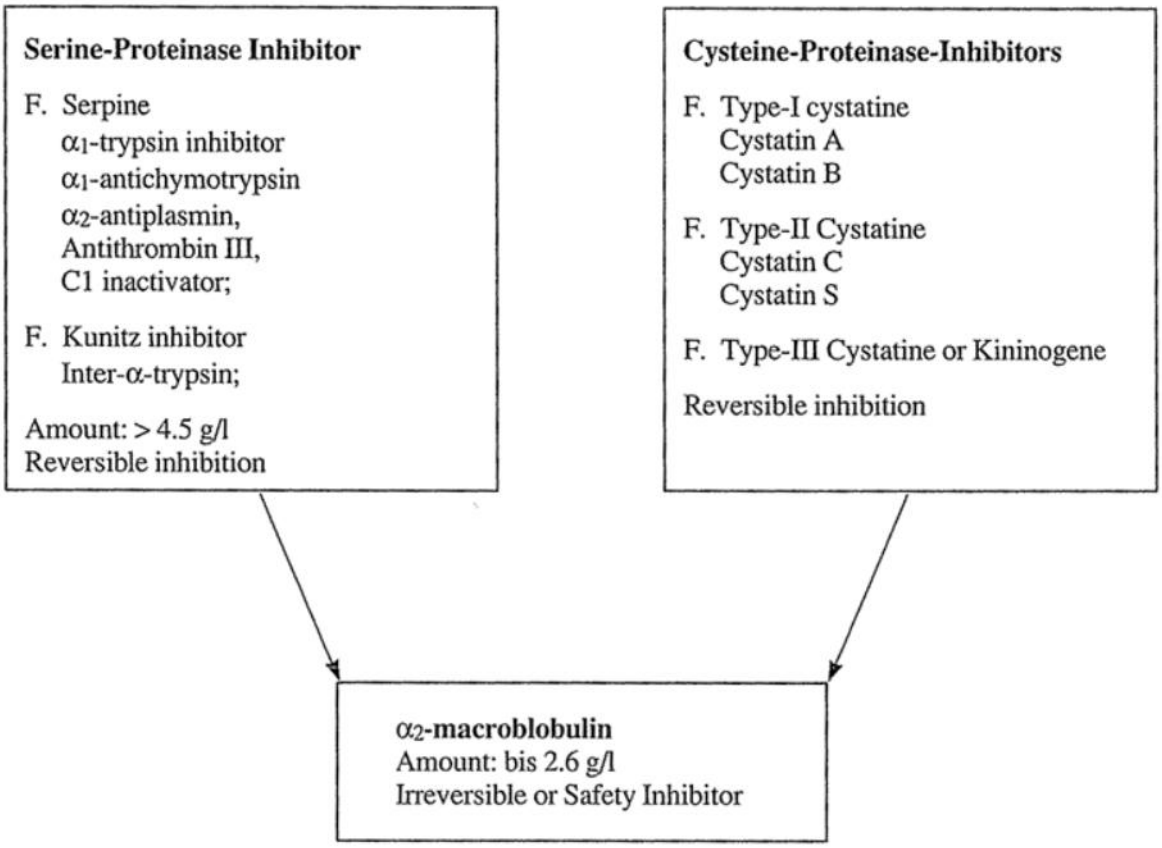

Fig. 1. Inhibitor interactions of proteolytic enzymes in the body

that the differences in immunological and enzyme substrate measurements depend on enzyme-specific turnover numbers (Table 2). Turnover numbers in therapeutically used enzymes have values of 100–1000 conversions per second, and one must multiply the immunological values by the conversion rate.

On the other hand, the absorption rates from radioactive tests are probably too high. As markers bound by adsorption can dissociate in altered pH conditions, one cannot rule out that the micromolecular markers in intragastrically administered test enzymes are split off, absorbed, and combine with blood proteins. If this happens, one finds false-positive values in succeeding chromatographic separations. One must also consider interactions with endogenous inhibitors, which amount to more than 10% of the blood proteins (Fig. 1). The main groups are serpines and cystatines, which block proteolytic enzymes totally and/or reversibly, as well as α_2-macroglobulin, a safety inhibitor and physiological regulator that surrounds bound biocatalysts and controls/reduces inversely to the molecular weight of the test substrates the activity of enzymes used (Fig. 2). Finally, enzyme macromolecules are absorbed, but until now we have been unable to measure their absorption in a reliable way. This is why we must measure bioavailability by indirect methods. Effects normally examined in enzymes are alterations in catalytic blood activities after their enteric administration or other criteria, for example, in hydrolytic enzymes splitting reactions and reductions of endogeneous substrates.

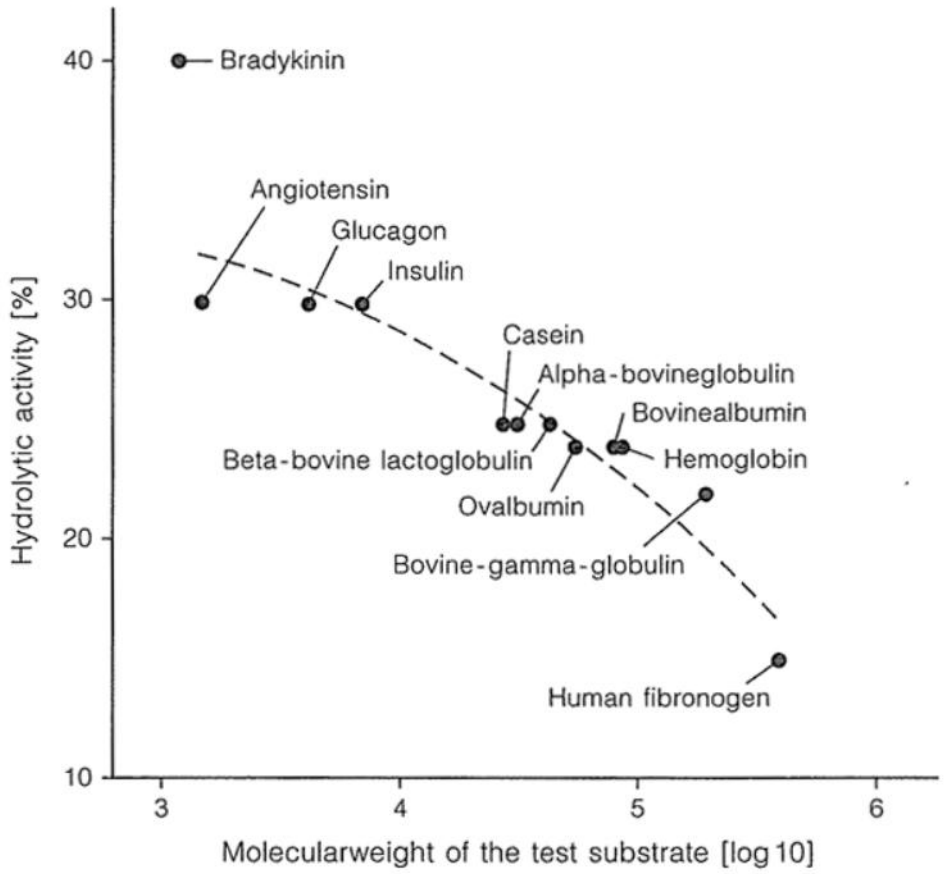

Fig. 2. Hydrolytic activity of the α_2-macroglobulin–Serratia protease complex and free enzymes depending on the molecular weight of the substrate

Results in Experimental Allergic Arthritis

Animal experiments show dose-dependent systemic effects in enterically administered enzyme drugs. In the Glynn model of allergic arthritis rabbits are sensitized by repeated stomach tube feedings with immunogenic substances (e.g., ovalbumin) and after developing high titers of antibodies are injected intra-articularly with the antigen. One finds various symptoms of an immune complex dependent inflammatory process inside the joints 18 h after beginning the reaction (Table 3). For example, (a), the cell number within synovial fluid increases, (b), the synovia layers are thickened, (c), the mesothelial cells proliferate, (d), cells immigrate from the articular soft tissue, (e), there are hyperemias, and (f), leukocytes aggregate.

Table 3. Study criteria in experimental allergic arthritis in rabbits

Parameter	Maximum score (range)
Number of cells in synovial fluid	0–3
Thickening of the synovial membrane	0–2
Mesothelial cell proliferation	0–2
Cell infiltration in the articular soft tissue	0–2
Increase in hyperemia	0–2
Increase in leukocyte aggregation	0–2

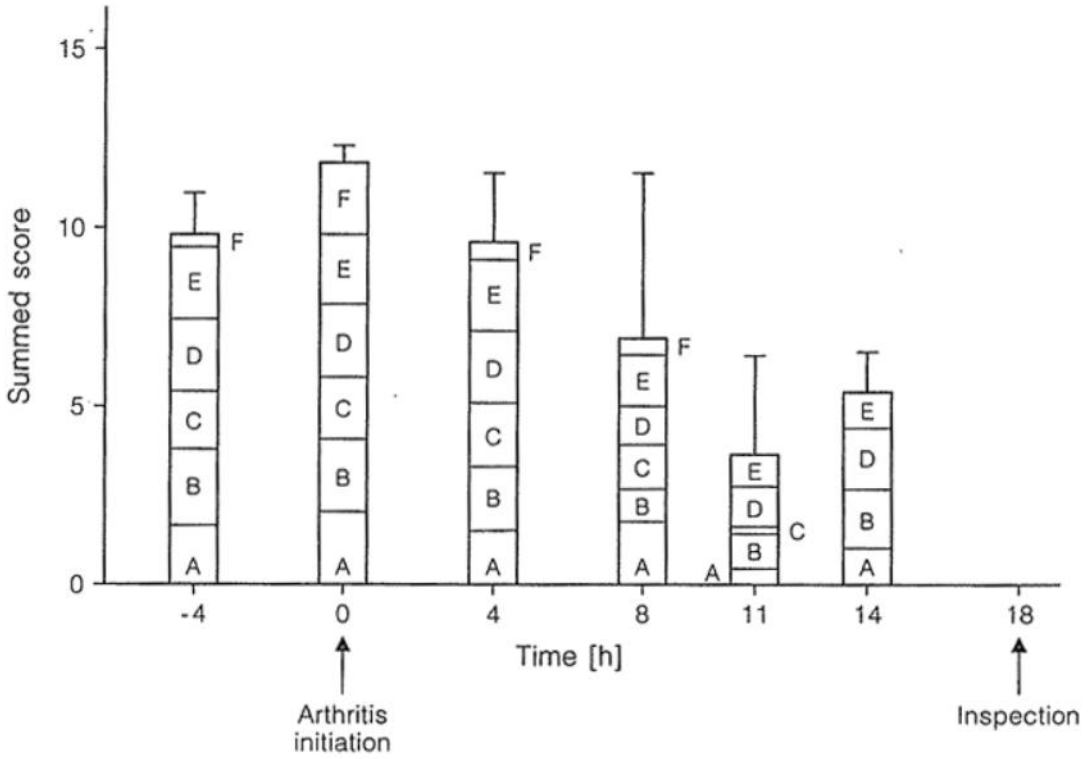

Fig. 3. Change in summed scores of histological parameters in experimental allergic arthritis in rabbits with respect to time of administration of 1.0 g suspended Wobenzyme-drug mixture by gavage

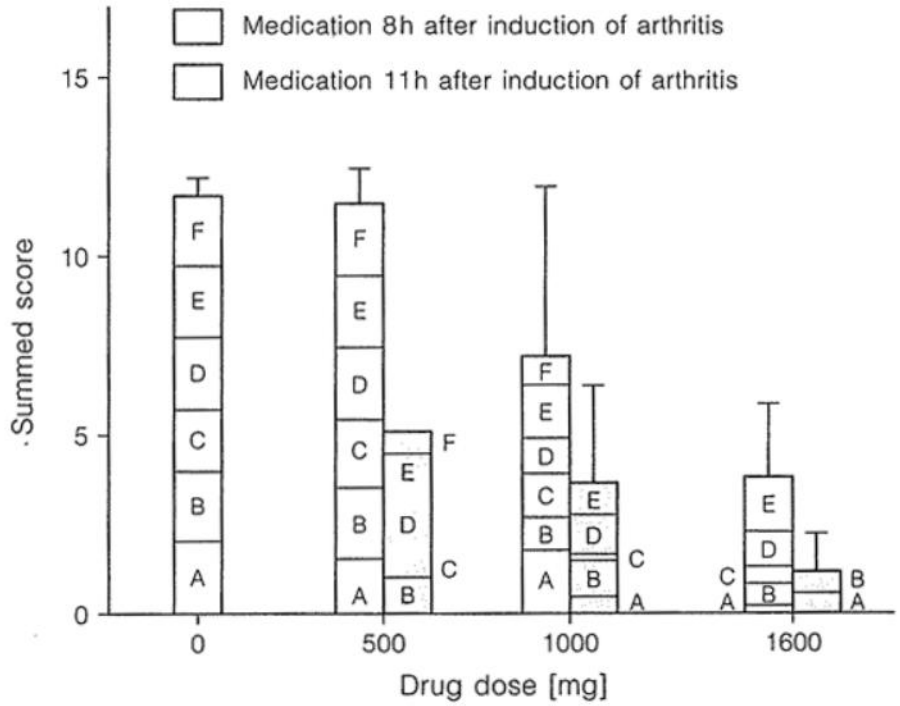

Fig. 4. Effect of the dose of Wobenzyme-drug mixture administered by gavage 8 and 12 h after induction of experimental allergic arthritis in rabbits on the summed scores of histological parameters investigated in the knee joint. *A*, Number of cells within the synovial fluid; *B*, thickening of the synovial membrane; *C*, proliferation of mesothelium; *D*, state of hyperemia; *E*, intensity of infiltrating cells; *F*, state of leukocyte aggregation

In a histological study based on scoring the immune complex induced changes Steffen and coworkers [2] found an average summed score of nearly 12.0 in untreated rabbits (Fig. 3). Scores were lower when the animals were treated with single doses of 1.0 g of an enzymatic combination drug by gavage at different times (4 h before and 4, 8, 11, 14 h after start of inflammation). The shorter the duration was between medication and inspection, the fewer symptoms were seen. In the opinion of the authors this was due to enzyme-induced cleavages of immune complexes, which develop as a modified Arthus reaction in the intra-articular blood vessels. The positive correlation between the posttreatment time intervals and the intensity of symptoms (expressed in the increased scores) resulted from drug elimination and the pathogenic effects induced by new formed toxic immune complexes. Using different doses of the same drug, administered 8 or 11 h after initiating the experimental arthritis Steffen and coworkers [4] observed both dose dependent and medication time dependent effects (Fig. 4). The shorter the exposure time was, the higher the antiinflammatory effects, and, the lower dose had fewer effects than the higher one.

Results in Experimental Glomerulonephritis

Glomerulonephritis was induced experimentally in rabbits by repeated intravenous injections of preformed immune complexes (three times at 12-h intervals, 5 ml dispersion of high-dose immune complexes suspended in physiological salt solution), Steffen and Menzel [2] observed sedimentations of the toxic agent 24 h after the last immune complex injection within the glomeruli. Examination of the glomerular distribution of immunofluorescence allowed differentiation of three patterns (Fig. 5) malpighian tufts without immunofluorescence (type I), glomeruli with relics of immunofluorescence (type II), and, Bowman's capsules totally filled with fluorescent immune aggregates (type III). Evaluation of 100 malpighian tufts selected at random from different renal areas showed most of the untreated animals to have type III immunofluorescent patterns (Fig. 6). Treated animals received 1.6 g of an enzyme combination drug dissolved in 5 ml water administered intragastrically by stomach tube 12 h after the last immune complex injection. The conclusion that can be drawn from these experiments is that, intragastrically administered, catalytically active enzymes are probably absorbed as active substances.

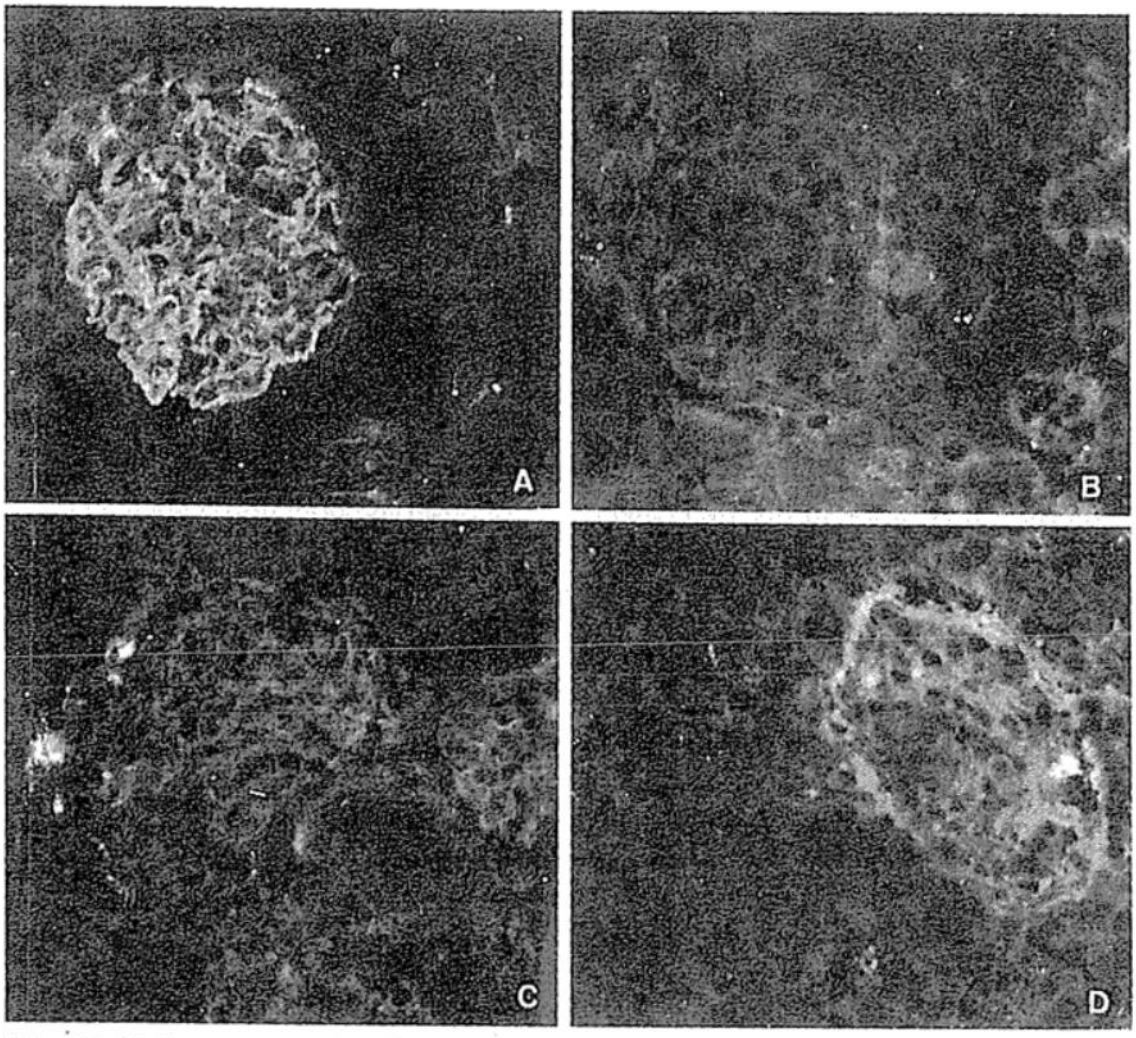

Fig. 5A–D. Immune complex fluorescence pattern in the renal glomeruli of rabbits after the intravenous administration of preformed antigen–antibody complexes. From [3]

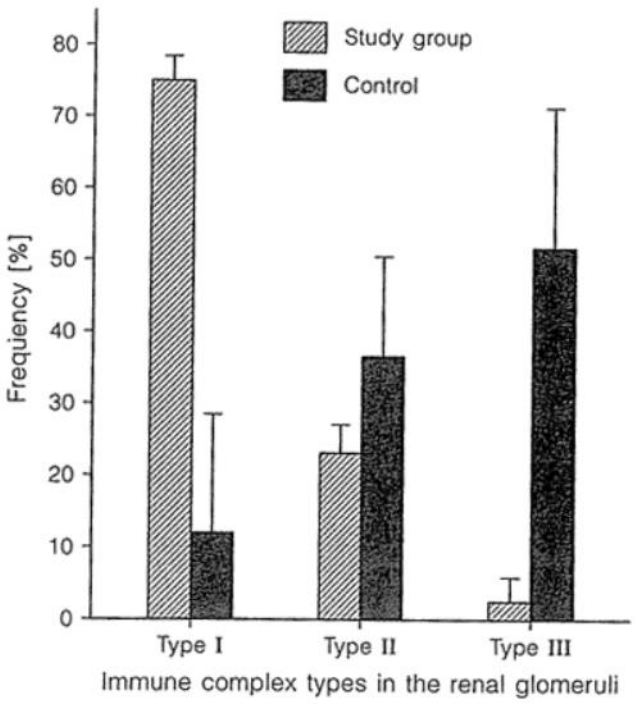

Fig. 6. Frequency of the three types of immune complex fluorescence found in the renal tissue of rabbits who 12 h before organ extraction had received 1.6 g Wobenzyme mixture in aqueous suspension and in control rabbits who had received the same amount of suspension (5 ml water)

Experiments in Human Volunteers

As these hydrolytic remedies are used in man, one should know something of their bioavailability in man. This is measured principally by activity changes in the relevant enzymic drug. Kleine and Van Schaik (unpublished data) examined proteolytic activity in healthy volunteers and observed a circadian rhythm which was correlated with daily mobility (Fig. 7). During the normal daytime when we are awake, values are higher than during normal night time and reach a minimum at 1–5 A.M. When the same persons swallowed four enteric-coated tablets, of which one, two or all were enzyme-containing remedies, the hydrolytic serum activity measured in blood samples taken before single medication at 9 A.M. and 11 P.M. increased in a dose-dependent manner (Fig. 8). With regard to the differences Kleine and Van Schaik found dose-dependent increases and prolongations of raised serum values. In addition, the reduction in elevated activity titers started in each

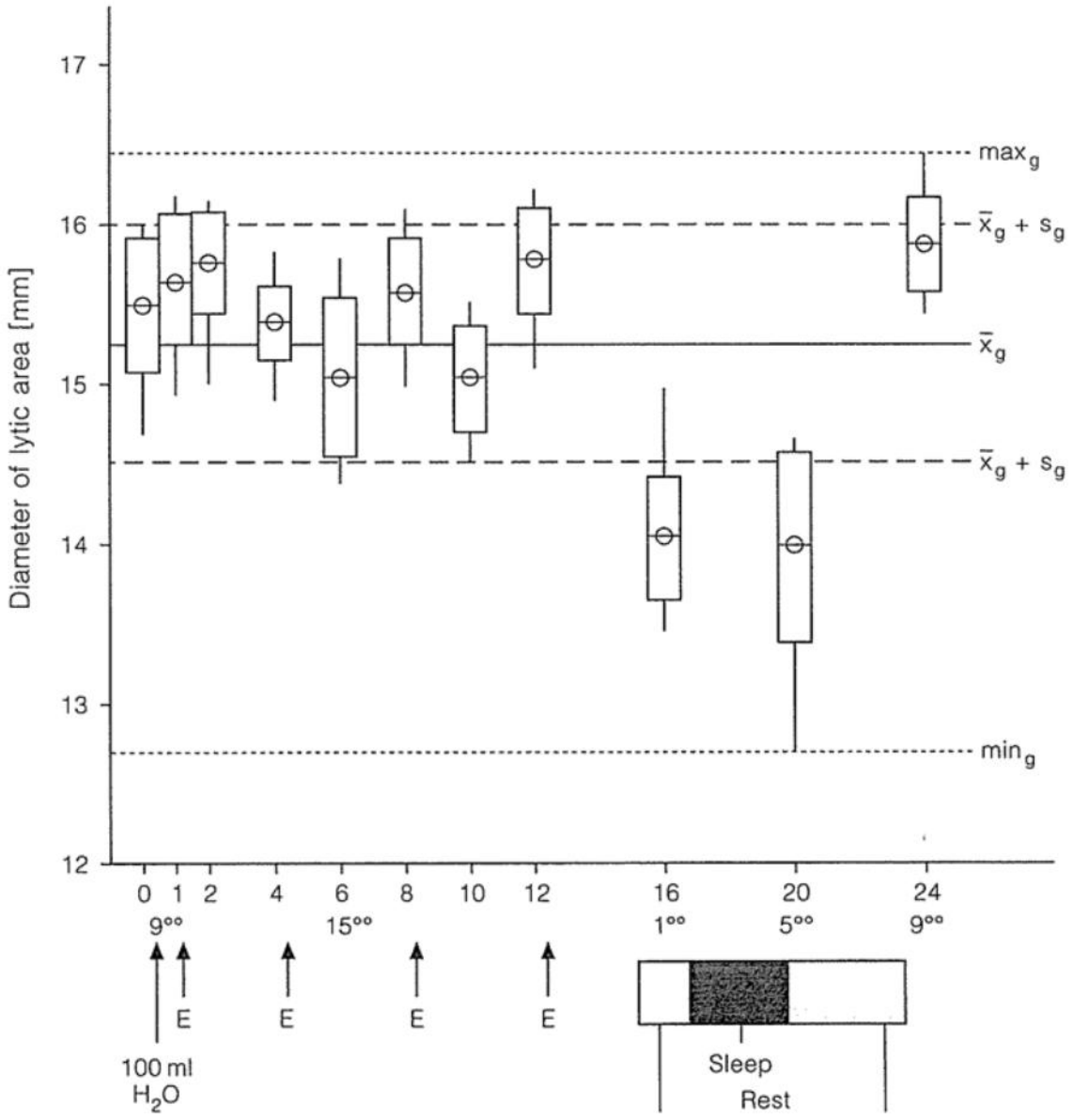

Fig. 7. Course of hydrolytic serum activity over 24 h in 12 approx. 25-year-old subjects. *E*, eating

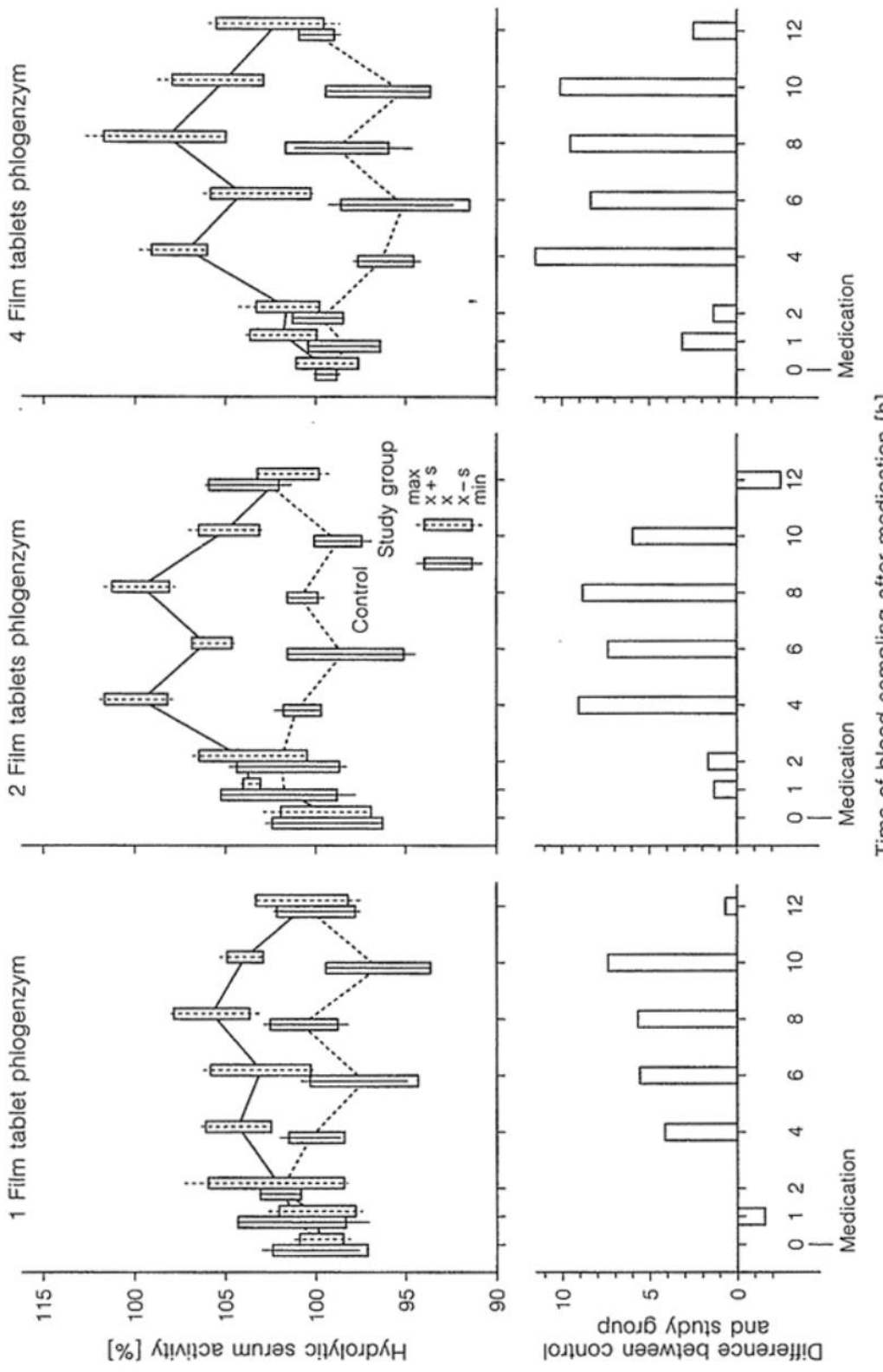

Fig. 8. Changes in hydrolytic serum activity in study subjects who received various doses of Phlogenzyme. Blood samples were taken via a chronically positioned catheter at 2-h intervals. The figure shows the percentages as relating to the zero value as well as the differences between the treatment and the course of hydrolytic activity without enzymes

test group about 10 h after medication and was normal in samples taken 24 h after swallowing the tablets.

Neuhold [1] treated volunteers with four enteric-coated tablets of a different enzyme combination drug, three times at 3-h intervals, and found hydrolytic serum activity to increase asymptomatically. Depending on the substrate used in plate test systems, these values were 20%–30% higher than the values before medication (Fig. 9). These findings indicate that catalytically active agents as used in therapy are probably absorbed by receptor-mediated processes.

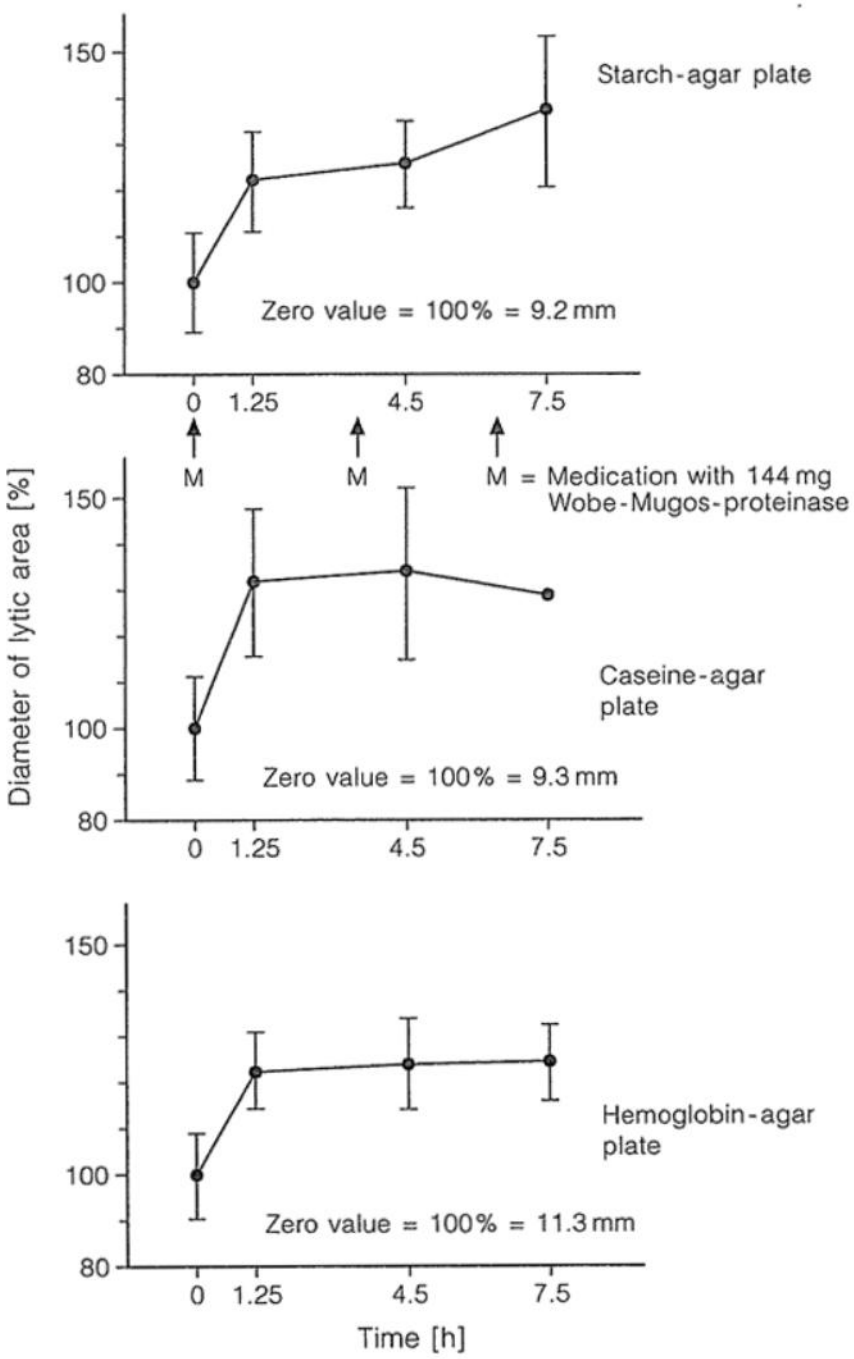

Fig. 9. Changes in hydrolytic serum activity after repeated intake of four tablets of Wobemugos with various test substrates

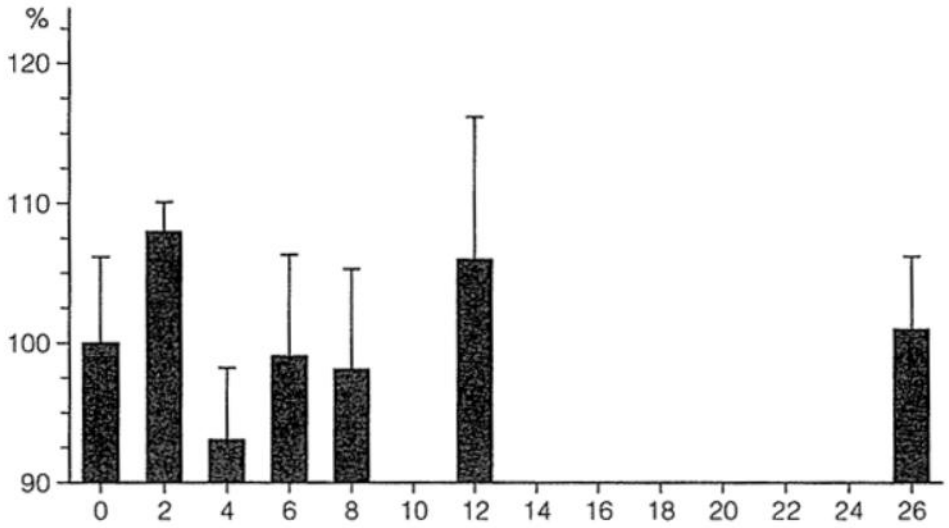

Fig. 10. Changes in hydrolytic serum activity based on the zero value in healthy study subjects after intake of 12.5 g Wobenzym hydrolase mixture

Kleine and Van Schaik (unpublished) examined changes in blood inhibitor titers to obtain information on the humoral interactions of orally administered enzyme and whether these effects can be used as a measurement of absorption processes. Subjects were healthy volunteers who had their last meal 12 h before the start of the experiment, and who for the first 14 h of the trial received nothing to eat. Eight

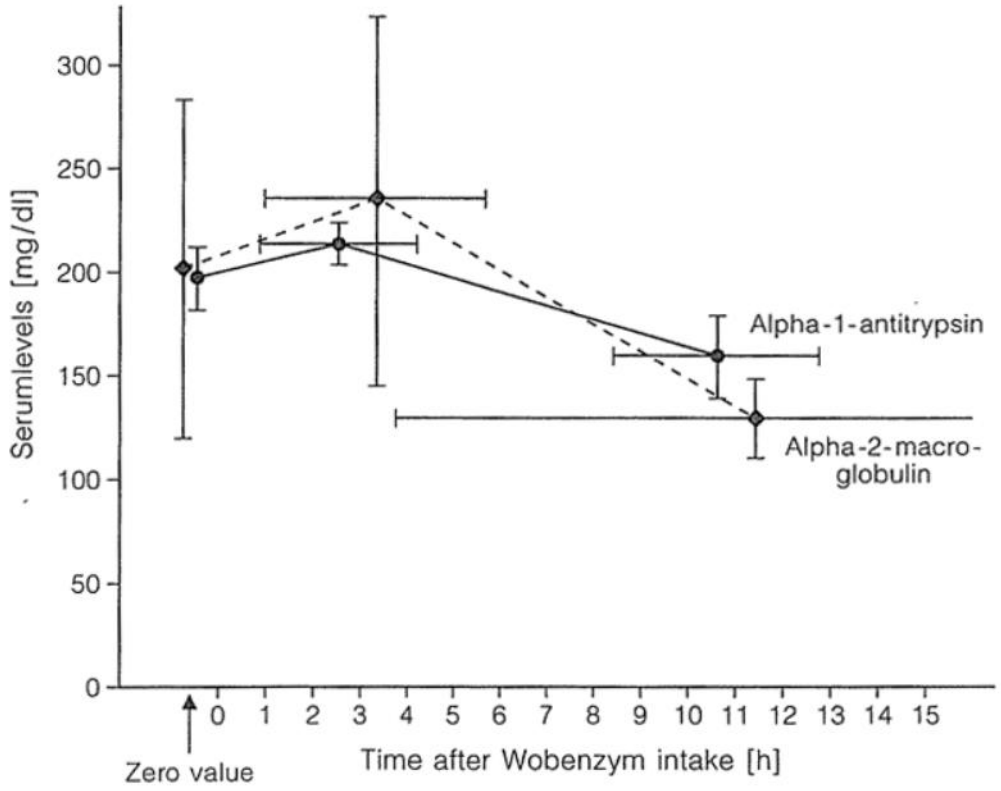

Fig. 11. The maximal and minimal α_1-antitrypsin and α_2-macroglobulin levels in healthy subjects who have taken 12.5 g Wobenzyme hydrolase mixture

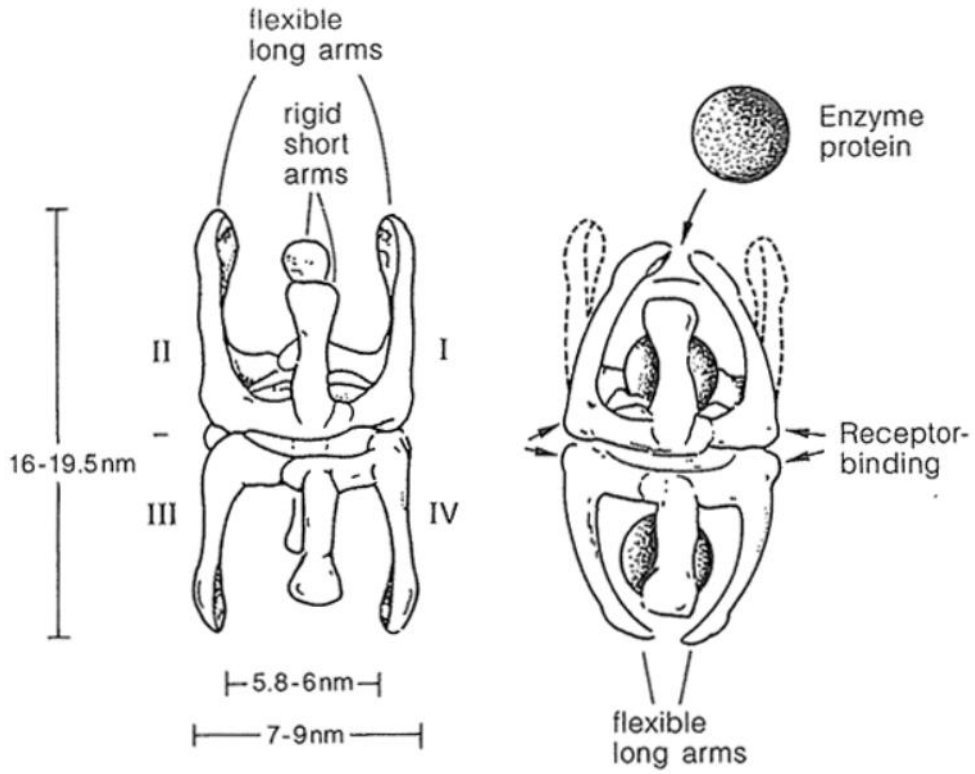

Fig. 12. The structural changes in the α_2-macroglobulin molecule are identical through the binding of one or two enzyme molecules. In both cases the „fast" form is created from the slowly eliminated form relatively quickly, phagocytosed by the cells of the mononuclear phagocytic system

cubital blood samples were taken from both arms just before swallowing 12.5 g active substance of an enzyme combination drug and at 2-h intervals thereafter. Proteolytic serum activity and α_1-antitrypsin and α_2-macroglobulin titers were measured by different plate test systems. With regard to the minima and maxima there were typical curves: (a) The protelytic activity increased initially and reached a maximum 2 h after the beginning. Thereafter it declined to a minimum 4–5 h after medication and increased again to a maximum 8 h after the beginning of the experiment. Twenty-four hours after intake the proteolytic serum levels were normal (Fig. 10). (b) In contrast to the hydrolytic activity the titers of α_1-antitrypsin and α_2-macroglobulin were initially increased (Fig. 11). Regarding the highest and lowest values in individual curves α_1-antitrypsin reached its maximum at 10% above baseline 2.5 h after the beginning. The maximum of α_2-macroglobulin at 20% above baseline was reached 1 h later. Its minimum was reached about 8 h after the maximum. The minimum was about 25% lower than the maximum for α_1-antitrypsin and about 40 lower for α_2-macroglobulin. (c) For α_1-antitrypsin there is a 1:1 relationship to proteolytic enzymes: in α_2-macroglobulin (Fig. 12) the stoichiometry is either 1:2 or 1:1. In both endogenous substances the binding to enzymes accelerates their elimination. That means, probably more than 25% of the substance is absorbed.

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