CLINICAL ENZYMOLOGY

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History

Clinical enzymology was born in 1908 when Wohlgemuth discovered the increase in alpha-amylase activity in serum and urine in acute pancreatitis [1]. Apparently, the time was ripe: the coincidence with Garrod's concept of the 'inborn errors of metabolism' (1909) is conspicuous [2]. However, diagnostic enzymology developed only slowly, despite the discovery of the increase in lipase activity concomitant with amylase activity in serum during acute pancreatitis by Rona in 1915 [3]. Other digestive enzymes were then proposed for diagnostic purposes, mainly in malignancies of the gastrointestinal tract, but they failed to meet the expectations of them. The next step occurred about 20 years later, when Robinson [4] and Kay [5] introduced the determination of alkaline phosphatases in serum for the diagnosis of bone and liver diseases. Again ten years later the clinical value of acid phosphatases in serum was first appreciated by the Gutman's in bone metastases from carcinoma of the prostate [6].

At that time the essential methods for the routine use of enzyme determinations in the serum existed, namely, the introduction of photometers into clinical laboratories by Heilmeyer [7], and the invention of the optical test by Warburg [8]. Further slow progress may have been partially due to the environmental circumstances which impeded investigation and interrupted the communication between scientists of different countries. Otherwise it is hardly conceivable that the significance of Warburg's and Christian's discovery in 1943 [9] of the elevation of glycolytic enzymes in the serum of tumor-bearing animals would have needed five years and more before it dawned upon clinical medicine. The systematic work of Sibley and Lehninger [10], first published in 1949, extended the data published by Warburg and his co-workers and stimulated investigations in many places.

The definitive break-through took place simultaneously in the USA and in Italy with the publications on transaminases by Karmen, Wroblewski and La Due [11], and De Ritis and his co-workers [12], respectively. The obvious improvement in the diagnosis of myocardial infarction and acute viral hepatitis led to persistent endeavours in the investigation of a wide variety of enzymes and their diagnostic value. These in turn resulted in an exponentially growing literature on the subject, which reached rather unmanageable proportions in the late sixties. Out of the bulk of enzymes then on trial, no more than a good dozen emerged as choice diagnostic aids.

Some of the enzymes listed in table 1 are now measured in virtually all hospitals and in many medical offices throughout the world. With others, however, there are considerable regional differences of usage: ICDH [13] for example, seems to be an enzyme of Anglo-Saxon interest, OCT [14] has hardly passed over the borders of the Scandinavian countries and GLDH [15] is virtually confined to laboratories in Austria and Germany.

On the whole, enzyme determinations in serum have attained an assured place in clinical practice, and much empirical knowledge on their significance has accumulated.

Pathophysiology

In contrast to the practical significance of enzyme levels in the plasma, the present knowledge of how they come about is still relatively fragmentary. A given enzyme activity in the plasma can be considered as the result of the rates of inflow, distribution and

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Table 1

Name	EC Number	Abbreviation
Acid phosphatase	3.1.3.2	SP
Alanine aminotransferase	2.6.1.2	GPT
Aldolase	4.1.2.13	ALD
Alkaline phosphatase	3.1.3.1	AP
α-Amylase	3.2.1.1	Amylase
Aspartate aminotransferase	2.6.1.1	GOT
Cholinesterase	3.1.1.8	CHE
Creatine kinase	2.7.3.2	CPK
Glutamate dehydrogenase ($NAD(P)^+$)	1.4.1.3	GLDH
γ-Glutamyl transferase	2.3.2.2	γ-GT
Isocitrate dehydrogenase ($NADP^+$)	1.1.1.42	ICDH
Lactate dehydrogenase	1.1.1.27	LDH
L-Leucin arylamidase	3.4.1.–	LAP
5-Nucleotidase	3.1.3.5	5-N
Ornithin carbamoyltransferase	2.1.3.3	OCT
Triacylglycerol lipase	3.1.1.3	Lipase

outflow, as outlined in the simplified diagram on the fate of cellular enzymes in the extracellular space (fig.1).

The starting-point in this process is the concentration of cellular enzymes within their cells of origin, which vary to a considerable extent, according to the type of cells and their metabolic functions [16–18]. The cellular enzyme patterns, well known for the majority of tissues, are fairly constant in normal circumstances, though affected even physiologically by developmental, nutritional, hormonal and other factors [19,20]. Under pathological conditions, additional variations occur by disturbances and reactive changes in the intracellular protein turnover [21].

The enzyme pattern in the serum depends primarily on the concentration gradient between the intra- and extracellular space [22].

The concentration gradients of the enzymes

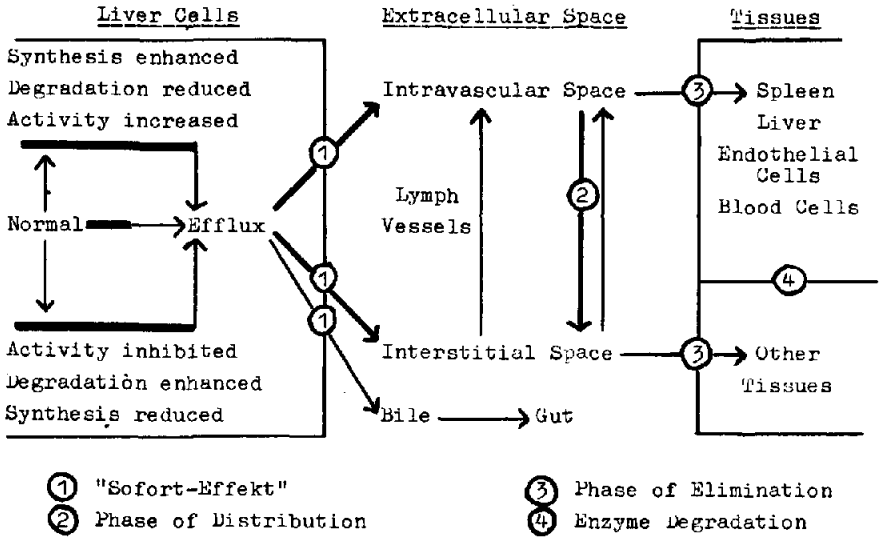


Fig.1. Scheme of enzyme release, distribution and elimination.

between the cells and their environment are so steep that their maintenance requires a considerable portion of the energy-yield of the cell. The complex structure of cellular membranes, serving the opposing functions of the preservation of cellular integrity on the one hand, and of the continuous orderly exchange of a variety of substances on the other, are therefore very vulnerable. It is well-known that the release of potassium and other low molecular weight cell constituents occurs at an early stage of cellular injury. It came as a surprise, however, that simultaneously high molecular weight cellular enzymes likewise begin to leak out. The first investigators of enzyme leakage, e.g. Warburg [23] or Zierler [24] showed this clearly by their experiments with cell suspensions and isolated muscle.

Despite this evidence a tedious discussion lasted for years concerning whether enzyme elevations in the serum must be considered as always being indicative of cellular necrosis. Just the contrary could be learned from clinical experience, e.g. in acute hepatitis or in right heart failure. Moreover, one glance at the pathological enzyme patterns in the serum reveals that they very seldom reflect exactly the pattern of the afflicted tissue, but are distorted by various factors [22,25–29].

Some of these factors have been elucidated, mainly by studies on the isolated perfused liver. This model offers several advantages; the anatomical integrity of the organ is maintained, and many functional properties are known to persist for an adequate time; and the experimental conditions can be controlled and varied without disturbance form the reactions of the whole body. The significance of the intracellular

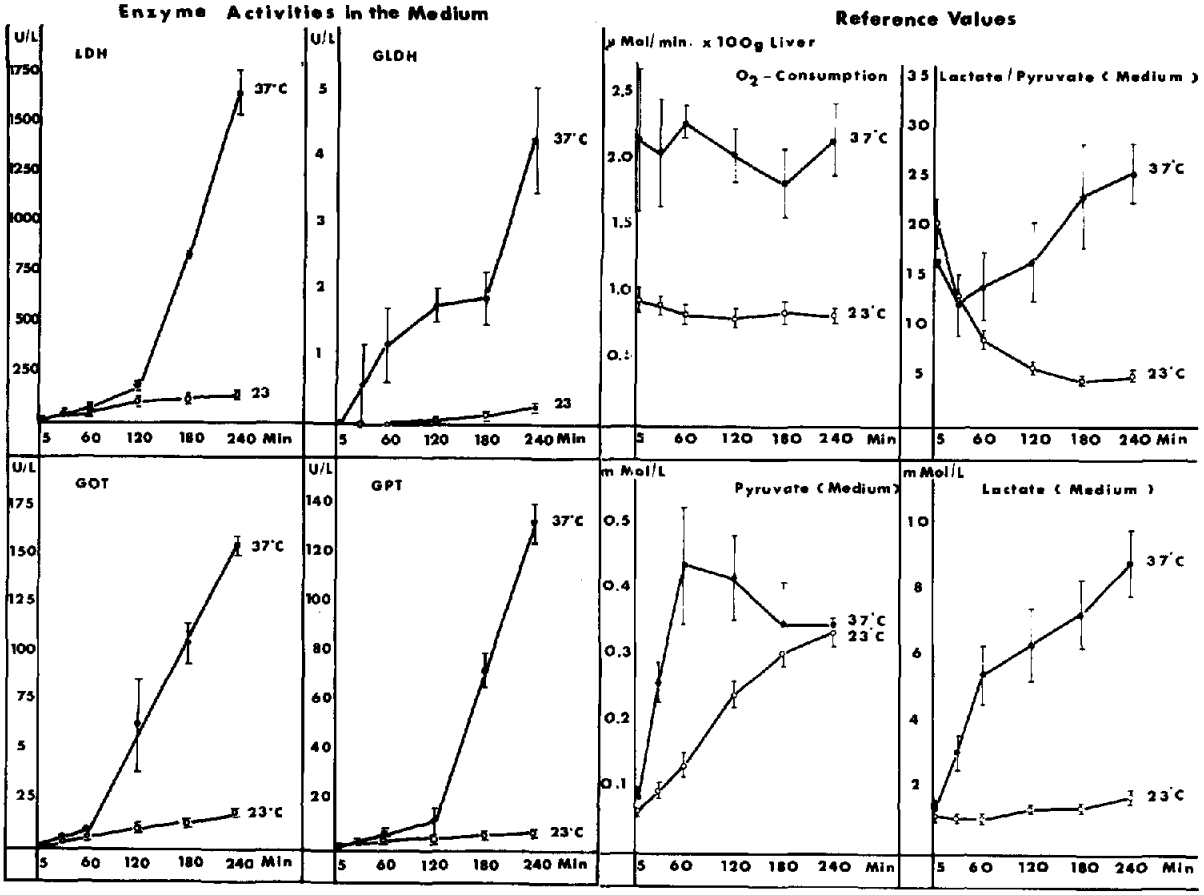


Fig.2. Effect of temperature. Fed rats: flow-constant perfusion; (○), 23°C (n = 10); (●) 37°C (n = 2).

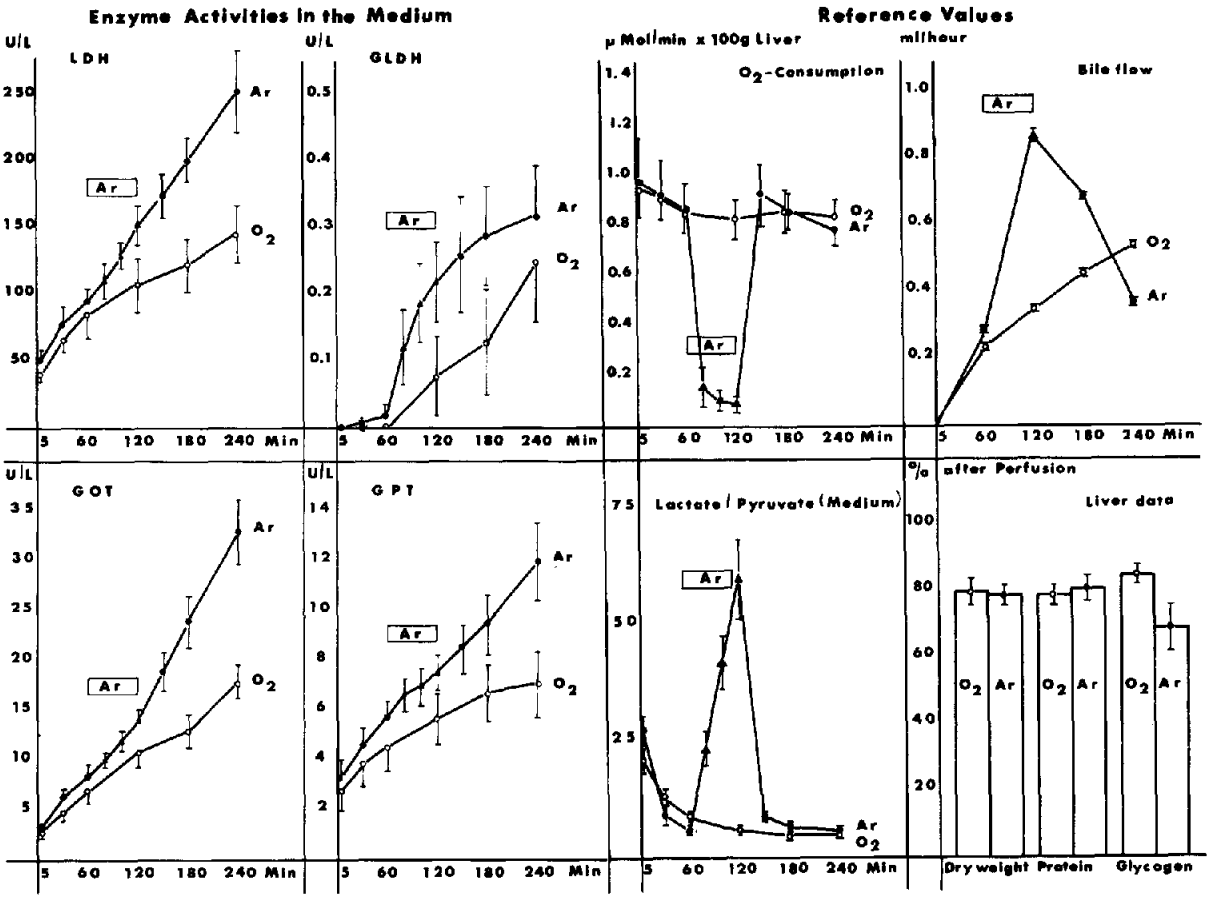


Fig.3. Effect of anoxia during perfusion. Fed rats: 23°C; (○), 4 h O_2 (n = 10); (○-▲), 1 h O_2 – 1 h Ar – 2 h O_2 (n = 4).

localisation of enzymes could be demonstrated: the leakage of mitochondrial enzymes and isoenzymes is delayed and is much less than that of the cytosolic ones [26,30–33]. It ceases again with about one third of these enzymes still in place, when the disintegration of the dead cells begins, whereas the efflux of cytosolic enzymes goes on up to equilibrium with the environment [34]. Comparison with morphology shows that the release of these mitochondrial proteins is in fact a sympton of necrobiosis. Thus the relation of cytosolic and mitochondrial enzymes in the serum can be used diagnostically for the estimation of the severity of single cell damage, independent of the absolute enzyme levels which are a measure of the number of cells involved and thus of the size of the damaged area of tissue [22,35]. The molecular weight, which also affects the release rate of enzymes, is of minor diagnostic importance; only insofar as for a given pathological state the release rate of small enzymes is higher than that of the large molecules. So the former are more sensitive indicators of cellular injury in general than the latter [30]. The various causes of the 'leakiness' of the cells result in three major deficiencies:

(1) Disproportion between requirement and supply of oxygen.

(2) Disproportion between requirement and supply of metabolizable substrates.

(3) Incapability of the cell to meet its requirements of energy by utilizing the supply of oxygen and substrates.

Fig.2 demonstrates how relative low oxygenation due to an increased requirement with elevation of temperature leads to an augmented release of enzymes from

Table 2
Effect of the nutritional state on the isolated rat liver after 4 hours of hemoglobin-free perfusion at 23°C

	Fed rats (n = 10)	Fasted rats (n = 6)
Glycogen (mg/g liver)	44.7 ± 2.7	0.41 ± 0.18
Lactate/pyruvate (liver)	4.2 ± 0.8	4.3 ± 0.5
ATP/ADP (liver)	4.06 ± 1.09	4.03 ± 1.15
Oxygen consumption (μmol/min × 100 g liver)	0.84 ± 0.23	0.89 ± 0.27
Glucose (perfusion medium) (mmol/l)	6.6 ± 0.6	5.9 ± 0.28

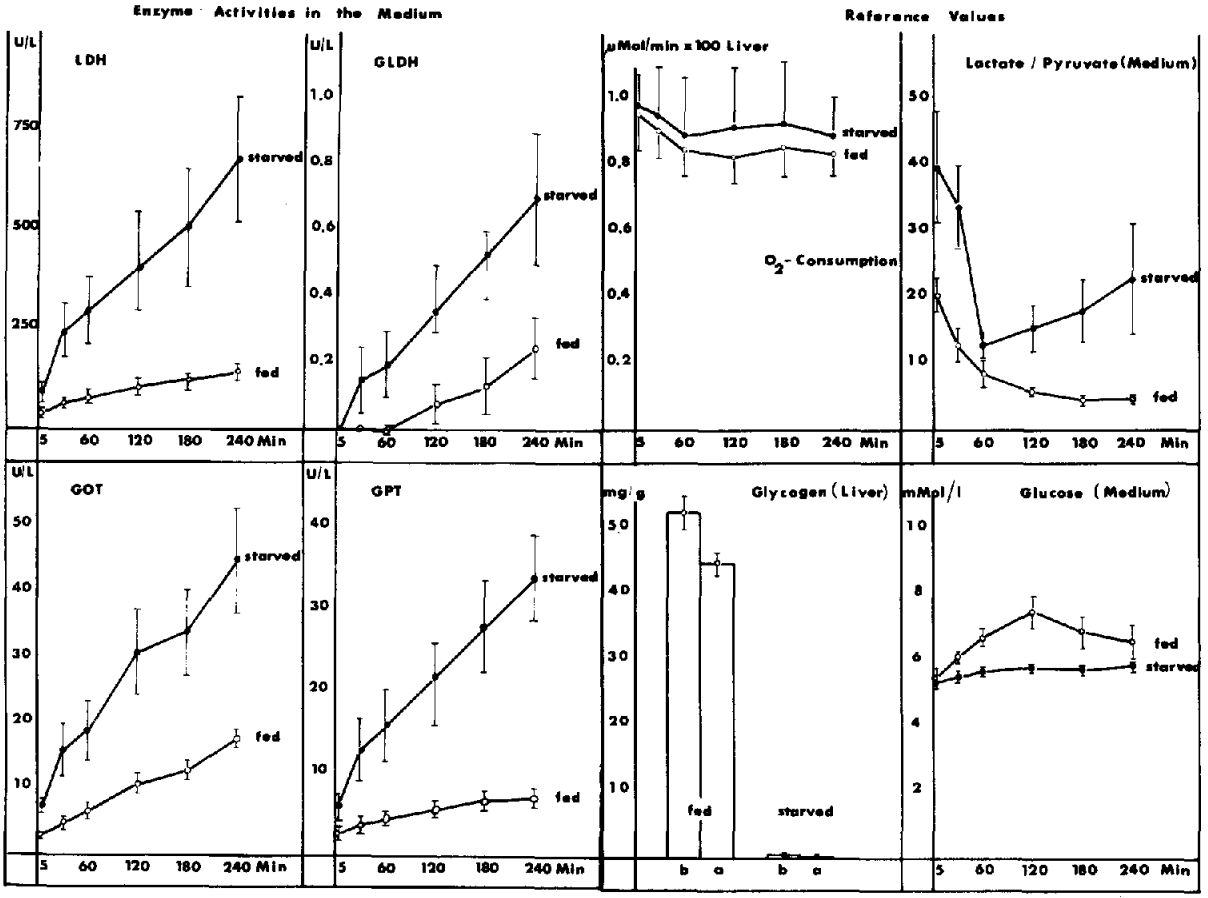


Fig.4. Effect of the nutritional state. Flow-constant: 23°C; (○), fed rats (n = 10); (●), ⩾ 15 starved rats (n = 6).

the perfused liver. This occurs immediately with mitochondrial (and acinocentral) GLDH, later with partly mitochondrial GOT, and even more delayed with cytosolic GPT and LDH. Though oxygen consumption is twice as high at 37°C as it is at 23°C, the relative oxygen shortage can clearly be read from the continually increasing lactate/pyruvate ratio [36]. Fig.3 shows that temporary absolute lack of oxygen also leads to a steep rise in enzyme activities in the perfusion medium, which persists (with the exception of GLDH) after the restitution of oxygen supply.

The significance of the nutritional state of the cells is shown in fig.4. The release rates from the livers of fasted rats are between 2.5-fold and 5-fold higher than from fed rats, though oxygen consumption is similar in both conditions, and extracellular glucose levels are very well maintained by the glycogen-depleted livers. The same is true for the lactate/pyruvate and the ATP/ADP ratios in the liver [36] (table 2). The histological appearance of the liver tissue is likewise virtually normal. These results among many others show that the release of cellular enzymes is one of the most sensitive parameters for the recognition of minute disturbances of cellular integrity.

When increased permeability has been produced previously by a variety of causes, enzyme leakage from the perfused liver was considerably aggravated (fig.5) [36]. In comparing the different types of injury not only quantitative variations can be noticed, but there are also conspicuous qualitative differences of the enzyme patterns in the perfusion medium. These can be attributed on the one hand to different involvement of subcellular structures with the different toxic substances, thus proving again the importance of the intracellular localization of enzymes (fig.6) [36]. On the other hand, the heterogeneity of

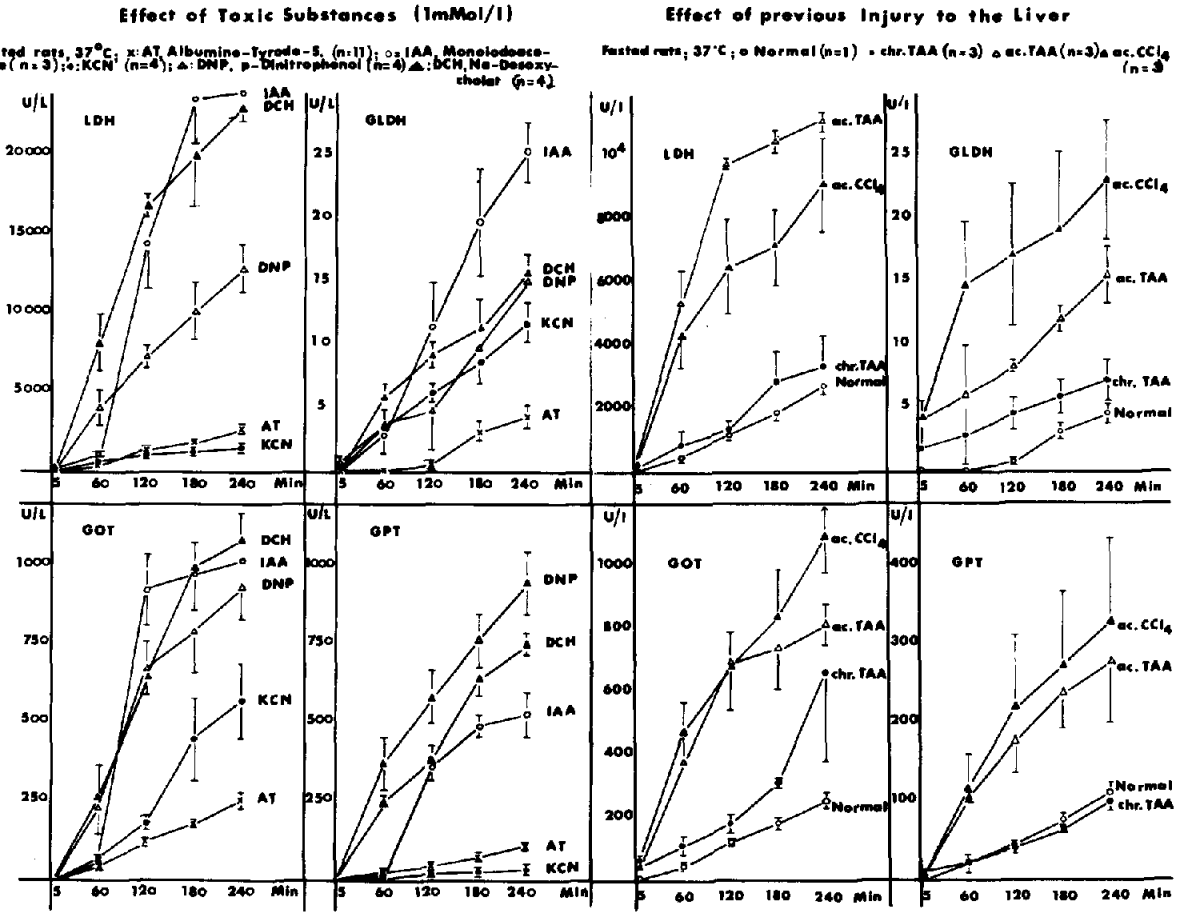


Fig.5. Enzyme activities in the medium.

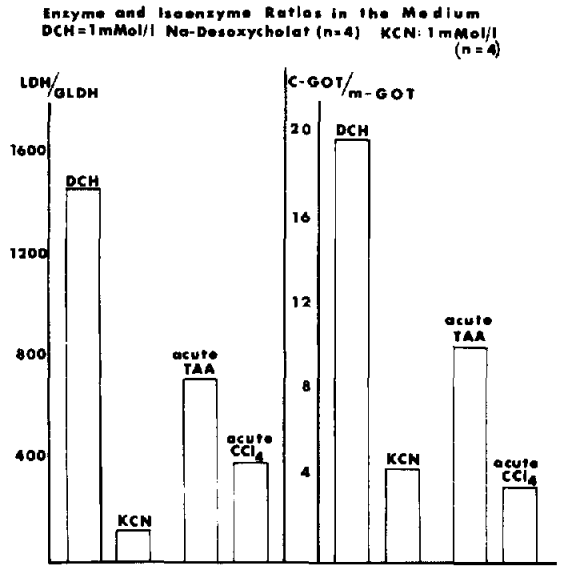


Fig.6. 'Membrane lesion' vs. 'necrobiosis'.

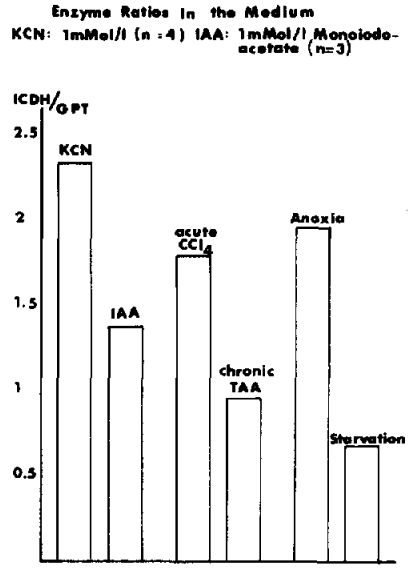


Fig.7. Central vs. peripheral or diffuse damage.

enzyme distribution throughout the hepatic lobule affects the release rates in proportion to the cell damage in the periphery or in the central area of the lobule (fig.7). This also shows the different patterns with lack of oxygen or glycogen depletion [36]. Both connections can be seen either histologically or deduced from the sites of action of the injurious agents.

Although at present the mechanisms of enzyme release are still unknown, the complexity of conditions and processes involved is beyond doubt. Subtle diagnostic enzymology presupposes some insight into this subject. After being released, cellular enzymes enter body fluids. Viewed from this aspect, the releasing cells can be divided into five groups: cells with direct contact to the intravascular space (e.g. blood cells), cells with direct contact to the intravascular and the interstitial space (e.g. spleen cells), cells with direct contact to the interstitial space only (e.g. muscle cells), cells with direct contact to the interstitial and the transcellular space (e.g. renal tubular cells) and finally, cells with direct contact to all three compartments (e.g. liver cells, which therefore have been chosen as an example in fig.1). Those cellular enzymes which are released into the transcellular space (urine, bile, intestinal contents, etc.) emerging from the cells on the inner and outer surfaces of the body, can be left out from further consideration because they are lost as regards measurement in serum. For the remaining portion, the initial course is different but finally they become distributed all over the extracellular space. This distribution occurs slightly faster from the intravascular to the interstitial space, than in the reverse direction via the lymphatic vessels. Nevertheless, in both ways distribution is rapid. It accounts for the first rapid phase of the well-known biphasic disappearance of injected enzymes (fig.8) [37,38,52,56]. The investigation of

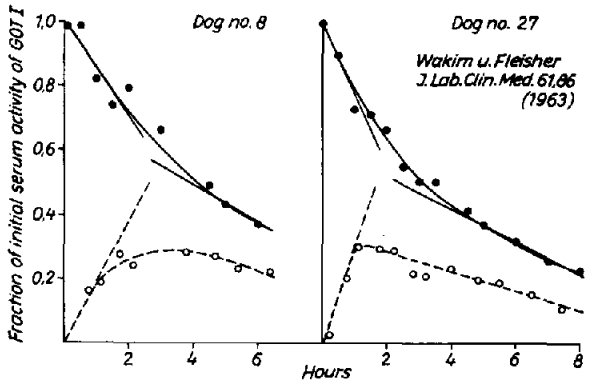


Fig.8. Fate of enzymes in body fluids. (●—●) Activity in serum. (○—○) Activity in lymph.

enzyme elimination from the circulation began early with the application of tissue extracts and more or less purified enzymes [38,41]. Here the fundamental work of Fleisher and Wakim [37,42–44,56] must be mentioned. They studied the rates of disappearance of transaminases and showed the great difference between GPT and GOT, and between the cytosolic and the mitochondrial isoenzyme of the latter. Additionally they tried to find out about the site of the elimination of circulating enzymes by a series of considered experiments. However, the question of whether enzymes gradually loose their activity, thus escaping detection and only simulating disappearance, or whether their molecules are in fact removed from the circulation, remained open.

The problem could be solved by the use of radioactively labelled enzymes which would remain traceable after inactivation and even after resumption into cells or excretion. With regard to in vivo experiments labelling of ε-amino groups with [^{14}C]acetate [45,46] was found to be more suitable than labelling by ^{131}iodine [47–51]. Not only is the yield of catalytically active enzyme after labelling many times better, but, aside from the expected minor alteration of the electrophoretic mobility, kinetic and molecular properties remain unaltered; further, the stability is excellent in vitro as well as in vivo. The latter, especially, is an indispensable quality in injection experiments [30].

Many of the conclusions of Fleisher and Wakim and others who worked with unlabelled enzymes could be confirmed. In addition, proof was furnished that enzymes are eliminated in a catalytically active form; after distribution, the catalytic activity and the radioactivity of an injected enzyme decrease in parallel. This is true in serum as well as in lymph, and it demonstrates that elimination takes place from both the intravascular and the interstitial space (fig.9) [52].

The half-lives of cellular enzymes in the circulation seem to have molecule-specific and species-specific properties. This was shown in animals as well as in peracutely ill patients. The half-life of an individual enzyme is also independent of sex, age, most disease states and its actual level in the plasma [53]. Recently, however, evidence has been reported that very high doses of some drugs may have an effect on the half-life [54].

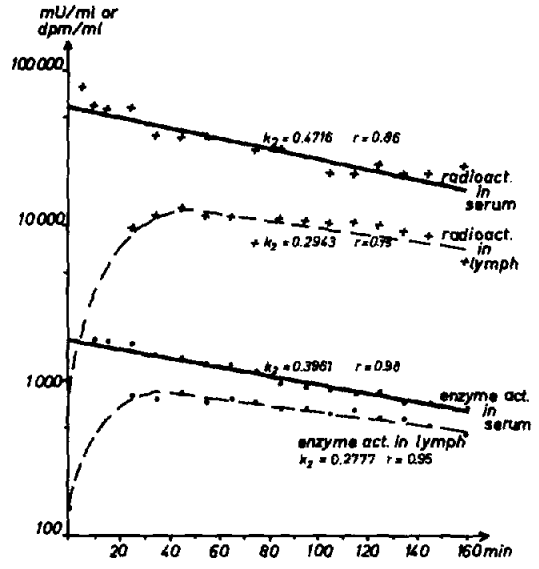


Fig.9. Radioactivity and enzyme activity in serum and lymph in the dog (H_{54}) after i.v. injection of [^{14}C]LDH_5.

There is no correlation between half-life and molecular weight, as can be read from the very different disappearance rates of the LDH-isoenzymes which have virtually the same molecular weight (fig.10) [54,55]. However, the half-lives may depend, at least partially, on the charge of the enzyme molecules. Substitution of the side chains of LDH-5 with polar groups results in a shorter half-life, whereas substitution with non-polar groups leaves it unaltered or retards elimination. (fig.11) [54,55].

The site of final excretion or elimination of circulating enzymes has been under investigation and discussion for more than 20 years. Former studies have shown, that neither the removal of liver or spleen nor of the kidneys or the intestinal tract affects the elimination rates [38–40,42,56,57]. It was then believed that enzyme elimination might be a function of the reticulo-endothelial system on the basis of indirect evidence before the era of labelled enzymes [37,42,44,58]. With ^{14}C-acetylated LDH it was found that not the reticulo-endothelial system but the parenchymal cells of most tissues are able to incorporate circulating enzyme molecules. The rate of incorporation was shown to be higher in organs with a rapid protein turnover than in organs with a less active protein metabolism [52].

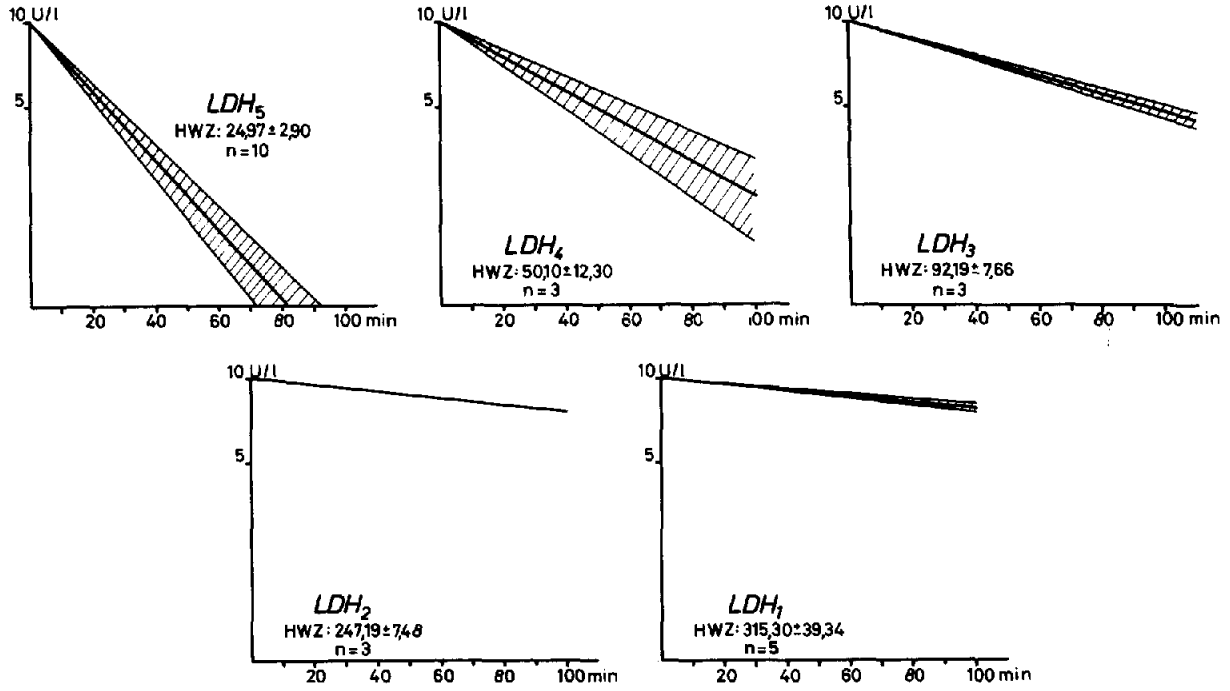


Fig.10. Half-life of LDH isoenzymes in the circulation of the rat.

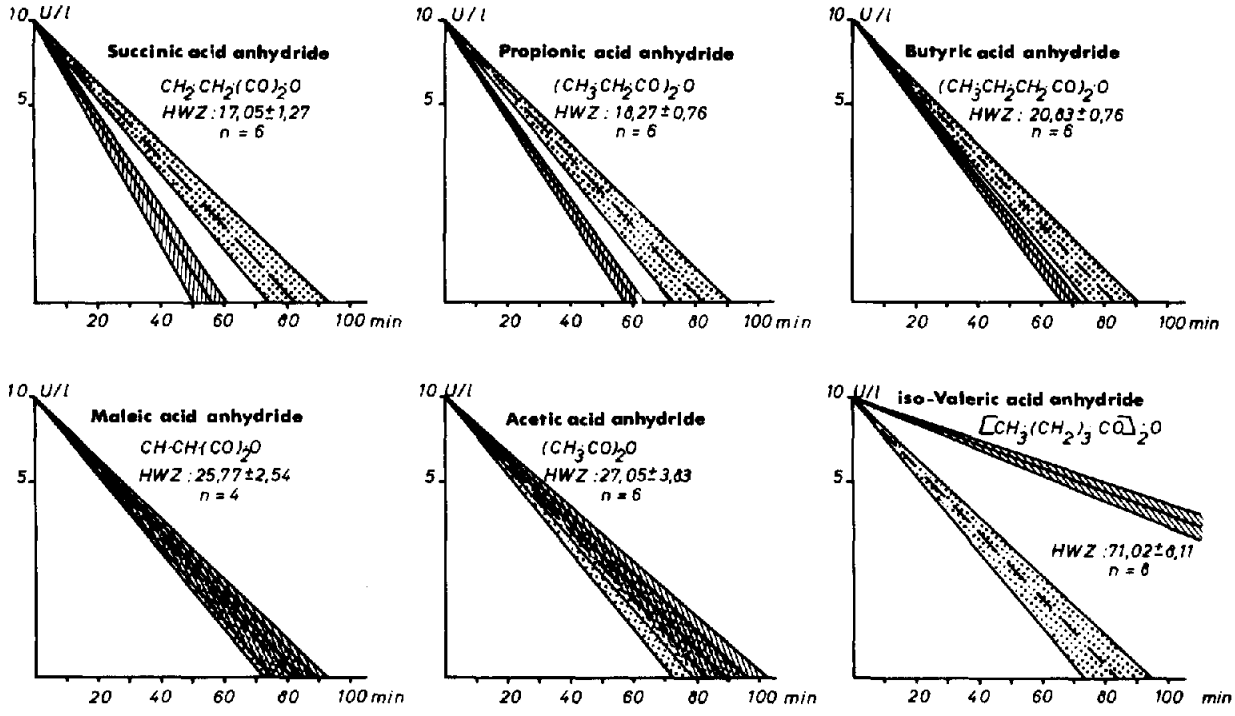


Fig.11. Effect of acylation of the ε-amino groups by different compounds on the half-life of LDH_{M4} in the circulation of the rat.

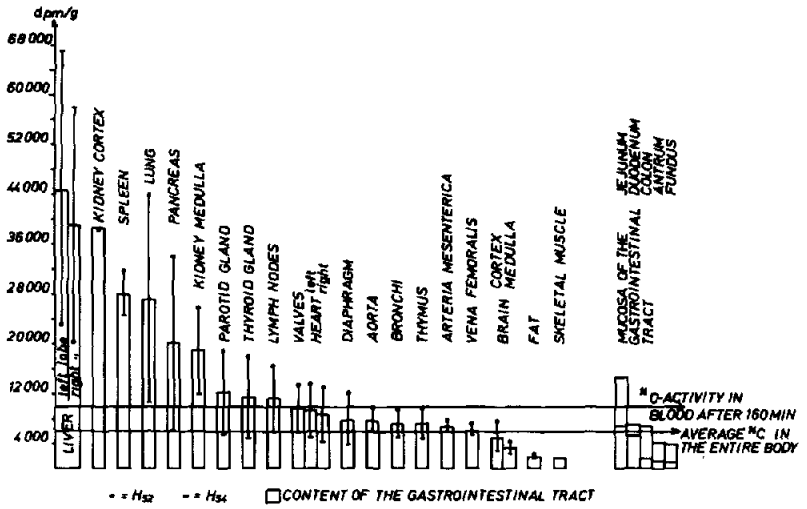


Fig.12. Concentration of radioactivity in dog organs 160 min after i.v. injection of [^{14}C]LDH$_5$ (dpm/g organ).

Fig.12 shows the concentration of radioactivity in various tissues after injection of labelled LDH-5 as compared to its concentration in the blood and to the average body contents. The sequence of contributory organs is different when the size of the organ is accounted for and the respective radioactivities are expressed a percentage of the original dose applied (fig.13). It came as a surprise that the cells of the reticulo-endothelial and the lymphatic system, e.g. the Kupffer cells in the liver or the lymphocytes are not accentuated in autoradiography, but the parenchyma of the organs and among blood cells the granular leucocytes only are accentuated [52].

A considerable portion of the injected radioactivity, but virtually no enzyme activity is found in urine and bile within the first 8 hours after injection of LDH. It can be concluded from experiments in dogs and rats that these findings in urine and bile are attributable to non-dialyzable split-products of the enzyme molecule after partial degradation in the littoral parenchymal cells of urinary and biliary tract, which exhibit a considerable accumulation of

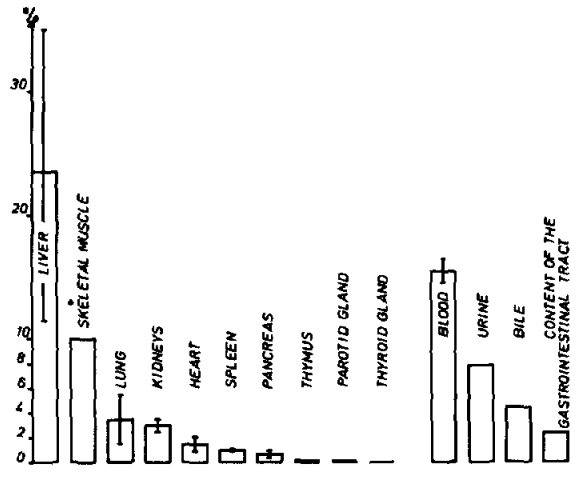


Fig.13. Radioactivity distribution after 160 min in organs and body fluids in dogs; expressed in percent of injected dose (H_{52} and H_{54}).

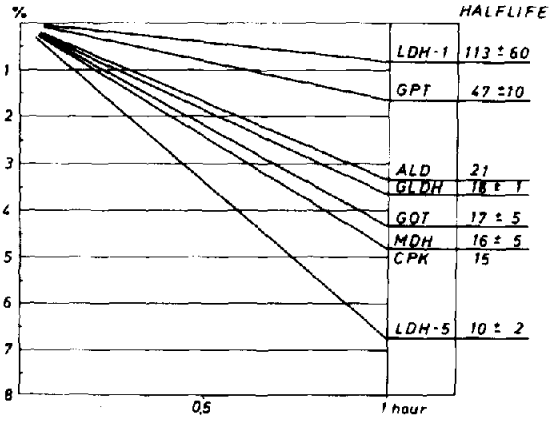
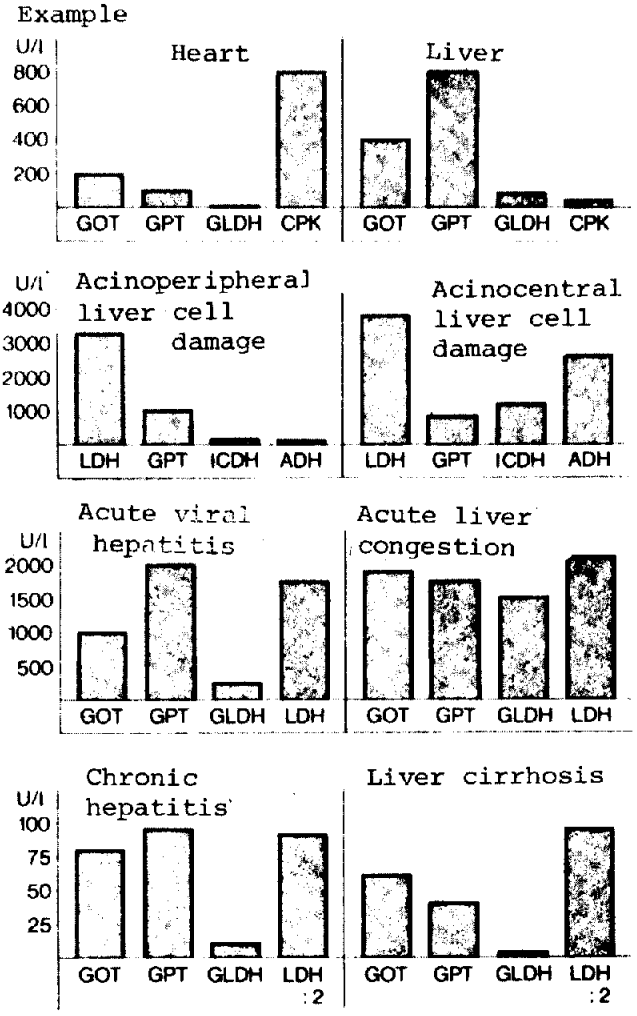


Fig.14. Enzyme elimination in human: plasma disappearance rates.

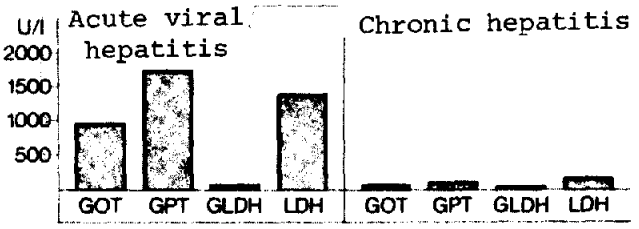
radioactivity in autoradiographic pictures [52]. Even low molecular weight enzymes, e.g. amylase, are eliminated from the circulation by the kidneys into the urine in the catalytically active form only in small proportions. Most enzyme activities in bile and urine, originate from the littoral cells themselves and not from the plasma [38,39,59–61]. The ideas about enzyme elimination are still fragmentary [62]. Nevertheless, the well-defined different half-lives of enzymes in the plasma are of major diagnostic significance (fig.14) [53].

Considering an individual enzyme, the constancy of its disappearance rate allows one to assume that modifications of its actual level in the plasma reflect only the alterations of inflow. Diagnostic enzymology in general is founded on this assumption. Considering enzyme patterns in the serum, the different half-lives of the respective enzymes contribute a great deal to the distortion of the original tissue pattern.

For the interpretation of enzyme patterns under diagnostic aspects the analysis of the distortion is just as weighty as e.g. the notion of the tissue patterns, the normal values in the serum, or the reliability of the methods. The use, which can be made of the understanding of the prerequisites and processes forming enzyme patterns in the serum is outlined and exemplified in fig.15 [35].

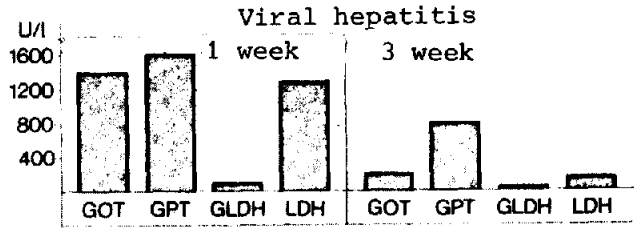


Basis	Application in serum
Enzyme patterns of tissues	Recognition of the organ of origin by organ-specific ratios of enzymes
Distribution of enzymes in the tissues	Recognition of the localization of cell damage by the ratios of enzymes with different distribution in the tissue
Intracellular localization of enzymes	Recognition of the severity of single cell damage by the ratios between cytosolic and mitochondrial enzymes
Alteration of the enzyme patterns of the organs with protracted cellular damage	Recognition of the stage of a chronic disease by the ratios of enzymes with altered activity to enzymes with unaltered activity in the tissue



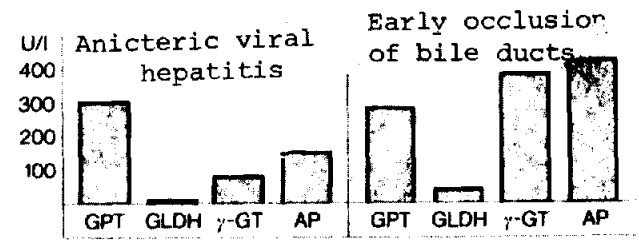
Release rates of enzymes

Recognition of the extent and the acuteness of cell damage in a distinct organ by the height of enzymes



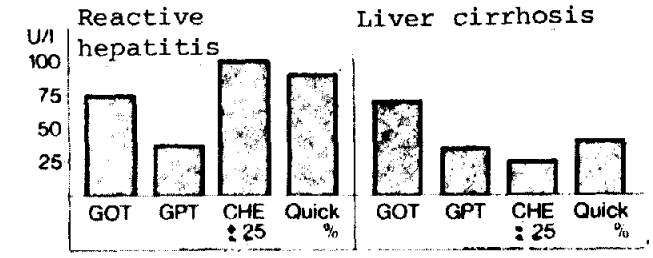
Half life of enzymes in the extracellular space

Recognition of the stage of an acute illness by the ratios of short-living to long-living enzymes



Reactively enhanced synthesis of membrane-bound "cholestasis-indicating" enzymes concomitant with disturbed cell permeability

Recognition of the degree of cholestasis and of reactive changes by the ratios between "cholestasis-indicating" enzymes and other cell enzymes



Impairment of the synthesis of secreted enzymes

Recognition of the reduction of the functional liver mass by the decrease of plasma-specific enzymes

Fig.15. Application of pathophysiology to enzyme diagnostics in serum.

Diagnostic application

Any diagnostic method is expected to indicate, reliably, sensitively and specifically, deviations from the normal state at a negligible risk to the patient and demanding as little as possible of him. These demands are largely met by enzyme determinations in the serum. The reliability, sensitivity and specificity of the respective methods are fair to excellent, and the patients are only minimally inconvenienced. From the diagnostic point of view enzyme leakage is in itself a very sensitive sign of disorder. The range of pathological changes compatible with life is far greater than with other clinico-chemical parameters. A hundred times normal levels are not uncommon and even the thousand-fold has been found (fig.16). Thus, possible gradations between the extremes and the normal are unusually fine, giving much facility to differentiation.

The diagnostic specificity of the alterations of enzyme activities in the serum is, however, much less, at least at first sight. Among the enzymes used in practice, there are very few organ specific ones and no disease-specific ones at all.

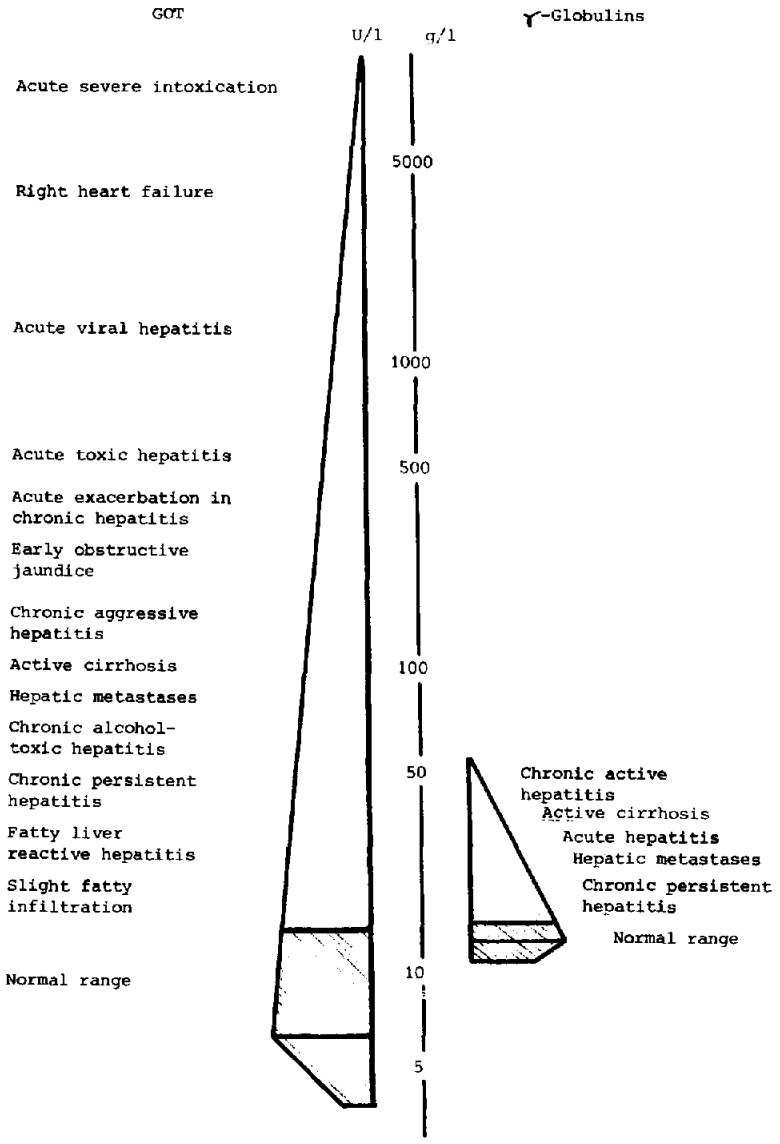


Fig.16. Degree of elevation of cellular enzymes in serum as compared to that of other plasma constituents, exemplified by GOT activity vs. γ-globulin concentration in liver disease.

The diagnostic specificity can be greatly enhanced yet by benefiting from the notions of physiology and pathophysiology of enzymes at hand [12,22,63–65].

From the enzyme patterns of tissues the 'leading enzymes' for the recognition of the affected organ can be derived [65]. As can be read from table 3 scarcely one single enzyme is sufficient, but in many cases advantage must be taken from the different

Table 3

'Leading enzymes' for organ diagnosis

Liver and biliary system	GPT, GLDH, γ-GT, CHE, (AP)
Heart	CPK, GOT, LDH (Isoenzyme 1)
Skeletal muscle	CPK, ALD, (GOT)
Bone	AP, SP
Blood	LDH
Pancreas	Amylase, Lipase
Prostate	SP (tartrate inhibitable)

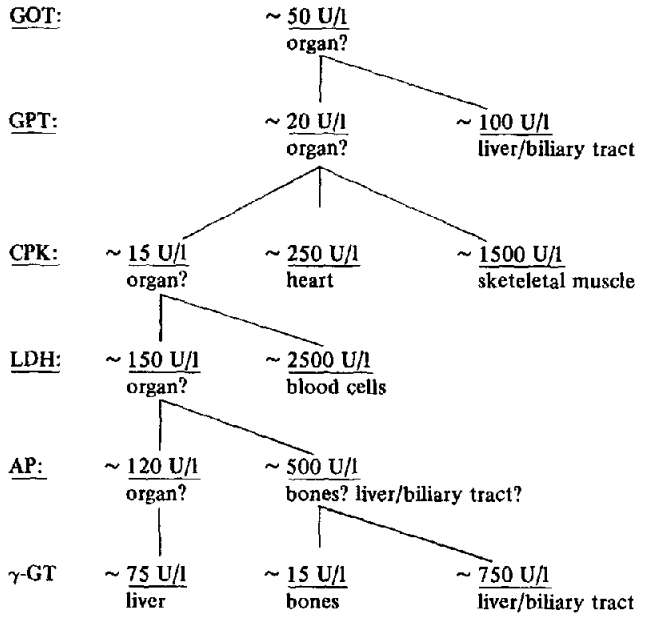


Fig.17. Enzyme ratios in serum for the diagnosis of the injured organ.

ratios of more or less unspecific or only group-specific enzymes and isoenzymes in the various organ systems.

Fig.17 gives an example how to reach organ differentiation, proceeding from the assumption that the activity of GOT in the serum is only about three times elevated, which is a rather ambiguous value. In reality, organ diagnosis is by no means the main object of enzyme determinations in the serum; it cannot be, because of the limitations of their applicability. They are rather useless in diseases of those organs, which are not listed on table 3, e.g. diseases of the kidneys, the respiratory tract, the endocrine glands, etc. [66–72]. In the diseases of these organ systems enzyme alterations in the serum also occur sometimes. But only the secondary affliction of organs, which are accessible for diagnostic enzymology in general, are thus manifested. This is especially true in malignancies: they exhibit characteristic enzyme patterns in the serum only in the case of metastases in the liver or the skeletal system. Even carcinoma of the prostate cannot be reliably detected by the estimation of the prostatic isoenzyme of acid phosphatase before the tumor has penetrated the capsule of the gland [73]. This is, however, partly due to unsatisfactory assay methods.

As for other small organs, e.g. heart or pancreas, only acute cellular damage leads to distinct elevations of enzymes in the serum. This confines the application of enzyme determinations virtually to myocardial infarction on the one hand and acute pancreatitis or the acute relapses of chronic pancreatitis on the other. Among the disorders of the hemopoetic system, it is primarily untreated pernicious anemia with its extremely high LDH activity, which is a rewarding object of enzyme diagnosis in the serum. With the diseases of skeletal muscles and bones elevated enzyme activities may arouse suspicion of an illness or can serve to find carriers of hereditary disorders, but they don't contribute much to differential diagnosis [66–72].

On the other hand, in nearly all diseases mentioned, periodical enzyme measurements for the follow-up of the course, for the evaluation of desirable and undesirable effects of treatment, peculiarly of drugs, and for the early detection of complications involving other organs, can be carried out easily and advantageously [74].

Up to now, diagnostic enzymology flourishes most in hepatology. Here, several favorable prerequisites coincide: the size of the organ, the absence of a

basal membrane around the hepatic sinusoids, and the multifarious enzyme equipment of the parenchymal cells. The former two qualities account for the sensitivity by which even minute hepatocellular damage is indicated by the elevation of cellular enzymes of hepatic origin in the serum. Thus enzyme determinations are most suitable as screening tests for hitherto subclinical hepatic disorders, such as chronic inflammations and toxic injuries, be that alcohol or industrial poisons or drugs with hepatotoxic side-effects. Fig.18 demonstrates the high incidence of abnormal findings with the four 'screening' enzymes in chronic inflammatory diseases of the liver [72,75]. The rich enzyme equipment of the liver permits differential diagnosis to be advanced into considerable details. That is why nearly all the examples in fig.15 are taken from liver pathology. An adequate set of enzymes for differential diagnosis of the disorders of the liver and the biliary tract contains some, whose extracellular increase indicates disturbances of cellular permeability, (e.g. GOT, GPT, GLDH, LDH,) others, whose elevations in the serum indicates not only raised permeability, but enhanced synthesis of these membrane-bound enzymes [76–82]. This cellular reaction is frequently provoked by or combined with cholestasis, which can be evaluated by a concomitant rise of, for example, γ-GT and AP even in the non-jaundiced patient. The third group of enzymes used in hepatology comprises CHE and clotting factors

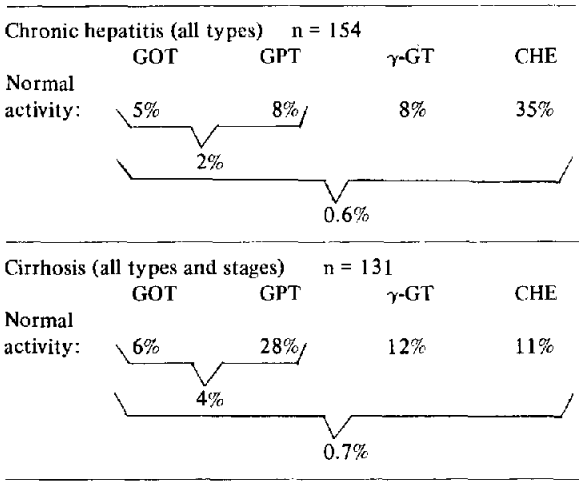


Fig.18. Sensitivity of a small enzyme pattern with chronic liver diseases.

which are synthesized in the liver and secreted into the plasma. The decrease of these plasma-specific enzymes indicates reduction of the synthesizing and secreting capacity or of the number of liver cells or of both [64,68,70,72,74].

By enzyme determinations in the serum in liver disease various functions can thus be examined and information as to the extent, the severity, the stage and the type of cellular damage can be derived by analysing the distortion of the enzyme pattern in the serum (see fig.15) [35,83].

Clinical experience and comparison with other, especially morphological findings have shown characteristic enzyme patterns to belong to distinct liver diseases, their successive stages and their different etiology.

Fig.19 gives an example for the differential-diagnostic procedure starting again with the ambiguous GOT activity of about 50 U/l. Although the given numbers are schematized and deviation may be considerable in individual cases, the diagnostic accuracy is as high as 80–90%. This is not worse than with other more taxing and more risky diagnostic methods.

Present problems and outlook

After the rapid progress of clinical enzymology during the '50s and '60s, recently, a feeling of saturation and even symptoms of fatigue have been noticed in this intermediate field between applied biochemistry and medicine. Its achievements can be considered to be fairly satisfactory only with hepatology.

A predominant concern of today's clinical enzymologists is the improvement of routine and reference assay methods and their standardization on national and international levels.

A second field of present investigation deals likewise with methodology, but rather with its fundamentals. The principle of determination of enzymes by their respective catalytic activities, so dependent upon the conditions of assay – though generally specific and sensitive enough – might be replaced in future by utilizing the antigenic qualities of the enzyme proteins for measurement. At present, assays of this type are in use either for isoenzyme differentiation or for enzymatic analysis of other compounds,

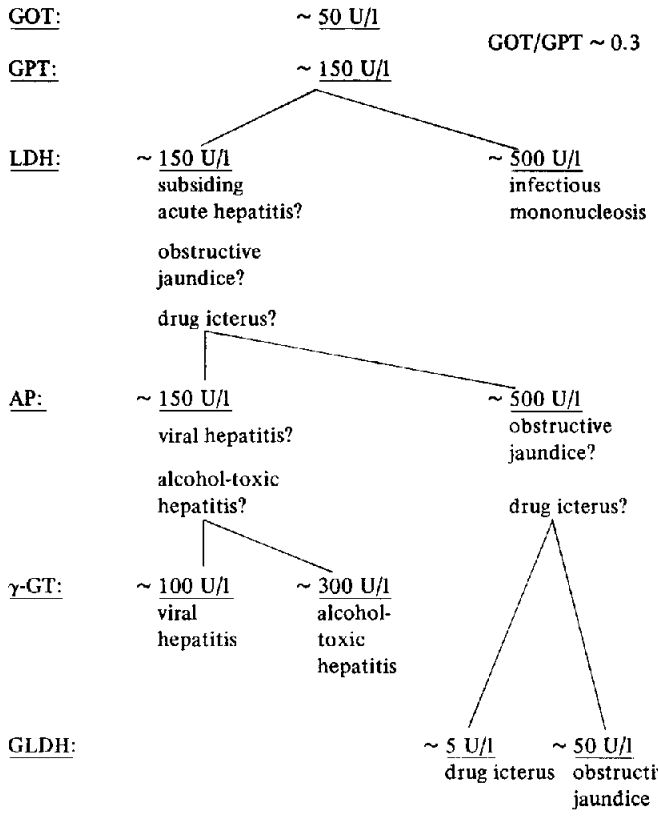


Fig.19. Enzyme ratios in serum for the diagnosis of the type of liver injury.

e.g. hormones. Probably, enzyme immunoassays in a restricted sense will facilitate or even make possible to enlarge the diagnostic set by such enzymes, which would give significant information in disease states, but whose activity determinations are too tricky for routine.

The search for further enzymes which might complement the enzyme patterns used hitherto – tiny in view of the real number of enzymes – constitutes a third area of work. Under clinical aspects, only such enzymes that are likely to supply new information and fill genuine gaps in today's diagnostic possibilities are worth investigating. Therefore, success in this field presupposes endeavors in biochemistry, physiology and pathophysiology as well. More than the metabolic function of an enzyme is taken into consideration, the cellular origin, its site of function, its molecular forms and, last but not least, its behavior in distinct disorders, are the main points of interest. It appears that by now the easily accessible enzymes are exploited for medicine. The investigation of special enzymes, e.g. of the cell surface, of the replication apparatus, or of the antibody formation system, will, of course, be difficult, but seems promising. There is no reason for fatigue or resignation, on the contrary, a challenge for co-operation in further developmental work.

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